



Full Length Article

Meta-Analysis of the Impact of Garlic Supplementation on Aspects of Egg Yolk Quality Characteristics of Laying Hens

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Abstract

Phytogenic feed additives (PFAs) have been highlighted to increase animal performance and product quality. However, the effect of garlic (*Allium sativum* L), one of such PFAs on the internal egg quality indices of laying hens is less clear. As a result, this meta-analysis investigated the impact of garlic supplementation on egg yolk weight (EYW) and egg yolk cholesterol (EYC) content in laying hens. A search carried out in three online databases yielded five hundred and ten studies of which fifteen passed the predefined selection conditions for the meta-analysis. Inclusion criteria were information on moderator variables (hen's age, layer strains, presentation form, treatment duration, inclusion level, and the number of hens used for the study), and outcome measures (EYW and EYC) with their corresponding measures of variance. All analyses were performed in OpenMEE software. Results indicate that addition of garlic to layer diets increased EYW by 0.063 g [95% confidence interval (CI) = 95% CI: -0.051, 0.176] compared to zero, though the difference was not statistically significant. Similarly, garlic supplementation reduced EYC concentrations by 0.641 mg/g yolk (95% CI: -0.059, -0.424) in comparison with controls, taking publication bias and heterogeneity into consideration. Subgroup and meta-regression analyses revealed that modifiers had impact on the results and conclusion. In conclusion, our results suggest that garlic supplementation improved egg yolk quality parameters in laying hens. © 2022 Friends Science Publishers

Keywords: Egg quality; Phytogenics; Garlic; Layers; Meta-analysis

Introduction

The world population is increasing and is expected to double by 2050 (UN 2013). This calls for an increase in the supply of animal products to prevent food scarcity in the future. One such animal product is the egg, which is high in essential nutrients. However, the consumer's concerns about egg consumption are linked to issues such as high cholesterol and saturated fatty acids content which are risk factors for the occurrence of cardiovascular diseases (CVD), diabetes and colon cancer in humans (John *et al.* 2009). Besides, eating to satisfy hunger or reduce the incidence of nutrient deficiency diseases; consumers now regard food as a vital channel for maintaining good health (Hasler 2000). It has been recommended that people at high risk of CVD should limit their cholesterol intake to less than 200 mg/day, while those at low risk should reduce their cholesterol intake to a level below 300 mg per day (Krauss *et al.* 2001). About two-thirds of the dry matter in egg yolk is low-density lipoprotein (Li-Chan *et al.* 1995).

As a result of the growing adverse consequences of high cholesterol and saturated fat intakes on human health, new initiatives in the livestock and pharmaceutical industries are attempting to promote the use of plant-based products in

improving animal product quality. Tropical countries have a rich plant diversity possessing medicinal and nutritional properties that have the potential to lower cholesterol levels in animal products (Ogbuewu *et al.* 2019). Garlic, a tropical medicinal plant, contains beneficial bioactive compounds that may be capable of lowering the high saturated fat contents in animal products (Hayat *et al.* 2016; Kimura *et al.* 2017). The influence of addition of garlic to layer diets has been extensively researched, with variable results (Canogullari *et al.* 2009; Mahmoud *et al.* 2010; Khan *et al.* 2008). Meta-analysis, statistical analysis that combines the findings of multiple scientific articles to increase statistical power, rectify conflicting findings, open new research areas, and generates new research insights, has been advocated for in resolving conflicting evidence (Ogbuewu *et al.* 2021). This study aimed to explore the impact of garlic additive on egg yolk parameters in laying hens using meta-analysis.

Materials and Methods

Development of database

Scopus, AGORA, and Google Scholar databases were systematically searched for scientific articles that assessed

the effect of diets with and without garlic feed additive on yolk parameters quality in layers. The search was executed using the combinations of several search terms and keywords. Included studies satisfied the following predefined selection conditions: the study evaluated egg yolk quality parameters in layers. Included article must at least record one of the variables of interest (EYC or EYW) in healthy laying hens. In addition, included study provided quantitative data on EYC and/or EYW, and has a measure of dispersion (SE or SD). Papers that reviewed the effect of garlic on yolk quality indices in layers were discarded. Studies not in laying hens were removed. Studies not in our measured outcomes were excluded. Studies that mixed garlic with other feed additives were also excluded. Five hundred and ten articles were identified from the systematic search performed on three online databases and fifteen scientific articles satisfied the predefined eligibility conditions as illustrated in Fig. 1.

Statistical analysis

Datasets of the 15 scientific articles that satisfied the predefined eligibility conditions were used. Mean effect size was expressed as standardised mean difference (SMD) with 95% CI. All analyses were performed in OpenMEE software (Wallace *et al.* 2016). The existence of publication bias was examined using Rosenberg's fail-safe number (Nfs) and funnel graph. Mean effect estimate was deemed robust in spite of the existence of publication bias when $Nfs > 5 * n + 10$ (Jennions *et al.* 2013), where n is number of studies. Heterogeneity was computed using standard methods. Studies considered to have undue influence on the SMD were detected using sensitivity analysis (Wallace *et al.* 2016). Subgroup analysis was restricted to treatment duration (6–22 weeks), inclusion level (1–80 g garlic/kg feed), presentation form (powder, oil, and extract), layer strains (Hisex, Dekalb white, Native desi, Lohmann, Isa Brown, Aryan, White Leghorn, Tetra-SL, White Novogen, SHSY-type brown, Hyline white and Rosa 1), hen's age (18–85 weeks) and the number of layers used for the study (40–240). Modifiers used in subanalysis were also used in meta-regression analysis. Based on the values used by the authors whose studies were used for the analysis, the studied moderators were categorised as follows: treatment duration (≤ 10 and >10 weeks), inclusion level (≤ 10 g per kg ration and >10 g per kg ration), hen's age (≤ 30 weeks and > 30 weeks), and number of layers utilised for the trials (< 100 and > 100) based the values used by the authors whose studies were used for the analysis. Mean effect size is said to be significant when the confidence interval does not include zero. Meta-regression and heterogeneity were deemed significant at $p < 0.05$.

Results

The characteristics of the 15 studies included in the meta-

analysis are presented in Table 1. Results showed that birds fed garlic had numerically higher EYW (SMD = 0.063 g; - 0.051, 0.176; $I^2 = 36.24\%$, $P = 0.021$; Fig. 2) compared to controls. Stratification analyses reporting the impact of garlic supplementation on EYW are described in Table 2. Studied moderators had no statistically significant influence on EYW. Results revealed that Poltowicz and Wesyk (2006) had an undue influence on the pooled result, and its exclusion solved the problem of between-study variance (SMD = 0.072 g; -0.058, 0.201; $I^2 = 0\%$, $P = 0.699$; Fig. 3). We found significant linear relationship between layer strain/breed and garlic feed additive, and layer breed/strain explained about 65% of the heterogeneity (Table 3). Funnel plot was near symmetry and showed minimal evidence of publication bias (Fig. 4).

Garlic supplementation significantly reduced yolk cholesterol content (SMD = -0.641 mg/g yolk; -0.059, -0.424; $I^2 = 77.85\%$; $P < 0.001$; Fig. 5) relative to controls, with evidence of significant between-study variance ($I^2 = 77.85\%$; $Q_B = 12.51$; $P < 0.001$; Fig. 5). We were unable to analyse the effect of garlic on low-density and high-density lipoprotein cholesterol content on egg yolk in this meta-analysis because of insufficient published data. Restricted subgroup analysis as illustrated in Table 2 showed that layers fed garlic powder, garlic oil, and extract had significantly lower EYC compared to zero. However, the magnitude of response was highest in garlic powder followed by garlic oil and extract. Chickens aged less than 30 weeks or more at the beginning of feeding trial offered diets having garlic at ≤ 10 and > 10 g/kg feed for ≤ 10 and > 10 weeks had significantly decreased egg yolk cholesterol levels in comparison with controls. Similarly, Novogen white, Rosa 1, and native desi layers from experiments that used < 100 and > 100 birds in their study had significantly lower egg yolk cholesterol levels than controls. The problem of significant heterogeneity was not solved by sensitivity and subgroup analyses. However, meta-regression found significant linear relationship between EYC and layer strains ($Q_B = 0.652$, $P = 0.002$), and layer strains reduced the I^2 value by 10% (Table 3). Funnel plot demonstrated minimal evidence of publication bias (Fig. 6) and the Nfs for the database is 484 is higher than 145 needed to declare the SMD robust.

Discussion

Five hundred and ten candidate primary studies were identified and retrieved for the experiment, of which fifteen studies comprising fulfilled the eligibility conditions for inclusion in the study. The publications utilized in the investigation span 15 years and ranged from 2004 to 2019. The studies were conducted in 10 countries that cut across three continents (Asia, Africa, and Europe).

Animal products with lower cholesterol and triacylglycerol contents command a higher price compared to those with higher fat contents. Given this, the livestock

Table 1: Characteristics of studies used for investigation

References	Country	Explanatory moderator variables						Outcomes
		Layer strain	Hen's age (week)	Garlic form	Inclusion level (g/kg feed)	Treatment duration (week)	Number of birds	
Adebiyi <i>et al.</i> (2017)	Nigeria	Isa Brown	≤ 30	Powder	> 10	> 10	< 100	EYC
Alfadhli <i>et al.</i> (2012)	Iraq	Lohmann brown	≤ 30	Oil	≤ 10	≤ 10	< 100	EYW
Alfadhli <i>et al.</i> (2012)	Iraq	Lohmann brown	≤ 30	Oil	≤ 10	≤ 10	< 100	EYW
Alfadhli <i>et al.</i> (2012)	Iraq	Lohmann brown	≤ 30	Oil	≤ 10	≤ 10	< 100	EYW
Alfadhli <i>et al.</i> (2012)	Iraq	Lohmann brown	≤ 30	Oil	≤ 10	≤ 10	< 100	EYW
Alfadhli <i>et al.</i> (2012)	Iraq	Lohmann brown	≤ 30	Oil	≤ 10	≤ 10	< 100	EYW
Behnamifar <i>et al.</i> (2015)	Iran	Tetra-SL	> 30	Extract	≤ 10	≤ 10	< 100	EYW, EYC
Canogullari <i>et al.</i> (2009)	Turkey	Hyline white	> 30	Powder	≤ 10	> 10	> 100	EYW
Canogullari <i>et al.</i> (2009)	Turkey	Hyline white	> 30	Powder	≤ 10	> 10	> 100	EYW
Canogullari <i>et al.</i> (2009)	Turkey	Hyline white	> 30	Powder	> 10	> 10	> 100	EYW
Hadj Ayed <i>et al.</i> (2018)	Tunisia	White Novogen	≤ 30	Powder	≤ 10	≤ 10	> 100	EYW, EYC
Hadj Ayed <i>et al.</i> (2018)	Tunisia	White Novogen	≤ 30	Powder	≤ 10	≤ 10	> 100	EYC
Hadj Ayed <i>et al.</i> (2018)	Tunisia	White Novogen	≤ 30	Powder	≤ 10	≤ 10	> 100	EYC
Hadj Ayed <i>et al.</i> (2018)	Tunisia	White Novogen	≤ 30	Powder	≤ 10	≤ 10	> 100	EYC
Hatice and Muhlis (2012)	Turkey	Lohmann white	> 30	Powder	> 10	> 10	> 100	EYC
Hatice and Muhlis (2012)	Turkey	Lohmann white	> 30	Powder	> 10	> 10	> 100	EYC
Hatice and Muhlis (2012)	Turkey	Lohmann white	> 30	Powder	> 10	> 10	> 100	EYC
Khan <i>et al.</i> (2008)	Pakistan	Native desi	≤ 30	Powder	> 10	≤ 10	< 100	EYC
Khan <i>et al.</i> (2008)	Pakistan	Native desi	≤ 30	Powder	> 10	≤ 10	< 100	EYC
Khan <i>et al.</i> (2008)	Pakistan	Native desi	≤ 30	Powder	> 10	≤ 10	< 100	EYC
Kolawole & Folake (2019)	Nigeria	Isa-Brown	≤ 30	Powder	≤ 10	> 10	< 100	EYW
Kolawole & Folake (2019)	Nigeria	Isa-Brown	≤ 30	Powder	≤ 10	> 10	< 100	EYW
Kolawole & Folake (2019)	Nigeria	Isa-Brown	≤ 30	Powder	> 10	> 10	< 100	EYW
Mahmoud <i>et al.</i> (2010)	Jordan	Hi-sex	> 30	Extract	≤ 10	≤ 10	< 100	EYW, EYC
Mahmoud <i>et al.</i> (2010)	Jordan	Hi-sex	> 30	Extract	≤ 10	≤ 10	< 100	EYW, EYC
Mahmoud <i>et al.</i> (2010)	Jordan	Hi-sex	> 30	Extract	≤ 10	≤ 10	< 100	EYW
Mottaghtalab and Taraz (2004)	Iran	Aryan	> 30	Powder	≤ 10	≤ 10	> 100	EYC
Olobatoke and Mulugeta (2011)	SA	Dekalb white	≤ 30	Powder	≤ 10	≤ 10	< 100	EYW
Olobatoke and Mulugeta (2011)	SA	Dekalb white	≤ 30	Powder	≤ 10	≤ 10	< 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Oil	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Extract	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Oil	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Extract	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Oil	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Oil	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Oil	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Extract	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Oil	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Extract	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Oil	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Extract	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Oil	≤ 10	≤ 10	> 100	EYW
Poltowicz and Wesyk (2006)	Poland	Rosa 1	> 30	Extract	≤ 10	≤ 10	> 100	EYW
Rahimi <i>et al.</i> (2006)	Iran	White Leghorn	> 30	Powder	> 10	≤ 10	< 100	EYC
Tesfaheywet <i>et al.</i> (2017)	Ethiopia	White Leghorn	> 30	Powder	≤ 10	> 10	> 100	EYC
Tesfaheywet <i>et al.</i> (2017)	Ethiopia	White Leghorn	> 30	Powder	> 10	> 10	> 100	EYC
Tesfaheywet <i>et al.</i> (2017)	Ethiopia	White Leghorn	> 30	Powder	> 10	> 10	> 100	EYC
Yalcin <i>et al.</i> (2006)	Turkey	SHSY-type brown	≤ 30	Powder	≤ 10	> 10	> 100	EYW, EYC
Yalcin <i>et al.</i> (2006)	Turkey	SHSY-type brown	≤ 30	Powder	≤ 10	> 10	> 100	EYW, EYC

SA = South Africa; d = day; EYW = Egg yolk weight; EYC = Egg yolk cholesterol

industry is in dire need of natural and safe products with the potential to enhance animal product quality. Hence, it has become pertinent to ascertain the efficacy of tropical medicinal plants in improving animal product quality. In the current study, we employed meta-analysis to ascertain the impact of diets with and without garlic on egg yolk quality indices in laying hens using fifteen peer-reviewed studies. Pharmacological active ingredients present in garlic have been reported to possess a wide range of biological activities

in animal models and humans (Hayat *et al.* 2016; Kimura *et al.* 2017). Pooled results showed that feeding garlic to layers did not increase EYW when compared to controls which disagree with the findings of Rahimi *et al.* (2006) that garlic intake significantly increased EYW in layers. The observed claim by Rahimi *et al.* (2006) that garlic significantly increased yolk size could be attributed to the layer breed used as the present meta-analysis found a significant relationship between breed and EYW. The comparable

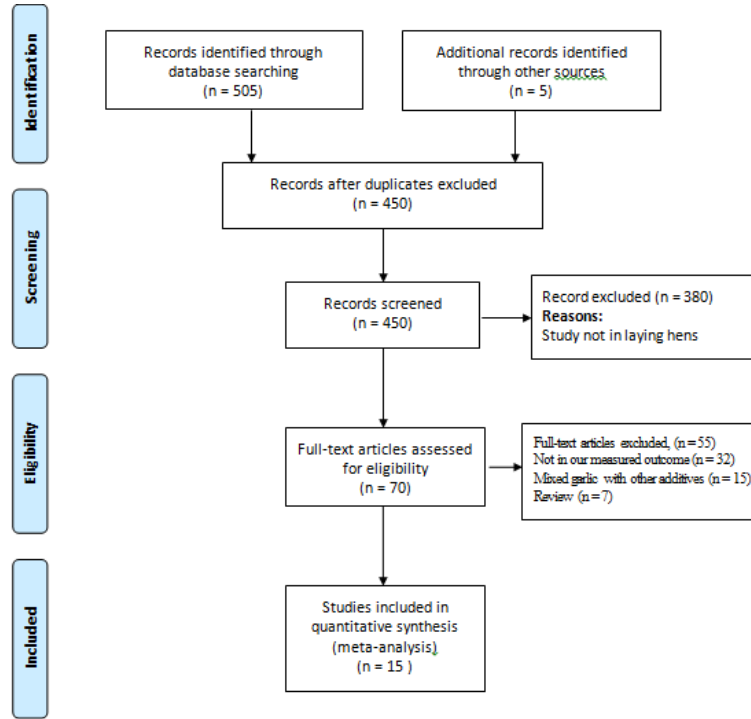


Fig. 1: Article selection flow chart

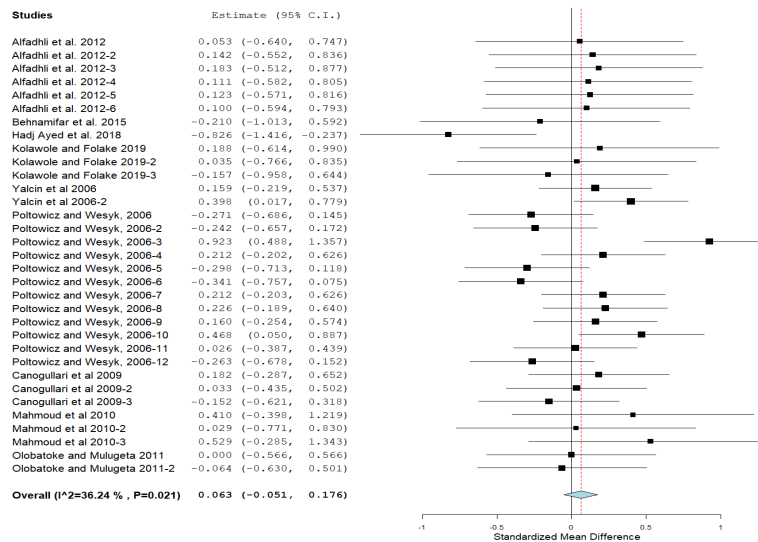


Fig. 2: Forest plot of effect of garlic on egg yolk weight

EYW can be ascribed to the capacity of garlic supplement to support vitellogenins synthesis and secretion in the liver, and its subsequent transport to the ovary where they are rapidly absorbed by the developing oocytes.

Evidence abounds both in animal and human studies that hepatic and plasma cholesterol concentrations are affected by diet types (Yalcin *et al.* 2006). Cholesterol synthesis in chickens occurs principally in the liver, with the highest amount found in the egg yolk (Ryś *et al.* 1996). In the present analysis, we found significantly reduced yolk

cholesterol concentrations in layers offered diets containing varying doses of garlic when compared to the control hens, which contrasted with the increased EYC levels reported by Tesfahyewet *et al.* (2017) in laying chickens offered rations having garlic at 1.0 and 6.0%. The observed difference could be linked to low analytical power arising from the use of small sample size in statistical analysis which according to Smith and Bryant (1975) limits the possibility of ascertaining the real effect of an intervention. One advantage of meta-analysis over the primary study is that

Table 2: Subgroup analysis of the effect of garlic supplementation on egg yolk weight and cholesterol content

Subgroups	Egg yolk weight						Egg yolk cholesterol level					
	SMD	95% CI	SE	P-val*	I ² (%)	P-val ⁺	SMD	95% CI	SE	P-val*	I ² (%)	P-val ⁺
Age of chickens (weeks)												
≤ 30	0.078	-0.109, 0.265	0.096	0.413	11.83	0.329	-0.618	-0.900, -0.336	0.144	< 0.001	82.32	<0.001
> 30	0.062	-0.082, 0.206	0.073	0.396	46.82	0.010	-0.721	-0.942, -0.502	0.112	< 0.001	16.84	0.301
Presentation form												
Powder	0.020	-0.166, 0.206	0.095	0.832	26.35	0.193	-0.723	-1.035, -0.412	0.159	< 0.001	81.81	<0.001
Extract	0.048	-0.163, 0.259	0.108	0.655	40.20	0.090	-0.335	-0.573, -0.097	0.121	0.006	14.15	0.324
Oil	0.120	-0.089, 0.328	0.106	0.260	47.32	0.035	-0.680	-1.092, -0.269	0.210	0.001	64.07	0.062
Inclusion level (g/kg feed)												
≤ 10	0.072	-0.051, 0.195	0.063	0.250	41.54	0.010	-0.628	-0.882, -0.374	0.130	< 0.001	75.11	<0.001
> 10	-0.057	-0.415, 0.301	0.183	0.755	0.000	0.952	-0.672	-1.090, -0.255	0.213	0.002	82.48	<0.001
Treatment duration (weeks)												
≤ 10	0.085	-0.110, 0.279	0.099	0.393	56.53	0.002	-0.611	-0.809, -0.413	0.101	< 0.001	51.32	0.009
> 10	0.052	-0.074, 0.178	0.064	0.418	0.000	0.687	-0.668	-1.120, -0.216	0.230	0.004	88.45	<0.001
Number of birds												
< 100	0.085	-0.099, 0.269	0.094	0.363	0.000	0.999	-0.889	-1.267, -0.511	0.193	< 0.001	20.20	0.276
> 100	0.047	-0.120, 0.214	0.085	0.583	63.77	<0.001	-0.578	-0.825, -0.331	0.126	< 0.001	82.19	<0.001
Chicken strain												
Lohmann	0.119	-0.165, 0.402	0.144	0.412	0.000	1.000	-0.697	-1.506, 0.111	0.412	0.091	85.60	0.001
Isa-Brown	0.022	-0.441, 0.484	0.236	0.926	0.000	0.836						
Rosa 1	0.066	-0.146, 0.277	0.108	0.543	67.55	<0.001	-0.467	-0.750, -0.184	0.144	0.001	63.03	0.019
Hyline white	0.021	-0.250, 0.29 2	0.138	0.878	0.000	0.614						
Hisex	0.320	-0.146, 0.786	0.238	0.179	0.000	0.668						
White Novogen							-0.589	-0.879, -0.299	0.148	< 0.001	0.000	0.579
Native desi							-1.169	-1.814, -0.523	0.329	< 0.001	26.40	0.257
White leghorn							-0.238	-1.065, 0.59 0	0.42 2	0.574	92.68	<0.001

SMD = standardised mean difference, * = probability value for the SMD, I² = Inconsistency index; + = probability value for the I² – statistic; SE = standard error; CI = confidence interval

Table 3: Relationships between moderators and yolk characteristics in laying hens

Covariates	df	Yolk weight			Yolk cholesterol levels		
		Q _M	P-value	R ² (%)	Q _M	P-val	R ² (%)
Hens' age	1.000	0.020	0.997	0.000	0.210	0.651	0.000
Layer strain	9.000	0.650	0.002	65.00	11.00	0.045	10.00
Garlic form	2.000	0.560	0.755	0.000	1.300	0.522	0.000
Inclusion level	1.000	0.330	0.565	0.000	0.010	0.909	0.000
Treatment duration	1.000	0.120	0.727	0.000	0.010	0.931	0.000
Number of birds	1.000	0.091	0.767	0.000	1.410	0.236	1.000

R²: Heterogeneity accounted by moderators; df: degree of freedom; Q_M: Test of moderators

meta-analysis increases sample sizes by pooling the results of several individual primary studies thus increasing the chance of detecting the true effect of an intervention. The mechanism of action of garlic in lowering EYC contents in laying hens is not clear. However, there are some hypotheses that phytochemicals may have down-regulatory effects on the hepatic hydroxymethylglutaryl-CoA reductase, the key enzyme in cholesterol biosynthesis in the hepatocytes (Nelson *et al.* 2008). One possible mechanism which garlic lower egg yolk cholesterol content by inhibiting cholesterol biosynthesis in the liver and increasing bile acid excretion as free cholesterol through the faeces (Nelson *et al.* 2008).

Subanalysis showed that garlic powder had the highest magnitude of effect size, followed by garlic oil and extract. This indicates that garlic powder and oil are more effective in lowering EYC content. As a result, it is suggested that farmers consider using garlic powder to improve egg yolk cholesterol content. Results also noticed differences in genetics within the layer strains investigated in this meta-

analysis with Lohmann brown and White Novogen fed diets supplemented with garlic laying eggs with lower EYC content than the Rosa 1, suggesting that farmers may consider using Lohmann and White Novogen strains for improved egg yolk quality. We found evidence of significant heterogeneity across the articles included in this data synthesis study as demonstrated by the I² statistic and sensitivity analysis fixed the issue of heterogeneity. However, sensitivity analysis did not solve the challenge of heterogeneity for EYC. Meta-regression test showed that layer strain/breed reduced the I² value by 10–65%, implying the capacity of garlic to reduce yolk cholesterol levels may differ amongst layer strains. No relationship was found between outcomes and other studied modifiers, implying absence linear relationship between yolk quality parameters and studied moderators.

This data synthesis included studies carried in ten study countries spanning three continents and the conclusion drawn from this analysis are valid. Publication bias threatens the conclusions drawn in meta-analysis and it

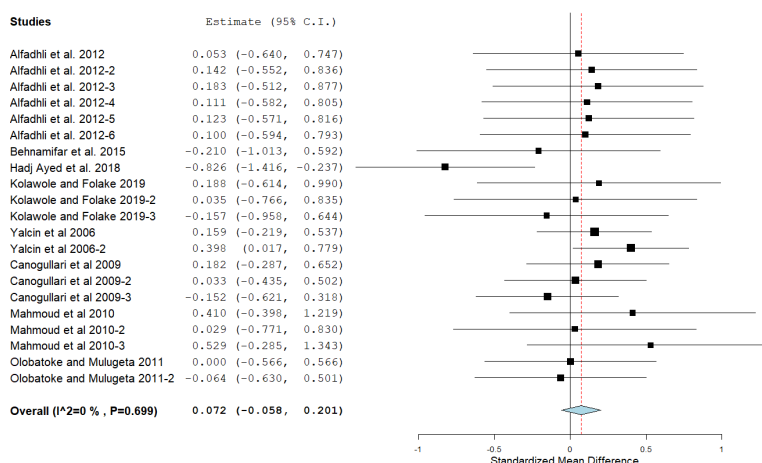


Fig. 3: Forest plot of effect of garlic on egg yolk weight after removal of a study considered to have undue influence on the SMD.

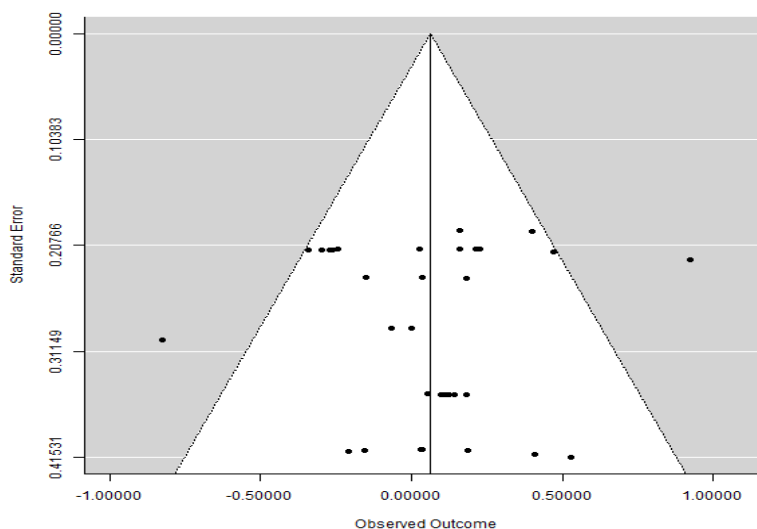


Fig. 4: Funnel plot of effect of garlic on egg yolk weight

usually arises because of journal editors' preference to publish studies with significant positive findings, while rejecting studies with no significant results (Dickerson 2005). In this study, there is presence of publication bias. However, this is not a problem as Nfs suggests that a relatively large number of negative trials or unpublished results would be needed to change the significant effect of garlic on EYC.

Conclusion

Our results suggest the ability of garlic to lower EYC content in laying hens, thus positioning garlic as a feed additive with the potential for industrial application and commercialization in the poultry industry. In addition, studies that examined the impact of garlic additive on low- and high-density lipoprotein cholesterol are lacking in the literature, and more research is needed in

this area. Results found that layer strain/breed explained about 10–65% of variations between studies analysed, implying the cholesterol-lowering ability of garlic on egg yolk depends on the layer strain used in the study. Chickens offered garlic at ≤ 10 and > 10 g/kg feed had comparable egg yolk parameters when compared to controls. As a result, more research effort should be directed to ascertain the most effective dosage schedule that optimizes egg yolk cholesterol level in layers using quadratic optimization model regression. Stratification analysis found differences in cholesterol lowering ability among the three forms of garlic additives analysed, with garlic powder having the best cholesterol-reducing effect, followed by garlic oil. It was also discovered that Lohmann laid eggs with the lowest yolk cholesterol content followed by White Novogen and Rosa 1. For improved yolk cholesterol content, farmers may be advised to use Lohmann layers.

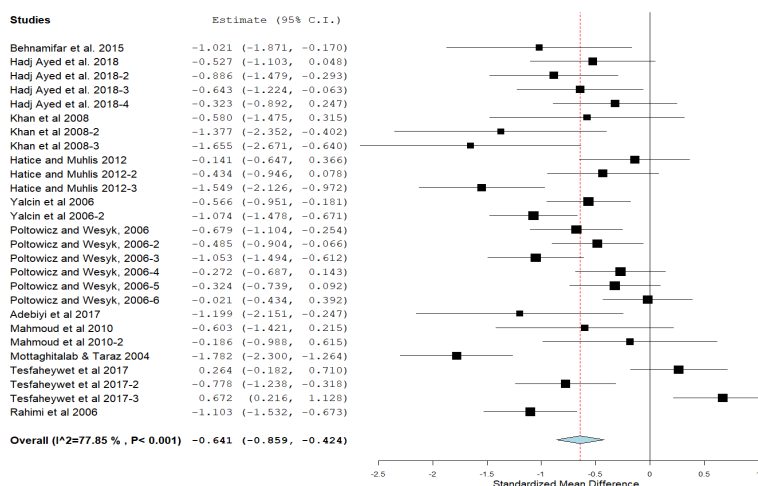


Fig. 5: Forest plot of effect of garlic on egg yolk cholesterol content

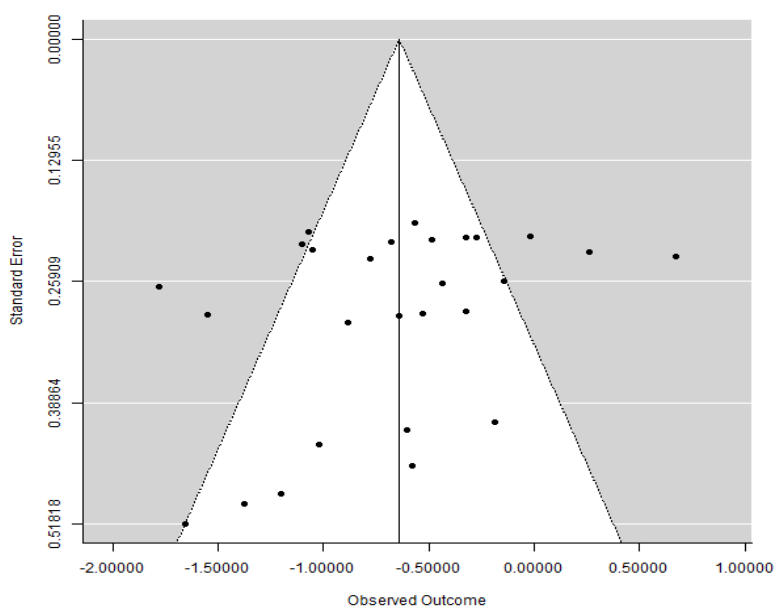


Fig. 6: Funnel plot of effect of garlic on egg yolk cholesterol content

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Author Contributions

IPO and CAM conceived the study, statistically analysed the data and wrote the manuscript. The final manuscript was read and approved by the authors.

Conflicts of Interest

Authors had no conflict of interest to declare.

Data Availability

All the data used for the meta-analysis are fully available as Figs and tables without restriction.

Ethics Approval

There is no ethical approval letter since this is a meta-analytic study.

References

Adebijei FG, AD Ologhobo, IO Adejumo (2017). Modulation of cholesterol in laying chickens fed sun-dried garlic powder. *J Exp Agric Intl* 19:1-7

- Alfadhli MKM, AT Wali, HK Shukair, MJ Ahmed (2012). Effect of garlic oil in some qualitative characteristics of laying hens eggs. *Intl J Adv Biol Res* 2:653–656
- Ayed MH, A Aïssa, M Noumi (2018). A comparative study between the effects of feed inclusion with garlic (*Allium sativum*), cloves and turmeric (*Curcuma longa*) rhizome powder on laying hens' performance and egg quality. *Iran J Appl Anim Sci* 8:693–701
- Behnamifar A, S Rahimi, MAK Torshizi (2015). Effect of probiotic, thyme, garlic and caraway herbal extracts on the quality and quantity of eggs, blood parameters, intestinal bacterial population and histomorphology in laying hens. *J Med Plants By-prod* 1:121–128
- Canogullari S, M Karaman, Z Erdogan, M Baylan, A Kucukgul, V Duzguner, AK Ozugur (2009). Effect of garlic powder on egg yolk and serum cholesterol and performance of laying hens. *Bull Vet Inst Pulawy* 53:515–519
- Dickerson K (2005). Publication bias: Recognizing the problem, understanding its origins and scope, and preventing harm. In: *Publication Bias in Meta-analysis: Prevention, Assessment, and Adjustments*, pp:11–34. Rothstein HR, AJ Sutton, M Borenstein (Eds.). Chichester, UK
- Hadj Ayed M, A Aïssa, M Noumi (2018). A comparative study between the effects of feed inclusion with garlic (*Allium sativum*), cloves and turmeric (*Curcuma longa*) rhizome powder on laying hens' performance and egg quality. *Iran J Appl Anim Sci* 8: 693–701
- Hasler CM (2000). The changing face of functional foods. *J Amer Coll Nut* 19:499–506
- Hatice K, M Muhlis (2012). Effect of inclusion of garlic (*Allium sativum*) powder at different levels and copper into diets of hens on performance, egg quality traits and yolk cholesterol content. *Intl J Poult Sci* 11:114–119
- Hayat S, Z Cheng, H Ahmad, M Ali, X Chen, M Wang (2016). Garlic, from remedy to stimulant: Evaluation of antifungal potential reveals diversity in phytoalexin allicin content among garlic cultivars; allicin containing aqueous garlic extracts trigger antioxidants. *Front Plant Sci* 7:1235
- Jennions MD, CJ Lortie, MS Rosenberg, HR Rothstein (2013). Chapter 14: Publication and related bias. In: *Handbook of meta-analysis in Ecology and Evolution* Koricheva J, J Gurevitch, K Mengersen. (Eds.), pp:207–236. Princeton University Press, Princeton and London
- John C, L Thomas, K Patrick (2009). *The European Society of Cardiology Textbook of Cardiovascular Medicine*, p:257. Blackwell Publishing, Oxford, UK
- Khan SH, S Hasan, R Sardar, MA Anjum (2008). Effect of dietary garlic powder on cholesterol concentration in native Desi laying hens. *Amer J Food Tech* 3:207–213
- Kimura S, YC Tung, MH Pan, NW Su, YJ Lai, KC Cheng (2017). Black garlic: A critical review of its production, bioactivity, and application. *J Food Drug Analgesic* 25:62–70
- Kolawole FSO, AO Folake (2019). Egg traits and productive performance of Isa-brown laying hens fed garlic supplemented diets. *Asian J Appl Sci* 7:398–403
- Krauss RM, RH Eckel, B Howard (2001). A Statement for healthcare professionals from the nutrition committee of the American Heart Association. *J Nutr* 131:132–146
- Li-Chan EC, WD Powrie, S Nakai (1995). The chemistry of eggs and egg products. In: *Egg Science and Technology*, pp:1–71. William JS, JC Owen (Eds.). CRC Press, Boca Raton, USA
- Mahmoud KZ, SM Gharaibeh, HA Zakaria, AM Qatramiz (2010). Garlic (*Allium sativum*) supplementation: Influence on egg production, quality, and yolk cholesterol level in layer hens. *Asian-Aust J Anim Sci* 23:1503–1509
- Mottaghitab M, Z Taraz (2004). Effects of garlic (*Allium sativum*) on egg yolk and blood serum cholesterol in Aryan breed laying hens. *Brit Poult Sci* 43:42–43
- Nelson DL, AL Lehninger, MM Cox (2008). *Lehninger Principles of Biochemistry*, Macmillan Learning, New York, USA
- Ogbuewu IP, VMO Okoro, EF Mbajorgu, CA Mbajorgu (2019). Beneficial effect of garlic (*Allium sativum*) in livestock and poultry nutrition: A review. *Agric Res* 8:411–426
- Ogbuewu IP, VMO Okoro, CA Mbajorgu (2021). Meta-analysis of the responses of laying hens to phytoantic (*Allium sativum*) supplementation. *Anim Feed Sci Tech* 275:114866
- Olobatoko RY, SD Mulugeta (2011). Effect of dietary garlic powder on layer performance, fecal bacterial load, and egg quality. *Poult Sci* 90:665–670
- Poltowicz K, S Wesyk (2006). Effect of garlic oil added to feed and water of laying hens on table egg quality. *12th European Poultry Conference, at Verona, Italy*. Book of abstracts: 150; Abstracts & Proceedings CD: 10359. Verona, Italy. Available from <https://www.cabi.org/Uploads/animal-science/worlds-poultry-science-association/WPSA-italy-2006/10359.pdf>
- Rahimi SH, A Rafiei, H Lotfollahian, A Afshamaderi (2006). Influence of combined usage of garlic powder and copper on egg yolk cholesterol concentration in laying hens. *J Vet Res* 63:1–6
- Ryś R, M Kuchta, J Koreleski, Z Zegarek (1996). The influence of nutritional factors on the cholesterol content in hen's egg yolk. *Sci Ann Zootechn* 23:167–186
- Smith RJ, RG Bryant (1975). Metal substitution in carbonic anhydrase: A halide ion probe study. *Biochem Biophys Res Comm* 66:1281–1286
- Tesfaheywet Z, A Meseret, A Negassi, U Mengistu (2017) Effects of supplementation of different levels of garlic (*Allium sativum*) on selected blood profile and immunity of white leghorn chickens. *Biotech Anim Husb* 33:333–348
- United Nation's report (2013). "World population projected to reach 9.6 billion by 2050 – UN report". UN News Centre. June 14, 2013. <https://news.un.org/en/story/2013/06/442212>. Accessed June 16, 2020
- Wallace BC, MJ Lajeunesse, G Dietz, IJ Dahabreh, TA Trikalinos, CH Schmid, J Gurevitch (2016). OpenMEE: Intuitive, open-source software for meta-analysis in ecology and evolutionary biology. *Methods Ecol Evol* 8:941–947
- Yalcin S, EE Onbasilar, Z Reisli, S Yalcin (2006). Effect of garlic powder on the performance, egg traits and blood parameters of laying hens. *J Sci Food Agric* 86:1336–1339