Dietary Phytogenic Extracts Favorably Influence Productivity, Egg Quality, Blood Constituents, Antioxidant and Immunological Parameters of Laying Hens: A Meta-Analysis

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Abstract: The present study aimed to assess the impact of dietary phytogenic extracts on laying hen productivity, egg quality, blood constituents, immunological, and pathological parameters through a meta-analytical approach. A total of 28 articles (119 data points) reporting the influence of dietary phytogenic extracts on the productive performance, egg quality, blood constituents, immunological, and antioxidant parameters of laying hens were embedded into a database. Statistical analysis was performed using a mixed model, with different studies treated as random effects and phytogenic extract levels treated as fixed effects. This meta-analysis revealed that dietary phytogenic extracts quadratically ($p < 0.05$) improved egg production and egg mass as well as decreased ($p < 0.05$) the feed conversion ratio (FCR) with no adverse effect on egg weight and egg quality. Feed intake and egg yolk percentage tended to increase linearly ($p < 0.1$). Total serum cholesterol and low-density lipoprotein (LDL) declined quadratically ($p < 0.001$ and $p < 0.05$, respectively), high-density lipoprotein (HDL) increased linearly ($p < 0.001$), and malondialdehyde (MDA) decreased linearly ($p < 0.01$), with increasing levels of dietary phytogenic extract. In addition, immunoglobulin G (IgG), immunoglobulin A (IgA), glutathione peroxidase (GSH-Px), and total superoxide dismutase (TSOD) increased linearly ($p < 0.05$) in line with the increase in dietary phytogenic extract level. It was concluded that the inclusion of phytogenic extracts in the diet of laying hens had a positive effect on productive performance, feed efficiency, egg mass, immunity, and antioxidant activity without interfering with egg quality. The optimum level of feed phytogenic extract for egg production and feed efficiency was determined to be around 300 mg/kg feed.

Keywords: bioactive compounds; egg; polyphenol; laying hens; meta-analysis
1. Introduction

The implementation of regulations prohibiting the application of antibiotics as growth promoters and growing concerns over the safety of poultry products have increased interest in the use of plant-based alternative feed additives. Phytophagic feed additives obtained from herbal plant extracts are commonly used in poultry, particularly in laying hens. Phytophagics, also known as phytobiotics, have beneficial effects on gut health and performance due to the presence of bioactive compounds such as polyphenols with antimicrobial, antioxidative, immunomodulatory, and anti-inflammatory properties [1,2]. Polyphenol compounds are the most widely produced plant bioactive compounds that serve to protect plants from the pests and UV radiation that can be found in plant parts, including the fruit, seeds, roots, bark, and leaves [3]. Flavonoids, phenolic acids, tannins, oligomeric proanthocyanidins, alkylresorcinols, avenanthramides, and lignans are some of the most well-known polyphenol groups [4].

Numerous studies on the diet of laying hens have confirmed the beneficial impact of phytophagic extracts on productive performance, egg quality, oxidative status, and immune system. However, several studies have also discovered a negligible effect of phytophagic extracts supplementation on the productive parameters or egg quality [5,6]. On the other hand, they may only produce a negative impact on poultry when added to diets at high levels [7,8]. For instance, a phenolic group such as tannins exhibits anti-nutritional properties at high concentrations. High levels of tannins (more than 10 g/kg feed) from plant extracts in the poultry diet can precipitate the protein and reduce fat digestion by binding bile salts or inactivating digestive enzymes [4,9,10]. Although several qualitative review articles have discussed differences in the responses of laying hens to dietary phytophagic extracts and a meta-analysis approach in broiler chickens [11,12], no meta-analysis has been performed to date in laying hens to quantify these differences. Therefore, the current meta-analysis study aimed to assess the impact of dietary phytophagic extracts on laying productivity, egg quality, blood constituents, and antioxidant and immunological parameters.

2. Materials and Methods

2.1. Database Development

Ethical approval was not required to conduct a meta-analysis study. The articles discussing the application of phytophagic extracts in laying hens were retrieved from scientific electronic databases such as Scopus, Web of Science, Crossref, Pub Med, Science Direct, and Google Scholar. To assist in the article selection process, search keywords such as “phytogenic”, “extract”, “phenolic”, “flavonoid”, and “laying hens” were applied. The following criteria were used to select articles for inclusion in the database: (a) performing in vivo trials on laying hens, (b) exclusively employing phytophagic extracts in the diets, (c) administration of extracts only through feed and without other confounding treatments, (d) reporting laying hens’ performance, egg quality, and blood parameters, and (e) the articles were written in English.

A total of 200 articles were initially found from the search engines based on the title and abstract of the article (Figure 1). The titles and abstracts of the articles were then screened based on the above-mentioned criteria, and 50 articles were eliminated for being improper, such as duplicate articles, review articles, or not being written in English. Finally, a total of 28 articles were added to the database for the meta-analysis after reviewing the substance including the data presentation, type of treatment, parameters observed, number of chickens, and proper statistical criteria.

2.2. Extraction and Description of Data

The information from the 28 selected articles is summarised in Table 1, including the authors’ names, publication year, strain, number and age of laying hens, extract level, plant name, plant part extracted, phytophagic content, and extract solvent type. Meanwhile, the variables included in the database were laying hen performances (egg production, feed intake, feed conversion ratio (FCR), egg weight, egg mass), egg qualities (eggshell thickness,
eggshell strength, eggshell weight, egg yolk weight, albumen weight, Haugh unit, egg index, egg yolk colour, egg yolk cholesterol), and blood serum parameters (albumin, total protein, glycogen, aspartate aminotransferase (AST), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), total superoxide dismutase (TSOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA)). Due to the limited number of studies, data on intestinal morphology and gut microbial population were not included in the database. Prior to the data processing, the parameter data were converted into similar units of measurement. The phytogenic extract level was reported in milligrams per kilogram of diet (mg/kg).

The articles (Table 1) were published between 2013 and 2022. A total of 7275 laying hens with a majority of Lohmann (28.6%) and Hy-line strain (25.0%) were used in the study. The plant materials used were leaf, bulb, seed, and peel that were extracted using water, ethanol, and petroleum ether as solvents. However, the types of solvent and plant material were not mentioned in several articles and/or were commercially used and were thus recorded as “unknown” in Table 1. The phytogenic extract levels ranged from 0 to 1000 mg/kg and were fed to laying hens aged 19 to 74 weeks.

2.3. Statistical Analysis

A mixed-model approach was used to analyze the data [13], which was performed in SAS® On Demand for Academics using the MIXED PROC procedure. The different studies were treated as random effects and the phytogenic extract levels were treated as fixed effects. The statistical model was as follows:

\[ Y_{ij} = \beta_0 + \beta_1 X_{ij} + \beta_2 X_{ij}^2 + s_i + b_i X_{ij} + \epsilon_{ij} \]
where $Y_{ij} =$ dependent variable, $\beta_0 =$ intercept in studies, $\beta_1 =$ coefficient of linear regression, $\beta_2 =$ coefficient of quadratic regression, $X_{ij} =$ continuous variable predictor value (extract level), $b_i =$ random effect of study $i$ on the regression coefficient of $Y$ on $X$, $s_i =$ random effect of study, and $e_{ij} =$ unexplained residual error.

Because the variable study contained no quantitative data, it was defined in the class expression. The corresponding linear regression model was used when the quadratic regression model was not significant. The $P$-value, the Akaike information criterion (AIC), and root mean square error (RMSE) were applied in the statistical model. The effect of treatment was considered significant at $p$-value $< 0.05$ and tended to be significant at $p < 0.1$.

**Table 1.** Studies descriptions included in the database.

| Author            | Source             | Main Bioactive Compound                                                                 | Extract Level (mg/kg) | Chicken Breeds | Number of Birds | Age (Week) |
|-------------------|--------------------|----------------------------------------------------------------------------------------|-----------------------|----------------|----------------|------------|
| Rahman et al. [8] | Mentha piperita    | menthol,menthone,menthyl acetate                                                      | 0–200                | Babcock        | 252            | 21–30      |
| Oh et al. [14]    | Diospyros kaki L.  | caffeic, p-coumaric, ferulic, gallic acids, tannins, terpenoids, naphthoquinones     | 0–750                | Hy-lyne brown | 120            | 50–56      |
| Liu et al. [15]   | commercial product | quercetin                                                                              | 0–600                | Hessian        | 240            | 28–36      |
| Ying et al. [16]  | commercial product | quercetin                                                                              | 0–600                | Hessian        | 240            | 29–38      |
| Alagawany et al. [17] | Yucca schidigera | yuccaols, resveratrol                                                                   | 0–150                | Hi-sex-brown   | 96             | 36–52      |
| Ahmed et al. [18] | Olea europaea L.   | hydroxytyrosol, vanillin, rutin, caffeic acid, catechin                                | 0–150                | Bandarah       | 360            | 24–42      |
| Iskender et al. [19] | commercial product | hesperidin, naringin, quercetin                                                        | 0–500                | Lohmann white  | 96             | 29–40      |
| Damaziak et al. [20] | Allium sativum, Allium cepa L. | thymol, carvacrol (Thymus vulgaris); anethole, limonene, fenchone, estragole, safrole, camphene (Foeniculum vulgare) | 0–40                | Hy-Line        | 200            | 26–38      |
| Sun et al. [21]   | grape seed         | procyanidins, proanthocyanidins                                                         | 0–150                | Hy-Line brown  | 640            | 25–33      |
| Vakili and Heravi [22] | Thymus vulgaris, Foeniculum vulgare | thymol, carvacrol (Thymus vulgaris); anethole, limonene, fenchone, estragole, safrole, camphene (Foeniculum vulgare) | 0–40                | Hy-Line        | 200            | 26–38      |
| Park et al. [23]  | Trigonella foenum-graecum L. | 4-hydroxy isoleucine, trigonelline, carotenoids, coumarins, saponins                  | 0–1000               | Hy-Line brown  | 96             | 36–52      |
| Simitzis et al. [24] | commercial product | quercetin                                                                              | 0–700                | Lohmann brown-classic | 192            | 70–74      |
| Damaziak et al. [25] | Zingiber officinale, Thymus vulgaris | gingerol, shaloa (Zingiber officinale); borneol, thymol, carvacrol (Thymus vulgaris) | 0–32                | ISA brown      | 216            | 19–35      |
| Xie et al. [26]   | Lonicera confusa, Astragali radix | luteolin, chlorogenic acid, caffeic acid (Lonicera confusa); astragaloside, formononetin, calycosin (Astragali radix) | 0–1000               | Lohmann pink-shell | 1440           | 52–64      |
| Song et al. [27]  | Camelia oleifera   | glucuronic acid, xyllose, rhamnose, methyl pentose                                      | 0–500                | Hy-Line brown  | 180            | 26–38      |
| Huang et al. [28] | Camellia sinensis (L.) O. Ktze. | theanine, theobromine, caffeine, catechins                                              | 0–300                | Lohmann brown  | 240            | 30–38      |
| Huang et al. [29] | Rhizoma drynariae   | naringin, neooricitrin, triterpenes, phenylpropanoids                                  | 0–200                | Lohmann pink-shell | 216            | 54–67      |
3. Results

3.1. Productive Performances and Egg Quality

Dietary phytogenic extract quadratically increased \( (p < 0.05) \) egg production (Figure 2) and egg mass, and it quadratically \( (p < 0.05) \) decreased FCR. Feed intake tended to increase linearly \( (p < 0.1) \); however, the inclusion of phytogenic extracts did not affect the egg weight (Table 2). Based on the egg production and FCR parameters, the optimum phytogenic extract levels for laying hens were 292 mg/kg and 313 mg/kg feed, respectively. In general, the administration of phytogenic extracts did not affect the egg qualities (eggshell weight, eggshell thickness, eggshell strength, egg yolk colour, egg index, albumen weight, Haugh unit). However, the egg yolk weight percentage tended to increase linearly \( (p < 0.1) \) (Table 3).

![Figure 2. Quadratic equation of dietary phytogenic extract (mg/kg) to egg production (%).](image-url)
Table 2. Regression equations for the impact of phytogenic extract levels on productive performances of laying hens.

| Parameter                        | n   | Intercept | SE Intercept | Slope    | SE Slope | p-Value | RMSE  | AIC   | Model | Trend |
|----------------------------------|-----|-----------|--------------|----------|----------|---------|-------|-------|-------|-------|
| Egg production (%)               | 72  | 83        | 1.77         | 0.0234   | 0.007055 | 0.02    | 7.25  | 457   | Q     | Positive |
| Feed intake (g/hen/day)          | 97  | 112       | 2.32         | 0.00315  | 0.00177  | 0.08    | 6.16  | 576   | L     | Positive |
| FCR                              | 94  | 2.1       | 0.049        | -0.00027 | 0.000185 | <0.001  | 0.21  | -36   | Q     | Negative |
| Egg weight (g/egg)               | 102 | 61.2      | 0.78         | 0.000948 | 0.000639 | 0.14    | 2.21  | 349   | L     | - |
| Egg mass (g/hen/day)             | 101 | 49.6      | 2.47         | 0.0119   | 0.00388  |         |       |       |       |       |

n, treatment number; RMSE, root mean square error; AIC, Akaike information criterion; SE, standard error; Q, quadratic; L, linear; FCR, feed conversion ratio.

Table 3. Regression equations for the impact of phytogenic extract levels on the egg quality of laying hens.

| Parameter                        | n   | Intercept | SE Intercept | Slope    | SE Slope | p-Value | RMSE  | AIC   | Model | Trend |
|----------------------------------|-----|-----------|--------------|----------|----------|---------|-------|-------|-------|-------|
| Eggshell thickness (mm)          | 99  | 0.36      | 0.0056       | 0.000111 | 0.0000777| 0.16    | 0.04  | 422   | L     | - |
| Eggshell strength (Newton)       | 92  | 37.4      | 0.91         | 0.00119  | 0.00118  | 0.32    | 5.86  | 514   | L     | - |
| Albumen weight (%)               | 21  | 60.8      | 1.88         | -0.00022 | 0.00155  | 0.89    | 2.41  | 93.3  | L     | - |
| Egg yolk weight (%)              | 42  | 27.2      | 1.21         | 0.000672 | 0.000367 | 0.08    | 1.31  | 155   | L     | Positive |
| Eggshell weight (%)              | 33  | 12.7      | 0.66         | 0.00102  | 0.001104 | 0.37    | 1.32  | 102   | L     | - |
| Haugh unit                       | 119 | 85        | 1.41         | 0.00167  | 0.00157  | 0.29    | 7.34  | 559   | L     | - |

n, treatment number; RMSE, root mean square error; AIC, Akaike information criterion; SE, standard error; L, linear.

3.2. Blood Constituents and Egg Yolk Cholesterol

The effect of phytogenic extracts on blood constituents and egg yolk cholesterol concentration is presented in Table 4. Serum cholesterol and LDL concentrations declined quadratically (p < 0.001 and p < 0.05, respectively) with increasing dietary phytogenic extract levels, whereas HDL concentration increased linearly (p < 0.05). Meanwhile, egg yolk cholesterol concentration tended to decrease linearly (p < 0.1). On the other hand, phytogenic extracts supplementation did not affect total protein, glucose, albumin, ALT, and AST.

Table 4. Regression equations for the impact of phytogenic extract levels on egg yolk cholesterol and blood parameters of laying hens.

| Parameter                        | n   | Intercept | SE Intercept | Slope    | SE Slope | p-Value | RMSE  | AIC   | Model | Trend |
|----------------------------------|-----|-----------|--------------|----------|----------|---------|-------|-------|-------|-------|
| Egg yolk cholesterol (mg/g)      | 20  | 13.4      | 0.9          | -0.0132  | 0.00676  | 0.08    | 4.04  | 118   | L     | Negative |
| Serum cholesterol (mg/dL)        | 54  | 151       | 7.73         | -0.168   | 0.0318   |         |       |       |       |       |
| LDL (mg/dL)                      | 36  | 50.7      | 8.49         | -0.0473  | 0.0128   |         |       |       |       |       |
| HDL (mg/dL)                      | 37  | 34        | 7.38         | 0.00657  | 0.0028   | 0.03    | 12.6  | 284   | L     | Positive |
| Total protein (g/L)              | 42  | 54        | 2.44         | -0.00396 | 0.00515  | 0.45    | 11.9  | 288   | L     | - |
| Albumin (g/dL)                   | 21  | 2.33      | 0.13         | -0.00028 | 0.000328 | 0.4     | 0.31  | 9     | L     | - |
| AST (U/L)                        | 26  | 205       | 21.1         | -0.0292  | 0.0279   | 0.31    | 41.1  | 246   | L     | - |
| ALT (U/L)                        | 23  | 2.64      | 0.69         | -0.00028 | 0.00103  | 0.8     | 0.97  | 66.1  | L     | - |

n, treatment number; RMSE, root mean square error; AIC, Akaike information criterion; SE, standard error; Q, quadratic; L, linear; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

3.3. Immunological and Antioxidant Parameters

The relationship between phytogenic extract level and immunological and antioxidant parameters is presented in Table 5. The IgM concentration was not affected by the addition.
of the phytogenic extract. However, the IgG, IgA, TSOD, and GSH-Px concentrations increased linearly ($p < 0.05$) with an increase in the dietary phytogenic extract level. Similarly, the concentration of MDA decreased linearly ($p < 0.01$) with increasing levels of phytogenic extracts.

**Table 5.** Regression equations for the impact of phytogenic extract levels on immunological and antioxidant parameters of laying hens.

| Parameter          | n    | Intercept | SE Intercept | Slope   | SE Slope | p-Value | RMSE | AIC  | Model | Trend |
|--------------------|------|-----------|--------------|---------|----------|---------|------|------|-------|-------|
| IgG (mg/dL)       | 22   | 3.56      | 0.75         | 0.00176 | 0.000593 | 0.01    | 0.74 | 38.7 | L     | Positive |
| IgM (mg/dL)       | 20   | 33.2      | 10.92        | 0.01073 | 0.01103  | 0.36    | 13.6 | 106.4| Q     | -     |
| IgA (mg/dL)       | 21   | 38.6      | 15.9         | 0.0158  | 0.00388  | 0.002   | 4.84 | 94.6 | L     | Positive |
| TSOD (U/mL)       | 33   | 194       | 18.8         | 0.0491  | 0.018    | 0.01    | 32.7 | 310  | L     | Positive |
| GSH-Px (U/mL)     | 28   | 7.56      | 0.86         | 0.0029  | 0.00122  | 0.03    | 7.4  | 160  | L     | Positive |
| MDA (nmol/mL)     | 21   | 4.21      | 0.16         | -0.00093| 0.00024  | 0.002   | 1.44 | 59.3 | L     | Negative |

$n$, treatment number; RMSE, root mean square error; AIC, Akaike information criterion; SE, standard error; Q, quadratic; L, linear; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; TSOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

### 4. Discussion

Phytogenics, also known as phytobiotics, are plant bioactive compounds that have beneficial effects on gastrointestinal health and the performance of poultry due to the presence of phytogenic compounds such as polyphenols with antioxidant and immunomodulatory properties [1,41]. Various studies have been conducted to assess the efficacy of phytogenic feed additives in laying hens to minimise antibiotic use. Beneficial effects on productive performance and egg quality were obtained by supplementing the laying hen’s diet with *Pinus massoniana* [40], *Curcuma longa* [32], *Geranium thunbergia*, and *Mentha arvensi* [42]. However, egg production, FCR, and egg weight were not increased with the addition of dietary *Thymus vulgaris* L. [43] and *Mentha piperita* [8]. Sharma et al. [6] stated that the administration of garlic and thyme to the diets of laying hens did not increase egg weight.

Our meta-analysis study generally revealed that dietary phytogenic extracts showed a positive effect on productivity, blood metabolites, and immunological and antioxidant parameters with no adverse effect on egg quality. Phytogenic compounds improved poultry performance by increasing digestive enzyme secretion, lowering the number of pathogenic bacteria in the digestive tract, or modulating intestinal morphology functions [4]. Previously, Iqbal et al. [44] and Tellez-Isaias et al. [45] confirmed that polyphenols can suppress several bacterial pathogens, including *Salmonella enteritidis* and *E. coli*. Similar results have shown that quercetin inclusion, one of the flavonoid compounds, enhanced the productive performance of laying hens due to its ability to reduce intestinal pathogenic bacteria [5,46]. This claim was supported by Mutlu et al. [47] who stated that the inclusion of quercetin can reduce coliforms in the cecal of laying quail and increase the *lactobacilli* population. In addition, the inclusion of curcumin has been reported to induce antibacterial activity through the inhibition of bacterial cell proliferation by interfering with the GTPase of the FtsZ protofilament activity, which was critically involved in bacterial cell division and survival [48]. In the case of gut health, Abdel-Moneim et al. [5] and Prihambodo et al. [11] revealed that flavonoids in herbal plants have a favorable effect on the digestive tract of poultry. They argued that flavonoids have antioxidant properties and can enhance the function of the small intestine in nutritional absorption. Other phytogenic compounds, such as genistein and hesperidin, also had a beneficial effect on gut morphology, including villus density, crypt depth, and villus height [49]. Then, higher villi increase the surface area of the intestine and improve nutrient absorption, whereas deeper crypts promote rapid villi renewal in response to pathogen-induced inflammation [50]. However, the limitations of this meta-analysis have not been able to confirm gut health due to the limited number of studies related to gut morphology and gut microbial populations of laying hens.
Based on the present meta-analysis, TSOD, GSH-Px, IgG, and IgA increased linearly in line with the increasing levels of dietary phytogenic extract. Under oxidative stress, the poultry body is unable to efficiently eliminate excess free radicals, particularly reactive nitrogen species and reactive oxygen species. Enzymatic mechanisms, including triad catalase, GSH-Px, and TSOD, are one mechanism for the removal of these free radicals [51,52]. Meanwhile, polyphenols are external antioxidants that serve as the first defense for cells against excessive free radical production and protect their constituents from oxidative damage. Among all polyphenols, flavonoids are the most effective at eliminating free radicals and preventing their negative effects [53,54]. For instance, naringenin and naringin have strong scavenging activity for lipid peroxidation inhibitors [55]. Furthermore, rutin, hesperidin, and genistein supplementation were found to improve GSH-Px, SOD, and T-AOC activity and decrease MDA serum concentrations [56,57]. Thus, these findings suggest that rutin, genistein, and hesperidin have the capacity to stimulate antioxidant enzymes, reduce oxidative stress, and further reduce MDA concentration in the blood. Generally, the mechanisms of polyphenol in protecting the cells from free radical oxidation include the activation of antioxidant enzymes (e.g., SOD, GPH-Px), pro-oxidant enzymes inhibition such as xanthine oxidase, direct cleaning of ROS by donating electrons, and an increase in the antioxidant activity of antioxidant substances (e.g., ascorbate, tocopherol) [58]. Phyto
genic extracts also provide promising immunotherapy due to the considerable increase in IgG and IgA concentrations. Recent studies have found that polyphenol modulates immune cell activity by binding to cellular receptors, modulating cell signalling pathways, and thus controlling host immunological responses [59]. For instance, tea polyphenols and curcumin raised the total antibody-secreting cells in the spleen and significantly improved immunoglobulin levels and humoral immune response [60,61]. Meanwhile, according to Abd El Latif et al. [62], the increase in immunoglobulin value following the addition of herbal plant supplementation rich in flavonoids prolonged the activity of other antioxidant properties such as vitamin C.

This meta-analysis revealed that supplementing the diets of laying hens with phyto
genic extracts lowered serum cholesterol, LDL, and improved HDL. Flavonoids can also reduce low-density lipoprotein cholesterol peroxidation by minimizing plasma and membrane lipid oxidation [63]. Zhou et al. [64] found that using flavonoid baicalein feed additive for broilers can reduce serum cholesterol and LDL. Wang et al. [60] reported that tea polyphenols reduced TC and LDL levels in serum due to increased cholesterol excretion through the excreta. The liver produces endogenous cholesterol and is transferred to extra-hepatic tissues by LDL. Meanwhile, HDL transports cholesterol from peripheral tissues to the liver before excreting it via the bile pathway [65]. In addition, polyphenols induce the expression of the cholesterol enzyme 7-alpha hydroxylase, which controls the bile synthesis and homeostasis of cholesterol and inhibits the activity of hydroxyl-3-methyl-glutaryl-CoA as a limiting enzyme for cholesterol synthesis [66,67]. Moreover, egg cholesterol deposition is closely related to plasma triglyceride level, total cholesterol, and LDL [68]. Our meta-
analysis approach confirmed the presence of lower levels of TC and LDL in plasma, as well as a linear tendency to decrease egg yolk cholesterol levels with higher dietary levels of phytogenic extracts.

Generally, while polyphenolic compounds have a beneficial impact at a certain level, several studies have reported a negative effect on the performance of poultry when polyphenols were added to poultry diets at high levels [7]. This decline in poultry performance may be attributed to the decreased digestion of fats and proteins through the binding of bile salts and/or inactivation of digestive enzymes. Meanwhile, the presence of polyphenol substances such as condensed tannins, which bind bile salts and restrict fat digestion, and the ability of polyphenols to bind endogenous proteins to form insoluble complexes may be related to the inhibition of digestive enzymes [5,9]. Therefore, the optimal level of dietary phytogenic extract identified through this meta-analysis approach can be considered to avoid these negative effects on laying hens.
5. Conclusions

The current meta-analysis confirms that the inclusion of phytogenic extracts in laying hens aged 19–74 weeks has a positive effect on productive performance, feed efficiency, and egg mass without interfering with egg quality. The optimum level of dietary phytogenic extract for egg production and feed efficiency is around 300 mg/kg diet. The phytogenic extracts have beneficial effects as antioxidant and immunomodulating agents demonstrated by an increase in TSOD, GSH-Px, IgA, IgG, and a decrease in oxidation products (MDA) in serum.

Author Contributions: Conceptualization, A.D., S. and E.O.; methodology, A.J.; software, A.D.; validation, A.J. and E.O.; formal analysis, A.D. and A.J; investigation, S. and E.O.; resources, A.D.; data curation, A.J.; writing—original draft preparation, A.D., W.H., D.M.S. and R.M.; writing—review and editing, S., A.J. and E.O.; visualization, W.H.; supervision, S. and E.O.; project administration, D.M.S. and R.M.; funding acquisition, A.J. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially provided by the Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors express gratitude to all members of Poultry Nutrition Division at IPB University and Ondokuz Mayas University for their assistance and support in the completion of this study.

Conflicts of Interest: The authors declare no conflict of interest.

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