META-ANALYSIS OF THE POSITIVE EFFECT OF GINGER FEED ADDITIVE ON HEALTH AND PRODUCTION INDICES OF LAYING HENS

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Abstract. Meta-analysis of the effects of ginger (*Zingiber officinale*) supplementation on outcome measures (health and production indices) in laying hens was investigated. A database of eighteen studies on the subject was systematically compiled based on set inclusion criteria and were used for the analysis. Outcome measures were pooled using a random-effects model and quantified using standardised mean difference (SMD) at 95% confidence interval (CI). The pooled results indicate that ginger supplementation improved blood antioxidant markers in laying hens. The results also showed that layers on ginger supplementation had significantly better plasma cholesterol concentrations (SMD = -1.140 mg/dl, 95% CI: -1.622 to -0.658) and hen day egg production (SMD = 0.217%, 95% CI: 0.080 to 0.354) compared to the controls. In addition, layers on ginger supplementation had significantly higher egg mass (SMD = 0.245 g egg/hen/day, 95% CI: 0.017 to 0.473) and egg weight (SMD = 0.096 g, 95% CI: 0.029 to 0.163) when compared to the controls, taking cognizance of heterogeneity. Meta-regression found that studied moderators explained most of the sources of variation. This meta-analysis suggests that ginger could improve health indices, hen day egg production, egg mass and egg weight in laying hens.
Keywords: *layers, blood characteristics, egg production, egg quality, data synthesis, meta-regression*

Introduction

The global demand for animal proteins is on the increase due to the rapid growth in human population. To meet up the increasing demand for high-quality animal products, the modern egg production industry needs to develop strategies to improve laying performance at a reduced feed to egg ratio. Feed to egg ratio, calculated as the quantity of feed consumed per unit of eggs is a vital production trait as feed contributes about 60 – 70% of the overall production costs in the egg production industry. Hence, optimizing feed efficiency is the key to a sustainable and profitable egg production enterprise. One of the methods to achieve this is the use of phytogenics. Phytogenics are products derived from plants and included in animal feed to enhance growth rate and productivity. Ginger (*Zingiber officinale*), is a flowering plant widely cultivated in the tropics and is belong to the family Zingiberaceae. It is used as a spice in human food preparation; and is rich in beneficial bioactive compounds and essential oil (Ahn et al., 2002; Akoachere et al., 2002; Dragland et al., 2003). Studies have shown that ginger has antioxidant activity and increases blood flow and nutrient uptake in chickens (Incharoen and Yamauchi, 2009; Kothari et al., 2019). Though the positive effects of ginger supplementation in laying hens have been reported (Incharoen and Yamauchi, 2009; Akbarian et al., 2011), there is consensus that many of these studies lack consistency, as

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results differ greatly from one research station to another (Windisch et al., 2008; Okoro, 2016; Gurbuz and Salih, 2018; Kumar et al., 2019; Ogbuewu and Mbajiorgu, 2020).

Resolving this conflict requires pooling results of individual studies on the effects of ginger on health and productive indices in laying hens using a meta-analysis approach. Meta-analysis is a statistical means of resolving conflicting research results using a quantitative approach. It aggregates data and determine treatment effects that ordinarily may not be detected by the individual study (Van Houwelingen et al., 2002). However, there is scanty information on the effect of ginger feed additive on health and productivity of laying hens using meta-analysis in the literature, hence this research. This meta-analysis is targeted at furnishing the egg production industry and poultry nutritionists of the needed information on the effects of ginger feed additive and explanatory variables (ginger presentation form, supplementation level and duration, chicken age, and breed/strain/lines) on health and productive indices of laying hens.

Materials and methods

Search strategy and inclusion criteria

A literature search of peer-reviewed original articles on the effect of ginger intervention on health and productive markers of laying hens was performed in Scopus, Google Scholar and AGORA using different search queries (OR, AND, $, and *) and keywords (ginger, "laying hens, egg production, antioxidant and blood markers). The reference lists of relevant papers identified during the search were manually screened for eligible studies. We conducted a manual search of papers published in offline journals. The included papers met the following criteria: (i) the title was on the effect of ginger on all, or any of the outcome measures: feed intake (FI), feed to egg ratio (F:E), blood makers (glutathione peroxidase, total antioxidant capacity, malondialdehyde or superoxide dismutase, packed cell volume, red blood cell, cholesterol, total protein, calcium or phosphorus), egg production and egg quality in laying hens. (ii) studies were peer-reviewed and published in English, (iii) included studies that have both control and experimental treatments, and administered ginger as extract, oil, or powder, (iv) diet is free of growth promoters and (v) trial reported the mean, number of animals used, and a measure of dispersion. Identified studies were independently assessed for eligibility and disparity was resolved by consensus. The Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) flow chart as presented in *Fig. 1* summarizes the search details.

Exclusion criteria

100 papers identified were excluded and five were excluded for being duplicates (*Fig. 1*). Fifty-five studies were also removed from the study for the title not being on any of the measured outcomes and study not published in English, mixed ginger with other plant materials and reported in animal other laying hens. In addition, 22 papers were removed from the remaining 40 studies for lack of randomization and being a review articles.

Data extraction and synthesis

Data presented in graphs were extracted using a ruler while studies that provided standard error (SE) instead of the standard deviation (SD), the SD values were computed

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from the SE using a standard formula (Koricheva et al., 2013). Data on the number of hens included in the control and treatment group, first author's surname, publication year and study location were extracted from each study that met the set inclusion criteria. Information on blood and antioxidant markers such as glutathione peroxidase (GSH-Px), total antioxidant capacity (TAOC), malondialdehyde (MDA) and superoxide dismutase (SOD), performance [feed intake (FI), feed to egg ration (F:E), hen day egg production (HDEP) and egg quality indices] and blood parameters [packed cell volume (PCV), red blood cell (RBC), cholesterol, total protein (TP), calcium (Ca) and phosphorus (P)] of laying hens in control and treatment group were also extracted. In addition, data on covariates (breeds, age of the birds at the beginning of the experiment, presentation form of ginger, duration of ginger supplementation and ginger supplementation levels) of hens in the control and treatment group were also extracted. We contacted 14 authors to supply missing information on housing conditions (natural or controlled environment), essential amino acids (lysine and methionine), cage dimensions, number of hens per cage, ambient temperature and rearing type (deep litter or battery cage), building type (closed or open-sided) however, no response was received from any of the authors and therefore, the influence of these predictor variables on performance of laying hens on ginger intervention was not analysis.

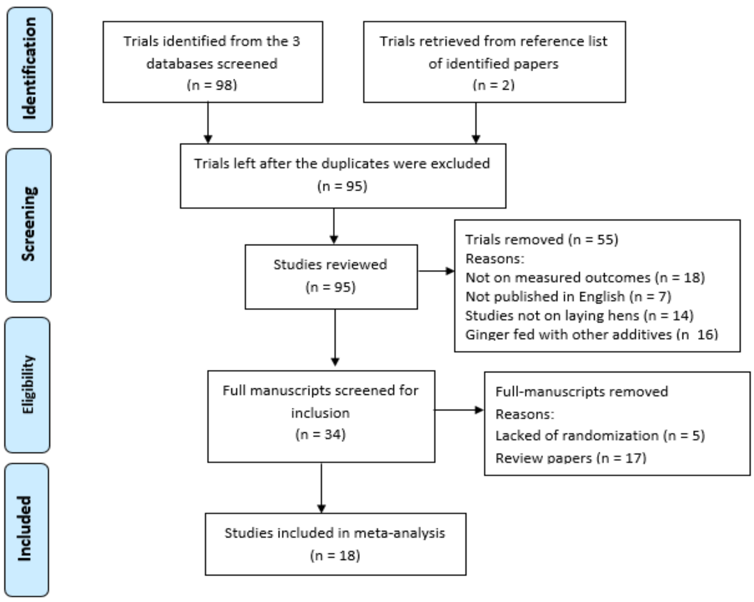

Figure 1. *Flow diagram of the article selection steps*

Statistical analysis

Forest plots and funnel graphs were built from dataset extracted from the 18 studies that met the set inclusion criteria using OpenMEE software (Wallace et al., 2016). Map was generated in Tableau software (version 2020.2). Bar graphs and pie charts were generated in SPSS 20.0 (SPSS, 2019). Publication bas as determined using funnel graphs and Rosenberg's fail-safe number (Nfs). Meta-analysis results were considered robust in the presence of publication bias when Nfs > 5 (n = number of layers in each treatment group) + 10 (Jennions et al., 2013; Ogbuewu et al., 2020). Publication bias was not performed on outcomes with less than 10 studies (Egger et al., 1997). Heterogeneity

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across trials was computed using the Cochran Q test and I^2 – index (Higgins et al., 2003; Higgins and Deeks, 2011). Subgroup analysis, meta-regression and funnel graph were not conducted in measured outcomes with less than 10 studies because of the report that test power from such analysis is usually low (Borenstein et al., 2009; Higgins and Green, 2009). We performed meta-regression in studies with non-significant heterogeneity test because non-significant test for heterogeneity does not guarantee homogeneity across studies used in meta-analysis (Thompson and Higgins, 2002). The diamond at the bottom of the forest plot represents the overall mean estimation (SMD = 0) and is said to be significant when the CI did not include zero (Koricheva et al., 2013). The points to the left of the line of no effect (i.e. where SMD = 0) denote a reduction in the measured outcome and the opposite is the case when the points are to the right of the line of no effect.

Results

Study characteristics

The summary of the 18 studies with 3600 hens that met the set inclusion criteria is presented in *Tables 1-5*. The studies included in this meta-analysis were conducted in 10 locations drawn from four continents: North America (n = 1), Europe (n = 2), Asia (n = 11) and Africa (n = 4) as shown in *Fig. 2* and *Fig. 3*. Out of the 18 articles used for the meta-analysis, 3 studies (16.67%) each were conducted in Nigeria, Iran and China, whereas 2 studies (11.11%) each were performed in Sudan and Japan as described in *Fig. 2*. The articles used for the analysis were published between 2009 and 2020, and span 12 years (*Fig. 4*).

Table 1. *Number of studies and hens included in the analysis*

Outcomes	T^n	Datasets	T^a	T^b	T^c
Antioxidant status					
Glutathione peroxidase (× 10^2 U/mL)	2	9	435	840	1275
Malondialdehyde (nmol/mL)	3	11	570	1110	1680
Superoxide dismutase (U/mL)	3	10	570	975	1545
Total antioxidant capacity (U/mL)	3	11	570	1110	1680
Blood indices					
Packed cell volume (%)	2	4	32	72	104
RBC count	2	4	32	72	104
Cholesterol (mg/dl)	7	14	271	537	808
Phosphorus (mg/dl)	2	5	38	96	134
Calcium (mg/dl)	2	5	38	96	134
Total protein (g/dl)	3	5	350	390	740
Performance					
Feed intake (g/hen/day)	14	29	663	1444	2107
Feed to egg ratios (g/g)	12	26	589	1308	1897
Egg Traits					
Hen day egg production (%)	15	30	921	1660	2581
Egg mass (g egg/hen/day)	10	20	527	1144	1671
Egg weight (g)	13	28	949	1713	2662
Egg quality traits					
Haugh unit	10	21	490	934	1424
Egg yolk weight (g)	8	19	327	628	955
Egg yolk cholesterol (mg/g yolk)	3	8	64	168	232
Egg shell thickness (mm)	10	21	490	934	1424
Egg shell weight (g)	5	12	106	238	344

T^n – number of studies included in the meta-analysis; T^a – Number of hens used in the control groups; T^b – Number of hens used in the treatment groups; T^c – Total of number of hens used for the analysis

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Table 2. *Overview of articles used to assess antioxidant ability of ginger in laying hens*

References	Location	Covariates					Outcome
		Breed	Hen's age (week)	PF	DS (day)	SL (%)	
An et al. (2019)	China	Hyline	25	Extract	49	0, 0.1	1, 2, 3, 4
Zhao et al. (2011)	Canada	Hyline	27	Powder	35	0, 0.5	1, 2, 3, 4
Zhao et al. (2011)	Canada	Hyline	27	Powder	35	0, 1	1, 2, 3, 4
Zhao et al. (2011)	Canada	Hyline	27	Powder	35	0, 1.5	1, 2, 3, 4
Zhao et al. (2011)	Canada	Hyline	27	Powder	35	0, 2	1, 2, 3, 4
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 0.5	1, 2, 3, 4
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 1	1, 2, 3, 4
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 1.5	1, 2, 3, 4
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 2	1, 2, 3, 4
Yang et al. (2017)	China	Hyline	40	Powder	28	0, 1	2, 4
Yang et al. (2017)	China	Hyline	40	Powder	56	0, 1	2, 3, 4

1- GSH-Px; 2 - MDA; 3 - SOD; 4 – TAC; PF – presentation form; DS – duration of supplementation; SL – supplementation level

Table 3. *Summary of studies used to assess the effect of ginger on feed intake and FE ratios in laying hens*

References	Location	Covariates					Outcome
		Breed	Hen's age (week)	PF	DS (day)	SL (%)	
Akbarian et al. (2011)	Iran	Hyline	30	Powder	56	0, 0.25	1, 2
Akbarian et al. (2011)	Iran	Hyline	30	Powder	56	0, 5	1, 2
Akbarian et al. (2011)	Iran	Hyline	30	Powder	56	0, 0.75	1, 2
Akanbi et al. (2020)	Nigeria	Isa Brown	24	Powder	56	0, 3	1
Gurbuz and Salih (2018)	Turkey	Atak-S	25	Powder	56	0, 1	1, 2
Gurbuz and Salih (2018)	Turkey	Atak-S	25	Powder	56	0, 2	1, 2
Gurbuz and Salih (2018)	Turkey	Atak-S	25	Powder	56	0, 3	1, 2
Incharoen and Yamauchi (2009)	Japan	Leghorn	24	Powder	140	0, 1	1, 2
Incharoen and Yamauchi (2009)	Japan	Leghorn	24	Powder	140	0, 5	1, 2
Kumar et al. (2019)	India	Leghorn	28	Powder	84	0., 0.5	1, 2
Kumar et al. (2019)	India	Leghorn	28	Powder	84	0, 1	1, 2
Malekizadeh et al. (2012)	Iran	Leghorn	103	Powder	63	0, 1	1, 2
Malekizadeh et al. (2012)	Iran	Leghorn	103	Powder	63	0, 3	1, 2
Nasiroleslami and Torki (2010)	Iran	Lohmann		Oil	42	0, 0.03	1, 2
Kumar et al. (2019)	India	Leghorn	28	Powder	84	0., 0.5	1, 2
Okoro (2016)	Nigeria	nr	73	Powder	49	0, 0.5	1, 2
Okoro (2016)	Nigeria	nr	73	Powder	49	0, 1	1, 2
Wen et al. (2019)	China	Hyline	40	Extract	56	0, 0.01	1, 2
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 0.5	1, 2
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 1	1, 2
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 1.5	1, 2
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 2	1, 2
Zomrawi et al. (2014b)	Sudan	Hisex	27	Powder	nr	0, 0.5	1, 2
Zomrawi et al. (2014b)	Sudan	Hisex	27	Powder	nr	0, 1	1, 2
Zomrawi et al. (2014b)	Sudan	Hisex	27	Powder	nr	0, 1.5	1, 2
Yang et al. (2017)	China	Hyline	40	Powder	28	0, 1	1, 2
Yang et al. (2017)	China	Hyline	40	Powder	56	0, 1	1, 2
Sittiya et al. (2017)	Japan	Sonia	53	Powder	56	0, 0.005	1
Sittiya et al. (2017)	Japan	Sonia	53	Powder	63	0, 0.005	1
Soliman and Kamel (2020)	SA	Hisex	35	Powder	70	0, 1	1, 2

SA-Saudi Arabia; nr - not reported; 1 – Feed Intake; 2 - Feed to egg ratio

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Table 4. *Summary of studies included to assess the impact of ginger on blood markers in laying hens*

References	Location	Covariates					Outcome
		Breed	Hen's age (week)	PF	DS (day)	SL (%)	
Akanbi et al. (2020)	Nigeria	Isa Brown	24	Powder	56	0, 3	1, 2, 3, 7
Zomrawi et al. (2014b)	Sudan	Hisex	27	Powder	nr	0, 0.5	1, 2, 3, 4, 5, 6, 7
Zomrawi et al. (2014b)	Sudan	Hisex	27	Powder	nr	0, 1	1, 2, 3, 4, 5, 6, 7
Zomrawi et al. (2014b)	Sudan	Hisex	27	Powder	nr	0, 1.5	1, 2, 3, 4, 5, 6, 7
Malekizadeh et al. (2012)	Iran	Leghorn	103	Powder	63	0, 1	4, 5, 6
Malekizadeh et al. (2012)	Iran	Leghorn	103	Powder	63	0, 3	4, 5, 6
Akbarian et al. (2011)	Iran	Hyline	30	Powder	56	0, 0.25	6
Akbarian et al. (2011)	Iran	Hyline	30	Powder	56	0, 5	6
Akbarian et al. (2011)	Iran	Hyline	30	Powder	56	0, 0.75	6
Nasiroleslami and Torki (2010)	Iran	Lohmann		Oil	42	0, 300	6, 7
Okoro (2016)	Nigeria	nr	73	Powder	49	0, 0.5	6
Okoro (2016)	Nigeria	nr	73	Powder	49	0, 1	6
Wen et al. (2019)	China	Hyline	40	Extract	28	0, 0.01	6
Wen et al. (2019)	China	Hyline	40	Extract	56	0, 0.01	6
Soliman and Kamel (2020)	SA	Hisex	35	Powder	70	0, 1	6

1- Hb, 2- PCV; 3- RBC; 4- Calcium; 5- Phosphorus; 6- Cholesterol; 7- Total protein

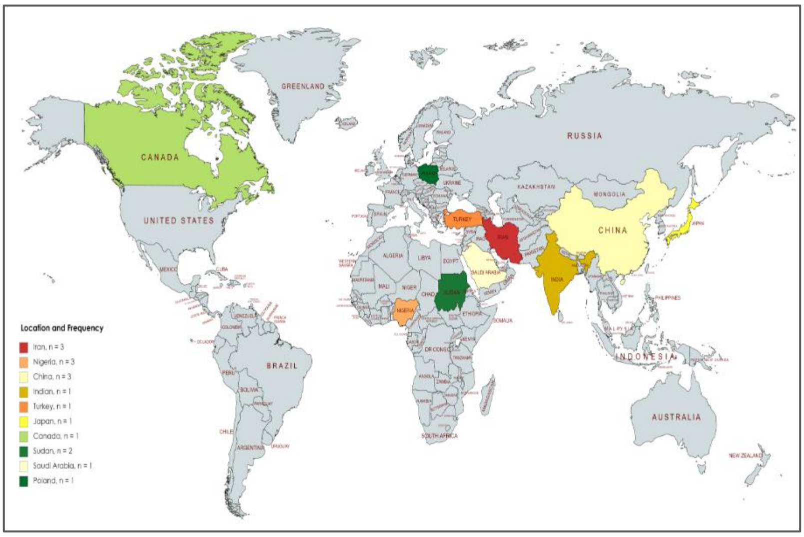

Figure 2. *Study location of papers used for the analysis*

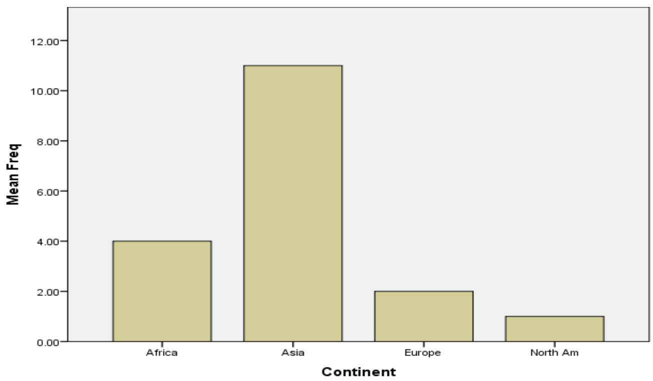

Figure 3. *Bar graph for study continent used for analysis*

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Table 5. *Overview of studies used to explore the impact of ginger on laying performance in hens*

References	Location	Covariates					Outcome
		Breed	Hen's age (week)	PF	DS (d)	SL (%)	
Akbarian et al. (2011)	Iran	Hyline	30	Powder	56	0, 0.25	1, 2, 7, 8
Akbarian et al. (2011)	Iran	Hyline	30	Powder	56	0, 0..5	1, 2, 7, 8
Akbarian et al. (2011)	Iran	Hyline	30	Powder	56	0, 0.75	1, 2, 7, 8
Akanbi et al. (2020)	Nigeria	Isa-brown	24	Powder	56	0, 3	1
An et al. (2019)	China	Hyline	25	Extract	49	0, 0.1	1
Gurbuz and Salih (2018)	Turkey	Atak-S	25	Powder	56	0, 1	1, 2, 3, 4, 5, 6, 8
Gurbuz and Salih (2018)	Turkey	Atak-S	25	Powder	56	0, 2	1, 2, 3, 4, 5, 6, 8
Gurbuz and Salih (2018)	Turkey	Atak-S	25	Powder	56	0, 3	1, 2, 3, 4, 5, 6, 8
Incharoen and Yamauchi (2009)	Japan	Leghorn	24	Powder	84	0, 1	1, 3, 4, 5
Incharoen and Yamauchi (2009)	Japan	Leghorn	24	Powder	84	0, 5	1, 3, 4, 5
Kumar et al. (2019)	India	Leghorn	28	Powder	49	0, 0.5	1, 2, 3
Kumar et al. (2019)	India	Leghorn	28	Powder	49	0, 1	1, 2, 3
Malekizadeh et al. (2012)	Iran	Leghorn	103	Powder	63	0, 1	1, 2, 3
Malekizadeh et al. (2012)	Iran	Leghorn	103	Powder	63	0, 3	1,2, 3
Nasiroleslami and Torki (2010)	Iran	Lohmann	nr	Oil	42	0, 0.03	1, 3, 4, 5, 6
Okoro (2016)	Nigeria	nr	73	Powder	49	0, 0.5	1, 2, 4, 5, 7, 8
Okoro (2016)	Nigeria	nr	73	Powder	49	0, 1	1, 2, 4, 5, 7, 8
Wen et al. (2019)	China	Hyline	40	Extract	28	0, 0.01	1, 3, 4, 5
Wen et al. (2019)	China	Hyline	40	Extract	28	0, 0.01	4, 5
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 0.5	1, 2, 3
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 1	1, 2, 3
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 1.5	1, 2, 3
Zhao et al. (2011)	Canada	Hyline	27	Powder	70	0, 2	1, 2, 3
Zomrawi et al. (2014b)	Sudan	Hisex	27	Powder	nr	0, 0.5	1, 2, 4, 5, 6, 7, 8
Zomrawi et al. (2014b)	Sudan	Hisex	27	Powder	nr	0, 1	1, 2, 4, 5, 6, 7, 8
Zomrawi et al. (2014b)	Sudan	Hisex	27	Powder	nr	0, 1.5	1, 2, 4, 5, 6, 7, 8
Yang et al. (2017)	China	Hyline	40	Powder	28	0, 1	1, 2, 3, 4, 5, 8
Yang et al. (2017)	China	Hyline	40	Powder	56	0, 1	1, 2, 3, 4, 5, 8
Sittiya et al. (2017)	Japan	Sonia	53	Powder	56	0, 0.005	1, 3, 4, 5, 6, 8
Sittiya et al. (2017)	Japan	Sonia	53	Powder	63	0, 0.005	1, 3, 4, 5, 6, 8
Soliman and Kamel (2020)	SA	Hisex	35	Powder	70	0, 1	1, 2, 3
Damaziak et al. (2018)	Poland	Isa brown	16	Extract	112	0, 0.04	2, 4, 5, 8
Udeh et al. (2018)	Nigeria	Isa brown	35	Powder	30	0, 2.5	2, 4, 6, 8
Udeh et al. (2018)	Nigeria	Isa brown	35	Powder	30	0, 2.5	2, 4, 6, 8
Udeh et al. (2018)	Nigeria	Isa brown	35	Powder	30	0, 2.5	2, 4, 6, 8

1- HDEP; 2- EW; 3- EM; 4- HU; 5- EST; 6- ESW; 7- EYC; 8- EYW

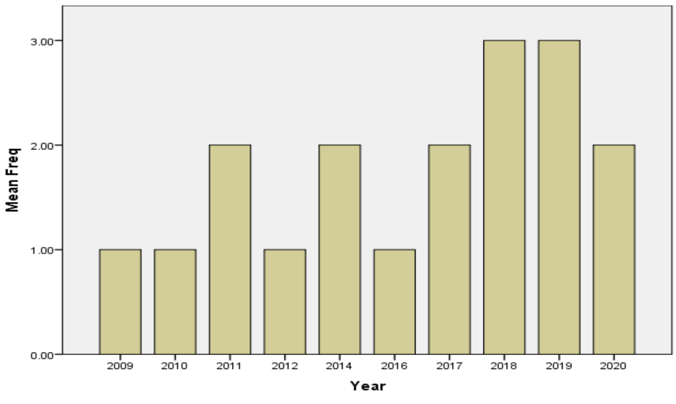

Figure 4. *Bar graph for the years the studies included in the analysis were published*

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Antioxidant capacity

Pooled estimate of the effect of ginger on blood antioxidant markers as presented in *Table 6* suggests that ginger intervention significantly increased GSH-Px (SMD = 9.485 U/mL; 95% CI: 9.148 to 9.822), TAOC (SMD = 10.03 U/mL; 95% CI: 9.466 to 10.593), SOD (SMD = 9.642 U/mL; 95% CI: 9.388 to 9.895) and reduced MDA (SMD = -2.092 nmol/mL; 95% CI: -2.863 to -1.320) in laying hens compared to controls. There was significant heterogeneity amongst the articles used to determine the impact of ginger intervention on the concentrations of MDA (I^2 = 98.62; p < 0.001) and TAOC (I^2 = 79.99; p < 0.001) in the plasma of laying hens. Studies used to evaluate the impact of ginger intervention on blood antioxidant markers were less than 10 studies (*Table 1*), as a result, we could not proceed to determine the relationships between blood antioxidant outcomes and our chosen covariates using stratified subgroup and meta-regression analysis.

Table 6. *Blood GSH-Px, TAOC, MDA and SOD in laying birds administered ginger*

Outcome	SMD	95% CI		SE	P-value	Q	df	I^2 (%)	*P-value
		Lower	Upper						
GSH-Px, U/mL	9.485	9.148	9.822	0.172	0.001	12.96	8	38.28	0.113
MDA, nmol/mL	-2.092	-2.863	-1.320	0.394	<0 .001	276.34	10	98.62	< 0.001
SOD, U/mL	9.642	9.388	9.895	0.129	<0 .001	4.475	9	0.00	0.877
TAOC, U/mL	10.03	9.466	10.593	0.288	< 0.001	49.98	10	79.99	< 0.001

*P-val – Probability value for heterogeneity; SE – Standard error; I^2 – Heterogeneity; GSH-Px - Glutathione Peroxidase; TAOC - Total Antioxidant Capacity; MDA – Malondialdehyde; SOD - Superoxide Dismutase

Blood markers

Table 7 indicated significant effect of ginger intervention on RBC (SMD = 1.573 × 10^{12}/L; 95% CI: 0.078 to 3.067) and plasma cholesterol level (SMD = -1.140 mg/dl; 95% CI: -1.622 to -0.658) of laying hens in treatment and control groups. Results indicate that ginger supplementation had similar PCV (SMD = 0.130%; 95% CI: -0.426 to 0.691), calcium (SMD = 0.690 mg/dl; 95% CI -0.051 to 1.143), phosphorus (SMD = 0.480 mg/dl; 95% CI: -0.460 to 1.420) and total protein (SMD = 0.498 mg/dl; 95% CI: -0.151 to 1.147) with the controls.

Table 7. *Aspects of blood markers in laying birds administered ginger*

Outcome	SMD	95% CI		SE	P - val	Q	df	I^2 (%)	*P-val
		Lower	Upper						
Packed cell volume (%)	0.13	-0.426	0.691	0.285	0.642	8.27	3	63.74	0.041
RBC (× 10^{12}/L)	1.573	0.078	3.067	0.763	0.039	43.80	3	93.15	< .001
Calcium (mg/dl)	0.690	-0.051	1.431	0.378	0.068	24.37	4	83.59	< .001
Phosphorus (mg/dl)	0.480	-0.460	1.420	0.480	0.317	38.75	4	89.68	< .001
Cholesterol (mg/dl)	-1.140	-1.622	-0.658	0.246	< .001	153.25	13	91.52	< .001
Total protein (g/dl)	0.498	-0.151	1.147	0.331	0.133	37.23	4	89.26	< .001

P-val – Probability value for heterogeneity; SE – Standard error; SMD – Standardised Mean Difference; I^2 – Heterogeneity; RBC- Red blood cell

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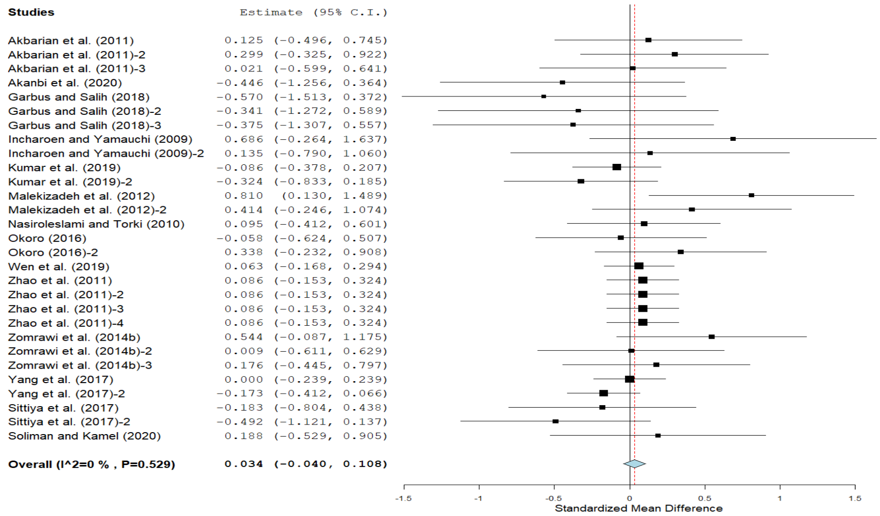
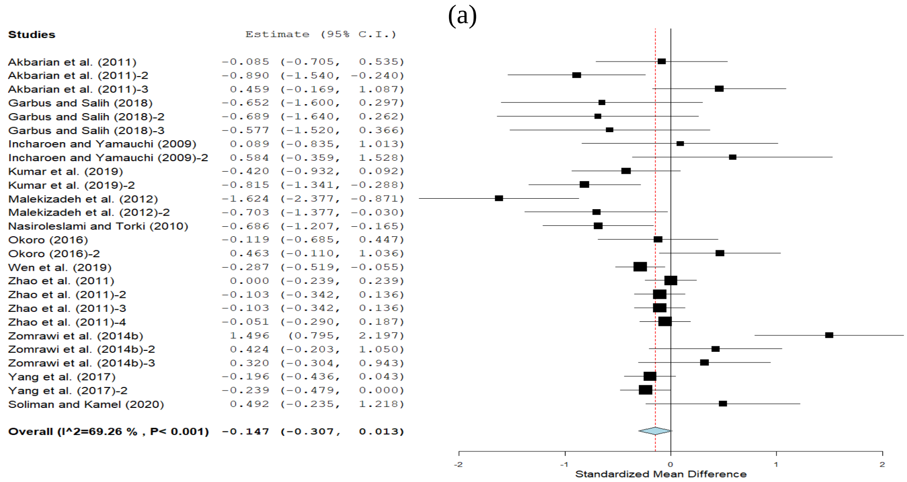
Feed intake and the ratio of feed to egg

The meta-analysis of the influence of ginger intervention on feed intake as presented in *Table 1* was computed with 14 publications that comprised 29 datasets and 2107 layers (663 hens for the control and 1444 hens for the treatment group). Twelve (12) publications with 26 datasets having 1897 layers that comprised 589 hens for the control group and 1308 hens for the treatment groups were used to assess the impact of ginger intervention on feed to egg ratio. The overall pooled result indicated that ginger intervention had no significant influence on feed intake (SMD = 0.034 g/hen/day; 95% CI: -0.040 to 0.108; *Fig. 5a*) and feed to egg ratio (SMD = -0.147; 95% CI: -0.307 to 0.013 *Fig. 5b*). There is evidence of significant heterogeneity ($I^2 = 69.26\%$, $p < 0.001$; *Fig. 5b*) among the articles used to examine the influence of ginger on feed to egg ratios in laying hens. Also, meta-regression analysis results (*Table 8*) indicated that hen's age, duration of supplementation and breed were significant predictors of treatment effect and accounted for the majority of the sources of variations.



(a)



(b)

***Figure 5.** Forest plot of the effect of ginger on: (a) feed intake and (b) feed to egg ratio. The thick vertical line is the line of no effect where the mean effect size is equal to zero. The dotted vertical line depicts the point estimate with the diamond at the bottom representing the 95% CI for the overall estimate. The point estimate is considered significant when the CI did not include zero*

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Table 8. *The relationships between the study moderators and ginger treatment*

Parameters	Moderators	Q_B	df	P-val	R^2 (%)
F:E ratio	Supplementation duration (day)	53.80	8	< .001	100.00
	Hen's age (week)	22.90	8	0 .004	55.44
	Supplementation level (%)	5.78	10	0.833	0.00
	Breed	49.00	5	< .001	99.96
HDEP (%)	Supplementation duration (day)	40.00	7	< .001	100.00
	Hen's age (week)	46.69	9	< .001	100.00
	Supplementation level (%)	6.52	11	0.836	0.00
	Breed	56.19	7	< .001	100.00
	Presentation form	0.001	1	0.981	0.00
Egg mass (g egg/hen/day)	Supplementation duration (day)	19.66	6	0.003	55.55
	Hen's age (week)	56.55	7	< .001	91.72
	Supplementation level (%)	3.50	8	0.900	0.00
	Breed	19.46	6	0.003	52.93
	Presentation form	0.046	2	0.977	0.00
Egg weight (g)	Supplementation duration (day)	9.79	8	0.280	99.99
	Hen's age (week)	11.74	8	0.163	100.00
	Supplementation level (%)	19.62	13	0.105	100.00
	Breed	5.02	4	0.285	0.00
	Presentation form	8.11	1	0.004	100.00
Haugh unit	Supplementation duration (day)	8.09	8	0.425	5.29
	Hen's age (week)	13.24	7	0.066	33.46
	Supplementation level (%)	22.10	11	0.024	57.74
	Breed	10.79	6	0.095	28.70
	Presentation form	15.47	2	< .001	62.33
Shell thickness (mm)	Supplementation duration (day)	36.50	8	< .001	74.66
	Hen's age (week)	57.79	7	< .001	91.08
	Supplementation level (%)	13.68	11	0.251	5.99
	Breed	56.18	6	< .001	90.94
	Presentation form	1.18	2	0.556	0.00

Q_B - Coefficient of covariates; df - Degree of freedom; p-val - Probability value; R^2 - Percentage of heterogeneity explained by the studied covariates

Egg production outcomes

A meta-analysis of 15 studies (*Table 1*) with 30 datasets having 2581 layers (921 and 1660 for control and ginger treatment, respectively) indicate that ginger significantly increased HDEP when compared with the controls (SMD = 0.217%; 95% CI: 0.080 to 0.354; *Fig. 6a*) with evidence of significant heterogeneity across studies included in the analysis (I^2 = 65.47%, p < 0.001; *Fig. 6a*). Meta-regression analysis (*Table 8*) results showed that hen's age, breed and duration of ginger supplementation had significant predictors of the treatment effect. The R^2 index which is used to quantify the proportion of variance explained by covariates explained majority of the heterogeneity. Furthermore, the point estimate of 10 studies, 20 datasets (*Table 1*), with 1671 layers having 527 hens for control and 1144 hens for the treatment group indicate that ginger significantly increased EM in comparison with controls (SMD = 0.245 g egg/hen/day, 95% CI: 0.017 to 0.473; *Fig. 6b*) with evidence of significant heterogeneity (I^2 = 83.64%, p < 0.001; *Fig. 6b*). *Table 8* indicated that chicken breed, hen's age and duration of ginger

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DOI: http://dx.doi.org/10.15666/aeer/2002_17411761

supplementation were predictors of the treatment effect and accounted for most of the heterogeneity. On the other hand, meta-analysis of 13 publications with 28 datasets (*Table 1*) indicate that ginger intervention increased EW (SMD = 0.096 g, 95% CI: 0.029 to 0.163; *Fig. 6c*) compared to the control, with proof of substantial heterogeneity (I^2 = 0%, p = 0.499; *Fig. 6c*). We also found significant relationships between ginger treatment and ginger presentation form for EW as presented in *Table 8*.

Egg quality characteristics

Overall pooled estimate of 10 articles having 21 datasets with 1424 laying chickens (i.e. 490 and 934 hens for control and treatment group, respectively) as described in *Table 1* showed that birds ginger supplementation had comparable HU (SMD = 0.123; 95% CI: -0.079 to 0.326; *Fig. 7a*) with the controls with evidence of significant heterogeneity (I^2 = 74.08%, p < 0.001; *Fig. 7a*). Meta-regression analysis of the explanatory moderator variable found that dosage (supplementation level) and ginger presentation form were predictors of the impact of ginger on HU (*Table 8*). Similarly, eight articles with 19 datasets comprising 955 layers (i.e. 327 hens for the control and 628 hens for treatment group) revealed that ginger intervention had no effect on egg yolk weight compared with the control, SMD = 0.063 g; 95% CI: -0.104 to 0.229; *Fig. 7b*) without evidence of significant heterogeneity across studies (I^2 = 47.13%, p = 0.012; *Fig. 7b*). Pooled effect estimate of 3 studies having 8 datasets, with 232 laying hens having 64 and 168 birds for control and ginger group, respectively (*Fig. 7c*) showed that ginger supplementation did not affect egg yolk cholesterol level when compared with the control (SMD = -0.049 mg/g yolk; 95% CI: -0.610 to 0.512) with evidence of significant heterogeneity (I^2 = 84.37%, p < 0.001; *Fig. 7c*). Ten publications having 21 datasets with 1424 layers (i.e. 490 for the control group and 934 for the treatment group) were used analyze the impact of ginger treatment on eggshell thickness (*Table 1*). Results revealed that birds on ginger supplement had similar eggshell thickness with control group (SMD = -0.029 mm; 95% CI: - 0.266 to 0.209; *Fig. 8a*) with evidence of substantial heterogeneity (I^2 = 81.28%; p < 0.001; *Fig. 8a*). *Table 8* found that breed, duration of ginger supplementation and hen's age were significant predictors of the treatment effect and explained the majority of the between-study variance. Also, the mean effect estimate of 5 trials comprising 12 datasets with 344 layers (106 for the control group and 238 for the treatment group) showed that layers on ginger had comparable ESW with the controls (SMD = 0.213 g; 95% CI: -0.051 to 0.477; *Fig. 8b*) without evidence of significant heterogeneity (I^2 = 50.14%, p = 0.024; *Fig. 8b*) across the 5 studies used for the meta-analysis.

Analysis of publication bias

Publication bias is a common issue in a meta-analysis, as it may alter the summary effect estimate of an intervention. Funnel plots asymmetry appeared to be higher in meta-analyses that used few publications. In the present study, analysis of bias was not conducted in studies that assessed the impact of ginger intervention on antioxidant and blood outcomes in laying hens as the number of studies included for their computation was less than ten. Publication bias was assessed for FI. F:E, HDEP, EM, EW, HU and EST using funnel graphs (*Figs. 9a,b and 10a-e*). The funnel graphs obtained in the current study were near asymmetry suggesting the possibility of minimal publication biases, and this could be attributed to a small-study effect, variations in test procedures, study design,

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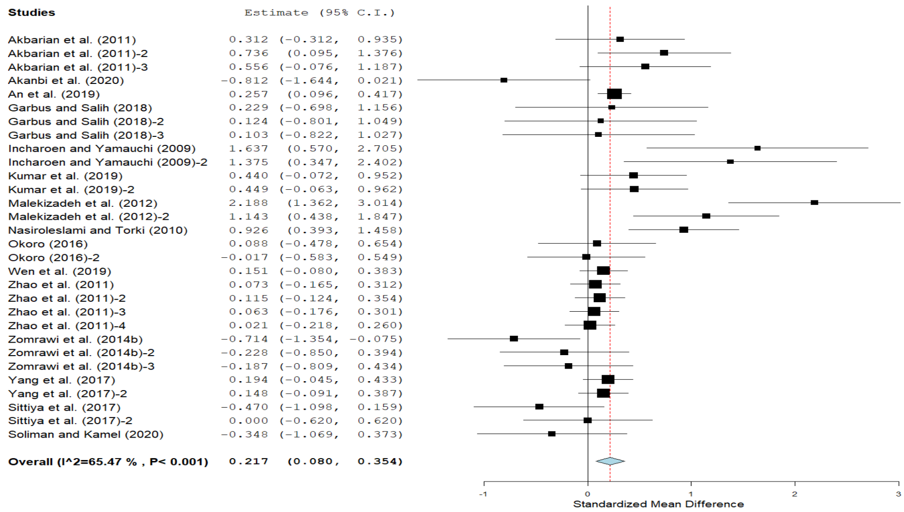
environmental factors and chance. This may also be due to language bias, as the current meta-analysis employed only studies published in English.



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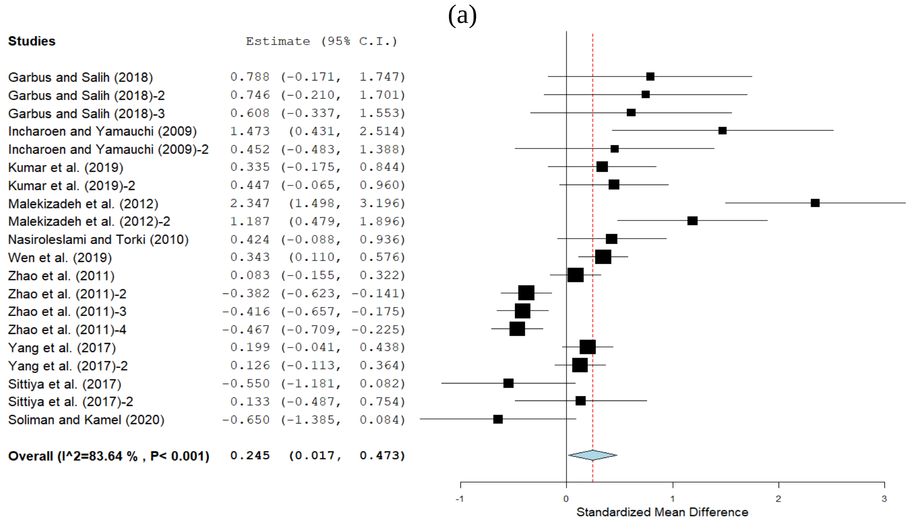

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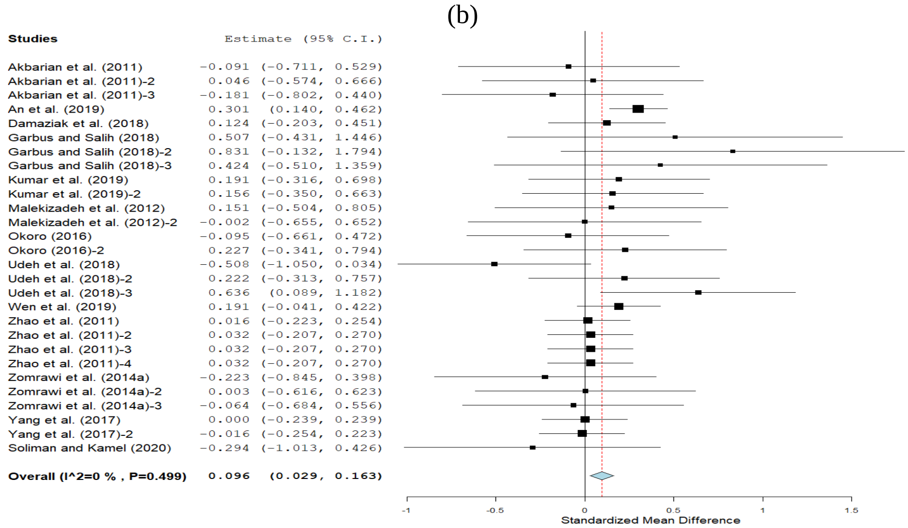

(c)

Figure 6. *Forest plot of the effect of ginger on: (a) HDEP (b) egg mass and (c) egg weight*

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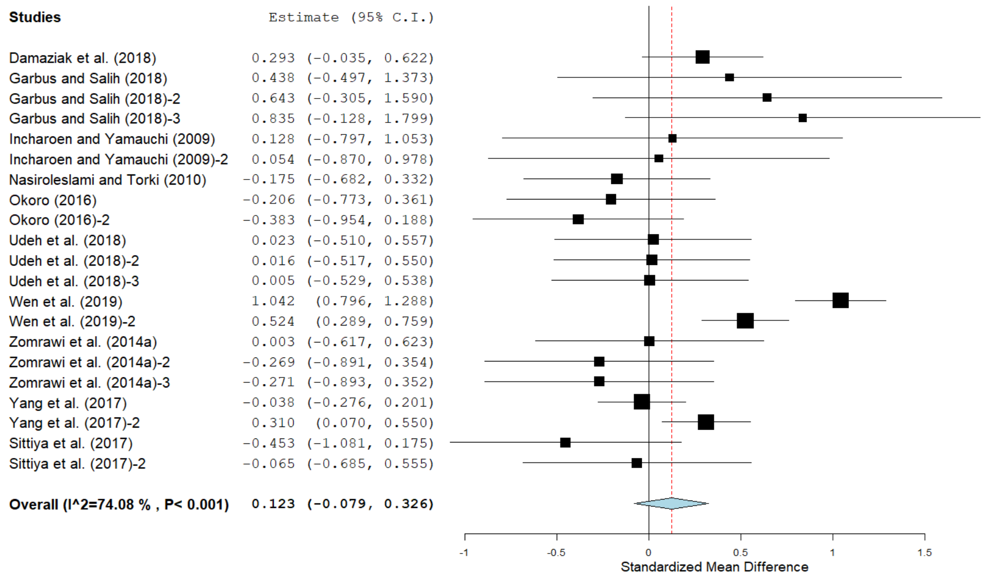

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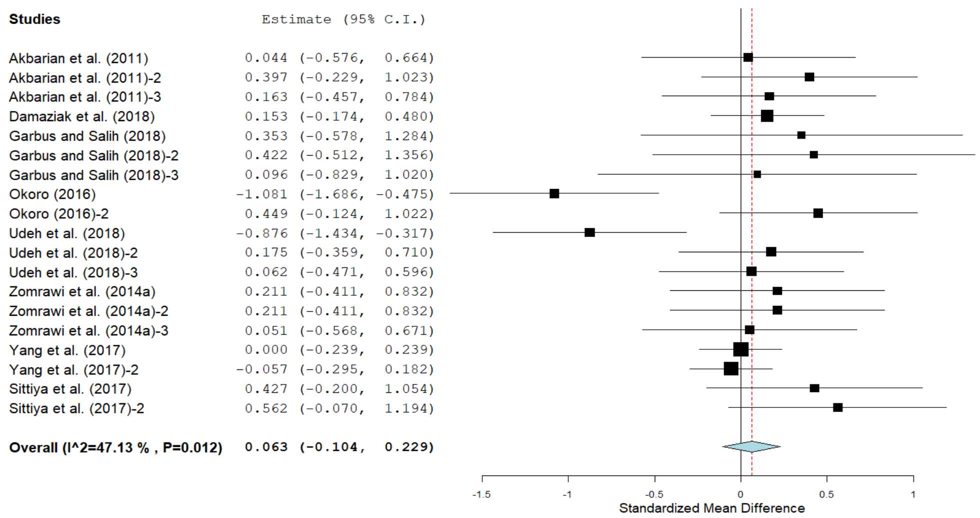

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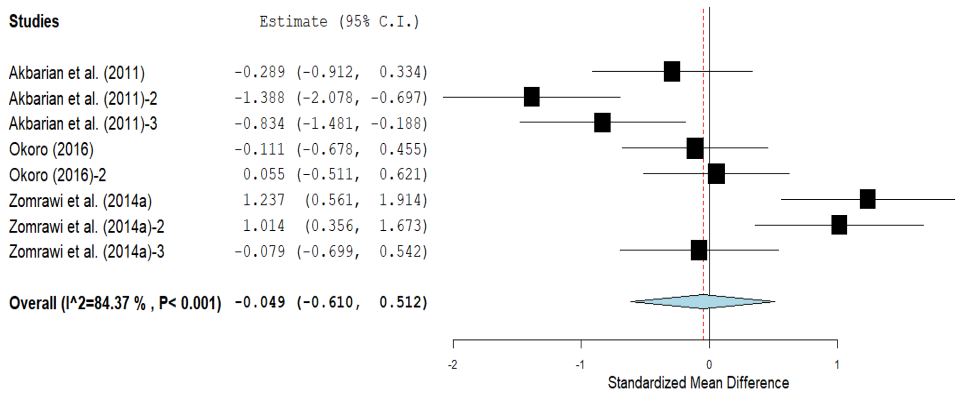

(c)

***Figure 7.** Forest plot of the effect of ginger on: (a) Haugh unit; (b) egg yolk weight and (c) egg yolk cholesterol*

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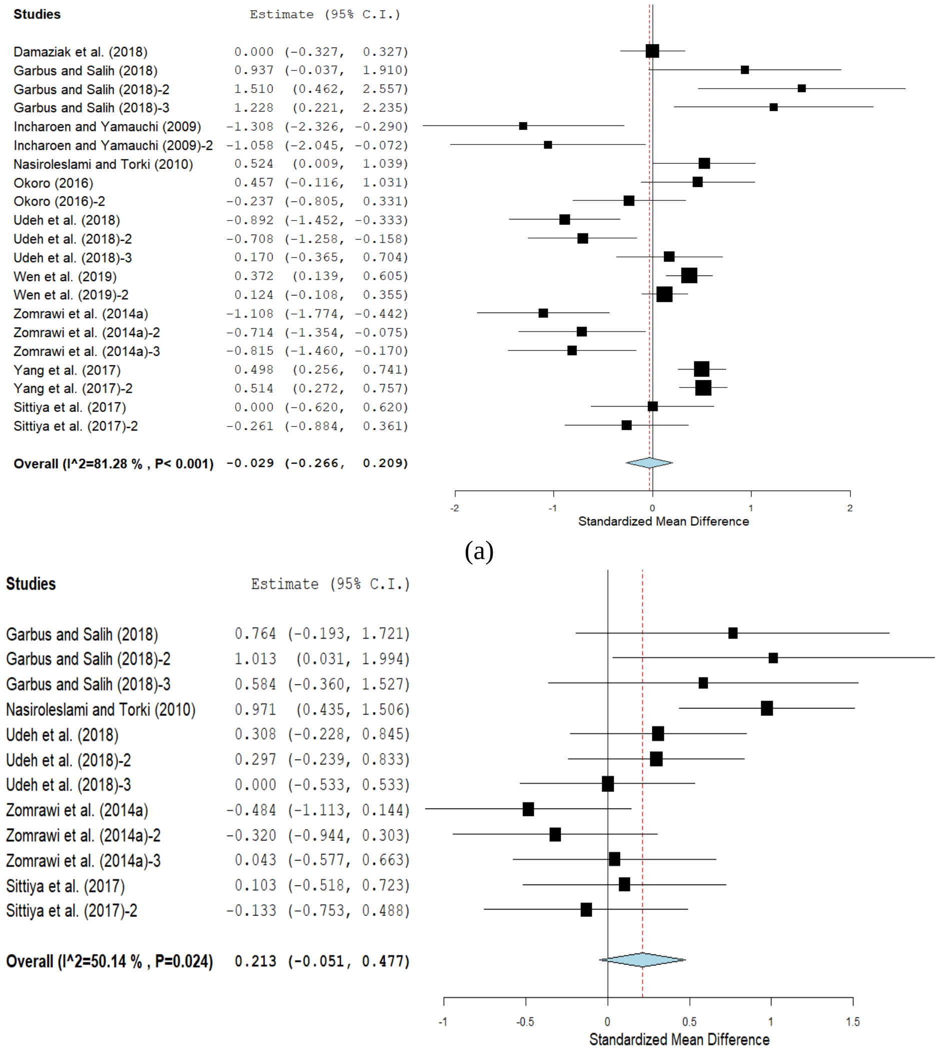
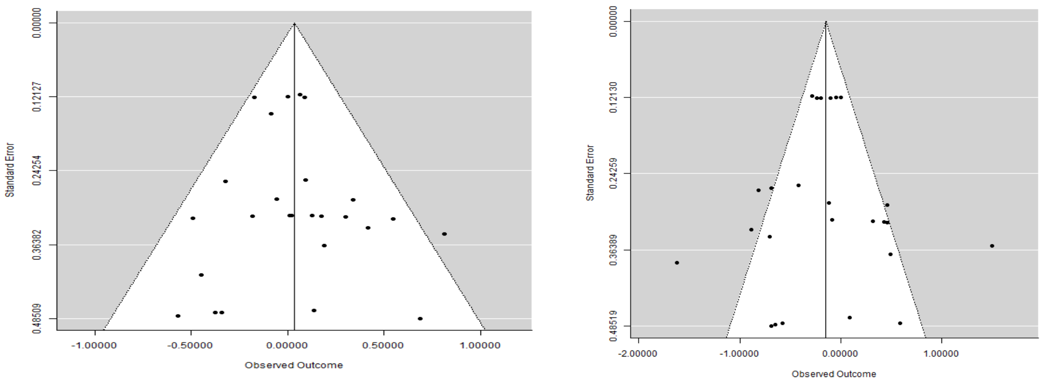


(a)

(b)

Figure 8. *Forest plot of the effect of ginger on: (a) egg shell thickness and (b) egg shell weight*



(A) (B)

Figure 9. *Funnel graphs of studies assessing the impact of ginger on: (A) feed intake and (B) feed to egg ratios in laying hens*

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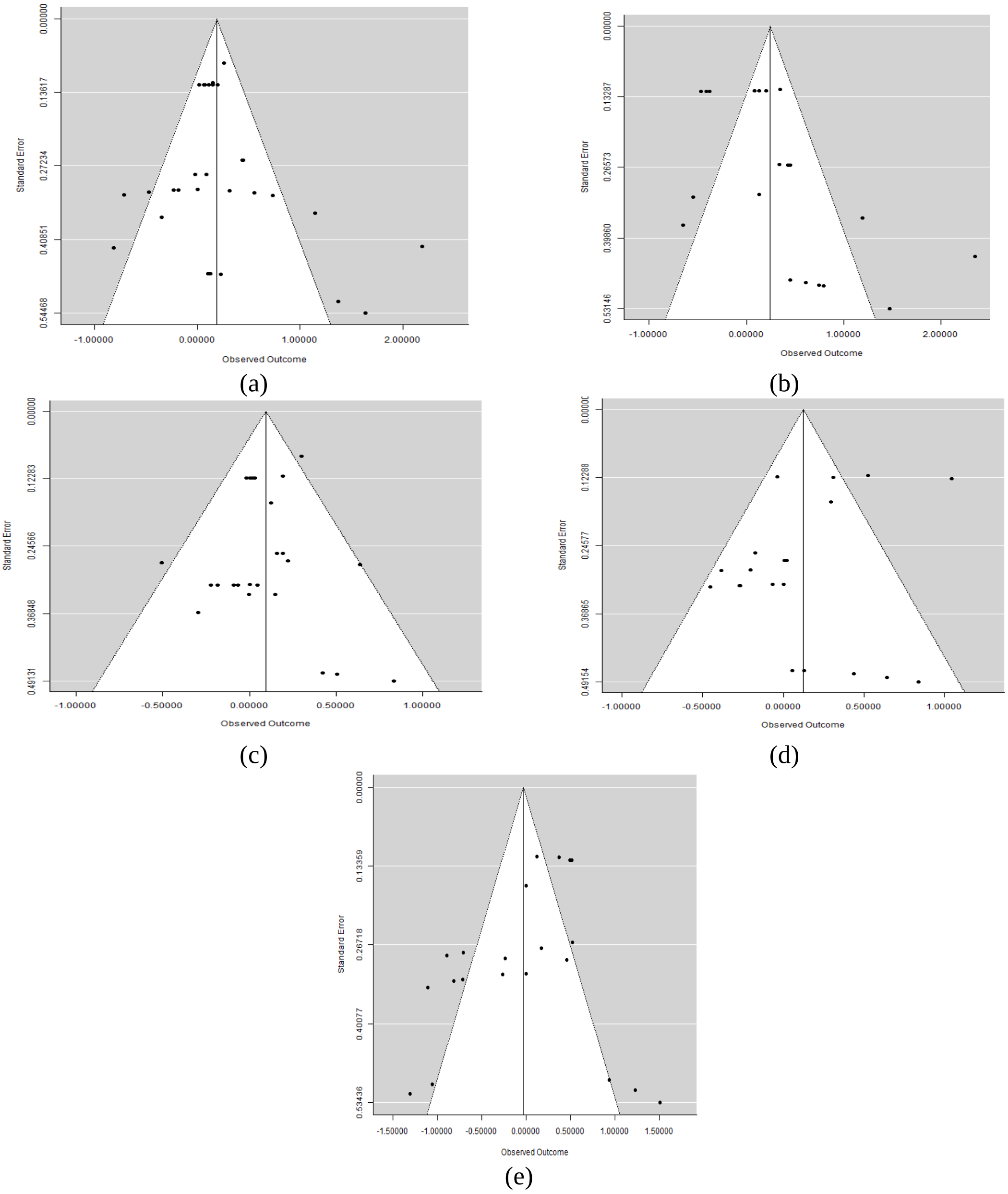

Figure 10. *Funnel graphs of studies assessing the impact of ginger on: (a) HDEP; (b) egg mass; (c) egg weight; (d) Haugh unit; and (e) egg shell thickness in laying hens*

Discussions

Ginger is known to possess several pharmacological actions including anti-inflammatory, anti-microbial and antioxidant properties (Ahn et al., 2002; Akoachere et al., 2002; Dragland et al., 2003). Ginger has been reported to enhance blood flow, feed intake and taste of feed, nutrient uptake and increase the secretion of digestive enzymes in chickens (Incharoen and Yamauchi, 2009; Kothari et al., 2019). Also, ginger has been shown to stimulate the flow of digestive enzymes in small laboratory animal models (Platel and Srinivasan, 1996; Platel and Srinivasan, 2000). Besides the pharmacological actions, ginger is also endowed with beneficial bioactive compounds whose composition and concentration are influenced by the season the rhizome was harvested, storage

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conditions and processing methods (Windisch et al., 2008). Our results indicate that ginger supplement had no effect on feed intake and feed to egg ratios in layers. Similar results have been reported in broiler chickens (Ogbuewu and Mbajiorgu, 2020) and layers (Incharoen and Yamauchi, 2009). Ogbuewu and Mbajiorgu (2020) have found ginger improves feed conversion ratio (FCR) in broiler chickens. However, the observed improved FCR was at variance with the results of this mea-analysis, and the observed disparity may be attributed to the different breeds used. There is presence of substantial heterogeneity amongst the 12 papers used to evaluate the effect of ginger supplement on feed to egg ratio, and meta-regression analysis suggested that hen's age, breed and duration of supplementation explained most of the variations.

The use of tropical medicinal plants with antimicrobial and antioxidant potentials in animal production is on the rise due to the restriction on the use of antibiotics in animal feed (Ahn et al., 2002; Dragland et al., 2003). Antioxidant function is routinely used to assess the physiological status of animals, and MDA, SOD, GSH-Px, and TAOC are the four common markers that reflect the antioxidant ability of chickens. The results of this meta-analysis revealed that ginger products increased the concentrations of GSH-Px, TAOC and SOD and reduced the concentrations of MDA in the plasma of laying hens. The significantly improved GSH-Px, TAOC, MDA and SOD concentration in laying hens on ginger intervention implied that ginger products can improve the antioxidant enzyme system of laying chickens, which is good for the health and productivity of layers. It is well known that cells under anaerobic environments are continuously faced with the dilemma of oxygen paradox. Cells need oxygen to work, but cell metabolites such as reactive oxygen species (ROS) may alter cell functions as well as threaten its survival. To avoid the adverse impacts of excessive generation of ROS, cells possess both enzymatic and non-enzymatic antioxidant defense systems that work together to prevent the harmful influences of ROS from aerobic mechanisms (Ogbuewu et al., 2010). The significantly increased plasma levels of GSH-Px and SOD in layers on ginger products over the controls indicate better cell functions through enhanced ability to mitigate the adverse effect of oxygen free radicals and the significantly reduced MDA implied lower oxidative degradation of lipids also called lipid peroxidation. On the other hand, the elevated blood TAOC that is composed of both enzymatic and non-enzymatic antioxidant defense systems suggests that ginger increased the blood antioxidant capacity of laying hens. Research has also shown that ginger is rich in bioactive substances such as gingerol and its derivatives (Fuhrman et al., 2000; Kota et al., 2008) known to have potent antioxidant ability (Ahn et al., 2002). The better antioxidant defense system of layers on ginger products as indicated by increased concentrations of GSH-Px, TAOC and SOD and reduced MDA in this meta-analysis could suggest the ability of ginger bioactive compounds to stimulate the secretion of both enzymatic and non-enzymatic antioxidant defense systems in laying hens (Fuhrman et al., 2000; Kota et al., 2008). The improved antioxidant markers as observed in layers on ginger treatment over the control layers also suggest the potential of ginger to enhance cell function by reducing the harmful effect of free radical attack through the stimulation of enzymatic and non-enzymatic antioxidant defense systems as corroborated by the increased concentrations of plasma TAOC.

Meta-analysis results of aspects of blood parameters of laying hens on ginger supplementation revealed that ginger significantly increased RBC count in laying hens. The significantly high RBC levels reported in layers on ginger supplementation over the controls suggest the high ability of the ginger treatment to support RBC production in the bone marrow leading to improved health indices and laying performance. In contrast,

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ginger had no significant influence on PCV, plasma calcium, phosphorus and total protein concentration in laying hens. Research has demonstrated the influence of medicinal plant-based diets on hepatic and plasma cholesterol concentration (Yalcin et al., 2006). Cholesterol biosynthesis and secretion in avian species occur principally in the liver (Ryś et al., 1996) and our pooled results showed significantly decreased plasma cholesterol value in laying hens on ginger intervention when compared with the controls. The mechanism of action behind the hypocholesterolemic property of ginger in laying birds is less clear. However, the observed reduction in plasma cholesterol concentration may be ascribed in part to increased uptake of cholesterol, one of the principal egg yolk precursors from the liver, the site of biosynthesis to the ovary where it is utilized for yolk synthesis under the influence of estrogen (Johnson, 2015). Besides the influence of ginger on blood variables as assessed in this study, it is important to note that blood parameters in chickens are strongly associated with the physiological state, genotype, age, environmental factors and sex (Ogbuewu et al., 2015). These factors were not evaluated in this meta-analysis because the requisite data needed to determine their influence on blood profiles of layers were not reported in most of the studies used for the analysis.

Results showed that birds on ginger intervention had higher HDEP with similar feed intake to the control. From an economic viewpoint, the success of the egg production enterprise lies in the total number of eggs laid (Sinha et al., 2018). Although the mechanism of action of ginger on egg production in chickens is not well understood, the observed higher HDEP in layers on ginger supplementation could be attributed to the activities of phytochemicals contained in ginger to enhance the secretion of gastric juices and digestive enzymes as well as increase blood circulation and nutrient uptake from the gut (Platel and Srinivasan, 2000; Ali et al., 2008; Incharoen and Yamauchi, 2009). Another likely explanation for the increased HDEP is the action of ginger to increase the synthesis and secretion of egg precursors such as egg yolk proteins (vitellogenins and apolipoprotein), triglycerides, phospholipids and cholesterol in the liver under the influence of estrogen (Johnson, 2015) leading to improved follicle development and release. Similarly, egg mass and weight were increased in layers on ginger treatment over the control, and this indicates the superiority of ginger over the control. The significantly increased egg mass and egg weight in layers on ginger over the controls may be due to the ability of ginger to balance intestinal microbiota and reduce the production of proinflammatory cytokines in the intestine owing to their antimicrobial properties, which in turn relieve intestinal challenge and immune stress, thus enhancing nutrient uptake of nutrient from the gastrointestinal tract. Even though our analysis found that ginger improved HDEP, egg mass and weight, the influence of other variables like housing temperature, lighting schedule and air velocity known to affect laying performance that are not analysed in this study may not be ruled out completely as these variables affect laying performance (Devi and Reddy, 2005; Parmar et al., 2006; Sinha et al., 2018). Haugh unit used as an index of albumen quality was not significantly influenced by ginger treatment likewise the egg yolk weight and egg yolk cholesterol. There are claims that ginger has positive effects on egg quality attributes in laying hens (Zhao et al., 2011; Okoro, 2016). The lack of significant effects of ginger on egg yolk weight and egg yolk cholesterol could be due to differences in preparation form (i.e. powder, extract and oil), inclusion level, hen’s age or breed of chickens used.

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Limitations and strengths of the analysis

This meta-analysis was limited to laying hen studies alone and may not apply to other animal species. The authors encountered few articles on the impact of ginger on antioxidant capacity indices, blood parameters and aspects of egg quality attributes, and therefore it was difficult to run subgroup and meta-regression analysis based on these few studies. The differences in analytical methods used by the individual studies may pose a constraint. Despite these constraints, the strength of this meta-analysis includes a systematic characterization of uncharacterized studies by aggregating data from different studies to resolve uncertainty, identify knowledge gaps, and create new insights on the effect of ginger intervention on health and production outcomes in laying hens.

Conclusion

The results of this study provided vital scientific insight into the positive effect of ginger intervention on health and production markers in laying hens. The results suggest that ginger significantly increased hen day egg production, egg weight and mass in laying hens when compared with the controls. The results also indicate that ginger did not affect feed intake, feed to egg ratio, eggshell thickness, eggshell weight, Haugh unit, egg yolk weight and yolk cholesterol content. Layers on ginger intervention experienced reduced plasma cholesterol content and enhanced red blood cell and blood antioxidant markers in comparison with the control. However, few studies were used for the blood and antioxidant marker assessment, thus these results need to be validated using a large number of studies. The studied covariates influenced the findings of this meta-analysis. Although laying performance in avian species were strongly related to lighting program and ambient temperatures, the evidence-based information on increased egg production and aspects of egg quality in laying hens on ginger intervention over the control may help poultry farmers, animal nutritionists and policymakers in making informed management decision on the use of ginger as feed additive in laying hens.

Implications for further research

Firstly, more research on the long-term effect of ginger intervention on health and laying performance are therefore needed, since the majority of the articles included in this analysis used layers at their first production cycle as this may increase the chance of the adoption of these findings in the commercial egg production enterprise. Secondly, the majority of the trials included in the analysis had missing data on housing type, lighting schedule, housing temperature, cage dimensions and number of layers included in each cell and rearing type (deep litter and battery cage). Thus, it has become vital for authors to report these variables known to influence egg production in laying chickens in their studies. Thirdly, none of the studies used for the analysis reported mortality or its absence. Future research should address this aspect. Fourthly, insufficient data prevented us from determining the effect of ginger presentation form (i.e. powder, extract and essential oil) on laying performance in hens in this meta-analysis. Thus, the current meta-analysis strengthens the call for more studies in this area.

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