

Dietary supplementation with tomato waste to improve performance and egg quality of laying hens: a meta-analysis

U.F. Handayani^{1,3}, A. Sofyan^{2,6,7,*}, D. Lestari³, M.M. Sholikin^{2,6}, W. Wulandari², M.A. Harahap², H. Herdian^{2,6,7}, H. Julendra², T. Okselni⁴ and M.E. Mahata⁵

¹ National Research and Innovation Agency, Post-doctoral of the Research Center for Animal Husbandry, 16915, Bogor, Indonesia

² National Research and Innovation Agency, Research Center for Animal Husbandry, 16915, Bogor, Indonesia

³ Universitas Muhammadiyah Kotabumi, Faculty of Agricultural and Animal Science, 34517, Kotabumi, Indonesia

⁴ National Research and Innovation Agency, Research Center for Pharmaceutical Ingredients and Traditional Medicine, 16915, Bogor, Indonesia

⁵ Universitas Andalas, Faculty of Animal Science, 25175, Padang, Indonesia

⁶ IPB University, Animal Feed and Nutrition Modelling Research Group (AFENUE), 16680, Bogor, Indonesia

⁷ National Research and Innovation Agency, Research Collaboration Center for Biomass and Biorefinery between BRIN and Universitas Padjadjaran, 16911, Bogor, Indonesia

KEY WORDS: cholesterol, laying hens, lycopene, meta-analysis, tomato waste

Received: 6 December 2022

Revised: 9 January 2023

Accepted: 20 January 2023

* Corresponding author:
e-mail: ahma053@brin.go.id

ABSTRACT. A meta-analysis was conducted to determine the effect of adding tomato waste to the diet of laying hens on their performance, egg quality, and blood parameters. A total of 22 articles were selected from five scientific databases such as Scopus, PubMed, ScienceDirect, Web of Science, and Directory of Open Access Journals. R software version 4.2.0, with supporting libraries, including *lme4* and *lsmeans* (least-squares means test), was used for modelling and statistical analysis. The results of meta-analysis showed that the addition of tomato waste to laying hen rations significantly increased feed intake ($P < 0.01$) and tended to correlate with a quadratic pattern of hen-day egg production ($P < 0.061$) and egg mass ($P < 0.075$); however, eggshell strength decreased significantly ($P < 0.01$). Our findings also demonstrated a significant effect on egg-yolk colour, as assessed by a quadratic pattern ($P < 0.01$). The lycopene content in egg yolk increased significantly ($P < 0.05$). Tomato waste supplementation had a significant ($P < 0.05$) effect on total triglyceride and cholesterol levels in blood serum, which followed a quadratic pattern. This meta-analysis has confirmed that feeding tomato waste to laying hens exerts positive effects on animal performance and egg quality.

Introduction

Lately, many people have come to understand that food is essential not only to satisfy appetite, but also to keep the body healthy. Animal products have always played a crucial role in human nutrition. Eggs are a source of protein widely consumed by humans. However, egg consumption is known to

increase cholesterol levels, which in turn increases the risk of heart disease and stroke in humans (Zhong et al., 2019). Eggs are also interesting in terms of developing their specific properties and nutritional value because they are highly responsive to the nutritional factors provided (Wang et al., 2017). An increasing amount of research has recently been conducted on waste-derived feed ingredients as

alternative feed components. Tomatoes are one of the most abundant agricultural products worldwide. Approximately 186 million tonnes of tomatoes are produced globally, with Asia accounting for 62.6% of total production and the rest being produced in the Americas (13.1%), Europe (12.2%), Africa (11.9%), and Oceania (0.2%) (FAOSTAT, 2022). Tomatoes serve as raw materials in many processed foods, including juices, canned fruits, and sauces. As a result, the tomato production and processing industries involve industrial waste due to the overproduction of fresh tomatoes or losses during processing (Laranjeira et al., 2022).

An interesting chemical compound called lycopene, contained in tomato waste, has attracted the attention of many researchers trying to maximize the utilisation of this residues. Lycopene is a natural pigment of the carotenoid family (Chauhan et al., 2011), which plants and microorganisms, such as fungi, bacteria, and algae, can synthesise (Rodriguez-Amaya, 2015; Reboul, 2019). Animals and humans in turn are not able to produce lycopene (Meroni and Raikos, 2018). Tomato powder contains 34.51 mg of lycopene in 100 g (Handayani et al., 2018) and may serve as a dietary supplement.

Lycopene can be used as an egg-yolk colouring agent that reduces cholesterol synthesis in the body. Carotenoid accumulation in egg yolk affects the intensity of its colour, whereas lycopene supplementation enhances a darker colour (Shevchenko et al., 2021). Lycopene supplementation in quail was shown to reduce blood serum and egg yolk cholesterol levels (Sahin et al., 2006). A positive effect of lycopene on lowering serum total cholesterol and low-density lipoprotein (LDL) levels, and increasing high-density lipoprotein (HDL) concentration was also reported in previous studies on rabbits (Verghese et al., 2008; Mulkalwar et al., 2012). Additionally, it was demonstrated that egg-yolk triglycerides could be reduced by lycopene supplementation (Hsu et al., 2015).

On the other hand, lycopene is known to reduce oxidative stress (Bin-Jumah et al., 2022) because it exerts a strong antioxidant effect (Jiang et al., 2015). Therefore, tomato waste, which is rich in lycopene, has a high potential to be a functional feed for laying hens, as it can supplement nutritional needs and improve egg quality by reducing their cholesterol levels and increasing egg yolk yellowness. Hence, the eggs produced would be healthier for consumption due to their low cholesterol levels, helping to overcome the phobia associated with high cholesterol concentrations. Furthermore, enriching the diet of hens with tomato waste had no adverse effect on their health;

on the contrary, tomato waste showed health-promoting effects, as it contains high concentrations of lycopene, acting as an antioxidant (Wang et al., 2022). Some studies showed that supplementation of laying hens' feed with tomato waste at concentrations of 9–12% reduced egg-yolk cholesterol and improved its colour (Hababashaka et al., 2014; Mahata et al., 2016). In contrast, other works reported no significant effect of tomato waste on lowering cholesterol levels in the blood and eggs of laying hens (Nobakht and Safamehr, 2007; Safamehr et al., 2011; Salajegheh et al., 2012; Jalalinasab et al., 2014; An et al., 2019). Furthermore, Jafari et al. (2006) found that feeding tomato pulp at a concentration of 15% reduced egg production of laying hens.

The wide range of differing results regarding the assessment of tomato addition effect prompted us to conduct a meta-analysis to summarise and comprehensively evaluate the effect of tomatoes in the diets of laying hens. This allowed the various results of previous studies to be summarised in a general manner. To the best of our knowledge, no meta-analysis studies have been carried out on this research topic. Therefore, using the meta-analysis method, the present study is the first to evaluate the effect of feeding tomato waste to laying hens on their production performance, egg quality, and blood parameters.

Material and methods

Literature search and study selection

This study discusses the effect of feeding tomato waste from cultivation or tomato processing by-products (e.g., tomato seeds, tomato peels, tomato pulp, tomato paste, or tomato pomace) on the production performance, egg quality, and blood parameters of laying hens. A literature search was conducted in several scientific databases (Scopus, PubMed, ScienceDirect, Web of Science, and Directory of Open Access Journals) using the keywords “laying hen” and “tomato”. The literature search had no publication year limit to identify all studies involving tomato administration to laying hens. This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009), as shown in Figure 1.

The literature was selected based on the following inclusion criteria: (1) *in vivo* studies published in English, (2) studies using tomatoes as animal feed, (3) studies with at least one response variable (including performance, egg quality, and blood parameters), and (4) studies

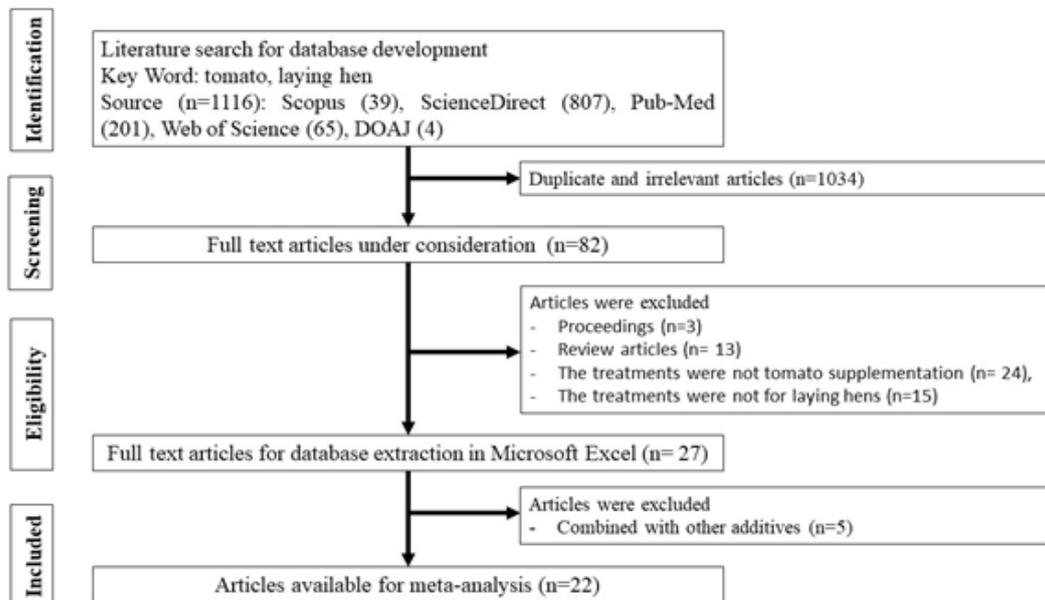


Figure 1. Flow chart of scientific literature search and selection for meta-analysis

providing complete details, such as broiler strain, number of replications, and age of bird (weeks). Exclusion criteria included proceedings, reviews, and studies that did not involve tomato supplementation or laying hens or where tomatoes were combined with additives.

Data from all included studies were extracted into a Microsoft Excel spreadsheet (Microsoft Co.,

Redmond, WA, USA). General information about the authors, country, animal strain, number of birds per replicate, study period, type of tomato waste, and diet composition is summarized in Table 1. Parameters compiled in the data were HDEP (hen-day egg production) (%), feed intake (g/hen/day), feed conversion ratio (FCR) (g/g), egg weight (g/egg), egg mass (g/hen/day), eggshell thickness (mm), eggshell

Table 1. Studies included in the meta-analysis database

| No. | References | Country | Strain | Birds in study | Period | Age of birds, week-old | Kind of tomato wastes | Level, % fed basis |
|-----|----------------------------|-------------|--------------------|----------------|--------|------------------------|------------------------------|--------------------|
| 1 | Akdemir et al., 2012 | Turkey | Lohman White | 90 | 90 | 49 | Tomato powder | 0–1 |
| 2 | An et al., 2019 | South Korea | Hy-Line Brown | 160 | 28 | 39 | Tomato paste | 0–1.7 |
| 3 | Bala et al., 2020 | Turkey | Lohman Brown | 90 | 30 | 30 | Tomato powder | 0–3.85 |
| 4 | Calislar and Uygur, 2010 | Turkey | Lohman Brown | 75 | – | 36 | Tomato pulp | 10–20 |
| 5 | Dotas et al., 1999 | Greece | Warren | 576 | 135 | 34 | Tomato pulp | 0–8 |
| 6 | Dotas et al., 1999 | Greece | Warren | 576 | 111 | 54 | Tomato pulp | 0–12 |
| 7 | Habanabashaka et al., 2014 | Rwanda | Rhode Island Red | 160 | 42 | 36 | Tomato waste (seed and peel) | 0–9 |
| 8 | Jafari et al., 2006 | Iran | Hy-Line | 288 | 84 | 27 | Tomato pulp | 0–15 |
| 9 | Jalalinasab et al., 2014 | Iran | Native laying hens | 180 | 84 | 28 | Tomato pomace | 8–16 |
| 10 | Mahata et al., 2016 | Indonesia | Isa Brown | 200 | 30 | 32 | Boiled tomato | 0–12 |
| 11 | Mahata et al., 2020 | Indonesia | Isa Brown | 200 | 60 | 32 | Boiled tomato | 0–12 |
| 12 | Mansoori et al., 2008 | Iran | White Leghorn | 144 | 70 | 54 | Tomato pomace | 0–10 |
| 13 | Nobakht and Safamehr, 2007 | Iran | Hy-Line | 182 | 63 | 65 | Tomato pomace | 0–10 |
| 14 | Orhan et al., 2021 | Turkey | Lohman | 150 | 84 | 20 | Tomato powder | 0–3 |
| 15 | Saed et al., 2018 | Iraq | Lohman Brown | 200 | – | 34 | Tomato pomace | 0–3 |
| 16 | Safamehr et al., 2011 | Iran | Hy-Line | 432 | 84 | 33 | Tomato pomace | 0–12 |
| 17 | Shahsavari, 2015 | Iran | Hy-Line | 540 | 84 | 36 | Tomato pulp | 0–5 |
| 18 | Tufarelli et al., 2022 | Iran | Hy-Line | 576 | 84 | 57 | Tomato pomace | 0–1.5 |
| 19 | Varzaru et al., 2021 | Romania | Tetra-SL | 90 | 28 | 42 | Tomato peel | 0–2 |
| 20 | Xue et al., 2013 | China | Hy-Line | 90 | 28 | 30 | Tomato extract | 0–0.02 |
| 21 | Yannakopoulos et al., 1992 | Greece | Harco-SL | 87 | 70 | 52 | Tomato seed | 0–15 |
| 22 | Rotolo et al., 2010 | Italy | Isa Brown | 20 | 14 | 20 | Tomato extract | 0–0.08 |
| 23 | Leke et al., 2015 | Indonesia | MB 405 | 100 | 56 | 36 | Tomato meal | 0–8 |

strength (kg/cm²), yolk weight (g/egg), eggshell weight (g/egg), albumen weight (g/egg), egg shape index (%), yolk index (%), yolk colour, Haugh units (HU), yolk cholesterol (mg/100 g), yolk fat (%), egg yolk lycopene (mg/100 g), and malondialdehyde (MDA) in egg yolk (µg/100 g). Additionally, several blood serum parameters, such as MDA (µmol/100 ml), total cholesterol (mg/dl), total triglycerides (mg/dl), LDL (mg/dl), HDL (mg/dl), and heterophil to lymphocyte (H/L) ratio were also considered. The units of measurement for each variable were manually converted if there was a difference between them.

Modelling and statistical analysis

The modelling aimed to determine the response of the specified parameters to tomato administration. R software version 4.2.0 with a code name “Vigorous Calisthenics”, with supporting libraries, including *lme4* and *lsmeans* (least-squares means test), was used for modelling and statistical analysis. Further, a linear mixed model (LMM) with linear and quadratic factors was applied to evaluate the effect of tomato addition to laying hens’ diet. The level of tomato supplementation was categorised as a fixed factor, and differences across studies were considered random factors (St-Pierre, 2001; Sauvant et al., 2008). The following LMM equation (1) was the basis for modelling the meta-data:

$$Y_{ijk} = \mu + S_i + \tau_j + S\tau_{ij} + \beta_1 X_{ij} + b_i X_{ij} + \beta_2 X_{ij}^2 + b_i X_{ij}^2 + e_{ijk} \quad (1),$$

where: Y_{ijk} – dependent variable, μ – overall mean, S_i – random effect of study i , assumed, $\sim N_{iid}(0, \sigma_S^2)$, τ_j – fixed effect of level j of factor τ_j , $S\tau_{ij}$ – random interaction between study i and level j of τ_j , factor assumed, $\sim N_{iid}(0, \sigma_{S\tau}^2)$, β_1 – overall value of the linear regression coefficient from Y to X (a fixed effect), β_2 – overall value of quadratic regression coefficient of Y for X (a fixed effect), X_{ij} and X_{ij}^2 – continuous value of the predictor variable (in linear and quadratic form, respectively), b_i – random effect of the study on the regression coefficient of Y for X, assumed, and e_{ijk} – residual error. $S\tau_{ij}$ and S_i are assumed to be random from the independent variables. Interactions between laying breeds (Br), treatment duration (Td), and tomato supplementation level (Tm) were calculated using a two-way test of variance. Least-squares means were used to test differences in tomato type for each parameter (Searle et al., 1980).

The model was tested for significance determination and validation. One-way analysis of variance (ANOVA) was used to determine significant values

of the parameter. Data with a P -value < 0.05 were considered a significant result, and a level of $0.05 < P$ -value < 0.1 was considered a tendency towards an effective result. Additionally, P_l was the P -value obtained from the linear constant (β_1) and P_q was the P -value obtained from the quadratic constant (β_2), the root means square error ($RMSE$) and Nakagawa determination coefficient (R^2) or $R_{GLMM}^{(c)2}$ were used for the validation test (Nakagawa et al., 2017; Nakagawa and Schielzeth, 2013; R Core Team, 2022). The following equations were used for $RMSE$ (2) and R^2 (3):

$$RMSE = \sqrt{\frac{\sum(O - P)^2}{NDP}} \quad (2),$$

$$R_{GLMM}^{(c)2} = \frac{(\sigma_f^2 + \sum(\sigma_v^2))}{(\sigma_f^2 + \sum(\sigma_v^2) + \sigma_e^2 + \sigma_d^2)} \quad (3),$$

where: O – actual value, P – estimated value, NDP – number of data points, σ_f^2 – variant of a fixed factor $\sum(\sigma_v^2)$ – sum of all variants of the component, σ_e^2 – variant due to the predictor dispersion, and σ_d^2 – specific distribution of the variant.

Results

Search results

The literature search in five databases produced 1116 studies, which were reduced to 82 after removing duplicate and irrelevant studies. These selected studies were screened for the articles that fulfilled the exclusion criteria, resulting in 27 studies. Subsequently, 5 studies were excluded, in which tomato was combined with other additives. A total of 23 studies (experiments) from 22 articles were finally included in meta-analysis. The literature search and study selection at each stage based on the PRISMA guidelines are shown in Figure 1.

Effects of tomato waste supplementation

Statistical descriptions of the individual parameters in the database are presented in Table 2. The effects of adding tomato waste to laying hens rations are summarised in Table 3. Feed intake was significantly increased by tomato waste addition to the ration of laying hens ($P < 0.01$; Table 3) and showed a linear pattern. The effect of tomato waste tended to correlate with a quadratic pattern of hen day egg production (HDEP) ($P < 0.061$; Table 3) and egg weight ($P < 0.075$; Table 3), with maximum levels of tomato waste of 7.1 and 8.6%, respectively,

Table 2. Descriptive statistics of meta-analysis data

| No. | Response variables | Unit | NDP | Mean | SD | Max. | Min. |
|---|--------------------------|--------------------|-----|--------|--------|---------|--------|
| 1 | Tomato level | % | 87 | 4.45 | 5.12 | 20.00 | 0.00 |
| Performance | | | | | | | |
| 2 | HDEP | % | 66 | 80.21 | 12.15 | 96.70 | 54.52 |
| 3 | egg mass | g/hen/d | 66 | 49.64 | 8.45 | 62.00 | 29.29 |
| 4 | feed intake | g/hen/d | 66 | 110.84 | 11.39 | 127.60 | 88.40 |
| 5 | FCR | | 66 | 2.27 | 0.42 | 3.32 | 1.64 |
| Egg quality | | | | | | | |
| 6 | egg weight | g/egg | 72 | 61.36 | 4.37 | 68.70 | 50.95 |
| 7 | eggshell thickness | mm | 53 | 0.45 | 0.49 | 4.00 | 0.30 |
| 8 | eggshell strength | kg/cm ² | 16 | 2.88 | 1.30 | 4.54 | 0.95 |
| 9 | eggshell weight | g/egg | 48 | 6.24 | 0.86 | 8.16 | 4.91 |
| 10 | yolk weight | g/egg | 32 | 15.03 | 2.79 | 18.75 | 9.10 |
| 11 | albumen weight | g/egg | 16 | 33.83 | 3.01 | 38.97 | 30.10 |
| 12 | egg shape index | % | 13 | 75.73 | 1.62 | 78.18 | 73.80 |
| 13 | yolk index | % | 19 | 42.26 | 7.99 | 55.00 | 27.26 |
| 14 | yolk color | | 58 | 8.41 | 3.52 | 14.53 | 1.58 |
| 15 | haugh unit | | 47 | 85.78 | 8.23 | 103.03 | 69.22 |
| 16 | yolk cholesterol | mg/100 g | 25 | 691.37 | 419.94 | 1382.00 | 147.71 |
| 17 | yolk fat | % | 11 | 41.33 | 13.63 | 56.24 | 26.27 |
| Carotenoid, antioxidant, and defence system | | | | | | | |
| 18 | lycopene in yolk | mg/100 g | 13 | 0.45 | 0.57 | 1.53 | 0.00 |
| 19 | malondialdehyde in yolk | µg/100 g | 11 | 0.31 | 0.14 | 0.68 | 0.20 |
| 20 | malondialdehyde in serum | µmol/100ml | 10 | 0.84 | 0.65 | 1.75 | 0.04 |
| Serum lipid | | | | | | | |
| 21 | triglyceride | mg/dl | 15 | 674.91 | 454.57 | 1471.18 | 181.67 |
| 22 | total cholesterol | mg/dl | 31 | 144.32 | 41.76 | 245.27 | 85.80 |
| 23 | HDL | mg/dl | 14 | 45.40 | 20.12 | 72.00 | 5.60 |
| 24 | LDL | mg/dl | 16 | 64.88 | 29.42 | 119.00 | 29.00 |
| Leukocyte count | | | | | | | |
| 25 | H/L ratio | | 13 | 0.62 | 0.81 | 3.07 | 0.13 |

FCR – feed conversion ratio, HDEP – hen-day egg production, HDL – high density lipoprotein LDL – low density lipoprotein, Max. – maximum value of each variable in dataset, Min. – minimum value of each variable in dataset, NDP – number of data points, SD – standard of deviation, H/L – heterophil to lymphocyte

resulting in the predicted values for HDEP of 82.5% and egg mass of 51.3 g/hen/day.

Regarding egg quality parameters, the addition of tomato waste to laying hens' feed significantly enhanced egg-yolk colour ($P < 0.05$; Table 3), following a quadratic pattern. The level of tomato waste sufficient to obtain optimum egg-yolk colour was 15.4% in the ration, yielding a predicted egg-yolk colour score of 9.8 (Roche fan), followed by a significant increase in the lycopene content in the egg yolk ($P < 0.05$; Table 3). However, this was also accompanied by a significantly reduced eggshell strength ($P < 0.05$; Table 3). Additionally, serum total triglyceride and cholesterol levels of laying hens were found to be significantly reduced ($P < 0.05$; Table 3), as demonstrated by a quadratic pattern with the lowest prediction value of 217.4 mg/dl for triglycerides and 124.2 mg/dl for cholesterol, with maximum tomato waste

levels at 11.6 and 7.3%, respectively. The H/L ratio was also significantly reduced by tomato waste supplementation ($P < 0.05$; Table 3), with a quadratic pattern at an optimal tomato waste level of 12.3%. The regression test also identified a significant interaction ($P < 0.05$; Table 3) between tomato waste administration level and laying hen breeds on feed conversion ratio (FCR) and egg yolk cholesterol parameters. In addition, a significant interaction ($P < 0.01$; Table 3) was found between breed of laying hens and duration of tomato waste addition.

Comparison of tomato waste and colouring pigment as supplements

The effects of adding tomato waste and colouring pigment as supplements on egg production and quality, as well as blood parameters of laying hens are compared in Table 4. There was a significant

Table 3. Regression equation of the effect of tomato waste addition (% of diet) on performance, egg quality, and blood parameters

| No. | Response variables | Unit | NDP | Intercept, μ | Slope (β_1 and β_2) ¹ | | P-value | RMSE | R ² | X | Y | Tm*Br | Tm*Td | Br*Td | Tm*Br*Td |
|---|--------------------------|--------------------|-----|------------------|--|----------|---------|-------|----------------|---------|--------|-------|-------|-------|----------|
| | | | | | value | SE | | | | optimum | | | | | |
| Performance | | | | | | | | | | | | | | | |
| 1 | HDEP | % | 61 | 81.40 | 2.71 | 0.299 | 0.174 | 0.093 | 1.79 | 0.97 | | 0.08 | 0.06 | 0.76 | 0.26 |
| | | | | | | -0.021 | 0.011 | 0.061 | | 7.10 | 82.50 | | | | |
| | | | | | HDEP, % = 81.4126 + 0.2994 X - 0.0211 X ² | | | | | | | | | | |
| 2 | egg mass | g/hen/d | 61 | 50.30 | 1.83 | 0.229 | 0.116 | 0.055 | 1.19 | 0.97 | | 0.28 | 0.30 | 0.85 | 0.57 |
| | | | | | | -0.013 | 0.007 | 0.075 | | 8.60 | 51.30 | | | | |
| | | | | | egg mass, g/hen/d = 50.3435 + 0.2289 X - 0.0133 X ² | | | | | | | | | | |
| 3 | feed intake | g/hen/d | 61 | 111.00 | 2.45 | 0.202 | 0.069 | 0.006 | 1.67 | 0.96 | - | 0.49 | 0.88 | 0.12 | 0.68 |
| 4 | FCR | | 61 | 2.20 | 0.09 | 0.004 | 0.004 | 0.223 | 0.09 | 0.93 | - | 0.02 | 0.34 | 0.24 | 0.43 |
| Egg quality | | | | | | | | | | | | | | | |
| 5 | egg weight | g/egg | 67 | 61.50 | 0.93 | 0.047 | 0.040 | 0.242 | 0.98 | 0.92 | - | 0.78 | 0.58 | 0.92 | 0.49 |
| 6 | eggshell thickness | mm | 48 | 0.52 | 0.11 | -0.014 | 0.015 | 0.375 | 0.51 | 0.03 | - | 0.35 | 0.97 | 0.94 | 0.96 |
| 7 | eggshell strength | kg/cm ² | 14 | 3.70 | 0.36 | -0.098 | 0.034 | 0.014 | 0.41 | 0.75 | - | 0.31 | | 0.89 | |
| 8 | eggshell weight | g/egg | 45 | 6.26 | 0.24 | -0.009 | 0.008 | 0.304 | 0.16 | 0.95 | - | 0.78 | | 0.84 | |
| 9 | yolk weight | g/egg | 30 | 15.10 | 1.10 | 0.0001 | 0.033 | 0.997 | 0.50 | 0.97 | - | - | | | |
| 10 | albumen weight | g/egg | 16 | 32.90 | 2.21 | 0.1 | 0.122 | 0.424 | 1.31 | 0.85 | - | - | | | |
| 11 | egg shape index | % | 11 | 75.10 | 0.76 | 0.038 | 0.020 | 0.103 | 0.20 | 0.97 | - | - | | | |
| 12 | yolk index | % | 17 | 41.70 | 4.11 | -0.053 | 0.065 | 0.428 | 0.73 | 0.99 | - | - | | | |
| 13 | yolk color | | 53 | 7.39 | 0.92 | 0.313 | 0.088 | 0.001 | 0.87 | 0.92 | | 0.21 | 0.99 | 0.14 | 0.91 |
| | | | | | | -0.010 | 0.005 | 0.067 | | 15.40 | 9.80 | | | | |
| | | | | | yolk color = 7.3915 + 0.3132 X - 0.0102 X ² | | | | | | | | | | |
| 14. | Haugh unit | | 45 | 86.60 | 2.24 | -0.128 | 0.105 | 0.240 | 2.16 | 0.90 | - | 0.43 | | 0.39 | |
| 15. | yolk cholesterol | mg/100 g | 25 | 739.00 | 167.00 | -0.024 | 5610 | 1.000 | 79.70 | 0.95 | - | 0.02 | | 0.002 | |
| 16. | yolk fat | % | 11 | 42.30 | 12.70 | 0.077 | 0.229 | 0.750 | 1.85 | 0.99 | - | - | | | |
| Carotenoid, antioxidant, and defence system | | | | | | | | | | | | | | | |
| 17 | lycopene in yolk | mg/100 g | 11 | 0.21 | 0.15 | 0.067 | 0.017 | 0.020 | 0.20 | 0.82 | - | - | | | |
| 18 | malondialdehyde in yolk | μ g/100 g | 10 | 0.38 | 0.06 | -0.059 | 0.035 | 0.130 | 0.11 | 0.28 | - | - | | | |
| 19 | malondialdehyde in serum | μ mol/100 ml | 10 | 0.98 | 0.33 | -0.110 | 0.060 | 0.130 | 0.17 | 0.89 | - | - | | | |
| Serum lipid | | | | | | | | | | | | | | | |
| 20 | triglyceride | mg/dl | 14 | 1137.00 | 195.00 | -159.000 | 55.600 | 0.018 | 141.00 | 0.87 | | | | | |
| | | | | | | 6.860 | 2.590 | 0.023 | | 11.60 | 217.40 | | | | |
| | | | | | triglyceride, mg/dl = 1137.3632 - 158.8381 X + 6.8562 X ² | | | | | | | | | | |
| 21 | total cholesterol | mg/dl | 30 | 150.00 | 15.70 | -7.130 | 3.380 | 0.047 | 22.60 | | | | | | |
| | | | | | | 0.485 | 0.213 | 0.034 | | 7.30 | 124.20 | | | | |
| | | | | | total cholesterol, mg/dl = 150.4424 - 7.13 X + 0.4851 X ² | | | | | | | | | | |
| 22 | HDL | mg/dl | 13 | 38.50 | 12.20 | 0.898 | 0.827 | 0.310 | 6.50 | 0.89 | - | - | | | |
| 23 | LDL | mg/dl | 15 | 75.20 | 13.80 | -1.440 | 1.680 | 0.410 | 18.40 | 0.52 | - | - | | | |
| Leukocyte count | | | | | | | | | | | | | | | |
| 24 | H/L ratio | | 12 | 1.85 | 0.61 | -0.311 | 0.082 | 0.005 | 0.20 | 0.957 | | | | | |
| | | | | | | 0.013 | 0.004 | 0.009 | | 12,30 | -0.10 | | | | |
| | | | | | H/L ratio = 1.8516 - 0.3112 X + 0.0126 X ² | | | | | | | | | | |

¹ the first row of each parameter is a linear coefficient (β_1) and the second row is a quadratic coefficient (β_2); FCR – feed conversion ratio, HDEP – hen-day egg production, HDL – high density lipoprotein, LDL – low density lipoprotein, NDP – number of data points, Br – laying hen breed, Td – treatment duration (days), Tm – level of tomato (%), R² – R squared Nakagawa for validate linear mixed model, RMSE – root mean square error, SE – standard error, H/L – heterophil to lymphocyte

Table 4. Effect of tomato waste addition compared to the addition of colouring pigment on performance, egg quality, and blood lipid parameters of laying hens

| No. | Response variables | Unit | NDP | Control | Pigment | Tomato waste | Vitamin C | SEM | P-value |
|---|--------------------------|--------------------|-----|---------------------|---------------------|---------------------|--------------------|--------|---------|
| Performance | | | | | | | | | |
| 1 | HDEP | % | 66 | 81.13 | 81.88 | 82.08 | | 1.50 | 0.71 |
| 2 | egg mass | g/hen/d | 66 | 50.02 ^a | 50.46 ^{ab} | 51.11 ^b | | 1.04 | 0.41 |
| 3 | feed intake | g/hen/d | 66 | 110.52 ^a | 108.81 ^a | 112.13 ^b | | 1.40 | 0.17 |
| 4 | FCR | | 66 | 2.24 | 2.19 | 2.23 | | 0.05 | 0.91 |
| Egg quality | | | | | | | | | |
| 5 | egg weight | g/egg | 72 | 61.43 | 61.38 | 61.87 | | 0.52 | 0.66 |
| 6 | eggshell thickness | mm | 53 | 0.62 | 0.35 | 0.39 | | 0.07 | 0.35 |
| 7 | eggshell strength | kg/cm ² | 16 | 3.44 | 2.81 | 3.16 | | 0.33 | <0.001 |
| 8 | eggshell weight | g/egg | 48 | 6.18 | 6.24 | 6.23 | | 0.12 | 0.83 |
| 9 | yolk weight | g/egg | 32 | 14.92 | 15.16 | 15.05 | | 0.49 | 0.18 |
| 10 | albumen weight | g/egg | 16 | 33.66 | | 33.38 | | 0.75 | 1.00 |
| 11 | egg shape index | % | 13 | 75.22 | 74.80 | 75.44 | | 0.45 | 0.53 |
| 12 | yolk index | % | 19 | 41.19 | 41.80 | 41.45 | | 1.83 | 0.28 |
| 13 | yolk color | | 58 | 7.20 ^a | 10.37 ^c | 8.94 ^b | | 0.46 | <0.001 |
| 14 | haugh unit | | 47 | 86.32 | 86.69 | 85.79 | | 1.20 | 0.94 |
| 15 | yolk cholesterol | mg/100 g | 25 | 745.73 | 729.72 | 735.69 | | 84.00 | 0.038 |
| 16 | yolk fat | % | 11 | 43.15 | | 42.16 | | 4.11 | 0.54 |
| Carotenoid, antioxidant, and defence system | | | | | | | | | |
| 17 | lycopene in yolk | mg/100 g | 13 | 0.10 ^{ab} | -0.11 ^a | 0.73 ^b | | 0.16 | 0.052 |
| 18 | malondialdehyde in serum | μmol/100 ml | 10 | 1.05 | 0.83 | 0.72 | | 0.04 | 0.30 |
| Serum lipid | | | | | | | | | |
| 19 | triglyceride | mg/dl | 15 | 938.34 | | 766.67 | 876.47 | 117.00 | 0.10 |
| 20 | total cholesterol | mg/dl | 31 | 154.63 | 130.43 | 134.89 | 112.03 | 7.50 | 0.72 |
| 21 | HDL | mg/dl | 14 | 34.48 | | 43.92 | 40.06 | 5.38 | 0.23 |
| 22 | LDL | mg/dl | 16 | 88 ^a | | 62.8 ^a | 44.00 ^a | 7.35 | 0.14 |
| Leukocyte count | | | | | | | | | |
| 23 | H/L ratio | | 13 | 1.56 ^b | | 0.77 ^a | 0.92 ^{ab} | 0.23 | <0.001 |

FCR – feed conversion ratio, HDEP – hen-day egg production, HDL – high density lipoprotein, LDL – low density lipoprotein, NDP – number of data points, SEM – standard error of the mean, H/L – heterophil to lymphocyte; ^{abc} – different letters in the same row have a significant difference with a 5% error degree

statistical increase in the egg-yolk colour index after treatment with tomato waste or colouring pigment. However, a lower colour index was obtained in the treatment with tomato waste than in the treatment with colouring pigment ($P < 0.001$). The H/L ratio in blood serum was also significantly ($P < 0.001$) affected by treatments. Further statistical analysis of the tomato waste treatment showed that the H/L ratio in the control group was higher than in the tomato waste group. However, significant differences were not recorded compared to the vitamin C group.

Discussion

Effect on laying hen performance

The results of the meta-analysis revealed that the administration of tomato waste tended to be

correlated with a quadratic pattern of HDEP and egg mass, even though feed intake increased consistently with a linear pattern (Table 3). The increases in HDEP and egg mass could be explained by the nutritional content of tomato waste, which is also high in crude fibre. However, the high fibre content of tomato waste was also reported to act as an anti-nutritional factor interfering with digestion and decreases the birds' capacity to absorb and utilise nutrients (Brenes et al., 2016; Mnisi et al., 2022). According to our findings, the crude fibre content in various tomato wastes was as high as 43.15% in tomato peels and seeds (Habanabashaka et al., 2014), 40.43% in tomato peels (Varzaru et al., 2021), 35% in tomato pulp (Jafari et al., 2006), and 29.75% in tomato pomace (Safamehr et al., 2011). Jha and Mishra (2021) argued that crude fibre acted as an anti-nutritional factor, negatively affecting feed

intake and nutrient digestibility. Fibre is a naturally occurring plant component associated with physiological, structural, and functional changes in the gastrointestinal tract (Deehan et al., 2022). Fibre in poultry diets affects intestinal morphology, digestive organ development, nutrient absorption, growth performance, and intestinal microbiota (Tejeda and Kim, 2021). Exceeding the maximum limit of dietary fibre content led to undigested feed ingredients, causing nutrient deficiency in the body of laying hens (Jha and Mishra, 2021). This was suspected to cause a decrease in egg production or egg mass weight after supplementing laying hens' diets with 7.1 or 8.6% tomato waste as reported in this meta-analysis (Table 3).

On the other hand, increasing tomato waste content in the feed also did not exert any adverse effect on palatability, i.e. the attractiveness of the feed that increases livestock appetite (Al-Souti et al., 2019). The latter authors found that palatability was strongly influenced by feed ingredients. Our findings indicated that the ingredients contained in tomato waste were acceptable for feeding laying hens.

Effect on egg quality

In most countries, egg yolks with a higher colour index are preferred (Rakonjac et al., 2014) as they are believed to be of better quality and healthier (Senbeta et al., 2015). We found that adding tomato waste to the ration increased egg colour (Table 3), which was assumed to be due to the presence of lycopene in tomato waste. Lycopene is one of the carotenoid compounds synthesized by plants (Reboul, 2019), which gives a characteristic red colour to fruits, vegetables and other plant parts (Cooperstone et al., 2016). The colour of the egg yolk was demonstrated to be affected by dietary ingredients, e.g. carotenoid compounds in the laying hens' diet strongly contributed to the intensity of their egg yolk colour (Rakonjac et al., 2014; Kaspers, 2016). The egg yolk colour index in the present meta-analysis ranged from 1.58 to 14.53, and exhibited a quadratic pattern (Table 3). This phenomenon was expected due to the high crude fibre content in tomato waste, which reduced the absorption of nutrients. Statistically, the lycopene content in egg yolk ($P < 0.05$; Table 3) increased significantly. These data demonstrated that lycopene in the diet could be deposited in egg yolks, thus, it was possible to enrich it with lycopene by adding lycopene-rich feedstuffs to the diet. The deposition of lycopene occurs probably due to the fact that structural lycopene is not oxidised and is potentially more easily absorbed in the digestive tract of laying hens. Additionally, the chemical structure of

carotenoid may affect its transport in the circulatory system and ultimately its deposition in the egg yolk (Olson et al., 2008).

Moreover, the administration of tomato waste to laying hens resulted in a negative trend for cholesterol and fat levels in the egg yolk; however, this effect was not statistically significant ($P > 0.05$; Table 3). In general, laying hens produce 300 mg of cholesterol per day in their livers and ovaries to cover their daily requirements, but cholesterol synthesised by the ovaries is rarely transferred to oocytes (Kim et al., 2004). In laying hens, egg cholesterol is synthesized in the liver and secreted into the blood in the form of very low-density lipoprotein (VLDL) particles (Kaspers, 2016). VLDL particles are internalized by vitellogenesis receptors on oocytes for follicular growth and yolk deposition (Kaspers, 2016). Therefore, the inhibition of the biosynthesis of cholesterol in the liver reduces the concentration of this compound in eggs (Kim et al., 2004).

Effect on blood parameters

This meta-analysis confirmed that the administration of tomato waste decreased serum total cholesterol and triglyceride levels in laying hens. Lycopene in tomatoes is thought to play a role in lowering cholesterol levels. According to data compiled from previous studies, lycopene has three possible mechanisms of action of reducing cholesterol concentration. In the first pathway, the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) is inhibited by the synthesis of mevalonate compounds (Fuhrman et al., 1997; Palozza et al., 2012; Alvi et al., 2017). Alvi et al. (2016) explained that HMG-CoA reductase was inhibited by lycopene in a reversible competitive manner by binding to almost half of the active enzyme sites, leading to a lower number of enzyme-substrate complexes. The second pathway involved increasing the cellular activity of the low-density lipoprotein (LDL) receptor, which could increase the amount of LDL degradation in blood serum (Fuhrman et al., 1997; Rao et al., 2006; Palozza et al., 2012). The number of LDL receptors on the surface of liver cells is increased by the inhibition of the activity of HMG-CoA reductase, thereby increasing LDL degradation to meet the cells' cholesterol requirements (Fuhrman et al., 1997). In the third pathway, the activity of acyl-coenzyme A cholesterol acyltransferase (ACAT) was demonstrated to be inhibited by cholesterol ester synthesis in the liver and other tissues (Elkin et al., 1999; Palozza et al., 2012). Cholesterol is absorbed in the small intestine as esterified cholesterol with the assistance of the

enzyme ACAT (Nguyen et al., 2012), which result in lower levels of cholesterol in the blood. The decrease in serum triglyceride levels is due to the ability of lycopene to inhibit the process of triglyceride synthesis in the liver, causing a significant reduction in blood triglycerides (Agarwal and Rao, 2000; Ševčíková et al., 2008; Hsu et al., 2015). Serum cholesterol concentrations in our analysis ranged from 85.80 to 245.27 mg/dl, which was considered a normal cholesterol level given the concentration range of 52–148 mg/dl determined in previous studies (Basmacioglu and Ergul, 2005).

The present meta-analysis demonstrated that lycopene contained in tomato waste did not affect serum LDL and HDL levels in laying hens. According to Hsu et al. (2015), the administration of commercial lycopene at doses of up to 18 mg/kg diet did not reduce blood serum LDL levels in laying quails. In contrast, Basuny et al. (2009) reported that the administration of 100–800 ppm of tomato lycopene or 200 ppm of commercial lycopene in the diet of rats significantly lowered serum LDL concentration in rats. A decrease in plasma cholesterol, triglyceride, and LDL levels after lycopene supplementation up to 200 mg/kg ration was shown in lambs (Jiang et al., 2015). Moreover, LDL levels in the present analysis ranged from 16.27 to 40.28 mg/dl, which was considered as normal LDL levels for laying hens given the classification from a previous study (Basmacioglu and Ergul, 2005), in which LDL concentrations < 130 mg/dl were considered normal.

Resistance of the body to environmental threats is a critical factor affecting the growth and productivity of poultry. The body's immunity is a sign of its ability to fight external factors, such as viruses and bacteria, and to protect cells and tissues against free radicals produced by the body. Indicators of the body's resistance in the form of chicken response to stress-causing factors can be determined in blood, e.g. the H/L ratio. Due to its simplicity and reliability, the measurement of the blood H/L ratio was the most common indicator of stress response in poultry (Post et al., 2003; Shakeri et al., 2014). The proportion of heterophils to lymphocytes in the blood was considered a potential stress biomarker in laying hens exposed to multiple stress conditions (Lee et al., 2022).

Malondialdehyde (MDA) level is also an essential indicator for determining the health status of chickens. MDA, which is the end product of the lipid peroxidation chain reaction, is utilised as a marker of oxidative stress. The higher the plasma MDA level, the higher the oxidative stress appearing in the cells of the body (Valko et al.,

2007). Oxidative stress usually occurs when the level of free radicals is higher than the concentration of antioxidants in the body, which results in cell damage. Lipid peroxidation is increased by free radicals, resulting in high MDA levels in the blood. Our meta-analysis clearly showed that lycopene contained in tomato waste could potentially prevent the occurrence of oxidative stress in laying hens. This was confirmed by a significant reduction in the H/L ratio and a negative trend in MDA levels. The antioxidant activity of the lycopene compound was correlated with its chemical structure containing 11 double bonds, which facilitated electron donation to radical atoms (Agarwal and Rao, 1998; Stahl and Sies, 2003; Tapiero et al., 2004; Gümüşay et al., 2015). Moreover, Shi et al. (1999) found that the antioxidant activity of lycopene was higher compared to β -carotene and α -tocopherol.

Conclusions

This meta-analysis has confirmed that feeding tomato waste to laying hens exerts positive effects on their performance and egg quality. The pigment present in tomato waste, i.e. lycopene, was transferred to the body, resulting in improved egg yolk colour, and reduced total triglycerides, cholesterol, and H/L ratio. The recommended level of tomato waste is 7.1% of the ration, resulting in maximised production at 82.5% HDEP, 51.3 g/hen/day of egg mass, yolk colour index of 9.1, 383.62 mg/dl triglycerides, 124.52 mg/dl cholesterol, and H/L ratio of 0.32.

Funding source

The first author would like to thank the National Research and Innovation Agency, Indonesia, for funding the study through the “Postdoctoral Program”, contract number 64/II/HK/2022.

Conflict of interest

The Authors declare that there is no conflict of interest.

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