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Research article

Meta-analysis of laying hen performance and egg quality characteristics in response to Spirulina supplementation

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Abstract

Because of its essential and beneficial nutritional content, the utilization of Spirulina in the layer chicken diet has received more attention nowadays. The meta-analysis in this study aimed to ascertain the efficacy of dietary Spirulina on feed intake (FI), feed conversion ratio (FCR), laying rate (LR), and egg quality in laying hens, including egg weight, eggshell thickness, yolk weight, yolk colour, haugh unit, and yolk cholesterol concentration. The analysis drew upon nineteen publications identified across PubMed, Scopus, and Google Scholar databases, collectively utilizing the varied search mechanisms therein. The data from these 19 trials underwent analysis using random-effects models within the OpenMEE software. The standardized mean difference (SMD) with its accompanying 95% confidence interval was unveiled as the result. The findings revealed a significant improvement in FCR (SMD = -2.233; p<0.001), LR (SMD=2.290; p<0.001), egg weight (SMD=1.551; p<0.001), eggshell thickness (SMD=1.223; p<0.001), yolk colour (SMD=10.659; p<0.001), haugh unit (SMD=2.189; p<0.001), and a reduction in yolk cholesterol concentration (SMD=-0.964; p<0.001). The results for FI (SMD=0.776; p=0.074) and yolk weight (SMD=0.367; p=0.075) were compared to the control group (dosage 0% in reference studies). The meta-analysis unveiled considerable variation among the included papers, indicating high heterogeneity. The meta-regression uncovered that the factors scrutinized in the research—namely, a hen's age, inclusion level, and duration of treatment—greatly impacted the divergences observed in this meta-analysis and substantially accounted for its outcomes. The current meta-analysis concluded that dietary supplementation of Spirulina in laying hens could improve the performance and egg quality characteristics.

Keywords: Egg quality, Performance, Layers, Spirulina, Supplementation

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INTRODUCTION

Spirulina, a blue-green microalgae, developed photosynthetic capabilities before any other organism and is regarded as the ancestor from which higher plants evolved (Mohan et al., 2014). *Spirulina platensis* and *Spirulina maxima* are two predominantly utilized species of Spirulina (Saranraj and Sivasakthi, 2014). Spirulina is gaining increased attention due to its nutritional and medicinal properties (Bondar et al., 2023; El-Shall et al., 2023). While Spirulina boasts an impressive protein profile with levels ranging from 53% to 67% of dry matter (Salgado et al., 2024) and supplying essential amino acids (Uzlasir et al., 2023) a noteworthy facet is its high protein content, which, according to various sources, constitutes the majority share of this superfood's nutritional breakdown. Moreover, Spirulina comprises vitamins, minerals, bioactive components, and phenolic compounds and is an excellent natural pigment source (Omri et al., 2019; Alagawany et al., 2021). In the last decade, the beneficial nutrients in Spirulina have received widespread attention. Researchers have conducted several recent research to examine the potential benefits of adding Spirulina to chicken diets in response to this growing trend (Eid Abdel-Moneim et al., 2021; Alghamdi et al., 2023).

Concerning laying hens, Spirulina can serve as an additional feed in the diet to boost egg production (Al-Otaibi et al., 2022), enhance egg quality (Omri et al., 2019), and strengthen immunity (Abbas et al., 2022). Furthermore, supplementing layer hen with Spirulina improves yolk colour intensity (Rey et al., 2021; Panaite et al., 2023) and alters the cholesterol content within eggs (Selim et al., 2018). Providing a diet incorporating Spirulina also plays a role as a microbial pathogenic property, potentially substituting for antibiotic growth promoters in the poultry industry (Fries-Craft et al., 2021).

To increase laying egg production, Spirulina's role as a feed supplement has attracted the attention of researchers. Indeed, many studies have investigated its impact (Ismail et al., 2023). Results showed a wide range of impacts—from no change (Zahroojian et al., 2013) to detrimental (Nia et al., 2021) and even beneficial outcomes (Samia et al., 2018). Given the varying data, drawing broad conclusions is a complex task. Several reviews on Spirulina's effect on layer chickens' diet tend to be descriptive explanations through reviews rather than numerical analysis (El-Shall et al., 2023).

Collecting and numerically analyzing past research aids in forming solid judgments. Meta-analysis is a stringent, number-based method—sifting past findings for sound insights into combined studies. It offers a thorough, merged view of available information (Russo, 2007; Ogbuewu and Mbajiorgu, 2022). We believe such an approach has not yet been employed for assessing Spirulina's impact on diets meant for layer hens. Hence, this study aimed to investigate the impact of integrating Spirulina into the diets of layer hens on egg production and quality through a meta-analytical methodology.

MATERIALS AND METHODS

Literature review, screening, and data extraction

A database was compiled from information gathered from Scopus, PubMed, and Google Scholar, focusing on research investigating the effects of the microalgae Spirulina. The parameters examined centered around the egg production and quality traits in laying hens. Employing Boolean operators (and/or), a systematic search was conducted using keywords such as "laying hens", "Spirulina", and "egg". The variables included in the search were feed intake (FI), feed conversion ratio (FCR), laying rate (LR), egg weight (EW), yolk weight (YW), shell thickness (ST), yolk colour (YC), Haugh unit (HU), and yolk cholesterol concentration (YCC). The search duration spanned three months, from November

2023 to January 2024. The feed used included *Spirulina platensis* and *Spirulina maxima*, along with various strains of laying hens. Pieces of information were extracted from various studies encompassed values of the variables, including standard deviation (SD) and standard error (SE) for each effect size.

Figure 1 illustrates the systematic search process. Nineteen out of 59 articles met the inclusion criteria for the dataset. Sixteen papers were removed due to data duplication, indicating that the same data had been published in multiple journals. Eighteen papers were excluded from the investigation for reasons such as being books or incomplete articles (n=4), having irrelevant content or variables (n=7), and involving a different animal type (n=7). Five of the remaining 25 studies were excluded due to insufficient data.

A database was constructed incorporating various details, including author names, study publication year, country of study, total number of experimental treatments, the overall count of hens involved in the experiments, strain or breed of laying hens, the age of hens when treatment commenced, duration of the treatment, specific type of micro-algae Spirulina used (*plantensis, maxima*), the level of Spirulina inclusion, and the extracted result of concern (FI, FCR, LR, or measures of egg quality) from each eligible article. Table 1 displays the studies considered in this current meta-analysis.

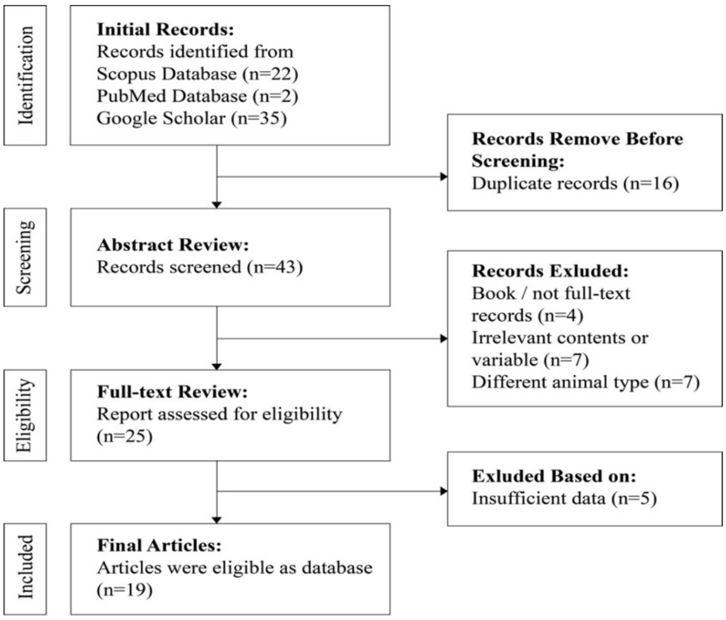

Figure 1 Overview of article selection process

Statistical analysis

Statistical analysis was conducted using OpenMEE Software, generating graphical representations in forest plots (Wallace et al., 2017). The standardized mean difference between the Spirulina intervention and control for continuous variables, along with a 95% confidence interval, was used to represent the results. The analysis was stratified based on covariate variables that were predetermined: hen's age at the beginning of Spirulina-treat (<50 weeks, >50 weeks), inclusion level of Spirulina (<1%, 1-5%, >5%), and treatment duration (<50 days, >50 days). In order to quantify the impact of the experimental variable, investigators calculated the average divergence between outcomes for the test and comparison collectives, standardizing this value by the standard mistake encompassing all participants randomly assigned to the various conditions. Heterogeneity was evaluated by employing the DerSimonian and Lard test, utilizing the Chi-square (Q) statistic, and assessing the Inconsistency index (I2) statistic (Higgins, 2003). The I2 statistic represents the percentage of variation in a meta-analysis attributed to the heterogeneity among studies. A sensitivity analysis was conducted to evaluate the appropriateness of employing a random-effects model approach in the meta-analysis, given the variability observed across the included studies. The influence of studies identified as deviant in the combined analysis was examined by removing one study at a time. A meta-regression was carried out to explore potential sources of heterogeneity, with the covariate variables utilized in the subgroup analysis employed as forecasters of impacts discerned between the studies. Subgroup analysis was skipped for strata with less than three effect sizes, and the diamond positioned at the bottom of the forest plot did not intersect with the line, indicating no effect (Koricheva et al., 2013). A fail-safe number (Nfs) was determined to identify potential publication bias from non-significant studies excluded from the analysis. A robustness indicator for the meta-analysis model was considered when Nfs exceeded five times the sample size (N) plus ten. The computation of Nfs (fail-safe number) followed the methodology proposed by Rosenthal (1979).

Table 1 Studies considered in the meta-analysis

No	Studies	Country	N	Total hen	Strain	Hen's age (weeks)	Spirulina	Duration, d	Level (%)	Result
1.	Zahroojian et al. (2011)	Iran	4	128	Hy-line W36	>50	Plantensis	<50	0-2.5	1,3,4,7
2.	Zahroojian et al. (2013)	Iran	4	128	Hy-line W36	>50	Plantensis	<50	0-2.5	1,2,3,4,5,7,8,9
3.	Ahammed et al. (2018)	Bangladesh	4	96	Shaver-579	>50	Plantensis	<50	0-0.3	3,4,7
4.	Hasan et al. (2018)	Bangladesh	3	30	White Leghorn	>50	Plantensis	<50	0-0.4	1,2,3,4
5.	Samia et al. (2018)	Egypt	3	90	Golden Montazah	<50	Plantensis	>50	0-0.3	1,2,3,4,5,6
6.	Selim et al. (2018)	Egypt	4	160	Norfa	<50	Plantensis	<50	0-0.3	1,2,3,4,5,8,9
7.	Omri et al. (2019)	Tunisia	3	45	Lohman White	<50	Plantensis	<50	0-2.5	1,2,3,4,5,6,7,8,9
8.	Curabay et al. 2021)	Turkey	3	60	Lohman LSL Classic	>50	Plantensis	<50	0-2	1,2,4,5
9.	Khan et al. (2021)	Egypt	4	120	Shaver-579	<50	Plantensis	<50	0-0.3	3,4,5,7,8
10.	Mardanpour et al. (2020)	Iran	4	192	Lohmann Selected Leghorn	>50	Plantensis	>50	0-3.5	1,2,3,4
11.	Nia et al. (2021)	Iran	4	192	Lohmann Selected Leghorn	>50	Plantensis	>50	0-0.9	1,2,3,4,5,7,8
12.	Rey et al. (2021)	Spain	3	54	White Leghorn, Rhode Island Red	<50	Plantensis	<50	0-3	4
13.	Tufarelli et al. (2021)	Iran	3	216	Hy-line W36	>50	Plantensis	>50	0-2	1,2,3,4,5,6,7,8,9
14.	Abbas et al. (2022)	Saudi Arabia	5	250	Hy-line W36	<50	Plantensis	>50	0-12	1,2,3,4,5,7,8
15.	Al-Otaibi et al. (2022)	Saudi Arabia	4	144	Hy-line W36	<50	Plantensis	<50	0-9	1,2,3,4,9
16.	Ismail et al. (2023)	Egypt	3	180	Sinai	<50	Plantensis	>50	0-0.08	1,3,4,9
17.	Panaite et al. (2023)	Romania	2	80	Lohman Brown	<50	Plantensis	<50	0-2	1,2,3,4,6,7,8
18.	Poveda-Víquez et al. (2023)	Costa Rica	4	80	Isa Brown	>50	Maxima	<50	0-6	2,4,7,9
19.	Wahyuni et al. (2023)	Indonesia	2	72	Isa Brown	>50	Plantensis	<50	0-0.3	5,7,8

Note: N = number of replication; 1 = feed intake; 2 = feed conversion ratio; 3 = laying rate; 4 = egg weight; 5 = shell thickness; 6 = yolk weight; 7 = yolk colour; 8 = haugh unit; 9 = yolk cholesterol concentration; d = days

RESULTS

Characteristics of the study and excluded studies

After the literature search illustrated in Figure 1, fifty-nine publications were identified. From these, nineteen articles fulfilled the designated criteria eligible for consideration in the analysis, and a comprehensive breakdown of them is outlined in Table 1. The articles chosen for the meta-analysis encompass a 12-year timeframe, starting from the earliest research published in 2011 and ending with the latest published in 2023. The cumulative data included in this study involve 2,317 laying hens across 236 comparisons.

Feed intake

Fourteen studies, encompassing 35 comparisons and involving 1,895 hens from the pool of 19 studies eligible for this study, were employed to investigate the impact of Spirulina on FI. The collective average estimate showed a noticeable impact of the treatment on FI (SMD = 0.776, 95% CI = -0.076 to 1.628; Table 3). Notably, considerable heterogeneity was observed among the 14 studies examined for the effect of dietary Spirulina on FI, as indicated by Cochran's Q (Q) = 407.08, I2 = 91.65%, p-value = <0.001 (Table 2), in comparison to the control groups. When breaking down the analysis based on covariates, no substantial impact on FI was identified, except for studies involving hens aged <50 weeks and those using an inclusion rate >5%, significantly increasing FI (Table 3). Meta-regression revealed that hen's age (coefficient of covariates (Q_M) = 11.50, df = 1, p-value = <0.001) and inclusion rate (Q_M = 38.9, df = 2, p-value = <0.001) functioned as notable indicators of the impact of Spirulina on FI, elucidating all sources of heterogeneity (Table 13).

Tabel 2 Heterogeneity result for the feed intake, feed conversion ratio, laying rate, egg quality characteristics, and yolk cholesterol concentration

Parameter	Q	df	Het. p-value	I^2
Feed intake	407.08	34	<0.001	91.65
Feed conversion ratio	445.36	32	<0.001	92.81
Laying rate	514.32	38	<0.001	92.61
Egg weight	518.72	45	<0.001	91.32
Shell thickness	186.89	24	<0.001	87.16
Yolk weight	14.12	6	0.028	57.51
Yolk Color	421.48	27	<0.001	93.59
Haugh unit	150.63	21	<0.001	86.06
Yolk cholesterol	101.97	17	<0.001	83.33

Note: Q = Cochran's Q; df = degree of freedom, Het. p-value = heterogeneity probability value; I^2 = measure of heterogeneity

Feed conversion ratio

The meta-analysis used Spirulina to evaluate the impact on FCR with 13 articles, incorporating 39 comparisons and involving 1,667 hens. The overall mean estimate indicated a clear impact of the intervention on FCR (SMD = -2.233, 95% CI = -3.215 to -1.250; Table 4). Notably, there was substantial heterogeneity, as evidenced by Cochran's Q (Q) = 445.36, I2 = 92.81%, and p-value = <0.001 (Table 2), compared to the control groups. Analysis based on covariates showed that all inclusions impact FCR, excluding studies utilizing an inclusion rate of 1-5%. All covariates with a p-value <0.05 exhibited a significant decrease in FCR (Table 4). Meta-regression revealed that the inclusion rate (Q_M = 6.98, df = 2, p-value = 0.030) and other variables significantly influenced the Spirulina effect on FCR, elucidating all sources of heterogeneity (Table 13).

Table 3 Subgroup analysis of the effect of Spirulina on feed intake in laying hens

Covariates	N	SMD	CI 95%		SE	p-value	Heterogeneity	
			Lower	Upper			I^2	p-value
Overall	35	0.776	-0.076	1.628	0.435	0.074	91.65	<0.001
Hen's age								
<50 weeks	17	3.493	1.821	5.165	0.853	<0.001	91.65	<0.001
>50 weeks	18	-0.353	-0.129	0.424	0.396	0.373	80.92	<0.001
Inclusion rate								
<1%	12	0.271	-1.199	1.741	0.750	0.710	92.72	<0.001
1-5 %	18	-0.143	-0.960	0.675	0.417	0.732	85.65	<0.001
>5%	5	17.169	9.368	24.970	3.980	<0.001	86.77	<0.001
Treatment duration								
<50 days	19	0.321	-0.886	1.529	0.616	0.602	90.73	<0.001
>50 days	16	1.245	-0.019	2.509	0.645	0.054	92.79	<0.001

Note: Estimate and I^2 were considered significant at p-value <0.05; N = Number of outcomes; SMD = standardized mean difference; CI = confidence interval; SE = standard error; I^2 = measure of heterogeneity

Table 4 Subgroup analysis of the effect of Spirulina on feed conversion ratio in laying hens

Covariates	N	SMD	CI 95%		SE	p-value	Heterogeneity	
			Lower	Upper			I^2	p-value
Overall	33	-2.233	-3.215	-1.250	0.501	<0.001	92.86	<0.001
Hen's age								
<50 weeks	15	-3.575	-5.360	-1.790	0.911	<0.001	95.60	<0.001
>50 weeks	18	-1.166	-2.177	-0.154	0.516	0.024	86.28	<0.001
Inclusion rate								
<1%	10	-3.723	-5.889	-1.557	1.105	<0.001	93.84	<0.001
1-5 %	17	-0.807	-1.764	0.151	0.489	0.099	89.31	<0.001
>5%	6	-7.315	-12.224	-2.405	2.505	0.004	94.16	<0.001
Treatment duration								
<50 days	19	-2.775	-4.178	-1.372	0.716	<0.001	93.04	<0.001
>50 days	14	-1.704	-3.095	-1.372	0.709	0.016	92.81	<0.001

Note: Estimate and I^2 were considered significant at p-value <0.05; N = Number of outcomes; SMD = standardized mean difference; CI = confidence interval; SE = standard error; I^2 = measure of heterogeneity

Laying rate

The meta-analysis incorporated studies that met the inclusion criteria of 15 out of 19, which evaluated the impact of Spirulina on LR. This meta-analysis involved 33 comparisons with 2,051 hens. The overall mean estimate showed clear evidence of a Spirulina effect on LR (SMD = 2.290, 95% CI = 1.342 to 3.238; Table 5). Indicated by Cochran's Q (Q) = 514.32, I2 = 92.61%, p-value = <0.001 (Table 2) showed significant heterogeneity among the 15 studies examined for the impact of dietary Spirulina on LR. Upon further analysis based on covariates, variables significantly influenced LR, except for studies involving hens aged >50 weeks, an inclusion rate of 1-5%, and a treatment duration of >50 days. All covariates with a p-value <0.05 demonstrated a significant increase in LR (Table 5). Meta-regression revealed that the inclusion rate (Q_M = 35.40, df = 2, p-value = <0.001) significantly predicted the Spirulina effect on LR, elucidating all sources of heterogeneity (Table 13).

Table 5 Subgroup analysis of the effect of Spirulina on laying rate in laying hens

Covariates	N	SMD	CI 95%		SE	p-value	Heterogeneity	
			Lower	Upper			I^2	p-value
Overall	39	2.290	1.342	3.238	0.484	<0.001	92.61	<0.001
Hen's age								
<50 weeks	20	2.290	1.342	3.238	0.484	<0.001	94.43	<0.001
>50 weeks	19	1.041	-0.148	2.230	0.607	0.086	89.01	<0.001
Inclusion rate								
<1%	18	2.015	0.574	3.457	0.735	0.006	92.84	<0.001
1-5 %	16	0.815	-0.301	1.932	0.570	0.152	90.50	<0.001
>5%	5	12.621	10.139	15.102	1.266	<0.001	0	0.460
Treatment duration								
<50 days	23	2.967	1.614	4.320	0.690	<0.001	93.09	<0.001
>50 days	16	1.486	0.115	2.856	0.699	0.034	91.94	<0.001

Note: Estimate and I^2 were considered significant at p-value <0.05; N = Number of outcomes; SMD = standardized mean difference; CI = confidence interval; SE = standard error; I^2 = measure of heterogeneity

Egg weight

A total of 46 comparisons with 2,245 hens were used in the meta-analysis study, which were identified from 17 out of 19. The overall average estimate indicated a distinct impact of the treatment on EW (SMD = 1.551, 95% CI = 0.773 to 2.328; Table 6). Significant heterogeneity was observed among the 17 studies examined for the effect of dietary Spirulina on EW, as evidenced by Cochran’s Q (Q) = 518.72, I2 = 91.32%, p-value <0.001; Table 2). Upon conducting a detailed analysis based on covariates, variables significantly influenced EW, except for studies involving hens aged >50 weeks and those utilizing an inclusion rate <1%. All covariates with a p-value <0.05 demonstrated a significant increase in EW (Table 6). Meta-regression identified that the hen's age (Q_M = 17.60, df = 1, p-value <0.001) was a crucial factor in determining the effect of Spirulina on EW, elucidating all sources of heterogeneity (Table 13).

Shell thickness

From the pool of 19 studies meeting the qualification criteria for the meta-analysis, only ten articles were selected, encompassing eight comparisons and involving 1,333 hens, to assess the influence of Spirulina inclusion on ST. The overall mean estimate indicated clear evidence of a Spirulina effect on ST (SMD = 1.223, 95% CI = 0.523 to 1.923; Table 7). Notably, there was considerable variability among the ten studies examined for the impact of Spirulina supplementation on ST, as indicated by Cochran’s Q (Q) = 186.89, I2 = 87.16%, p-value = <0.001 (Table 2), compared to the control groups. After conducting an in-depth analysis considering covariates, variables showed no significant impact on ST, except for studies involving hens aged <50 weeks, an inclusion rate >5%, and a treatment duration <50 days. The studies that included a hen's age <50 weeks, an inclusion rate >5%, and a treatment duration <50 days exhibited a significant increase in ST (Table 7). Meta-regression found that the hen's age (Q_M = 7.25, df = 1, p-value = 0.007) and inclusion rate (Q_M = 13.7, df = 1, p-value = 0.001) served as significant predictors of the Spirulina effect on ST, elucidating all sources of heterogeneity (Table 13).

Table 6 Subgroup analysis of the effect of Spirulina on egg weight in laying hens

Covariates	N	SMD	CI 95%		SE	p-value	Heterogeneity	
			Lower	Upper			I^2	p-value
Overall	46	1.551	0.773	2.328	0.397	<0.001	91.33	<0.001
Hen's age								
<50 weeks	22	4.226	2.885	5.566	0.684	<0.001	92.27	<0.001
>50 weeks	24	-0.110	-0.843	0.623	0.374	0.768	83.90	<0.001
Inclusion rate								
<1%	18	0.915	-0.203	2.034	0.571	0.109	92.04	<0.001
1-5 %	22	1.546	0.584	2.508	0.491	0.002	89.17	<0.001
>5%	6	49.481	20.481	78.481	14.796	<0.001	91.32	<0.001
Treatment duration								
<50 days	30	1.235	0.269	2.202	0.493	0.012	91.23	<0.001
>50 days	16	1.551	0.773	2.328	0.397	<0.001	91.32	<0.001

Note: Estimate and I^2 were considered significant at p-value <0.05; N = Number of outcomes; SMD = standardized mean difference; CI = confidence interval; SE = standard error; I^2 = measure of heterogeneity

Table 7 Subgroup analysis of the effect of Spirulina on shell thickness in laying hens

Covariates	N	SMD	CI 95%		SE	p-value	Heterogeneity	
			Lower	Upper			I^2	p-value
Overall	25	1.223	0.523	1.923	0.357	<0.001	87.16	<0.001
Hen's age								
<50 weeks	14	2.527	1.402	3.653	0.574	<0.001	91.67	<0.001
>50 weeks	11	0.053	-0.657	0.762	0.362	0.884	67.33	0.001
Inclusion rate								
<1%	12	1.008	-0.074	2.090	0.552	0.068	89.00	<0.001
1-5 %	10	0.611	-0.067	1.290	0.346	0.077	71.80	<0.001
>5%	3	6.885	3.834	9.837	1.532	<0.001	58.25	0.091
Treatment duration								
<50 days	14	1.464	0.396	2.533	0.545	0.007	87.20	<0.001
>50 days	11	0.923	-0.034	1.876	0.487	0.059	86.75	<0.001

Note: Estimate and I^2 were considered significant at p-value <0.05; N = Number of outcomes; SMD = standardized mean difference; CI = confidence interval; SE = standard error; I^2 = measure of heterogeneity

Yolk weight

From the pool of 19 studies that fulfilled the suitability criteria for participation in this study, four specifically chosen articles, which involved 7 comparisons and a total of 431 hens, were employed to examine the effects of Spirulina inclusion on YW. The overall mean estimate indicated an impact on YW (SMD = 0.367, 95% CI = -0.037 to 0.771; Table 8). Notably, a significant degree of heterogeneity was observed among the 4 studies analyzed for the effect of dietary Spirulina on YW, as indicated by Cochran's Q (Q) = 14.12, I2 = 57.51%, p-value = 0.028 (Table 2), when compared to the control groups. Following a thorough analysis of covariates, variables did not significantly influence YW (Table 8). Meta-regression failed to identify significant predictors for the impact of Spirulina on YW and did not clarify all sources of heterogeneity (Table 13).

Table 8 Subgroup analysis of the effect of Spirulina on yolk weight in laying hens

Covariates	N	SMD	CI 95%		SE	p-value	Heterogeneity	
			Lower	Upper			I^2	p-value
Overall	7	0.367	-0.037	0.771	0.206	0.075	57.51	0.028
Hen's age								
<50 weeks	2	0.270	-0.427	0.966	0.355	0.448	57.51	0.028
>50 weeks	5	0.403	-0.116	0.923	0.265	0.128	71.55	0.007
Inclusion rate								
<1%	2	0.274	-0.086	0.634	0.184	0.136	0	0.514
1-5 %	5	0.433	-0.233	1.099	0.340	0.202	70.48	0.009
Treatment duration								
<50 days	4	0.273	-0.047	0.593	0.163	0.094	0	0.927
>50 days	3	0.537	-0.537	1.610	0.548	0.327	57.51	0.028

Note: Estimate and I^2 were considered significant at p-value <0.05; N = Number of outcomes; SMD = standardized mean difference; CI = confidence interval; SE = standard error; I^2 = measure of heterogeneity

Yolk colour

Eleven selected articles, which include 12 comparisons and involve 1,407 hens from the pool of 19 studies meeting the suitability criteria, were chosen to investigate the effect of dietary Spirulina on YC. The remaining results indicated a conspicuous effect of the intervention on YC (SMD = 10.659, 95% CI = 7.696 to 13.622; Table 9). Remarkably, a considerable degree of heterogeneity was observed among the 10 studies analyzed for the effect of Spirulina inclusion on YC, as demonstrated by Cochran's Q (Q) = 421.48, I2 = 93.60%, p-value = <0.001 (Table 2), in comparison to the control groups. Upon conducting a detailed analysis based on covariates, variables significantly impacted YC, except for studies that used an inclusion rate >5% (Table 9). Despite this, meta-regression failed to identify significant predictors for the effect of Spirulina on YC and did not clarify all sources of heterogeneity (Table 13).

Table 9 Subgroup analysis of the effect of Spirulina on yolk colour in laying hens

Covariates	N	SMD	CI 95%		SE	p-value	Heterogeneity	
			Lower	Upper			I^2	p-value
Overall	28	10.659	7.696	13.622	1.512	<0.001	93.59	<0.001
Hen's age								
<50 weeks	10	21.060	15.141	26.978	3.020	<0.001	92.80	<0.001
>50 weeks	18	4.593	1.424	7.763	1.617	0.005	92.36	<0.001
Inclusion rate								
<1%	10	7.864	5.182	10.546	1.369	<0.001	85.78	<0.001
1-5 %	14	14.005	8.144	19.866	2.990	<0.001	95.03	<0.001
>5%	4	16.672	-1.006	34.350	9.020	0.065	95.92	<0.001
Treatment duration								
<50 days	19	13.944	9.157	18.731	2.442	<0.001	95.19	<0.001
>50 days	9	7.635	4.987	10.282	1.351	<0.001	93.59	<0.001

Note: Estimate and I^2 were considered significant at p-value <0.05; N = Number of outcomes; SMD = standardized mean difference; CI = confidence interval; SE = standard error; I^2 = measure of heterogeneity

Haugh unit

Nine articles, incorporating 28 comparisons and involving 1,263 hens from the pool of 19 studies meeting the criteria for inclusion in this study, were explicitly chosen to delve into the influence of dietary Spirulina on HU. The overall mean estimate unveiled a distinctive treatment impact on HU (SMD = 2.189, 95% CI = 1.297 to 3.081; Table 10). Remarkably, there was a significant level of heterogeneity among the 9 studies scrutinized for the effect of Spirulina inclusion on HU evident in Cochran's Q (Q) = 150.63, I2 = 86.60%, p-value = <0.001 (Table 2), when compared to the control groups. Upon conducting a meticulous analysis based on covariates, variables significantly shaped HU, except for studies employing hen's age >50 weeks (Table 10). All covariates, excluding the hen's age >50 weeks, exhibited a noteworthy surge in HU; Table 10. Meta-regression underscored that hen's age (Q_M = 7.10, df = 1, p-value = 0.008) emerged as a crucial predictor of the Spirulina effect on HU and elucidated all sources of heterogeneity (Table 13).

Table 10 Subgroup analysis of the effect of Spirulina on haugh unit in laying hens

Covariates	N	SMD	CI 95%		SE	p-value	Heterogeneity	
			Lower	Upper			I^2	p-value
Overall	22	2.189	1.297	3.081	0.455	<0.001	86.06	<0.001
Hen's age								
<50 weeks	13	3.113	2.035	4.191	0.550	<0.001	83.96	<0.001
>50 weeks	9	0.744	-0.667	2.155	0.720	0.301	85.15	<0.001
Inclusion rate								
<1%	10	1.985	0.683	3.287	0.664	0.003	81.17	<0.001
1-5 %	9	1.565	0.236	2.895	0.678	0.021	90.01	<0.001
>5%	3	5.191	3.659	6.723	0.782	<0.001	1.30	0.363
Treatment duration								
<50 days	13	1.630	0.321	2.940	0.668	0.015	90.13	<0.001
>50 days	9	2.855	1.854	3.855	0.510	<0.001	65.06	0.004

Note: Estimate and I^2 were considered significant at p-value <0.05; N = Number of outcomes; SMD = standardized mean difference; CI = confidence interval; SE = standard error; I^2 = measure of heterogeneity

Yolk cholesterol concentration

Seven research articles, with a total of 18 comparisons and 953 hens from the 19 eligible studies, were utilized to investigate how Spirulina in the diet affects YCC in laying hens. The overall conclusion from the data suggests a noticeable impact of the Spirulina treatment on reducing YCC (SMD = -0.964, 95% CI = -1.765 to -0.164; Table 11). Notably, there was a considerable level of variation among the 7 studies when assessing the impact of dietary Spirulina on YCC, indicated by Cochran's Q (Q) = 101.97, I2 = 83.33%, p-value = <0.001 (Table 2), in comparison to the control groups. Upon a closer examination of the data considering various factors, it was found that these variables did not significantly influence YCC, except for studies using an inclusion rate of 1-5% (Table 11). Studies incorporating an inclusion rate of 1-5% showed a significant decrease in YCC (Table 11). Furthermore, the meta-regression analysis identified that the inclusion rate (Q_M = 8.53, df = 2, p-value = 0.014) played a significant role in predicting the effect of Spirulina on YCC and helped explain all sources of variability (Table 13).

Table 11 Subgroup analysis of the effect of Spirulina on yolk cholesterol concentration in laying hens

Covariates	N	SMD	CI 95%		SE	p-value	Heterogeneity	
			Lower	Upper			I^2	p-value
Overall	18	-0.964	-1.765	-0.164	0.408	0.018	83.328	0.001
Hen's age								
<50 weeks	10	-1.515	-2.850	-0.179	0.681	0.026	88.01	<0.001
>50 weeks	8	-0.646	-1.575	0.284	0.484	0.173	73.95	<0.001
Inclusion rate								
<1%	5	-0.249	-0.820	0.323	0.292	0.394	0	0.884
1-5 %	10	-1.141	-2.017	-0.264	0.447	0.011	80.14	<0.001
>5%	2	-25.071	-55.961	5.819	15.760	0.112	96.01	<0.001
Treatment duration								
<50 days	14	-0.943	-1.927	0.041	0.502	0.060	84.32	<0.001
>50 days	4	-1.163	-2.636	0.310	0.752	0.122	82.92	0.001

Note: Estimate and I^2 were considered significant at p-value <0.05; N = Number of outcomes; CI = confidence interval; SE = standard error; I^2 = measure of heterogeneity

Publication bias analysis

The potential existence of publication bias was suggested by the funnel plots, with Rosenthal Nfs values exceeding the predetermined thresholds for considering the pooled effect size results robust. Specifically, the values for FI, FCR, LR, EW, ST, YW, YC, HU, and YCC were 225, 1,803, 2,528, 2,436, 328, 13, 3,929, 1,234, and 248, respectively. These values surpass the respective thresholds of 185, 175, 205, 240, 135, 45, 150, 120, and 100 (for FI, FCR, LR, EW, ST, YW, YC, HU, and YCC). Consequently, the meta-analysis suggests that publication bias is not a significant concern, as many studies that were not published would be necessary to alter the statistically significant impacts of Spirulina in the diet on the response variables (Table 12, Figure 2).

Table 12 Analysis of publication bias (Rosenthal fail-safe number)

Parameter	SMD	Observed significance	Target significance	Nfs number	n	Nfs>[5 (a) +10]
Feed intake	-	<0.001	0.05	225	35	185
FCR	-	<0.001	0.05	1803	33	175
Laying rate	-	<0.001	0.05	2528	39	205
Egg weight	-	<0.001	0.05	2436	46	240
Shell thickness	-	<0.001	0.05	328	25	135
Yolk weight	-	0.003	0.05	13	7	45
Yolk colour	-	<0.001	0.05	3929	28	150
Haugh unit	-	<0.001	0.05	1234	22	120
Yolk cholesterol concentration	-	<0.001	0.05	248	18	100

Note: SMD = standardized mean difference; n = number of outcomes; Nfs = fail-safe number

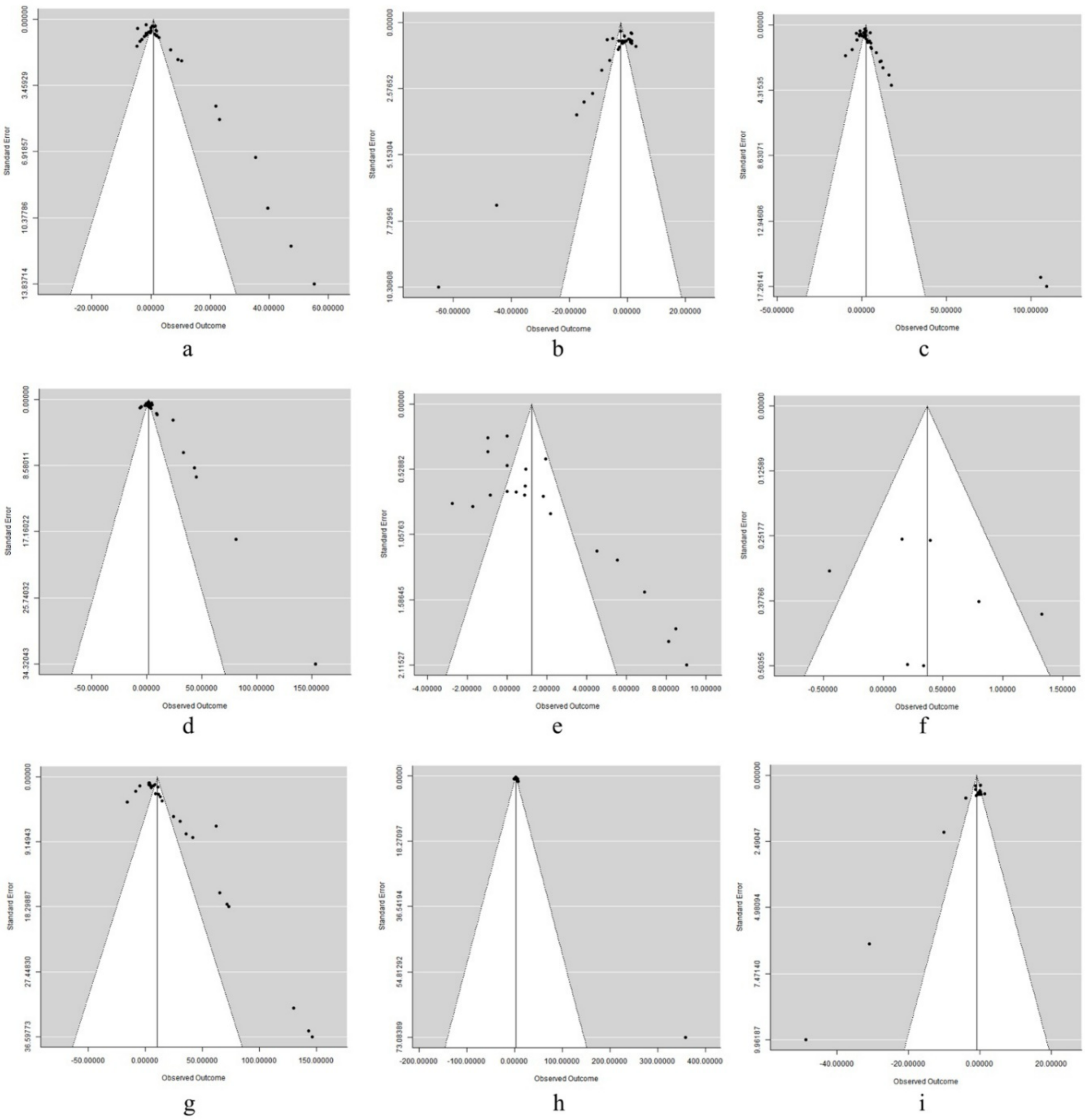

Figure 2 Funnel plots of the effects of Spirulina supplement on feed intake (a), feed conversion ratio (b), laying rate (c), egg weight (d), shell thickness (e), yolk weight (f), yolk colour (g), haugh unit (h), and yolk cholesterol concentration (i) in laying hens

DISCUSSION

In this investigation, the combined effect size results suggested that adding Spirulina to the diet of laying hens did not result in a statistically significant change in their FI parameter. This lack of a significant effect with Spirulina supplementation on laying hen FI was also corroborated by the studies of Tufarelli et al. (2021), Curabay et al. (2021) and Hasan et al. (2018). Moreover, previous research conducted by Zahroojian et al. (2011) noted that several levels of dietary Spirulina (1.5%, 2%, and 2.5%) did not influence FI in 63-week-old Hy-line W36 hens. Conversely, Abbas et al. (2022) reported an increase in FI with Spirulina supplementation at 6-12% doses, while a lower dose of 3% showed no difference in FI in 40-week-old Hy-Line W36 hens. The meta-regression analysis in this study also revealed that the moderate effects of inclusion rate and hen's age as moderators explain 81.56% and 21.61%, respectively, of the variability in studies related to the impact of Spirulina feeding on laying hens FI.

Table 13 Mixed-effect meta regression of moderators on response parameters

Parameter	Covariates	Q_M	df	p-value	R^2 index (%)
Feed intake	Hen's age	11.50	1	<0.001	21.61
	Inclusion rate	38.90	2	<0.001	81.56
	Treatment duration	0.63	1	0.428	0
FCR	Hen's age	0.29	1	0.590	0
	Inclusion rate	6.98	2	0.030	13.36
	Treatment duration	1.59	1	0.208	0
Parameter	Covariates	Q_M	df	p-value	R^2 index (%)
Laying rate	Hen's age	2.18	1	0.139	10.62
	Inclusion rate	35.40	2	<0.001	62.66
	Treatment duration	1.15	1	0.283	0
Egg weight	Hen's age	17.60	1	<0.001	33.11
	Inclusion rate	29.30	2	<0.001	0
	Treatment duration	0.64	1	0.425	0
Shell thickness	Hen's age	7.25	1	0.007	20.26
	Inclusion rate	13.70	2	0.001	38.01
	Treatment duration	0.03	1	0.865	0
Yolk weight	Hen's age	0.06	1	0.812	0
	Inclusion rate	0.10	2	0.755	0
	Treatment duration	0.25	1	0.616	0
Yolk colour	Hen's age	0.04	1	0.845	0
	Inclusion rate	3.33	2	0.189	0
	Treatment duration	2.17	1	0.140	0
Haugh unit	Hen's age	7.10	1	0.008	26.82
	Inclusion rate	6.67	2	0.036	20.25
	Treatment duration	2.16	1	0.142	6.00
Yolk cholesterol concentration	Hen's age	0.90	1	0.342	0
	Inclusion rate	8.53	2	0.014	0
	Treatment duration	0.06	1	0.803	0

Note: Q_M was considered significant at p-value <0.05; R^2 = amount of heterogeneity accounted for; df = degree of freedom; Q_M = coefficient of moderators; FCR = feed conversion ratio

The analysis of pooled effect sizes indicated that adding Spirulina significantly affected the FCR in laying hens. This significant impact of Spirulina use in laying hens was further supported by Zahroojian et al. (2013) and Panaite et al. (2023). Another study by Samia et al. (2018) demonstrated that Spirulina supplementation at 0.2% and 0.3% affected FCR in 29-week-old Golden Montazah laying hens. Abbas et al. (2022) also stated that using Spirulina at 6% and 9%

doses significantly reduced FCR in the Hy-line W36 strain. However, contrary results were reported by Omri et al. (2019), Mardanpour et al. (2020) and Poveda-Víquez et al. (2023), stating that the use of Spirulina in laying hen did not significantly influence FCR with doses of 1% until 5%. Meta-regression indicated that the inclusion rate as a moderator has a moderate effect, explaining 13.36% of the variation in studies related to the effect of dietary Spirulina on the laying hens' FCR.

Our findings revealed that layer hens fed by Spirulina as a supplement showed a significantly increased LR compared to the group that did not receive the supplement. Incorporating Spirulina into the diet at concentrations ranging from 3% to 9% was found by Al-Otaibi et al. (2022) to considerably boost the LR, with improvements of 5.96% to 11.17% relative to those in the control group. The observed increase in egg production in layers receiving Spirulina supplementation might be related to Spirulina's potential as an antioxidant. According to Abbas et al. (2022), this microalgae contains high amounts of polyphenolic and flavonoid compounds, collectively providing strong antioxidant properties. As a result, when laying hens were given increasing doses of Spirulina, their redox state increased linearly. Furthermore, the consumption of Spirulina has the potential to decrease inflammation owing to its phenolic and flavonoid components that can impede the formation of reactive oxygen species, thereby mitigating their effects on cellular endurance, as was documented by Kumar and Pandey (2013). Comparing our work, Zahroojian et al. (2013) noted that adding Spirulina up to a dose of 2.5% resulted in egg production results similar to controls in Hy-line 36, aged 63 weeks. Based on subgroup analysis, the variability among authors regarding the effect of Spirulina addition on LR might be due to the age of the chickens, the level of inclusion, and the duration of treatment.

Pooled estimation indicates that layers fed with Spirulina had numerically higher ST than the un-supplemental group. Selim et al. (2018) published similar findings, indicating that adding Spirulina to the diet at a concentration of 0.3% positively impacted ST. The increase in egg ST with Spirulina supplementation was also observed by Omri et al. (2019). Layers given Spirulina at a dose of 0.3% could increase ST by up to 34.71%. This could be associated with the macro mineral content present in Spirulina, mainly calcium (Abbas et al., 2022). The addition of Spirulina to laying hens had a significant effect on EW. Panaite et al. (2023) and Omri et al. (2019) documented similar results. Furthermore, according to Al-Otaibi et al. (2022), there was a good correlation between the dosage of Spirulina (3%, 6%, 9%) and the EW increase in 44-week-old Hy-line W36 layer chicken. The increased EW was presumed to be due to the high protein content in micro-algae Spirulina (Omri et al., 2019). However, Ahammed et al. (2018) and Nia et al. (2021) reported that supplementing Spirulina only affected EW at dosage levels of 0.1% and 0.9%. Meta-regression analysis showed that the hen's age and inclusion rate as moderators had moderate effects of 33.11% and 0%, respectively, on the variation in studies related to the effect of Spirulina supplementation on EW in laying hens.

The utilization of Spirulina did not have any impact on the YW in laying hens. The study conducted by Samia et al. (2018) found that the application of 0.2% and 0.3% did not impact the YW in Golden Montazah laying hens. In addition, Omri et al. (2019) found that including Spirulina at the dosage of 1.5% and 2.5% on laying diets did not influence the YW variable. The pooled effect size analysis showed that using Spirulina in a laying hen diet could increase the YC intensity. In the research conducted by Panaite et al. (2023), it was found that adding 2% Spirulina resulted in an almost twofold rise in YC value, reaching 172% compared to the control. Wahyuni et al. (2023) also showed similar results, including 0.3% Spirulina enhanced YC value by 9.60% compared to the control treatment. The vibrant yellow hue of egg yolk can be attained by including carotenoids in the diet. Nevertheless, laying hens cannot produce these colour pigments through synthesis metabolism. Therefore, external intervention is necessary in the diet. Spirulina is a source of phytopigments containing phycocillin, phycocyanin, and allophycocyanin (Khan et al., 2021). In addition, Pereira et al. (2021) asserted that Spirulina is rich in zeaxanthin, xanthophylls, and various carotenoid pigments, including beta-carotene. The high beta-carotene content will be converted to retinol and deposited into the egg yolk (Abbas et al., 2022).

The significantly higher HU score in the Spirulina-treated layer feed than the control indicates better protein digestion and utilization in the diet. Our findings align with the conclusions of prior studies that examined this topic, corroborating the results found by Omri et al. (2019) and Khan et al. (2021). Conversely, Zahroojian et al. (2013) and Selim et al. (2018) found that Spirulina supplementation in the diet did not affect HU scores in layer hen. The meta-analysis revealed a clear and noteworthy improvement in HU scores due to the inclusion of Spirulina. However, it was essential to acknowledge that other factors, such as chicken strain, age, and strain/age interaction, may also have an impact on HU scores (Williams, 1992). The divergent findings across authors regarding the effects of Spirulina supplementation might be attributed to the age of the hens and the rate at which it was included in their diet. The observed reduction in laying hens YCC aligned with the findings of Selim et al. (2018). They discovered that including 0.3% Spirulina in the diet of Norfa laying hens (White Leghorn×Fayoumi×White Baladi) efficiently decreased YCC by 13.97% compared to the control group. The presence of a significant amount of polyunsaturated fatty acids in Spirulina is the reason for this phenomenon (Selim et al., 2018). Omega-3 fatty acids enhance the function of lecithin cholesterol acyltransferase, an enzyme that converts cholesterol into cholesterol esters. As a result, most of the newly formed cholesterol esters are initially added to high-density lipoprotein (Vaysse-Boué et al., 2007). In addition, microalgae docosahexaenoic acid can hinder the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which results in a decrease in cholesterol synthesis and subsequently lowers the levels of cholesterol in the bloodstream (Chen et al., 2011). Contrary to our results, Omri et al. (2019) discovered that incorporating Spirulina into the diet of White Lohmann White laying hens had no impact on YCC. The variation in findings among these research could be attributed to disparities in the inclusion levels, as evidenced by the outcomes derived from meta-regression.

CONCLUSIONS

The findings derived from the meta-analysis of 19 studies indicate that the supplementation of Spirulina led to enhancements in various aspects, including FCR, LR, EW, ST, YC, and HU, while concurrently reducing YCC. Adding Spirulina to feed did not impact FI and YW. Specifically, incorporating lower concentrations of Spirulina (i.e., below 1%) as a dietary additive is more conducive to achieving a higher laying rate in layer hens. Subgroup analyses revealed that specific moderators, such as inclusion level, hen age, and treatment duration, influenced factors of the response variables. Compared to the control groups, the significant enhancements reported in egg production and quality attributes in laying hens fed with Spirulina-enriched diets can facilitate progress in policy and the sustainable utilization of Spirulina in the egg production industry. The result of this study recommend the use of spirulina in layer hen feed at concentrations of less than 1%.

AUTHOR CONTRIBUTIONS

Muhammad Maulana Sadid: Collection and selection of the articles, data curation, data analysis, visualization, and original draft preparation. Moh Sofi'ul Anam: Conceptualization, methodology, analytical software, data curation, original draft preparation, review, and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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