Supplementary n-3 fatty acids sources on performance and formation of omega-3 in egg of laying hens: a meta-analysis

A. Irawan ,[*,†,‡,1] N. Ningsih,[§] Hafizuddin,[‡,#] R. K. Rusli ,[‡,‖] W. P. S. Suprayogi,[*] N. Akhirini,[*] R. F. Hadi,[*] W. Setyono,[*] and A. Jayanegara [‡,¶]

[*]*Vocational Program of Animal Husbandry, Universitas Sebelas Maret, Surakarta, 57126, Indonesia;* [†]*Department of Animal and Rangeland Sciences, Corvallis, OR, 97331, USA;* [‡]*Animal Feed and Nutrition Modelling (AFENUE) Research Group, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia;* [§]*Department of Animal Science, Politeknik Negeri Jember, Jember, 68101, Indonesia;* [#]*Faculty of Veterinary Medicine, Universitas Syah Kuala, Banda Aceh, 23111, Indonesia;* [‖]*Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Padang, 25163 Indonesia; and* [¶]*Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia*

ABSTRACT A meta-analysis was performed to evaluate the effects of supplementary n-3 polyunsaturated fatty acids (**PUFA**) sources in the diet on the formation of some important n-3 PUFA contents in eggs and to assess factors contributing to the conversion efficiency of omega-3 in laying hens. A dataset was constructed from 34 studies examining the impact of dietary inclusion with ingredients rich in n-3 PUFA on fatty acids profile and production performance of laying hens. The eligibility criteria were developed to obtain studies reporting required information with sufficient quality. The mixed model methodology was employed where the "study" was set as random effects and fatty acid (**FA**) supplements as fixed effects. Several factors were included in the models as covariates. Discrete analysis for sources of FA was also performed to compare their effects on FA formation in eggs. Significant linear positive associations were observed between the concentration of α-linolenic acid (**ALA**), total n-3 PUFA, and the ratio of linoleic acid (**LA**) to ALA (LA/ALA) in diets with the formation of eicosapentaenoic acid (**EPA**), docosahexaenoic acid (**DHA**), total n-3 PUFA, and n6/n3 ratio in egg ($P < 0.05$) with different magnitudes. ALA and total n-3 PUFAs concentration had no relationship with cholesterol concentration, feed intake, and egg weight. Prediction models for DHA formation was higher for ALA as predictor variables (slope = 0.482; R^2 = 0.684) than n-3 PUFAs (slopes = 0.998, R^2 = 0.628). Significant interactions were found on the level of ALA × FA sources and n-3 PUFA × FA sources. Fish oil ($P = 0.0148$, $R^2 = 0.732$) improved the prediction equation to estimate DHA formation. To conclude, levels of ALA, n-3 PUFA, and the ratio of LA/ALA can be used as predictor variables to estimate the formation of n-3 fatty acids in eggs. It was confirmed that although all n-3 FA sources had a positive correlation on DHA and n-3 PUFA deposition, however, fish oil showed the highest prediction model for DHA formation across all FA sources included in the dataset.

Key words: docosahexaenoic acid, egg quality, fatty acid profile, laying hens, omega 3

2022 Poultry Science 101:101566
https://doi.org/10.1016/j.psj.2021.101566

INTRODUCTION

Demands for animal protein sources rich in functional properties are substantially increasing due to their health-associated benefits. As the most affordable protein source, there is increasing preference among individuals to consume eggs with higher content of n-3 polyunsaturated fatty acids (**PUFAs**), especially **DHA** (docosahexaenoic acids, C22:6 n-3) and **EPA** (eicosapentaenoic acid, C20:5 n-3) (Khan et al., 2021). Many efficacies emerge in the last decade to provide the positive impacts of EPA and DHA in preventing chronic diseases primarily related to cardiovascular and nervous system diseases (Mason et al., 2020). In humans, elevating EPA and DHA intakes strongly reduced cardiovascular morbidity and mortality events (Khan et al., 2021). Substantive evidence suggested that these essential fatty acids could lower the triglycerides concentration, stabilize membrane structure, and they are believed to have antithrombotic, anti-inflammatory as well as antiarrhythmic properties (Fraeye et al., 2012a). Therefore,

Received August 9, 2021.
Accepted October 10, 2021.

[1]Corresponding author: a.irawan@staff.uns.ac.id

there has been ongoing research agenda, especially for poultry nutritionists to enhance the content of n-3 beneficial PUFAs in the egg for people.

Dietary supplementation with sources of EPA, DHA, or their precursor for laying hens is a common strategy to enrich eggs with desired n-3 PUFAs. The main precursor for EPA and DHA is α-linolenic acid (18:3 n−3, **ALA**) which possesses enzymatic pathways for EPA and DHA biosynthesis (Alagawany et al., 2019). Some plants and animal-derived products are often supplemented to diet as a source of ALA, such as flaxseed, canola, linseed, sunflower seed, fish oil, microalgae, etc. However, several scientific reports were suggesting that conversion from ALA to EPA and DHA greatly differed depending on FA sources and the conversion to DHA are often limited due to different path of desaturation and elongation (Fraeye et al., 2012a). For instance, extruded flaxseed supplementation up to 9% diets resulted in ~150 mg DHA and 530 to 670 mg n-3 PUFAs in eggs (Huang et al., 2020) while *Aurantiochytrium limacinum* microalgae inclusion at 1% diet could produce 286 mg DHA and 626 mg n-3 PUFAs in a 60 g egg (Moran et al., 2019). Reports on DHA and n-3 PUFAs contents of egg using fish oil, microalgae, linseed oil, sunflower oil, and combination from different sources also varied between 50 and 290 mg/eggs (Coorey et al., 2015; Jing et al., 2017; Moran et al., 2019; Omri et al., 2019) depending on the source, levels, and form of supplemental FA.

One important reason regarding the discrepancies is because enzymes involved in the transformation processes compete to utilize their substrates (linoleic acid/LA and ALA), making the conversion efficiency different, although reports on these were not consistent. For instance, linearly increased DHA contents were reported by supplementing diets with increasing levels of soluble flaxseed (differ in LA/ALA) and fish oil (similar in LA/ALA) with the later showed higher conversion efficiency (Kralik et al., 2021; Lee et al., 2021). Interestingly, these studies suggested that ratios of LA/ALA in the diets did not affect conversion rate. On the other hand, Aguillón-Páez et al. (2020) in their studies using full-fat sunflower and flaxseed seed reported that LA/ALA ratio had a significant effect on DHA formation in eggs. In addition, the strain, length of feeding trials, and feed additive incorporation such as acidifier, enzyme, and antioxidants might also affected lipid metabolism and the output of DHA in eggs (Jia et al., 2008; Attia et al., 2013; Pérez et al., 2021; Lee et al., 2021). On the other hand, the effects of feeding hens with FA sources on production performance are conflicting, that is, there was a decrease in egg production (**EP**) and egg weight (**EW**) (Cufadar et al., 2016; Aguillón-Páez et al., 2020) where other experiments reported an increased on EP and EW (Dong et al., 2018; Westbrook and Cherian, 2019) and others mostly reported no effects on production parameters (Huang et al., 2018, 2020; Moran et al., 2019; Kralik et al., 2021).

Despite there are clear evidences that supplementary feeding with sources rich in ALA or DHA successfully increased DHA and n-3 PUFAs contents in eggs, however, very few studies provided empirical data on the factors affecting the conversion rates in egg. We hypothesized that types, sources, and levels of FA as well as hens and dietary factors might be contributed to the efficiency of PUFAs biosynthesis in eggs and hens' performance. Considering that empirical experiments exploring this area are increasing, it is possible to build a robust model for the efficiency of n-3 PUFAs formation in egg by employing a meta-analysis method. This approach is valuable to establish a valid statistical power from different individual studies. Moreover, it enables to identify covariates that may interfere with the response variables (Sauvant et al., 2008). Therefore, the present meta-analysis aimed to quantify the effects of different sources of dietary n-3 PUFAs on production performance and egg's fatty acids profile in laying hens. This meta-analysis also attempted to determine factors that may contribute to the formation efficiency of DHA and n-3 PUFAs in egg as the main outcome variables.

MATERIALS AND METHODS

Search Strategy and Selection Process

Digital scientific databases (Scopus, web of knowledge, and PubMed Central) were used to search articles published in peer-reviewed journals that reported the use of supplemental fatty acids in laying hens. The queries inputted into the databases were the combination of "fatty acid", "laying hens", "supplementation", "egg", and/or "omega 3". No limit on publication year was set but the search was conducted only in the English language. To assure the quality and appropriateness of papers, eligibility criteria were determined a priori as follows: 1) experiments should directly evaluate the use of fatty acids sources in the diet and report fatty acids profile in egg; 2) fatty acids profile should be quantitatively reported in feed or the supplemental source to possibly estimate the fatty acid contents supplemented to laying hens; 3) in egg, fatty acid profile might be reported in any measurement units but allowing to convert into a similar unit of measurement, preferably mg/egg; 4) used reliable and sufficient information on methodology. The process of study selection flow is presented in Figure 1.

All titles identified during the searching process were imported to the reference manager after exclusion of non-relevant documents (review article, book chapter, etc.). Processes of study selection were performed following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines (Liberati et al., 2009). Titles, abstracts, and full-text article evaluations were conducted by 2 independent investigators, resulting in a final list of titles potentially eligible for meta-analysis. Following this, the third researcher reviewed the proposed titles as the final studies to minimize publication bias. Studies with fatal flaws detected in the materials and methods sections would

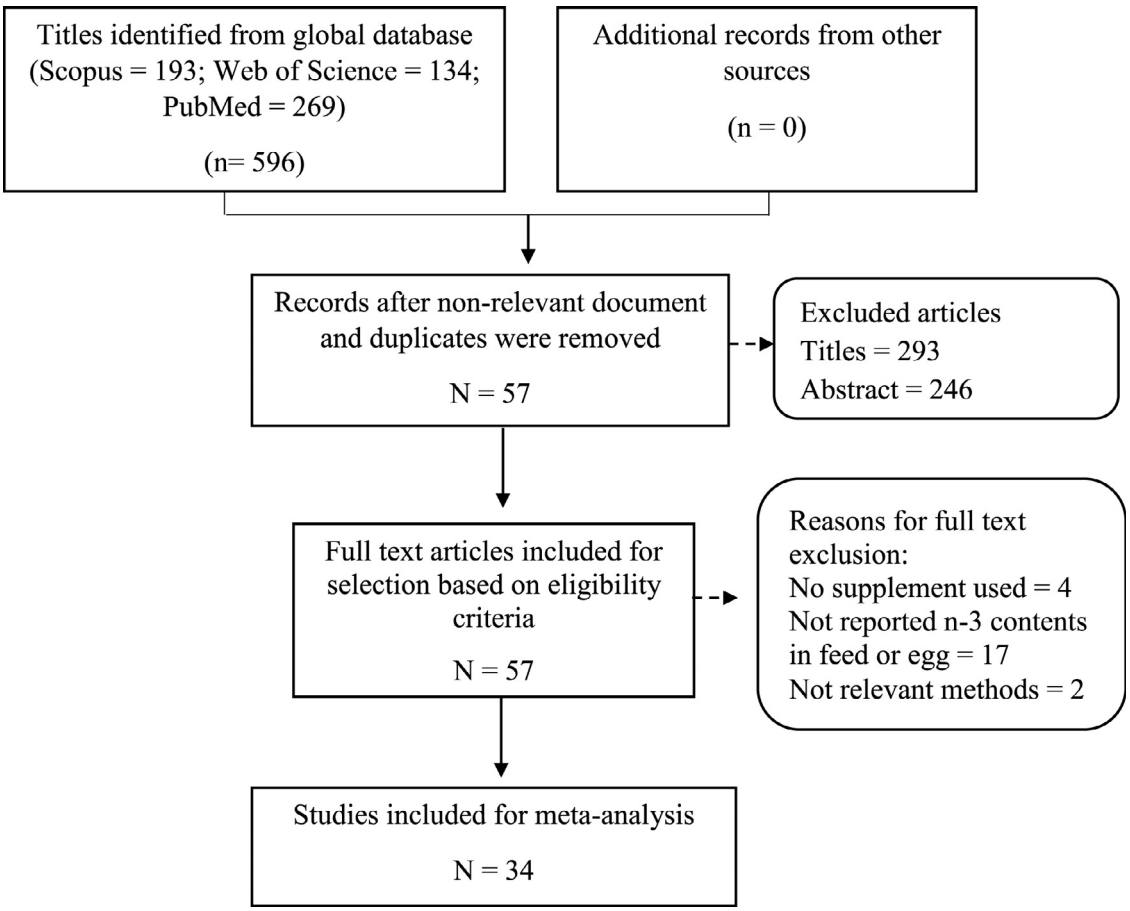

Figure 1. Flowchart of selection process of the articles used for meta-analysis.

not be considered to be included in the database. In addition, insufficient information on FA profiles was also being the reason to reject studies.

Database Construction

The eligible studies generated from the previous step as shown in Table 1 were extracted into a spreadsheet. The dataset consisted of a column representing the qualitative information (authors, journal, year, country of origin, strain of laying hens, type of diet, source of fatty acids supplement) and quantitative data including variables of interest (number of replication, number of birds, age, length of the experiment, nutrient specification, fatty acids profile of diet and/or material used as FA sources, production parameters, and fatty acids contents in egg). Coding for the strain of chickens and source of FAs as well as other potential sources of variances were established to include in the statistical models. Source of FAs was classified into 2 different columns as subgroup categories: the first column classified them as "oil" or "seed" where the second column categorized them into including their corresponding levels and also as #1 CON (control, diet without FA supplementation), #2 FLAX (flaxseed, seed, or ground), #3 FLO (flaxseed oil), #4 MA (microalgae), #5 FO (fish oil), #6 HSO (hemp seed oil and hemp omega), #7 LSO (linseed oil), #8 SFO (sunflower oil), and #9 SBO (soybean oil). Experiments using FA combination as treatments were coded as #10 MIX (mixtures) while other FA sources identified only in one study were classified as #11 OT (others). Corresponding levels for each FA source were provided.

Fatty acids of interest included in the dataset were linoleic acid (C18:2n-6, LA), ALA, EPA, DHA, total n-3 PUFAs, and n-6:n-3 ratio. When no information available in the papers, total n-6, total n-3 PUFAs and n-6/n3 ratio were calculated as follows: 1) total n-6 = C18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6; 2) total n-3 = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:6n-3; and 3) ratio of n-3:n-6 = total n-6:total n-3. In the papers, large variability existed for fatty acids data whereas provided differently, that is, as % of total FAs, % or g/kg of diets, and few papers only provided FA profiles in the material used as FA sources. These nonsimilar units were transformed to g/kg diet and mg/60 g egg, respectively. Computation was performed by using relevant information on articles such as total lipid, total FA content, egg weight, and yolk weight. Studies lack on these data was disregarded. The final dataset yields 43 × 198 data points.

Statistical Analysis

The present meta-analysis utilized the mixed model methodology (**LMM**) because this model suits to develop empirical modeling on biological response in the

experimental design. The models considered "study" as random effects and predictor variables of treatments as fixed effects (St-Pierre, 2001). Prior to statistical analysis, data distribution was observed through graphical visualization (scatter plots) to evaluate the data quality by identifying data points from experiments with extreme values. This way also helped to determine the statistical model (Polycarpo et al., 2017). The relationship of inter- and intrastudy was examined to control data quality and the biological nature of the database (Sauvant et al., 2008). In linear mixed model analysis, continuous data of fatty acids contents (g/kg feed) including LA, ALA, ratio of LA/ALA, DHA, EPA, and total n-3 PUFAs were the main model predictors while several factors such as strain, age, number of birds, study period, source of FA, and nutrient compositions were included in the models tested as covariates. Linear and quadratic models to predict the effects of independent variables (fatty acid content in the diets) were tested using the following model:

$$Yijk = \mu + si + \tau j + s\tau ij + B1Xij + biXij + BjXij + eijk$$

where Yijk is response variable tested, μ is overall intercept across all studies (fixed effect), si is random effect model for study I, τj is fixed effect for j level, $s\tau$ij is the interaction effects between i study and the j levels, B_1 is a slope for linear regression (fixed effect) of Y on X, Xij = value of the continuous predictor variable (specific fatty acids level), bi = random effect of study i on the slope of Y on X in study i, Bj = effect of j level of the discrete factor τ on the regression coefficient (fixed effect), and eijk is residual error. When a quadratic model was $P > 0.10$, the model was transformed into a linear model by taking out the B2Xij term. To facilitate multicovariates analysis, several interaction models between levels of specific FA and covariates were tested declared in the model. Model statistics used were P-value, root means square error (**RMSE**), AIC, and coefficient of determination (R^2). Significance was noted at $P < 0.05$ while tendency was stated at $0.05 < P < 0.1$. The number of replicates for each study was used as a weighting factor and was declared in WEIGHT statement of the model. The qualitative covariates were declared in the CLASS statement (Jayanegara et al., 2019).

Several covariates were removed from the models presented herein due to lack of significance ($P > 0.10$), including quadratic models. In addition to LMM, variance-covariance analysis was performed to compare different sources of FA used as a supplement (Irawan et al., 2021) according to the following model:

$$Yij = \mu + si + \tau j + s\tau ij + eij$$

where Yij = the predicted output for dependent variable Y, μ = overall mean, Si = random effect of i study, τj = fixed effect of the j level, Sτij = random interaction between i study and the j level, and eij = residual error. A significant effect was stated at $P < 0.05$ or there is a tendency when the P-value was between 0.05 and 0.10. Tukey-Kramer's test was used to indicate the least square means among categorical variables. In this model, 10 subgroup categories were created excluding #OT (other) category aforementioned to avoid confounding in practical operation. Several best-fitted predicted models were visualized using scatter plots that were created based on adjusted predicted Y values. The adjusted predicted outcomes (Y) were obtained by adding the predicted values with corresponding residual values (St-Pierre, 2001). The intercepts in the graphics were set as zero.

RESULTS

Description of the Database

The studies used for the present meta-analysis were 34 studies comprising 36 experiments and 198 observations with a total of 5,535 laying hens (Table 1). The articles were retrieved from international reputable journals, of which 10/34 were publication from Poultry Science, between 1991 and 2021, represented 17 countries of origin (Austria, Belgium, Brazil, Canada, China, Colombia, Croatia, Egypt, Italia, Italy, Mexico, Poland, South Korea, Tunisia, Turkey, UK, USA). There were 19 strains of laying hens described in the studies, with Hy-Line W-36 (11.8%), Lohmann white (14.7%), and Isa Brown (20.6%) were the most frequently used. The database described that the experiments were conducted in various ages between 18 and 67 wk whereas 26.5% (9/34) were conducted in a pre-peak laying period (18−25 wk), 14.7% were in peak production (26−35 wk) and post-peak production periods (36−50 wk), respectively while 32.4% of investigations were in late production periods (50−67 wk). They were evaluated in 18 to 35 d of experimental periods (38.2%), 42 to 84 d (50.0%), and the rests were in 112 to 168 d (10.2%), respectively.

The final database showed that fatty acids supplemented to diets were from oil sources, seeds or ground seeds, and microalgae. There were distinct variabilities of the supplemental fatty acids used to increase n-3 beneficial FAs in the egg where at least 31 different sources were recorded, either supplemented alone (based on levels) or in combination (based on levels and sources). We were aware that comparisons made from such heterogeny sources might result in low robust models. Therefore, we extracted and calculated the fatty acids profile of the diets used in the studies from available information indicated in the articles with emphasis on the levels of C18:2 n6 (linoleic acid, LA), C18:3 n3 (α-linolenic acid, ALA), C20:5 n-3 (Eicosapentaenoic acid, EPA), C22:6 n-3 (Docosahexaenoic acid, DHA), and total n-3 PUFA as predictor variables. However, studies reporting levels of EPA and DHA in the supplement materials and diets were not many (n = 10 studies with EPA = 36 observations and DHA = 37 observations, respectively). Thus, we utilized the concentration of ALA, ratio of LA/ALA, and total n-3 PUFA in the diets (g/bird/d) to develop reliable models.

A total of 26 of 34 studies (76.47%) used corn + soybean meal (**SBM**) as the main source of

Table 1. Description of studies included in the meta-analysis.

Study	Authors	Country	N[1]	Birds (n)	Strain	Age, week	Length, d	Type of diet	Sources	Levels[2]	FA calculation[3]
1	Cherian and Sim (1991)	Canada	4	40	White Leghorn	30	21	Wheat + SBM	flaxseed, canola seed	0-160	Diet
2	Schreiner et al. (2004)	Austria	4	72	White Leghorn	26	63	Corn + Wheat + SBM	seal blubber oil	0-90	Diet
3	Mazalli et al. (2004)	Brazil	13	300	Hy-Line W-36	46	140	Corn + Wheat + SBM	soybean oil, canola oil, sunflower oil, flaxseed oil, fish oil, flaxseed meal	0-250	Material
4	Carrillo-Domínguez et al. (2005)	Mexico	3	90	White leghorn	65	21	Sorghum + SBM	red crab meal	0-60	Material
5	Amini and Ruiz-Feria (2007)	UK	6	144	Shaver White	62	42	Corn + SBM	flaxseed	0-236	Diet
6	Celebi and Macit (2008)	Turkey	5	200	Isa Brown	67	56	Corn + Wheat + SBM	flaxseed oil, sunflower oil	0-75	Material
7	Rizzi et al. (2009)	Italy	7	126	Warren laying	28	42	Corn + SBM	linseed oil, fish oil, microalgae	19-49.5	Diet
8	Oliveira et al. (2010)	Brazil	4	1152	Dekalb White	54	30	Corn + SBM	sunflower oil, soybean oil, linseed oil	0-34	Diet
9	Wang and Huo (2010)	China	4	192	Newroman	29	70	Corn + SBM	sunflower seed, flaxseed	0-26	Diet
10	Antruejo et al. (2011)	UK	8	384	Brown Shaver	24	84	Corn + SBM	rapeseed, chia seed and oil, flaxseed seed and oil, oleic acid, soybean oil	0-250	Diet
11	Ebeid (2011)	Egypt	4	100	Hisex Brown	56	84	Corn + SBM	fish oil, linseed oil, vegetable oil	0-25	Diet
12	Goldberg et al. (2012)	Canada	6	48	White Bovan	19	56	Wheat + SBM	hemp seed, hemp seed oil	0-200	Diet
13	Poureslami et al. (2012)	Belgium	7	288	Isa Brown	45	35	Corn + SBM	soybean oil, linseed oil, flaxseed oil	0-30	Diet
14	Nain et al. (2012)	Canada	3	75	Lohmann White Leghorn	65	18	Corn + SBM	flaxseed, pea seed	0-150	Material
15	Lemahieu et al. (2015)	Belgium	5	72	Isa brown	29	21	Wheat + SBM	flaxseed, fish oil, I. galbana	0-14.3	Diet
16	Cherian and Quezada (2016)	USA	3	75	Lohmann brown	48	112	Corn + SBM	camelina full fat, flaxseed	0-100	Diet
17	Kim et al. (2016a)	USA	6	60	Shaver Leghorn	20	42	Corn + SBM	defatted microalgae	0-50	Material
18	Cufadar et al. (2016)	Turkey	4	60	Hy-Line W36	44	84	Corn + SBM	soybean oil	0-23	Material
19	Goldberg et al. (2016)	Canada	4	96	White Lohmann	60	28	Corn + SBM	canola meal, flaxseed oil	0-236	Diet
20	Kim et al. (2016b)	USA	13	90	Shaver leghorn	20	28	Corn + SBM	flaxseed oil, microalgae	0-18.5	Material
21	Zhang et al. (2017)	China	8	80	ISA brown	29	35	Corn + SBM	flaxseed, perilla seed, E. ulmoides seed, microalgae	0-115	Material
22	Ehr et al. (2013)	USA	12	132	Hy-Line W36	25	56	Corn + SBM	flaxseed meal and flaxseed oil	0-50	Diet
23	Kostogrys et al. (2017)	Poland	4	40	Isa Brown	26	84	Corn + SBM	sunflower oil, punicic oil	0-25	Diet
24	Jing et al. (2017)	Canada	5	40	Lohmann white	19	56	Barley + SBM	hemp seed oil, hemp omega	0-80	Diet
25	Huang et al. (2018)	UK	4	72	White Leghorn	58	56	Corn + SBM	flaxseed	0-225	Diet
26	Westbrook and Cherian (2019)	UK	4	72	Brown layer	51	120	Corn + SBM	flaxseed	0-10	Diet
27	Elkin et al. (2018)	USA	16	100	Hy-Line W36	18	84	Corn + SBM	sunflower oil, flaxseed oil	0-50	Diet
28	Moran et al. (2019)	Italia	4	360	Isa Brown	20	168	Corn + SBM	microalgae	0-10	Material
29	Omri et al. (2019)	Tunisia	3	60	Novogen White	27	31	Corn + SBM	linseed meal	0-45	Diet
30	Huang et al. (2020)	Canada	4	72	White Leghorn	58	56	Corn + SBM	flaxseed	0-90	Diet
31	Ngo Njembe et al. (2020)	Belgium	6	12	Isa Brown	24	22	Corn + SBM	flaxseed, R. heudelotii, pomegranate seed oil	0-150	Diet
32	Aguillón-Páez et al. (2020)	Colombia	3	150	Babcock Brown	27	56	Corn + SBM	flaxseed, sunflower (fullfat)	0-135	Diet
33	Lee et al. (2021)	South Korea	5	210	Hy-Line Brown	52	28	Corn + SBM	flaxseed oil	0-8	Diet
34	Kralik et al. (2021)	Croatia	6	540	Tetra SL	47	35	Corn + SBM	fish oil	0-15	Diet

[1]N = number of observations.
[2]Provided as g/kg diet.
[3]Fatty acids intakes (expressed in g/bird/d) were calculated either from dietary treatments (available in the dietary composition table) or material supplemented to diets by multiplying them to feed intake.

Table 2. Summary of descriptive statistics of the studies included in the meta-analysis.

Item	N[1]	Min	Max	Mean	SD[2]
Birds	198	12	1152	166.92	184.70
Age, week	198	18	67	35.87	15.45
Length, day	198	18	168	59.98	36.00
Oil supplementation, g/kg	136	0.00	120.00	21.86	21.65
Seed supplementation, g/kg	81	0.00	250.00	68.21	68.69
Nutrient composition of diets					
Metabolizable energy, kcal/kg	183	2,600.00	3,095.00	2,839.80	88.96
Ether extract, %	105	2.75	14.23	7.33	2.65
Crude protein, %	167	14.50	21.50	17.46	1.79
Lysine, %	113	0.64	1.13	0.88	0.13
Methionine, %	97	0.27	0.62	0.42	0.08
Performance					
Feed intake, g/d	198	90.80	137.30	108.23	11.87
Hen day production, %	121	53.50	99.80	90.40	10.44
Feed conversion	103	1.50	3.10	2.07	0.32
Egg weight, g	141	54.10	67.20	61.51	3.67
Egg yolk, g	106	13.10	19.60	15.96	1.39
Cholesterol, %	48	10.71	37.30	15.29	6.13
FA composition of diet, g/kg					
C18:2 n-6[3]	176	1.00	5.43	4.92	1.07
C18:3 n-3[4]	180	0.02	1.65	1.16	0.31
Ratio of n6:n3	176	0.26	11.72	7.05	0.12
C20:5 n-3[5]	36	0.01	4.60	0.91	0.13
C22:6 n-3[6]	37	0.01	5.70	1.59	0.17
Total n-3 PUFA[7]	198	0.04	7.81	5.24	1.09
Fatty acids intake, g/bird/d					
C18:3 n-3	194	0.10	7.90	1.02	1.26
C20:5 n-3	51	0.00	0.59	0.08	0.14
C22:6 n-3	53	0.00	0.73	0.14	0.21
Total n-3	198	0.01	7.90	1.11	1.24
Fatty acids composition of egg, mg/egg					
C18:2 n-6	153	200.84	2,898.78	1,017.86	496.69
C18:3 n-3	164	1.60	855.81	141.58	156.10
C20:5 n-3	132	0.10	55.59	5.61	9.19
C22:6 n-3	164	0.80	292.34	97.22	60.69
Total n-3 PUFA	182	2.17	1,006.26	254.93	199.25
n6/n3 ratio[4]	102	1.25	53.88	8.45	9.16

[1]N = number of observations.
[2]SD = standard of deviation.
[3]C18:2 n-6 = Linoleic acid (LA).
[4]C18:3 n-3 = a-linolenic acid (ALA).
[5]C20:5 n-3 = Eicosapentaenoic acid (EPA).
[6]C22:6 n-3 = Docosahexaenoic acid (DHA).
[7]Total n-3 PUFA was summed as C18:3n-3 + C20:5n-3 + C22:6n-3 or as indicated in the articles.

energy and protein, respectively, while the other studies used wheat + SBM (3 studies), corn + wheat + SBM (3 studies), barley + SBM (1 study), and sorghum + SBM (1 study). Information on nutrient composition of diets (metabolizable energy, ME; % ether extract, % crude protein, % lysine, % methionine) including fatty acids compositions (g/kg diet) are summarized in Table 2. Nutrient profiles of the experiments were in a normal range according to nutrient recommendations of most breeder companies or the National Research Council (National Research Council, 1994). Nevertheless, FA profiles were greatly different as indicated from large SD values which is reasonably due to the large variability of supplemental treatments.

Effects of Dietary Fatty Acids Levels

The results presented herein are based on linear models because most quadratic terms were not significant to predict response variables. The relationship between the concentration of ALA, n-3 PUFA, and the ratio of LA/ALA in the diet with production performance parameters in laying hens is reported in Table 3. These fatty acids concentrations were not related to feed intake and egg weight, and egg yolk. For egg production, the effects of increasing supplementary levels of ALA and total n-3 PUFA tended to be negative ($P < 0.10$) but the magnitude was very small ($R^2 = 0.003$). Concurrently, FCR tended to increase when levels of ALA increased ($P = 0.092$, $R^2 = 0.004$). Interaction effects from covariates (strain, period, age, and source of FA) are not provided because of the lack of significance on these production parameters.

On the other hand, responses of fatty acids content in egg were mostly influenced by independent variables tested (Table 4). In the initial models, we included all individual FA with a sufficient number of observations (sample size > 30), but then removed several predictor

Table 3. Regression equations of the production performance of laying hens with supplemental fatty acids on the diets as obtained from the mixed model analysis.

			Parameter estimates				Model statistics			
Response variable	Independent variable(g/kg)	N[1]	Intercept	SE[2] Intercept	Slope	SE Slope	*P*-value	RMSE[3]	AIC[4]	R^2
Feed intake, g/d	ALA[5]	180	100.15	0.94	−0.01	0.01	0.692	11.71	954	0.000
	LA/ALA	180	99.94	0.93	0.01	0.03	0.855	11.82	936	0.003
	n-3 PUFA[6]	180	100.08	0.85	−0.02	0.01	0.509	11.90	1,007	0.000
Egg production, %	ALA	99	77.69	1.13	−0.05	0.03	0.069	10.56	457	0.003
	LA/ALA	99	76.99	1.08	0.01	0.01	0.766	8.44	455	0.140
	n-3 PUFA	99	77.31	0.99	−0.04	0.02	0.094	10.46	545	0.003
Feed conversion	ALA	85	2.59	0.04	0.00	0.00	0.092	0.34	−4.2	0.004
	LA/ALA	85	1.73	0.64	0.00	0.00	0.929	0.32	−28.5	0.145
	n-3 PUFA	85	2.59	0.04	0.01	0.00	0.116	0.32	−13.7	0.001
Egg weight, g	ALA	119	66.11	0.63	0.00	0.01	0.996	3.79	467	0.029
	LA/ALA	119	66.11	0.37	-0.003	0.02	0.885	3.87	460	0.002
	n-3 PUFA	119	66.11	0.32	-0.01	0.01	0.731	3.68	495	0.001
Yolk, g	ALA	88	17.32	0.34	0.01	0.01	0.376	5.95	241	0.080
	LA/ALA	88	17.08	0.19	-0.01	0.01	0.457	5.98	234	0.070
	n-3 PUFA	88	17.08	0.17	-0.01	0.01	0.470	6.13	1241	0.020

[1]N = number of observations.
[2]SE = standard error.
[3]RMSE = root means square error.
[4]AIC = Akaike information criterion.
[5]LA = C18:2 n-6 (Linoleic acid).
[6]ALA = C18:3 n-3 (a-linolenic acid).

Table 4. Regression equations to estimate the egg's fatty acids composition of laying hens in responses to fatty acids composition of feed supplemented with n-3 PUFA sources.

			Parameter estimates				Model statistics				Interaction models		
Content of fatty acids in egg (mg/egg)	Independent variable	N[1]	Intercept	SE[2] Intercept	Slope	SE Slope	*P*-value	RMSE[3]	AIC[4]	R^2	level vs. strain	level vs. age	level vs. source
LA[5]	ALA	136	999.19	75.92	−1.12	0.55	0.043	410.04	1,504	0.342	0.966	0.976	0.814
	LA/ALA	136	1,031.48	42.26	2.98	1.59	0.065	413.06	1,507	0.288	0.982	0.566	0.003
	n-3 PUFA	136	1,071.69	40.05	0.15	1.47	0.919	495.79	1,689	0.558	0.485	0.215	<0.001
ALA[6]	ALA	142	22.71	32.19	2.73	0.39	<0.001	156.26	1,483	0.003	0.317	0.960	0.253
	LA/ALA	142	92.87	32.80	−0.21	1.02	0.839	154.82	1,523	0.090	0.882	0.868	<0.001
	n-3 PUFA	142	31.69	26.54	5.94	1.00	<0.001	141.86	1,713	0.628	0.079	0.001	<0.001
EPA[7]	ALA	110	14.96	2.63	−0.15	0.05	0.002	9.58	650	0.237	0.997	<0.001	0.307
	LA/ALA	110	8.78	1.88	−0.25	0.10	0.015	9.57	656	0.158	0.987	0.063	0.212
	n-3 PUFA	110	7.15	1.47	0.12	0.07	0.067	8.87	765	0.592	0.984	0.853	0.629
DHA[8]	ALA	142	88.11	7.88	0.48	0.12	<0.001	39.93	1,585	0.655	0.904	<0.001	0.544
	LA/ALA	142	112.85	8.70	−1.40	0.31	<0.001	53.93	1,536	0.083	0.525	0.301	0.769
	n-3 PUFA	142	88.82	10.27	0.99	0.34	0.005	60.21	1,800	0.628	0.003	<0.001	0.000
Total n-3 PUFA[9]	ALA	160	136.09	39.40	2.90	0.48	<0.001	134.39	1739	0.684	0.484	0.825	0.399
	LA/ALA	160	258.61	38.12	−0.37	1.25	0.003	186.84	1772	0.143	0.027	0.768	0.999
	n-3 PUFA	160	140.49	30.59	9.59	1.12	<0.001	185.04	1884	0.812	0.312	0.360	<.0001
Ratio of n6:n3[10]	ALA	89	7.16	2.21	−0.09	0.03	0.004	8.29	746	0.186	0.895	0.950	0.788
	LA/ALA	89	3.46	1.93	0.19	0.07	0.012	6.87	751	0.321	0.195	0.551	0.106
	n-3 PUFA	89	15.12	0.62	−0.15	0.10	<0.001	9.12	638	0.243	0.403	0.563	<0.001
Cholesterol, %	ALA	48	12.54	0.50	−0.01	0.00	0.201	1.36	149	0.012	0.221	0.614	0.021
	LA/ALA	48	12.27	0.47	−0.04	0.02	0.075	1.21	142	0.010	0.862	-	-
	n-3 PUFA	48	12.13	0.41	−0.03	0.02	0.181	1.39	141	0.000	0.835	0.570	0.003

[1]N = number of observations.
[2]SE = standard error.
[3]RMSE = root means square error.
[4]AIC = Akaike information criterion.
[5]LA = C18:2 n-6 (Linoleic acid).
[6]ALA = C18:3 n-3 (a-linolenic acid).
[7]EPA = C20:5 n-3 (Eicosapentaenoic acid).
[8]DHA = C22:6 n-3 (Docosahexaenoic acid).
[9]Total n-3 PUFA = total of n-3 polyunsaturated fatty acids (ALA, EPA, DHA).
[10]Ratio of n6:n3 was calculated as total n6 divided by total n3 FAs.

variables such as EPA and DHA because the sample sizes were low and weak to estimate response variables. Therefore, we retained prediction models with the concentration of ALA, total n-3 PUFAs, and the ratio of LA/ALA. Among predictor variables tested, concentration of ALA in the diets significantly predicted the LA ($P = 0.043$, $R^2 = 0.342$), ALA ($P < 0.0001$, $R^2 = 0.003$), EPA ($P = 0.002$, $R^2 = 0.237$), DHA ($P < 0.0001$, $R^2 = 0.449$), total n-3 PUFA ($P < 0.0001$, $R^2 = 0.528$), and n6/n3 ratio ($P = 0.004$, $R^2 = 0.186$) with large variability of coefficient of determinants. Responses of contents of ALA, DHA, total n-3 PUFAs, and n6/n3 ratio in egg were significantly related to the concentration of n-PUFAs in the diets ($P < 0.01$) with the highest degree of prediction on mg n-3 PUFAs concentration in egg ($P < 0.0001$, $R^2 = 0.812$). According to calculations based on the dataset, both ALA and total n-3 PUFAs concentration failed to predict egg's cholesterol concentration while increasing the ratio of LA/ALA in the diet tended to decreased cholesterol level in egg ($P = 0.075$). Contents of EPA, DHA, and total n-3 PUFAs in egg were also linearly decreased ($P < 0.01$) when the ratio of LA/ALA increased. As a predictor variable, concentration of ALA in the diets resulted in higher prediction models to DHA content in egg (slope = 0.482; $R^2 = 0.684$) when compared to levels of dietary total n-3 PUFAs (slopes = 0.998, $R^2 = 0.628$) (AIC = 1585 vs. 1800, respectively). The comparison for these equations is shown in Figure 2. Conversely, total n-3 PUFAs in diets also had a higher degree of prediction on n-3 PUFAs content in egg (slope = 9.59; $R^2 = 0.812$) that that of ALA levels in the diet (slope = 0.289; $R^2 = 0.528$).

Interaction Effects from Moderator Variables

A significant interaction between the dietary concentration of n-3 PUFA × FA sources to predict total n-3 PUFA and DHA contents (mg/60 g egg) was observed ($P < 0.01$). As shown in Figure 3, Two FA sources were chosen as the factors with the most significant effects to predict n-3 PUFA content in egg. Flaxseed oil is the best predictor for n-3 PUFA content in egg ($P < 0.0001$, $R^2 = 0.855$) while the combination of more than one FA sources resulted in lower estimate for n-3 PUFA content in egg ($P = 0.0281$, $R^2 = 0.369$). As the concentration of DHA (mg/ 60 g egg) was affected by the interaction between FA sources and levels of n-3 PUFA in the diets ($P < 0.0001$, Table 3), prediction models for several sources of FA were developed and are presented in Figure 4. Microalgae ($P < 0.0001$, $R^2 = 0.697$) and fish oil ($P = 0.0148$, $R^2 = 0.732$) are 2 groups of FA sources that could improve the prediction equation to estimate DHA content in egg while mixed source of FA lowered

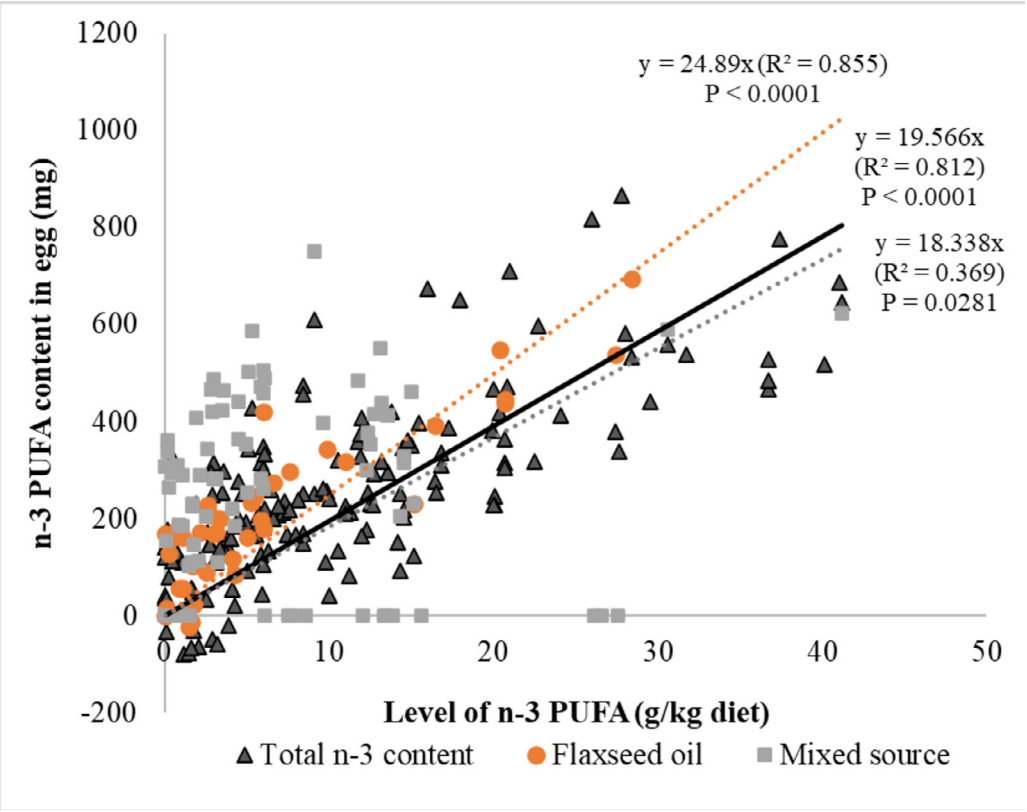

Figure 2. Relationship between levels of n-3 PUFA in the diets with some n-3 PUFA sources and the formation of total n-3 PUFA contents (mg) in egg of laying hens.

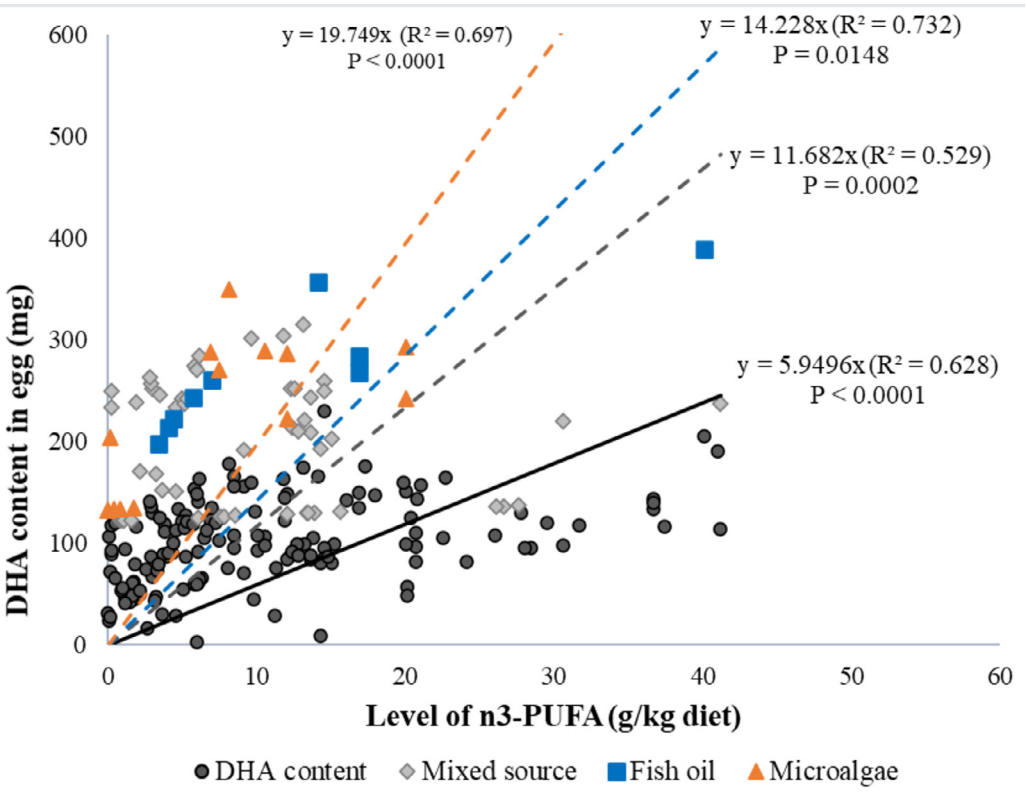

Figure 3. Relationship between levels of n-3 PUFA in the diets with some n-3 PUFA sources and the formation of DHA contents (mg) in egg of laying hens.

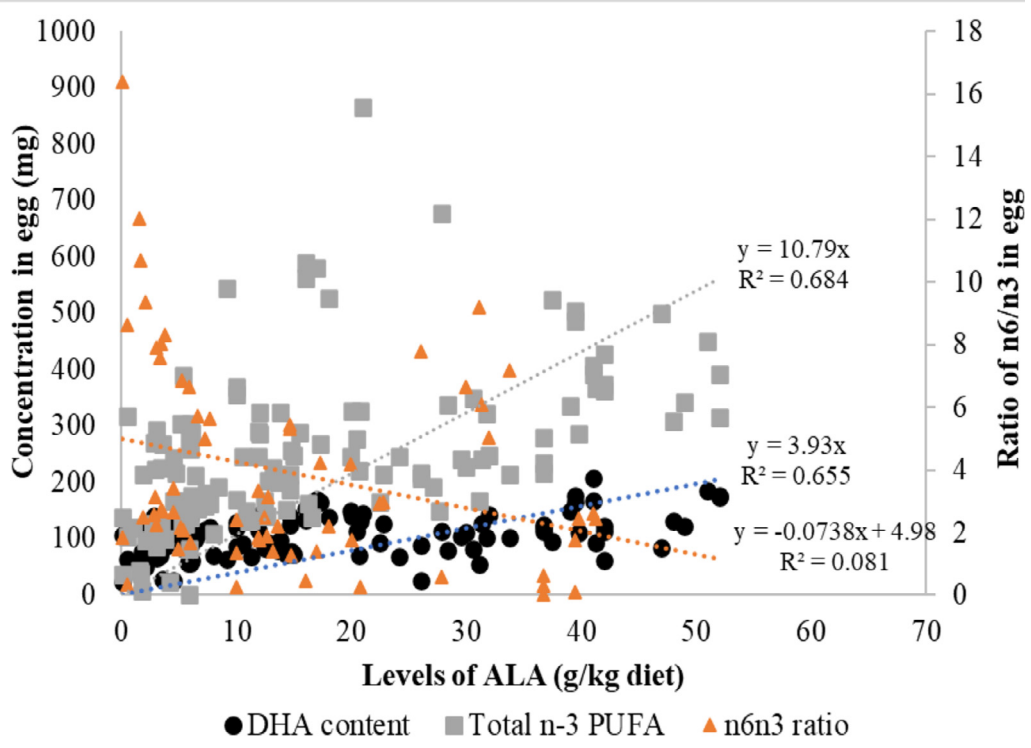

Figure 4. Relationship between levels of a-linolenic acid in the diets and contents of DHA, total n-3 PUFA, and ratio of n6:n3 in egg.

the model ($P = 0.0002$, $R^2 = 0.529$). The interaction effects between levels of ALA vs. age were significant on EPA and DHA contents in egg ($P < 0.0001$). Interaction effects between levels of n-3 PUFA vs. strain and between LA/ALA ratio vs. strain were also found to be significant on DHA content ($P = 0.003$) and total n-3 PUFA content in egg ($P = 0.027$).

Subgroup Analysis

Table 5 reports discrete analysis of fatty acids profile of egg as influenced by different sources of FA supplemented to diet. Overall, dietary inclusion with ingredients high in n-3 FA concentration present significant effects on fatty acids contents of egg ($P < 0.01$) but they did not influence cholesterol concentration in egg (%). Higher LA content in egg was found with sunflower oil supplementation ($P < 0.05$) while other supplement materials were similar with the control group. Supplementation with soybean oil resulted in a significantly lower on egg's ALA content ($P < 0.05$) but it significantly increased with flaxseed oil, flaxseed meal, or a mixture of several ingredients rich in n-3 PUFA contents when compared with the control ($P < 0.05$). Most of the FA sources did not change the level of EPA in egg except for fish oil supplementation which elevated the EPA ($P < 0.05$). DHA and total n-3 PUFA concentrations in egg

Table 5. Effects of the sources of supplemental fatty acids on egg' fatty acids composition (expressed as mg/egg) from laying hens.

	Fatty acids composition (mg/egg)[1]						
Source of supplemental FA	C18:2 n-6	C18:3 n-3	C20:5 n-3	C22:6 n-3	n-3 PUFA	n6/n3 ratio	Cholesterol, %
N[2]	137	137	109	137	155	87	44
Control	1030.35^{b}	52.70^{b}	2.56^{b}	65.31^{c}	133.35^{c}	11.91^{b}	15.81
Microalgae	1062.93^{b}	79.48^{b}	3.94b^{b}	131.90^{ab}	244.21^{b}	7.41b^{c}	15.89
Flaxseed (seed/meal)	990.87^{b}	257.03^{a}	8.70^{ab}	109.50^{b}	390.35^{a}	5.13^{c}	15.80
Flaxseed oil	947.10^{b}	227.27^{a}	4.69^{b}	121.45^{b}	379.60^{a}	3.92^{c}	16.83
Fish oil	1087.72^{b}	37.46^{b}	15.70^{a}	150.49^{a}	238.46^{b}	7.59^{bc}	15.61
Hemp seed oil	1132.12^{b}	157.91^{ab}	4.09^{b}	91.14^{bc}	309.96^{a}	-	15.56
Linseed oil	1137.13^{b}	140.09^{ab}	0.82^{b}	122.26^{b}	322.58^{a}	4.30^{c}	16.75
Mixture sources	1031.73^{b}	206.87^{a}	9.99^{ab}	119.41^{b}	354.51^{a}	3.01^{c}	15.95
Soybean oil	1143.57^{b}	2.45^{c}	2.00^{b}	47.74^{cd}	101.43^{c}	22.52^{a}	17.72
Sunflower oil	1402.05^{a}	29.33^{b}	2.88^{b}	33.56^{cd}	108.50^{c}	3.53^{c}	16.45
SEM	112.16	49.26	3.09	16.90	56.40	2.36	0.53
P-value	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	0.130

^{a,b,c}Means with different superscripts within the same row differ at $P < 0.05$.

[1]C18:2 n-6 = Linoleic acid.

[2]C18:3 n-3 = a-linolenic acid. 3C20:5 n-3 = Eicosapentaenoic acid. 4C22:6 n-3 = Docosahexaenoic acid. 5n-3 PUFA was summed as C18:3n-3 + C20:5n-3 + C22:6n-3 or as indicated in the articles. 6N = number of observations.

were significantly increased with diets supplemented with almost sources of FA ingredients such as microalgae, flaxseed, fish oil, linseed oil, and combination of these sources ($P < 0.05$) while sunflower oil and soybean oil failed to increase the DHA content in egg ($P > 0.05$). Concomitantly, these FA sources decreased the n6:n3 ratio in eggs ($P < 0.05$), except for fish oil which the n6:n3 did not changed compared to control.

DISCUSSION

Production of Fatty Acids Intake on Laying Hens' Performance

Despite it was postulated that enriching diets with omega 3 precursor is the most effective strategy to enhance functional FA properties in egg, the supplementation effects on production parameters must be well understood. Overall, responses of hens' performance corresponded with a large variety of fat and oil sources supplemented to individual study were generally not significant and were not age-, strain-, or trial period-dependents. However, we found that higher ALA intake tended to decrease egg production and FCR. This could be due to the decreased of CP or phosphorus (**P**) levels as PUFAs supplementation increased which is similar to the study of Aguillon-Paez et al. (2020) and Lee et al. (2021), respectively. Additionally, such minor effects might also be attributed to the effects of antinutritional factors (**ANFs**) presence in the supplemented ingredients as FA sources that had a negative consequence on palatability and nutrient digestion (Fraeye et al., 2012a; Aguillón-Páez et al., 2020; Lee et al., 2021). This might be expected when seeds or meal forms of FA sources were incorporated. Evidences regarding this reason are available from several previous studies. For instance, a recent experiment using 13.5% sunflower and flaxseed decreased egg production by 14.6% (Aguillón-Páez et al., 2020). Similarly, laying hens fed diets with 5% milled flaxseed resulted in BW decrease after 24 h (Ehr et al., 2017). Supplementing diet with 15% flaxseed was also reported to decrease egg production by 8.26% while concurrently increased FCR (Jia et al., 2008).

Although there was no report on the antinutritional content of the ingredients, however, cyanogenic glycosides and tannins are mostly presented in flaxseed that can impair respiration rate and stress in laying hens. Other ANFs in flaxseed are phytic acid and trypsin inhibitors that increased intestinal viscosity (Alzueta et al., 2003) reduced nutrient bioavailability (Goyal et al., 2014). These situations were reported to negatively impact laying performance and quality of eggs (Imran et al., 2015). In addition, sunflower meal was reported to contain chlorogenic (1.56%), quinic acids (0.48%) (Senkoylu and Dalet, 1999), and up to 3.5% phenolic acids that can inhibit trypsin and lipase activities as well as bind lysine and methionine (Aguillón-Páez et al., 2020). However, such negative effects somehow not appeared, probably due to the inclusion level or form of ingredients that directly influenced the nutrient composition of diets offered. Important steps to avoid the negative effect on ANFs in seed as n-3 FA sources are by mechanical processing such as extruding or heating or by enzyme supplementation (Westbrook and Cherian, 2019; Huang et al., 2020). In this regard, (Huang et al., 2020) reported that adding 9% extruded flaxseed did not affect egg production, egg weight, and feed intake.

Several recent studies using oils or soluble ingredients as sources of dietary FA have been suggested to not affect the performance parameters in laying hens. Supplementations with 0.8% flaxseed oil, 3.5% soybean oil + 1.5% fish oil, or 9% hemp seed oil were reported to not affecting hen day production, feed intake, FCR, egg weight, and egg quality parameters (Jing et al., 2017; Kralik et al., 2021; Lee et al., 2021). However, it is important to note that continuous exposure to diets supplemented with fish oil or ingredients containing a high gap of LA to ALA would decrease egg weight because recommended LA % in diets is required to maintain egg weight (Grobas et. al., 1999). It was reported that fish oil supplementation rich in ALA did not affect egg weight for 12 wk trial period but decreased the egg weight after 16 wk (Dong et al., 2018).

Effects of Types of Fatty Acids on n-3 PUFA Formation in Egg

Production of egg containing desired content of omega 3 is expected to continuously increase in the future, reasonably due to increasing consumers' demand for superior egg quality. Interests to increase daily intakes of functional properties available in egg have driven poultry nutritionists to improve deposition efficiency of omega 3 fatty acids in egg. It is well documented that the concentration of omega 3 in egg was successfully increased by adding fat or oil sources (Fraeye et al., 2012b; Alagawany et al., 2019). Empirical relationships between supplementation n-3 sources on egg's omega-3 contents are widely proven. However, in some aspects, it is not clear what factors contributed to the large variations of omega-3 formation among studies. Therefore, this meta-analysis attempted to systematically analyze aspects that hypothesized to interfere with the conversion efficiency of omega 3 in egg.

In the present meta-analysis, our findings revealed that increasing ALA levels in the diets linearly increased EPA, DHA, and total n-3 PUFAs and concomitantly decreased LA concentration in eggs. From the results, it can be interpreted that feeding 100 g/kg ALA would produce egg with ±136 mg DHA. However, we also found that increasing LA levels which represented by increasing LA to ALA proportion could also linearly decreased formation rates of EPA, DHA, and n-3 PUFAs formation. Though weaker, total n-3 PUFA content in the diet could also predict DHA formation because ALA predominantly composed the n-3 PUFA in many FA sources. This was supported by a previous

study reporting that excessive amounts of long-chain fatty acids other than ALA, EPA, and DHA lowered conversion efficiency (Cachaldora et al., 2008). To our knowledge, studies presenting conversion efficiency of specific FA on ALA, EPA, DHA, and n-3 PUFAs in eggs are limited. Most of previous authors often presented their dietary FA enrichment experiments without rate of LA conversion from feed to eggs. Our results suggested that the concentration of ALA, n-3 PUFA, and the ratio of LA/ALA can be used as a single predictor to estimate DHA and total n-3 PUFA formation in egg. In the models, these independent variables showed significant linear relationships with variable estimates, especially DHA and total n-3 PUFA. We found that the magnitude among predictor variables varied, with the concentration of ALA in diets better to predict DHA formation while total n-3 PUFA had a stronger prediction model to estimate the n-3 PUFA deposition in egg according to the coefficient of determinant.

Our findings were in agreement with available theory that ALA serves as the main precursor for EPA and DHA synthesis. When ingested by laying hens, Δ-6-desaturase enzymes desaturate the ALA by removing the hydrogen atom and then elongated by adding the carbons to form EPA. From this standpoint, EPA is further converted to DHA by elongation and desaturation processes (Fraeye et al., 2012b). The conversion efficiency of ALA into DHA varied among experimental setup, primarily caused by the proportion of LA included (Ehr et al., 2013). It is because Δ-6-desaturase can also utilize linoleic acid as substrate although the activity is lower than on ALA. Therefore, increasing the ratio of LA/ALA may disturb the DHA formation in egg due to substrate competition (Fraeye et al., 2012a). Several recent investigations support this reason. For example, decreasing LA while increasing ALA proportions from various FA sources linearly elevated DHA content in egg (Huang et al., 2018; Omri et al., 2019; Aguillón-Páez et al., 2020; Lee et al., 2021). In addition to LA/ALA proportions, other factors explaining the discrepancies of DHA deposition are the age of hens and the source of FA. Age was previously proposed to influence DHA conversion efficiency (Fraeye et al., 2012a) and this meta-analysis empirically confirmed that finding. Older hens are known to have larger liver which has more effective metabolism especially in the DHA formation from ALA sources.

Furthermore, this meta-analysis also emphasized that ingredients used as ALA sources explained different efficiency to form DHA and total n-3 PUFA. This could be attributed to a large variation in the fatty acids profile of each ingredient. As the most concentrated ALA source in comparison to FA sources (Harris et al., 2009), flaxseed oil supplementation had the most efficient source to produce n-3 PUFA in egg (Figure 2). On the other hand, fish oil is suggested to have the highest conversion efficiency in DHA formation (Figure 3). It is plausible because fish oil is rich in DHA content by nature. Comparison studies have demonstrated that DHA content of egg yolk was significantly greater with fish oil supplemented diet when compared with linseed oil and microalgae (Rizzi et al., 2009). Similarly, by comparing fish oil and linseed oil supplemented diets, (Ebeid, 2011) found that fish oil resulted in higher contents of EPA and DHA in egg. Most recent study also reported that inclusion of 1.5% fish oil produced 142 mg DHA/ 60 g egg (Kralik et al., 2021) which higher than 0.8% flaxseed oil (estimated DHA = 106 mg/ 60 g egg) (Lee et al., 2021) and 5 to 10% flaxseed with microalgae (DHA estimated = 76−89 mg/ 60 g egg) (Ngo Njembe et al., 2020). Our discrete analysis showed that fish oil supplementation resulted in the highest DHA content when compared to control and other sources of FA (Table 5).

Above all, it should be kept in mind that one major problem to include n-3 PUFA in the diets is its susceptibility to lipid oxidation (Faitarone et al., 2016) which directly affects egg quality and flavor. Increasing soluble flaxseed oil was reported to increase MDA concentration in egg (Lee et al., 2021). Several previous studies using fish oil and flaxseed were reported to increase lipid oxidation in egg (Hayat et al., 2010; Kralik et al., 2021). Thus, adding antioxidants together with n-3 PUFA sources has been proposed as an effective strategy to inhibit oxidation that could deteriorate egg and shorten the lifespan of egg (Huang et al., 2019; Omri et al., 2019; Sepidarkish et al., 2019).

CONCLUSIONS

The concentration of ALA, total n-3 PUFA, and the ratio of LA/ALA in diets had a linear relationship with EPA, DHA, total n-3 PUFA, and n6/n3 ratio and they can be used as predictor variables to estimate the formation of n-3 fatty acids in egg. Nevertheless, the associations were age and sources dependent. It was confirmed that although all n-3 FA sources supplemented to diets had a positive correlation for DHA and n-3 PUFA deposition, however, our study emphasized that fish oil had the highest prediction model for DHA formation in egg across all FA sources included in the dataset. In addition, our study suggested that increasing supplementary levels of ALA or n-3 PUFA in the diet might cause a detrimental effect on the production performance of laying hens. Therefore, a limit inclusion level must be set considering the presence of antinutritional factors in the material used.

ACKNOWLEDGMENTS

The authors thank the Institute of Research and Community Services (Lembaga Penelitian dan Pengabdian Kepada Masyarakat) Universitas Sebelas Maret, Indonesia for funding this study through the Indonesian Higher Education Research Collaboration grant scheme.

Ethics statement: This study was conducted using generated data from published experiments without directly used animals. Thus, it is not necessary to obtain approval from the Animal Ethics Committee of Universitas Sebelas Maret or related institution.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

Aguillón-Páez, Y. J., L. A. Romero, and G. J. Diaz. 2020. Effect of full-fat sunflower or flaxseed seeds dietary inclusion on performance, egg yolk fatty acid profile and egg quality in laying hens. Anim. Nutr. 6:179–184.

Alagawany, M., S. S. Elnesr, M. R. Farag, M. E. Abd El-Hack, A. F. Khafaga, A. E. Taha, R. Tiwari, M. Iqbal Yatoo, P. Bhatt, S. K. Khurana, and K. Dhama. 2019. Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. Animals 9:573.

Alzueta, C., M. L. Rodriguez, M. T. Cutuli, A. Rebole, L. T. Ortiz, C. Centeno, and J. Trevino. 2003. Effect of whole and demucilaged linseed in broiler chicken diets on digesta viscosity, nutrient utilisation and intestinal microflora. Br. Poult. Sci. 44:67–74.

Amini, K., and C. A. Ruiz-Feria. 2007. Evaluation of pearl millet and flaxseed effects on egg production and n-3 fatty acid content. Br. Poult. Sci. 48:661–668.

Antruejo, A., J. O. Azcona, P. T. Garcia, C. Gallinger, M. Rosmini, and R. Ayerza. 2011. Omega-3 enriched egg production: the effect of α -linolenic ω -3 fatty acid sources on laying hen performance and yolk lipid content and fatty acid composition. Br. Poult. Sci. 52:750–760.

Attia, Y. A., E. Abdul, Abd. El-Hamid, H. F. Ellakany, F. Bovera, M. A. Al-Harthi, and S. A. Ghazaly. 2013. Growing and Laying performance of japanese quail fed diet supplemented with different concentrations of acetic acid. Ital. J. Anim. Sci. 12:e37.

Cachaldora, P., P. García-Rebollar, C. Alvarez, J. Méndez, and J. C. De Blas. 2008. Double enrichment of chicken eggs with conjugated linoleic acid and n-3 fatty acids through dietary fat supplementation. Anim. Feed Sci. Technol. 144:315–326.

Carrillo-Domínguez, S., M. E. Carranco-Jauregui, R. M. Castillo-Domínguez, M. I. Castro-González, E. Avila-González, and F. Pérez-Gil. 2005. Cholesterol and n-3 and n-6 fatty acid content in eggs from laying hens fed with red crab meal (Pleuroncodes planipes). Poult. Sci. 84:167–172.

Celebi, S., and M. Macit. 2008. The effects of sources of supplemental fat on performance, egg quality, and fatty acid composition of egg yolk in laying hens. J. Sci. Food Agric. 88:1237–1243.

Cherian, G., and N. Quezada. 2016. Egg quality, fatty acid composition and immunoglobulin Y content in eggs from laying hens fed full fat camelina or flax seed. J. Anim. Sci. Biotechnol. 7:1–8.

CHERIAN, G., and J. S. SIM. 1991. Effect of feeding full fat flax and canola seeds to laying hens on the fatty acid composition of eggs, embryos, and newly hatched chicks. Poult. Sci. 70:917–922.

Coorey, R., A. Novinda, H. Williams, and V. Jayasena. 2015. Omega-3 fatty acid profile of eggs from laying hens fed diets supplemented with chia, fish oil, and flaxseed. J. Food Sci. 80:S180–S187.

Cufadar, Y., R. Göçmen, and G. Kanbur. 2016. The effect of replacing soya bean oil with glycerol in diets on performance, egg quality and egg fatty acid composition in laying hens. Animal 10:19–24.

Dong, X. F., S. Liu, and J. M. Tong. 2018. Comparative effect of dietary soybean oil, fish oil, and coconut oil on performance, egg quality and some blood parameters in laying hens. Poult. Sci. 97:2460–2472.

Ebeid, T. A. 2011. The impact of incorporation of n-3 fatty acids into eggs on ovarian follicular development, immune response, antioxidative status and tibial bone characteristics in aged laying hens. Animal 5:1554–1562.

Ehr, I. J., M. E. Persia, and E. A. Bobeck. 2013. Comparative omega-3 fatty acid enrichment of egg yolks from first-cycle laying hens fed flaxseed oil or ground flaxseed. Poult. Sci. 96:1–9.

Ehr, I. J., M. E. Persia, and E. A. Bobeck. 2017. Comparative omega-3 fatty acid enrichment of egg yolks from first-cycle laying hens fed flaxseed oil or ground flaxseed. Poult. Sci. 96:1791–1799.

Elkin, R. G., A. N. Kukorowski, Y. Ying, and K. J. Harvatine. 2018. Dietary high-oleic acid soybean oil dose dependently attenuates egg yolk content of n-3 polyunsaturated fatty acids in laying hens fed supplemental flaxseed oil. Lipids 53:235–249.

Faitarone, A. B. G., E. A. Garcia, R. O. Roca, E. N. Andrade, F. Vercese, and K. Pelicia. 2016. Yolk color and lipid oxidation of the eggs of commercial white layers fed diets supplemented with vegetable oils. Braz. J. Poult. Sci. 18:9–16.

Fraeye, I., C. Bruneel, C. Lemahieu, J. Buyse, K. Muylaert, and I. Foubert. 2012a. Dietary enrichment of eggs with omega-3 fatty acids: a review. Food Res. Int. 48:961–969.

Fraeye, I., C. Bruneel, C. Lemahieu, J. Buyse, K. Muylaert, and I. Foubert. 2012b. Dietary enrichment of eggs with omega-3 fatty acids : a review. FRIN 48:961–969.

Goldberg, E. M., N. Gakhar, D. Ryland, M. Aliani, R. A. Gibson, and J. D. House. 2012. Fatty acid profile and sensory characteristics of table eggs from laying hens fed hempseed and hempseed oil. J. Food Sci. 77:1–8.

Goldberg, E. M., D. Ryland, M. Aliani, and J. D. House. 2016. Interactions between canola meal and flaxseed oil in the diets of White Lohmann hens on fatty acid profile and sensory characteristics of table eggs. Poult. Sci. 95:1805–1812.

Goyal, A., V. Sharma, N. Upadhyay, S. Gill, and M. Sihag. 2014. Flax and flaxseed oil: an ancient medicine & modern functional food. J. Food Sci. Technol. 51:1633–1653.

Grobas, S., G. Mateos, and J. Mendez. 1999. Influence of dietary linoleic acid on production and weight of eggs and egg components in young brown hens. J. Appl. Poult. Res. 8:177–184.

Harris, W. S., D. Mozaffarian, E. Rimm, P. Kris-Etherton, L. L. Rudel, L. J. Appel, M. M. Engler, M. B. Engler, and F. Sacks. 2009. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association nutrition subcommittee of the council on nutrition, physical activity, and metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation 119:902–907.

Hayat, Z., G. Cherian, T. N. Pasha, F. M. Khattak, and M. A. Jabbar. 2010. Oxidative stability and lipid components of eggs from flax-fed hens: effect of dietary antioxidants and storage. Poult. Sci. 89:1285–1292.

Huang, J., Q. Hao, Q. Wang, Y. Wang, X. Wan, and Y. Zhou. 2019. Supplementation with green tea extract affects lipid metabolism and egg yolk lipid composition in laying hens. J. Appl. Poult. Res. 28:881–891.

Huang, S., B. Baurhoo, and A. Mustafa. 2018. Effects of extruded flaxseed on layer performance, nutrient retention and yolk fatty acid composition. Br. Poult. Sci. 59:463–469.

Huang, S., B. Baurhoo, and A. Mustafa. 2020. Effects of feeding extruded flaxseed on layer performance, total tract nutrient digestibility, and fatty acid concentrations of egg yolk, plasma and liver. J. Anim. Physiol. Anim. Nutr. (Berl). 104:1365–1374.

Imran, M., F. M. Anjum, M. Nadeem, N. Ahmad, M. K. Khan, Z. Mushtaq, and S. Hussain. 2015. Production of Bio-omega-3 eggs through the supplementation of extruded flaxseed meal in hen diet. Lipids Health Dis 14:1–9.

Irawan, A., C. Hidayat, A. Jayanegara, and A. Ratriyanto. 2021. Essential oils as growth-promoting additives on performance, nutrient digestibility, cecal microbes, and serum metabolites of broiler chickens: a meta-analysis. Anim. Biosci. 34:1499–1513.

Jayanegara, A., T. U. P. Sujarnoko, M. Ridla, M. Kondo, and M. Kreuzer. 2019. Silage quality as influenced by concentration and type of tannins present in the material ensiled: a meta-analysis. J. Anim. Physiol. Anim. Nutr. (Berl). 103:456–465.

Jia, W., B. A. Slominski, W. Guenter, A. Humphreys, and O. Jones. 2008. The effect of enzyme supplementation on egg production parameters and omega-3 fatty acid deposition in laying hens fed flaxseed and canola seed. Poult. Sci. 87:2005–2014.

Jing, M., S. Zhao, and J. D. House. 2017. Performance and tissue fatty acid profile of broiler chickens and laying hens fed hemp oil and HempOmegaTM. Poult. Sci. 96:1809–1819.

Khan, S. U., A. N. Lone, M. S. Khan, S. S. Virani, R. S. Blumenthal, K. Nasir, M. Miller, E. D. Michos, C. M. Ballantyne, W. E. Boden, and D. L. Bhatt. 2021. Effect ofomega-3 fatty acids on cardiovascular outcomes: a systematic review and meta-analysis. EClinica 38:100997.

Kim, J., M. Barcus, A. Magnuson, L. Tao, and X. G. Lei. 2016a. Supplemental defatted microalgae affects egg and tissue fatty acid composition differently in laying hens fed diets containing corn and flaxseed oil. J. Appl. Poult. Res. 25:528–538.

Kim, J., A. Magnuson, L. Tao, M. Barcus, and X. G. Lei. 2016b. Potential of combining flaxseed oil and microalgal biomass in

producing eggs-enriched with n - 3 fatty acids for meeting human needs. Algal Res 17:31–37.
Kostogrys, R. B., A. Filipiak-Florkiewicz, K. Dereń, A. Drahun, I. Czyżyńska-Cichoń, E. Cieślik, B. Szymczyk, and M. Franczyk-Żarów. 2017. Effect of dietary pomegranate seed oil on laying hen performance and physicochemical properties of eggs. Food Chem 221:1096–1103.
Kralik, G., Z. Kralik, M. Grcevic, O. Galovic, D. Hanzek, and E. Biazik. 2021. Fatty acid profile of eggs produced by laying hens fed diets containing different shares of fish oil. Poult. Sci. 100:101379.
Lee, S. H., Y. B. Kim, D.-H. Kim, D.-W. Lee, H.-G. Lee, R. Jha, and K.-W. Lee. 2021. Dietary soluble flaxseed oils as a source of omega-3 polyunsaturated fatty acids for laying hens. Poult. Sci. 100:101276.
Lemahieu, C., C. Bruneel, R. Termote-verhalle, K. Muylaert, I. Foubert, and J. Buyse. 2015. Dynamics of omega-3 long chain polyunsaturated fatty acid incorporation in egg yolk by autotrophic microalgal supplementation. Eur. J. Lipid Sci. Technol. 117:1391–1397.
Liberati, A., D. G. Altman, J. Tetzlaff, C. Mulrow, P. C. Gøtzsche, J. P. A. Ioannidis, M. Clarke, P. J. Devereaux, J. Kleijnen, and D. Moher. 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 339:b2700.
Mason, R. P., P. Libby, and D. L. Bhatt. 2020. Emerging mechanisms of cardiovascular protection for the omega-3 fatty acid eicosapentaenoic acid. Arterioscler. Thromb. Vasc. Biol. 40:1135–1147.
Mazalli, M. R., D. E. Faria, D. Salvador, and D. T. Ito. 2004. A comparison of the feeding value of different sources of fat for laying hens: 2. Lipid, cholesterol, and vitamin E profiles of egg yolk. J. Appl. Poult. Res. 13:280–290.
Moran, C. A., M. Morlacchini, J. D. Keegan, and G. Fusconi. 2019. Increasing the omega-3 content of hen's eggs through dietary supplementation with aurantiochytrium limacinum microalgae: effect of inclusion rate on the temporal pattern of docosahexaenoic acid enrichment, efficiency of transfer, and egg characteristics. J. Appl. Poult. Res. 28:329–338.
Nain, S., R. A. Renema, D. R. Korver, and M. J. Zuidhof. 2012. Characterization of the n-3 polyunsaturated fatty acid enrichment in laying hens fed an extruded flax enrichment source. Poult. Sci. 91:1720–1732.
National Research Council. 1994. Nutrient Requirements of Poultry. 9th ed. National Academy Press, Washington, DC.
Ngo Njembe, M. T., E. Dormal, C. Gardin, E. Mignolet, C. Debier, and Y. Larondelle. 2020. Effect of the dietary combination of flaxseed and Ricinodendron heudelotii or Punica granatum seed oil on the fatty acid profile of eggs. Food Chem. 344:128668.
Oliveira, D. D., N. C. Baião, S. V. Cançado, R. Grimaldi, M. R. Souza, L. J. C. Lara, and A. M. Q Lana. 2010. Effects of lipid sources in the diet of laying hens on the fatty acid profiles of egg yolks. Poult. Sci. 89:2484–2490.
Omri, B., R. Chalghoumi, L. Izzo, A. Ritieni, M. Lucarini, A. Durazzo, H. Abdouli, and A. Santini. 2019. Effect of dietary incorporation of linseed alone or together with tomato-red pepper mix on laying hens' egg yolk fatty acids profile and health lipid indexes. Nutrients 11:1–13.
Pérez, J. A., A. Castro, C. Rolo, A. Torres, R. Dorta-Guerra, N. G. Acosta, and C. Rodríguez. 2021. Fatty acid profiles and omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) biosynthesis capacity of three dual purpose chicken breeds. J. Food Compos. Anal. 102:104005.
Polycarpo, G. V., I. Andretta, M. Kipper, V. C. Cruz-Polycarpo, J. C. Dadalt, P. H. M. Rodrigues, and R. Albuquerque. 2017. Meta-analytic study of organic acids as an alternative performance-enhancing feed additive to antibiotics for broiler chickens. Poult. Sci. 96:3645–3653.
Poureslami, R., K. Raes, G. Huyghebaert, A. B. Batal, and S. De Smet. 2012. Egg yolk fatty acid profile in relation to dietary fatty acid concentrations. J. Sci. Food Agric. 92:366–372.
Rizzi, L., D. Bochicchio, A. Bargellini, P. Parazza, and M. Simioli. 2009. Effects of dietary microalgae, other lipid sources, inorganic selenium and iodine on yolk n-3 fatty acid composition, selenium content and quality of eggs in laying hens. J. Sci. Food Agric. 89:1775–1781.
Sauvant, D., P. Schmidely, and J. J. Daudin. 2008. Meta-analyses of experimental data in animal nutrition. Animal 3:1203–1214.
Schreiner, M., H. W. Hulan, E. Razzazi-Fazeli, J. Böhm, and C. Iben. 2004. Feeding laying hens seal blubber oil: effects on egg yolk incorporation, stereospecific distribution of omega-3 fatty acids, and sensory aspects. Poult. Sci. 83:462–473.
Senkoylu, N., and N. Dalet. 1999. Sunflower meal in poultry diets: a review. Worlds. Poult. Sci. J. 55:153–174.
Sepidarkish, M., M. Morvaridzadeh, M. Akbari-Fakhrabadi, A. Almasi-Hashiani, M. Rezaeinejad, and J. Heshmati. 2019. Effect of omega-3 fatty acid plus vitamin E Co-Supplementation on lipid profile: a systematic review and meta-analysis. Diabetes Metab. Syndr. Clin. Res. Rev. 13:1649–1656.
St-Pierre, N. R. 2001. Invited review : integrating quantitative findings from multiple studies using mixed model methodology 1. J. Dairy Sci. 84:741–755.
WANG, L.hua, and G.cheng HUO. 2010. The effects of dietary fatty acid pattern on layer's performance and egg quality. Agric. Sci. China 9:280–285.
Westbrook, L. A., and G. Cherian. 2019. Egg quality, fatty-acid composition and gastrointestinal morphology of layer hens fed whole flaxseed with enzyme supplementation. Br. Poult. Sci. 60:146–153.
Zhang, P., C. Tang, Z. Ding, H. Huang, and Y. Sun. 2017. Effects of simultaneous supplementation of laying hens with α-linolenic acid and eicosapentaenoic acid/docosahexaenoic acid resources on egg quality and n-3 fatty acid profile. Asian-Australas. J. Anim. Sci. 30:973–978.