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Research article

Effect of pepper *(Capsicum sp)* on productivity and egg quality of laying hens: a meta-analysis

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Abstract

The restriction on the use of antibiotics as a growth promotant in poultry diets, due to their adverse effects, has led to increased use of feed additives with natural ingredients such as medicinal plants and herbs. Pepper (Capsicum sp) is a potential feed additive as an antibiotic alternative in laying hen diet. This meta-analysis aimed to determine the effect of pepper supplementation on the performance and egg quality attributes of laying hens. The PubMed, Scopus, Google Scholar, and Science Direct databases were searched for peer-reviewed randomized controlled trials written in English. Information on the moderators (hen age at the beginning of the intervention, pepper preparation form, inclusion level of pepper, and treatment duration) were required for inclusion in the meta-analysis, as well an acceptable explanation of randomization, statistical analysis of egg production and quality, and associated variance measurements, such as standard deviation or standard error. The meta-analysis included 19 research papers that met the criteria. Open meta-analyst for ecology and evolution (OpenMEE) software was used for all analyses. The pooled results demonstrated that supplementing with pepper reduced feed intake (FI) by −0.44 g/day, increased hen day egg production (HDEP) by 0.71%, egg mass (EM) by 1.1g, eggshell thickness (EST) by 0.32 mm and egg yolk color (EYC) by 5.7 but had no effect on feed convertion ratio (FCR), egg weight (EW) and haugh unit (HU) compared to the control, after considering publication bias and heterogeneity. This meta-analysis indicates that pepper can be used as a feed additive in laying hens to increase egg production and quality.

Keywords: Feed additives, Laying hens performance, Pepper fruit, Yolk color

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INTRODUCTION

Several years ago, antibiotics were commonly used as feed additives in poultry diets to prevent diseases and maximize chicken production, with the aim of improving feed utilization and reducing mortality caused by pathogens (Muaz et al., 2018). Antibiotic growth promoters (AGPs) have been banned in the poultry sector because of antibiotic resistance in bacteria and public awareness of health and food safety concerns (Van et al., 2020; Ali et al., 2021). This circumstance has urged the poultry sector to seek alternatives to AGPs and more sustainable feed management solutions to enhance poultry performance (Abd El-Hack et al., 2022b). Using natural ingredients from herbs is an alternative to antibiotics to increase the productivity of laying hens (Diaz-Sanchez et al., 2015).

Pepper (*Capsicum* sp.) is a potential feed additive for laying hens. This Solanaceae fruit grows in tropical and humid areas. It includes several species, such as *Capsicum annum* and *Capsicum frutescens* (da Silveira Agostini-Costa et al., 2017; de Sá Mendes and Gonçalves, 2020). Dietary supplementation of pepper in laying hen diets was shown to increase egg production, egg weight, and feed conversion of laying hens (Abou-Elkhair et al., 2018). These results may be due to capsaicin compound present in pepper which can act as an antibacterial so as to improve gut health and productivity of laying hens (Gurnani et al., 2016; Chowdhury et al., 2020; Abd El-Hack et al., 2022b). Several studies have demostrated that pepper (*Capsicum* sp.) not only increases egg production but also egg quality of laying hens (Lokaewmanee et al., 2013; Abou-Elkhair et al., 2018; Saleh et al., 2021). Pepper is a source of carotenoids so it can be used as a natural yolk colorant that is safe and potentially healthier for egg consumers than synthetic or artificial colorants (Saleh et al., 2021).

Research on the supplementation of pepper to laying hen rations on productivity and egg quality has been widely conducted. However, the results showed different and ambiguous conclusion. So, meta-analysis is a sophisticated statistical tool for combining the data of disparate research to detect trends, overcome ambiguities, identify knowledge gaps, and develop new insights (Ogbuewu et al., 2021). This study aimed to determine the effect of feed with or without pepper fruit (*Capsicum* sp.) supplementation on egg productivity and quality characteristics using subgroup and meta-regression stratification analyses.

MATERIALS AND METHODS

Literature search and study selection

Studies that had investigated the effects of pepper fruit (*Capsicum sp*.) supplementation on productivity and egg quality characteristics were searched using several scientific web databases, including Scopus (www.scopus.com), PubMed. (pubmed.ncbi.nlm.nih.gov), ScienceDirect (www.sciencedirect.com), and Google Scholar (scholar.google.com). The literature search was not limited by time, and the search terms were “capsicum,” “pepper,” and “laying hens.” The inclusion criteria were a randomized study design that included treatment and control groups, average data from the treatment and control groups were provided, and their variability (standard deviation and standard error), and sample size. The articles included in this meta-analysis must provide data on hen day egg production (HDEP), feed intake (FI), egg weight (EW), feed conversion ratio (FCR), egg mass (EM), eggshell thickness (EST), haugh unit (HU) and egg yolk color (EYC). The explanatory variables in this study included the age of the laying hen, pepper form, pepper level, and duration of administration. Exclusion criteria were limited information regarding the experimental design used and its variability. Based on the searches conducted, 19 studies were identified that met the criteria shown in Figure 1 and Table 1.

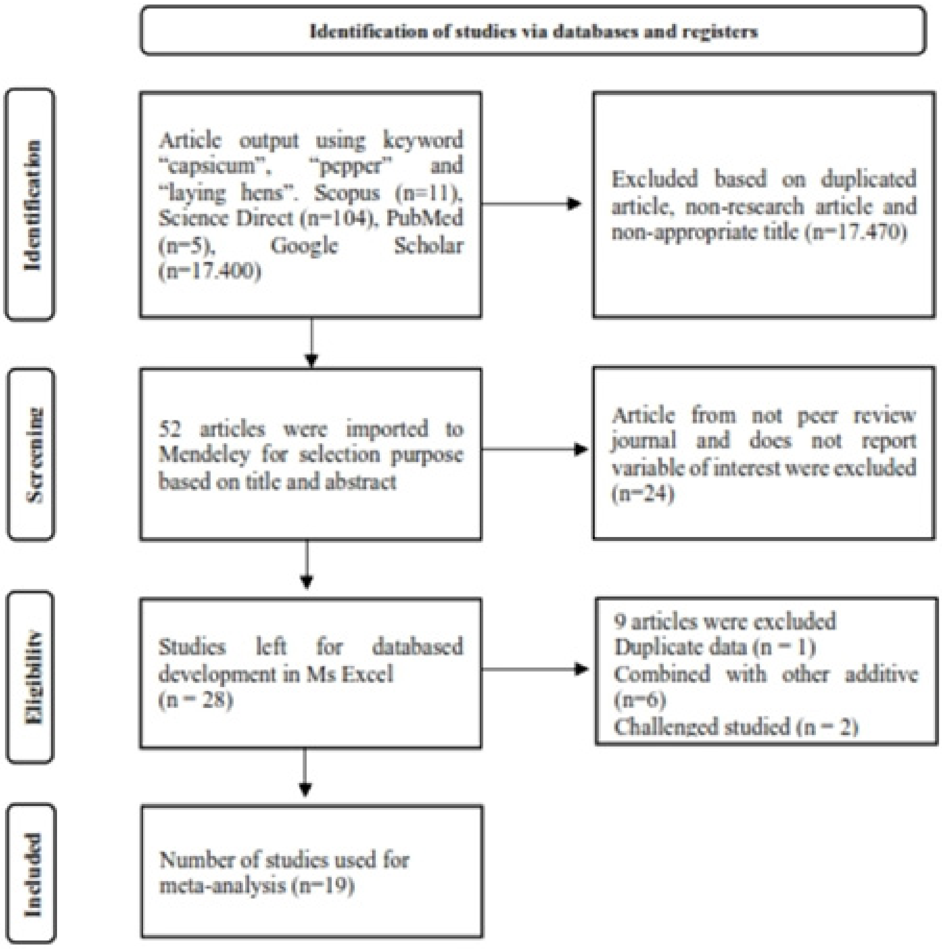

Figure 1 Flow charts of the articles selection process utilized for the meta-analysis

Table 1 Studies considered in the meta-analysis

No.	Reference	Country	Strain	Hen age (week)	Pepper	Form	Level (g/kg)	Duration (weeks)	Variables
1.	(Gurbuz et al., 2003)	Turkey	Hy-Line White	22	*Capsicum annuum* L.	Powder	0, 5, 10, 20, 30, 40	3	FI, HDEP, FCR, EW, EYC
2.	(Al-Harthi 2004)	Saudi Arabia	Hisex	20	*Capsicum annuum* L.	Powder	0, 1, 2, 3	24	HDEP, EW, EST,EYC
3.	(Rowghani et al., 2006)	Iran	Hy-Line White	25	*Capsicum annuum* L.	Powder	0, 5, 10, 20, 30	4	FI, HDEP, FCR, EW, EYC
4.	(Niu et al., 2008)	China	Lohman	53	*Capsicum annuum* L.	Extract	0, 1, 2, 4, 8	4	FI, HDEP, FCR, EW, HU, EYC
5.	(Lokaewmanee et al., 2009)	Japan	Charoen Pokphand brown	25	*Capsicum annuum* L.	Extract	0, 1	3	EW, EST, HU, EYC
6.	(Lokaewmanee et al., 2011)	Japan	Charoen Pokphand brown	25	*Capsicum annuum* L.	Extract	0, 1	3	HDEP, EW, EST, HU, EYC
7.	(Li et al., 2012)	China	Hy-Line Brown	30	*Capsicum frutescens*	Powder	0, 8	2	FI, HDEP, FCR, EW, EYC
8.	(Lokaewmanee et al., 2013)	Japan	Boris Brown	39	*Capsicum annuum* L.	Powder	0, 5	3	FI, HDEP, FCR, EM, EST, HU, EYC
9.	(Moeini et al., 2013)	Iran	Hy-Line W36	103	*Capsicum annuum* L.	Powder	0, 10, 30	9	FCR, EW, EM, HU
10.	(Aderemi et al., 2013)	Nigeria	Isa Brown	126	*Capsicum annuum* L.	Powder	0, 4	10	FI, HDEP, EW, HU
11.	(Abiodun et al., 2014)	Nigeria	Black Harco	20	*Capsicum annuum* L.	Powder	0, 40	6	FI, HDEP, EW, EST, HU, EYC
12.	(Shahsavari 2015)	Iran	Hy-Line W36	40	*Capsicum annuum* L.	Powder	0, 20	12	HDEP, FCR, EW, EST, HU, EYC
13.	(Rossi et al., 2015)	Brazil	Hy-Line W36	73	*Capsicum annuum* L.	Powder	0, 0.08, 0.13, 0.23	16	FI, HDEP, FCR, EW, EW, HU, EYC
14.	(de Oliveira et al., 2017)	Brazil	Hy-Line W36	95	*Capsicum annuum L.*	Extract	0, 6	4	FI, HDEP, FCR, EW, EST, HU, EYC
15.	(Spasevski et al., 2017)	Serbia	Lohman	38	*Capsicum annuum L.*	Powder	0, 15	4	EW, EYC
16.	(Abou-Elkhair et al., 2018)	Egypt	Lohman Brown	32	*Capsicum annuum* L.	Powder	0, 5	4	FI, HDEP, FCR, EW, EM, EST, HU, EYC
17.	(Sözcü 2019)	Turkey	Nick Chick White	78	*Capsicum annuum* L.	Powder	0, 5, 10, 15	10	FI, HDEP, FCR, EW, EM, EST, HU, EYC
18.	(Bala et al., 2020)	Turkey	Lohman Brown	30	*Capsicum annuum* L.	Powder	0, 40	4	HDEP, FCR, EW, EM, EST, HU, EYC
19.	(Saleh et al., 2021)	Egypt	Bovans	42	*Capsicum annuum* L.	Powder	0, 4	12	FI, HDEP, FCR, EW, EM, EST, EYC

Note: FI = feed intake; HDEP = hen day egg production; FCR = feed conversion ratio; EW = egg weight; EM = egg mass; EST = eggshell thickness; HU = haugh unit; EYC = egg yolk color

Data extraction

The form of the pepper, supplementation rate, duration of supplementation, and age of the hens were collected from all articles that satisfied the inclusion criteria. The mean and measures of variance (Standard deviation or Standard error) of the outcome variables of interest from the treatment and control groups, the first author's surname, and the year of publication were extracted. Standard error (SD) values were transformed to standard deviation (SD) using Higgins and Deeks' method (Higgins and Deeks, 2008): SD =SE ×√n, where n is the number of animals assigned to each treatment group. Furthermore, where an experiment provided supplementation levels in percentages (%) rather than grams per kilogram (g/kg), the percentage value was converted to grams per kilogram (g/kg) using the formula.: 1% =10 g/kg. We assessed each pepper group to the control group in feeding studies with several pepper groups. The data collected from the 19 studies that passed the selection criteria were transferred to a Microsoft Excel 2021 comma-separated value (CSV) file format, which is the appropriate file format for the analysis using Brown University's OpenMEE (Open meta-analyst for ecology and evolution) software.

Statistical Analysis

Data extracted from the articles were entered into a Microsoft Excel sheet, transformed into a CSV file, and analyzed using OpenMEE (Wallace et al., 2017). The variable data were provided as a standardized mean difference (SMD) among the pepper treatment and controls with a 95% confidence interval (CI). The following a priori moderator factors were included in the stratified analysis: hen age at the beginning of treatment, pepper form, pepper inclusion level, and treatment duration. The effect size was estimated as the difference between the means of the experimental and treatment groups divided by the overall standard deviation from which the variuos treatment groups were chosen. The Der Simonian and Lard test (Chi-square (Q) - statistic) and the Inconsistency index (I^2) - statistic Higgins are used to analyze heterogeneity (Higgins et al., 2003). The I^2 statistic is the percentage of variance in a meta-analysis attributable to study heterogeneity. Because heterogeneity exists in different degrees within each pooled study, a random-effects model (REM) was adopted for the meta-analysis. The robustness of the meta-analysis results was evaluated using a sensitivity assessment, which was carried out by eliminating a single study from the analysis each time it was conducted. Meta-regression was performed to determine the origins of the heterogeneity. In meta-regression, moderator characteristics used in the subgroup analysis were also used to predict the study effects. No subgroup analysis was conducted when an individual group had three or fewer impact sizes in the meta-analysis. A P-value <0.05 was considered significant. The results of the meta-regression of the effect of pepper level on egg yolk color showed a significant effect ($p<0.05$), so a visualisation of the effect of pepper (*Capsicum* sp) supplementation level on the difference in egg yolk color (%) between the control and the treatment was carried out.

RESULTS

Study characteristic

A total of 19 studies from nine countries were identified, with Iran (15.8%), Japan (15.8%), Turkey (15.8%), Brazil (10.5%), China (10.5%), Egypt (10.5%), Nigeria (10.5%), Saudi Arabia (5.3%) and Serbia (5.3%) (Table 1). Several laying hen strains were used, Hy-Line (47.4%) was the most prevalent followed by Lohman (21%). Two varieties of peppers were used. Pepper powder was utilized in 15 investigations, whereas pepper extract was used in four. A pepper extract was commonly utilized in tiny doses ranging from 0 to 8 g/kg diet, whereas pepper powder was included at >40 g/kg (Table 1). Asymmetrical funnel plots revealed the publication bias among the studies (Figure 2). As indicated in Table 2, the nutritional specifications were appropriate to the nutrient recommendation of NRC (1994).

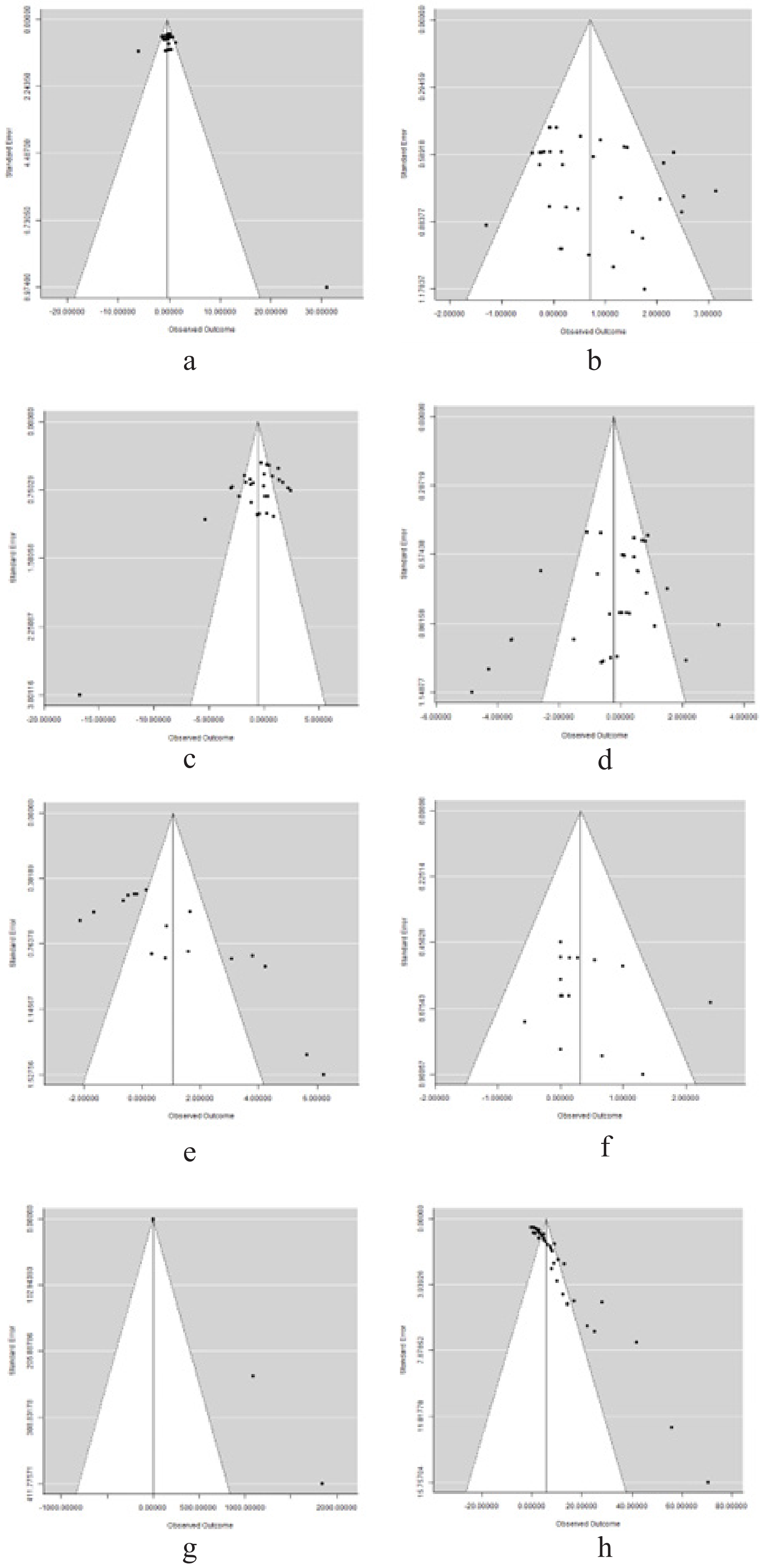

Figure 2 Funnel plots analysis on (a) feed intake (b) hen day egg production, (c) feed conversion ratio, (d) egg weight, (e) egg mass, (f) eggshell thickness, (g) haugh unit and (h) egg yolk color to detect publication bias between-study

Table 2 Descriptive statistics of nutrient specifications of the diets used in the meta-analysis

Nutrient	N	Mean	SD	Min	Max
ME (kcal/kg)	46	2743	81	2500	2896
Protein (%)	48	16.5	0.88	14.64	18.0
Methionine (%)	31	0.43	0.13	0.29	0.75
Met-Cys (%)	7	0.63	0.21	0.58	0.65
Lysine (%)	36	0.84	0.09	0.71	1.0
Calcium (%)	42	3.6	0.36	3.1	4.61
Phosphor (%)	8	0.61	0.06	0.55	0.71
Available Phosphor (%)	36	0.39	0.08	0.30	0.55

Note: N = number of samples; SD = standard deviation; Min = minimum; Max = maximum; ME = metabolizable energy

Feed intake

Twelve papers satisfied the inclusion criteria for the meta-analysis to evaluate the effect of supplementing with pepper on FI of laying hens (Table 3), according to the REM in the general SMD estimations revealed that the pepper treatment reduced FI (P < 0.05) (SMD = −0.44; 95% CI −0.81 to −0.07; P = 0.019). Pepper supplementation decreased FI of <50-week-old laying hens. Pepper in a powdered form reduced FI. A pepper supplementation rate of >9 g/kg in the ration and duration of administration >5 weeks also reduced FI.

Table 3 Subgroup analysis of the effect of pepper on FI in laying hens

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	29	-0.44	-0.81	-0.07	0.19	0.019	58.8	<0.001
Hen's age								
<50 week	17	-0.52	-1.0	0.00	0.27	0.052	59.2	0.001
>50 week	12	-0.36	-0.91	0.18	0.28	0.191	61.7	0.003
Form								
Powder	24	-0.52	-0.95	-0.09	0.22	0.017	62.0	<0.001
Extract	5	-0.14	-0.83	0.56	0.35	0.701	38.5	0.165
Inclusion rate								
<9 g/kg	19	-0.44	-0.94	0.06	0.26	0.086	68.4	<0.001
>9 g/kg	10	-0.49	-0.96	-0.02	0.24	0.041	16.0	0.296
Treatment duration								
<5 weeks	19	-0.22	-0.51	0.08	0.15	0.150	0.0	0.614
>5 weeks	10	-0.98	-1.9	-0.08	0.46	0.032	81.2	<0.001

Note: SMD and I^2 were considered significant at p <0.05; N = number of comparisons; SMD = standardized mean differences between the pepper treatment and controls; CI = confidence interval; *p*-value = probability value; SE = standard error; I^2 = heterogeneity level of the meta-analysis model

Hen day egg production

A meta-analysis was carried out using 16 articles to evaluate the effect of supplementing with pepper in the diet on hen HDEP of laying hens, and the estimated pooled mean effect was 0.71 (95% CI = 0.38 to 1.04) (Table 4). Supplementing the diet with pepper had an increasing effect on HDEP (P < 0.001). Pepper powder increased HDEP (P < 0.001), while the pepper extract from three studies had no effect. Moreover, the pepper treatment increased HDEP in <50-week-old laying hens (SMD = 0.86; 95% CI = 0.46 to 1.26; P < 0.001) with low heterogeneity (I^2 = 44.0%, P = 0.013).

Table 4 Subgroup analysis of the effect of pepper on HDEP in laying hens

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	35	0.71	0.38	1.0	0.17	<0.001	54.5	<0.001
Hen's age								
<50 week	23	0.86	0.46	1.3	0.20	<0.001	44.0	0.013
>50 week	12	0.49	-0.07	1.0	0.28	0.086	64.4	0.001
Form								
Powder	29	0.90	0.54	1.3	0.18	<0.001	51.8	<0.001
Extract	6	-0.15	-0.68	0.38	0.27	0.583	8.1	0.364
Inclusion rate								
<9 g/kg	23	0.61	0.23	0.99	0.19	0.002	51.9	0.002
>9 g/kg	12	0.93	0.27	1.6	0.33	0.006	59.5	0.004
Treatment duration								
<5 weeks	21	0.57	0.18	0.97	0.20	0.005	42.4	0.022
>5 weeks	14	0.90	0.33	1.5	0.29	0.002	66.5	<0.001

Note: SMD and I^2 were considered significant at p <0.05; N = number of comparisons; SMD = standardized mean differences between the pepper treatment and controls; CI = confidence interval; *p*-value = probability value; SE = standard error; I^2 = heterogeneity level of the meta-analysis model

Feed conversion ratio

As shown in Table 5, 13 studies with 31 comparisons were qualified to analyze the effect of the pepper treatment on FCR. According to the pooled effect estimates, pepper supplementation did not affect FCR (SMD = −0.58; 95% CI = −1.18 to 0.03; P = 0.062). There was 83.0% heterogeneity within studies (P < 0.001). Pepper supplementation decreased FCR of <50-week-old laying hens. Pepper powder decreased the FCR while the pepper extract increased FCR (P < 0.05).

Table 5 Subgroup analysis of the effect of pepper on FCR in laying hens

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	31	-0.58	-1.2	0.03	0.31	0.062	83.0	<0.001
Hen's age								
<50 week	18	-1.1	-1.9	-0.25	0.43	0.011	48.7	0.119
>50 week	13	0.04	-0.81	0.89	0.43	0.929	83.5	<0.001
Form								
Powder	26	-0.93	-1.6	-0.27	0.33	0.006	82.3	<0.001
Extract	5	1.1	0.12	2.1	0.51	0.028	65.2	0.022
Inclusion rate								
<9 g/kg	18	-0.60	-1.5	0.25	0.43	0.164	86.6	<0.001
>9 g/kg	13	-0.59	-1.4	0.23	0.42	0.159	74.1	<0.001
Treatment duration								
<5 weeks	20	-0.46	-1.3	0.39	0.44	0.291	83.9	<0.001
>5 weeks	11	-0.83	-1.7	0.02	0.43	0.057	81.9	<0.001

Note: SMD and I^2 were considered significant at p <0.05; N = number of comparisons; SMD = standardized mean differences between the pepper treatment and controls; CI = confidence interval; *p*-value = probability value; SE = standard error; I^2 = heterogeneity level of the meta-analysis model

Egg weight

Eighteen articles that assessed the effect of pepper supplementation on EW were used out of the 19 that passed the inclusion criteria for analysis (Table 6). The pooled SMD estimates revealed that dietary pepper supplementation did not affect laying hen EW (SMD = −0.24; 95% CI = −0.69 to 0.24; P = 0.227). In a restricted study based on pepper species, the pepper inclusion rate, treatment duration, hen age, and strain revealed no treatment effect on EW compared to the control. Pepper supplementation for <5 weeks decreased EW (P < 0.05).

Table 6 Subgroup analysis of the effect of pepper on EW in laying hens

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	36	-0.24	-0.69	0.20	0.23	0.288	72.6	<0.001
Hen's age								
<50 week	22	-0.57	-1.2	0.15	0.37	0.121	77.4	<0.001
>50 week	14	0.15	-0.32	0.61	0.24	0.541	54.7	0.007
Form								
Powder	29	-0.32	-0.87	0.24	0.28	0.263	77.1	<0,001
Extract	7	0.67	-0.42	0.55	0.25	0.790	0.0	0.585
Inclusion rate								
<9 g/kg	21	-0.13	-0.61	0.36	0.25	0.605	64.6	<0.001
>9 g/kg	15	-0.47	-1.3	0.42	0.46	0.301	80.3	<0.001
Treatment duration								
<5 weeks	20	-0.66	-1.3	0.01	0.34	0.054	73.5	<0.001
>5 weeks	16	0.19	-0.40	0.77	0.30	0.534	71.2	<0.001

Note: SMD and I^2 were considered significant at *p* <0.05; N = number of comparisons; SMD = standardized mean differences between the pepper treatment and controls; CI = confidence interval; *p*-value = probability value; SE = standard error; I^2 = heterogeneity level of the meta-analysis model

Egg mass

Assessing the effect of pepper supplementation on EM in laying hen eggs, 9 articles with 17 comparisons that met the eligibility rule for inclusion in the meta-analysis were used (Table 7). The Grand mean estimate revealed no evidence of treatment effect on EM (SMD = 1.1; 95% CI 0.2 to 1.9; *p*=0.016). Hens <50 weeks old showed an increase in egg mass when supplemented with pepper (p=0.027), while those >50 weeks old had no effect. Results of subgroup analysis of pepper form showed form powder increased EM. Moreover, supplementation at doses above 9 g/kg diet and for a duration of more than 5 weeks of treatment showed an increase in EM.

Table 7 Subgroup analysis of the effect of pepper on EM in laying hens

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	17	1.1	0.20	1.9	0.44	0.016	86.7	<0.001
Hen's age								
<50 week	8	1.0	-0.46	2.5	0.77	0.175	89.8	<0.001
>50 week	9	1.1	0.13	2.2	0.52	0.027	82.9	<0.001
Form								
Powder	16	1.0	0.14	1.9	0.46	0.024	87.3	<0.001
Extract	1	1.6	-0.02	3.2	0.81	NA	NA	NA
Inclusion rate								
<9 g/kg	11	0.42	-0.51	1.3	0.47	0.375	85.1	<0.001
>9 g/kg	6	2.1	0.78	3.5	0.69	0.002	76.8	<0.001
Treatment duration								
<5 weeks	6	0.30	-1.3	1.9	0.80	0.712	87.7	<0.001
>5 weeks	11	1.5	0.47	2.5	0.51	0.004	85.3	<0.001

Note: SMD and I^2 were considered significant at $p < 0.05$; N = number of comparisons; SMD = standardized mean differences between the pepper treatment and controls; CI = confidence interval; *p*-value = probability value; SE = standard error; I^2 = heterogeneity level of the meta-analysis model

Eggshell thickness

Eleven papers satisfied the inclusion criteria for the meta-analysis to evaluate the effect of supplementing with pepper on EST (Table 8), according to the REM in the general SMD estimations revealed that the pepper treatment increased EST (SMD = 0.32; 95% CI: 0.04 to 0.64; p =0.025). Restricted subgroup analysis on pepper preparation form also showed that powder form increased EST (p=0.014). In addition, sub-group analysis of the treatment duration showed that 5> weeks increased EST (p =0.011) in low heterogeneity (I^2 = 30.0.7%, Q = 0.170).

Table 8 Subgroup analysis of the effect of pepper on EST in laying hens

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	18	0.32	0.04	0.61	0.15	0.025	1.9	0.432
Hen's age								
<50 week	14	0.15	-0.17	0.47	0.16	0.356	0.00	1.0
>50 week	4	0.86	-0.20	1.9	0.54	0.113	69.6	0.02
Form								
Powder	15	0.39	0.08	0.70	0.16	0.014	8.58	0.357
Extract	3	-0.22	-1.1	0.66	0.45	0.619	0.00	0.825
Inclusion rate								
<9 g/kg	13	0.31	-0.08	0.70	0.20	0.114	19.3	0.249
>9 g/kg	5	0.39	-0.11	0.89	0.26	0.127	0	0.666
Treatment duration								
<5 weeks	8	0.03	-0.39	0.45	0.21	0.902	0	0.995
>5 weeks	10	0.60	0.14	1.1	0.24	0.011	30.0	0.170

Note: SMD and I^2 were considered significant at $p < 0.05$; N = number of comparisons; SMD = standardized mean differences between the pepper treatment and controls; CI = confidence interval; *p*-value = probability value; SE = standard error; I^2 = heterogeneity level of the meta-analysis model

Haugh unit

Grand estimates obtained from SMD suggested that pepper had no effect on HU of laying hens (SMD = 0.30, 95% CI: -0.22 to 0.82, p =0.261) (Table 9). In addition, the results of subgroup analysis of hen age, form preparation, inclusion rate and treatment duration showed the addition of pepper had no effect on HU.

Table 9 Subgroup analysis of the effect of pepper on HU in laying hens egg

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	24	0.30	-0.22	0.82	0.27	0.261	73.9	<0.001
Hen's age								
<50 week	10	0.37	-0.94	1.7	0.67	0.582	85.6	<0.001
>50 week	14	0.29	-0.15	0.73	0.23	0.200	49.0	0.020
Form								
Powder	17	0.49	-0.25	1.2	0.37	0.195	81.0	<0.001
Extract	7	-0.09	-0.57	0.39	0.24	0.703	0	0.975
Inclusion rate								
<9 g/kg	17	-0.03	-0.60	0.52	0.29	0.894	69.8	<0.001
>9 g/kg	7	1.1	0.10	2.1	0.52	0.032	72.3	0.001
Treatment duration								
<5 weeks	12	0.01	-0.72	0.75	0.38	0.970	72.8	<0.001
>5 weeks	12	0.58	-0.14	1.3	0.37	0.115	73.5	<0.001

Note: SMD and I^2 were considered significant at p <0.05; N = number of comparisons; SMD = standardized mean differences between the pepper treatment and controls; CI = confidence interval; *p*-value = probability value; SE = standard error; I^2 = heterogeneity level of the meta-analysis model

Egg yolk color

To investigate the effect of pepper supplementation on EYC of laying hen eggs, a meta-analysis was conducted with 19 publications, and the estimated pooled mean effect was 5.7 (95% CI = 4.5 to −6.9) (P <0.001). Overall, dietary pepper intake increased EYC (Table 10), although there was significant variability between studies (I^2 = 88.2, Q <0.001). The increase in EYC was consistent across all subgroups.

Table 10 Subgroup analysis of the effect of pepper on EYC in laying hen eggs

Covarities	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	38	5.7	4.5	6.9	0.61	<0.001	88.2	<0.001
Hen's age								
<50 week	27	7.8	6.0	9.7	0.94	<0.001	88.1	<0.001
>50 week	11	3.3	1.9	4.7	0.72	<0.001	87.9	<0.001
Form								
Powder	31	5.8	4.5	7.2	0.69	<0.001	89.2	<0.001
Extract	7	5.3	3.0	7.5	1.1	<0.001	76.6	<0.001
Inclusion rate								
<9 g/kg	29	5.3	3.9	6.8	0.74	<0.001	89.0	<0.001
>9 g/kg	9	6.9	4.6	9.3	1.2	<0.001	87.5	<0.001
Treatment duration								
<5 weeks	25	9.0	7.0	11.0	1.0	<0.001	86.5	<0.001
>5 weeks	13	3.0	1.7	4.2	0.65	<0.001	87.7	<0.001

Note: SMD and I^2 were considered significant at p <0.05; N = number of comparisons; SMD = standardized mean differences between the pepper treatment and controls; CI = confidence interval; *p*-value = probability value; SE = standard error; I^2 = heterogeneity level of the meta-analysis model

Publication bias

The funnel plots of the effect of dietary pepper treatment on all parameters in laying hens (Figure 2) indicate that the funnel plots were almost symmetrical. Because a substantial number of unpublished papers would be required to change the statistically significant results, the presence of publication bias was not a problem in this meta-analysis.

Meta-regression

Table 11 displays the findings of a meta-regression analysis of the effect of pepper inclusion level on egg quality and performance in laying hens. Considering the large number of pepper inclusion rates in the layer diet, we investigated the association between the Hedges' g effect size from the outcome variables and the inclusion levels of pepper in the diet as predictor variables in the meta-regression analysis. The meta-regression findings demonstrated that the inclusion level did not affect FI (P=922), HDEP (P=697), EW (P=626), EM (P=0.324), EST (P=0.668) and HU (P=0.248) but did affect EYC (P=0.017). Increasing the level of pepper in the extract drastically increased EYC ($y = 72.6x + 25.4$, $R^2 = 0.83$), while increasing the level in the powder slowly increased EYC ($y = 7.7x + 2.9$, $R^2 = 0.73$). The best effect was observed at 1–15 g/kg DM feed (Figure 3).

Table 11 Meta-regression analysis of the levels of pepper fruit on laying hens performance and egg quality (SMD)

Response Variable	Unit	N	Parameter Estimates				*p*-value
			Intercept	SE Intercept	Slope	SE Slope	
Feed intake	g/d	29	-0.46	0.26	0.02	0.18	0.922
HDEP	%	35	0.66	0.23	0.06	0.15	0.697
FCR	g/g	31	-0.46	0.44	-0.10	0.27	0.702
Egg weight	g	36	-0.14	0.31	-0.09	0.19	0.626
Egg mass	g	17	0.67	0.57	0.39	0.40	0.324
Eggshell thickness	mm	18	0.38	0.19	-0.06	0.13	0.668
Haugh unit		24	0.06	0.34	0.27	0.23	0.248
Egg yolk color		38	4.5	0.81	1.5	0.61	0.017

Note: SE = standard error; *p*-value = probability value; HDEP = hen day egg production; FCR = feed conversion ratio

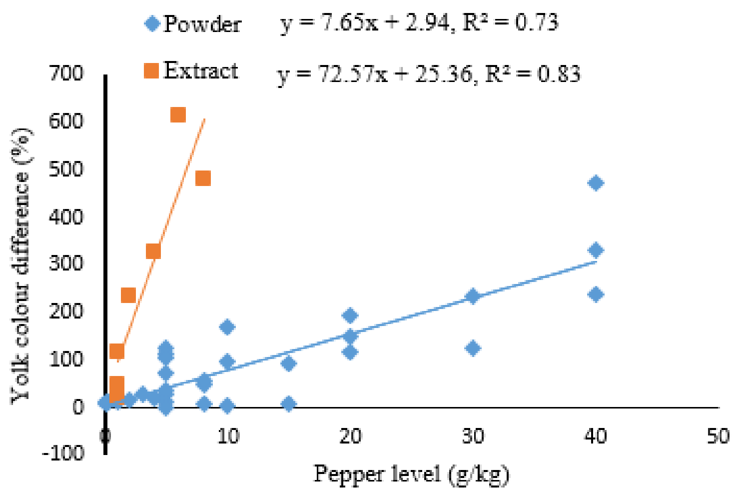

Figure 3 Effect level of pepper supplementation on the difference between control and treatment egg yolk color

DISCUSSION

The present egg production results provide a robust explanation of how pepper fruit supplementation increases HEDP and EM of laying hens. The results of a recent meta-analysis confirmed that pepper fruit is useful as an alternative antibiotic growth promoter with anti-bacterial and antioxidant properties (Abd El-Hack et al., 2022a). Capsaicin is the main alkaloid in pepper fruit, which is antioxidant and anti-bacterial (Rosca et al., 2020; Vidyarthi and Munglang 2020). Previous research has reported that capsaicin prevents inflammation, decreases the number of pathogens, and improves the balance of the intestinal ecosystem (Song et al., 2017). Capsaicin in pepper fruit is bactericidal against *Salmonella* and *E. coli* (Morrine et al., 2018). Enhancing the intestinal ecosystem increases chicken productivity and prevents pathogen infection (Pan and Yu, 2014; Xiang et al., 2022). The study by Lokaewmanee et al. (2013). (Lokaewmanee et al., 2013) reported that feeding red pepper increases the height of intestinal villi, villi area, cell area, and mitotic cells in the small intestine of laying hens. Increased villi height and villi area suggest an increase in nutrient absorption area in the intestine (Donaldson et al., 2021). Supplementing with pepper decreased FI in laying hens. The subgroup analysis showed that administering >9 g/kg of pepper in feed and duration of treatment of >5 weeks reduced the FI of laying hens. Capsaicin compounds in pepper fruit decrease feed consumption by affecting the nervous system through the transient receptor vanilloid potential (TRVP-1) activation pathway(Yoshioka et al., 1999; Yoshioka et al., 2004; Wang and Siemens 2015). The TRP protein is a non-specific phosphoinositide mediated by the Ca^{2+}-permeable channel (Minke, 2006). Capsaicin compounds in the digestive tract trigger vagal signals to appetite-regulatory centers in the brain. Capsaicin decreases feed consumption through increased secretion of glucagon-like peptide-1 (Ludy et al., 2012).

The meta-analysis revealed that supplementing with pepper did not affect the FCR. Interestingly, layer chickens aged <50 weeks that were supplemented with pepper had higher egg production and lower FCR values compared to chickens aged >50 weeks. This may be due to the increased responsiveness of young laying hens to changes in the gut microbiota. The gut microbiota in young chickens fluctuates greatly depending on the environmental conditions and the feed offered (Videnska et al., 2014). Moreover, In the post-peak production rate of high-intensity metabolism, laying hens gradually entered the late laying stage and occupied a long time in the whole production cycle. In the late stage of laying, laying hens were more susceptible to external factors due to the decline of ovarian function, and weakened resistance to stress and disease, which were often accompanied by low laying rate, low albumen height, poor eggshell quality, and a variety of diseases (Liu et al., 2013).

Moreover, the addition of pepper to the diet can increase EST which is an important parameter in the egg production chain. These results are consistent with Saleh et al. (2021) and Sözcü (2019). The findings may be associated with enhanced utilisation of certain nutrients, particularly minerals that play a crucial role in shell development (Platel and Srinivasan, 2004). Current research from Prakash and Srinivasan (2013) explained that the addition of capsaicin to rat feed can increase calcium uptake in the small intestine.

Pepper fruit supplementation also improves EYC, which is a consumer egg quality factor (Bovšková et al., 2014). Carotenoid deposition considerably improved the color of yolks (Kotrbácek et al., 2013; Ortiz et al., 2021; Panaite et al., 2021). Xanthophylls are the main contributors to chicken egg yolk pigmentation, responsible for 88–92% of the total carotenoids in egg yolk. Chickens have an almost exclusive aggregation because β-carotene is transformed into vitamin A or is metabolized (Surai et al., 2001; Kljak et al., 2021). The overall carotenoid concentration and ratio of the red carotenoids (astaxanthin, capsanthin, and canthaxanthin) to yellow carotenoids (zeaxanthin and lutein) affect the color of the egg yolk and its components (yellowness and redness). Supplementing with pepper increases yolk redness but does not affect Pepper in powder form had a greater effect on performance than the extract. The pepper extraction process largely determines the effectiveness of the pepper supplement in laying hens. The solvent used for extraction greatly affects the active compounds contained in the extract (Bae et al., 2012; Bacon et al., 2017; Salamatullah et al., 2022). Pepper is dissolved in a non-polar solvent to remove the pigments (carotenoids) and active compounds, such as phenols, flavonoids, and capsaicin, are dissolved in polar solvents (Bae et al., 2012; Dang et al., 2014; Nascimento et al., 2014). These are possible explanations for the increased performance after supplementing with pepper powder.

CONCLUSIONS

This meta-analysis provided critical scientific insight into the beneficial effects of pepper on laying hens. In conclusion, diets treated with pepper showed higher egg production and egg yolk color parameters than controls. Moreover, the subgroup analyses demonstrate that the moderators tested (hen age at trial start, pepper form, inclusion level, and supplementation duration) impacted components of the response variables. This meta-analysis may be useful to egg producers, veterinarians, and policymakers while deciding on the use of pepper in laying hens diet.

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AUTHOR CONTRIBUTIONS

Muhammad Fathin Hanif: Collected and selected the articles, analyzed the data and drafted the manuscript. Bambang Ariyadi: Drafted and modified the manuscript. Muhlisin: Drafted and modified the manuscript. Ali Agus: Designed study concepts and modified the manuscript.

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