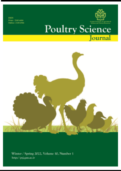

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Flavonoids as Dietary Additives in Laying Hens: A Meta-analysis of Production Performance, Egg Quality, Liver, and Antioxidant Enzyme Profile

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Abstract
In this study, a meta-analysis was employed to evaluate the effects of dietary flavonoids on laying hens. A database of flavonoids was developed from published journal papers in which flavonoids were supplemented to laying hens at various doses and then monitored for production performance, egg quality, liver condition, and antioxidant enzyme profile. A total of 22 journal papers were included in the database, and statistically analyzed using a mixed-model methodology in which random effects consisted of the different studies and fixed effects were the dosages of flavonoids. The model statistics were p-value and Akaike information criterion. The significance of an effect was assumed at $P < 0.05$. The results showed that dietary addition of flavonoids linearly decreased ($P < 0.001$) feed intake. The laying rate was not affected by the addition of flavonoids, while the FCR tended to linearly decrease ($P < 0.1$). The addition of flavonoids linearly reduced egg and yolk cholesterol (both at $P < 0.001$), and linearly increased egg mass ($P < 0.001$), egg strength ($P < 0.05$) and yolk color ($P < 0.001$). Further, a linear decreasing response was observed in the liver triglyceride, and cholesterol concentrations (both at $P < 0.001$) after flavonoid supplementation. Superoxide dismutase showed a quadratic response ($P < 0.01$) following the addition of flavonoids. In conclusion, dietary supplementation of flavonoids is able to favourably modulate production performance and egg quality of laying hens.

Introduction

Secondary metabolites, commonly referred to as phytochemicals, are the naturally present complex compounds in plants. These compounds have various benefits for the plants, animals humans, ,and the environment. Some phytochemicals, however, have anti-nutritional properties that hinder digestion and utilization of nutrients in the digestive tract of animals (Takahama and Hirota, 2018; Panda and Shinde, 2017). The phytochemicals classified as anti-nutrients include flavonoids, tannins, saponins and alkaloids (Kunatsa *et al*., 2020). In recent years, these anti-nutritional groups have been avoided due to reduced nutrient absorption in livestock. However, these compounds have become an exciting topic for research because phytochemicals can be used as antibacterial (Prihambodo *et al*., 2019), anti-tumour, anti-inflammatory, and antiviral substances in an appropriate dosage (Raina *et al*., 2014)

Flavonoids are one of the largest groups of phytochemicals in plants with numerous benefits. They are often referred to as health-promoting biomolecules and functional ingredients because of their role in improving health and prevention of

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chronic diseases. They have often been used in recent decades in livestock and human nutrition (Kamboh *et al*., 2015). A previous meta-analysis (Prihambodo *et al*., 2020) has reported positive effects of flavonoids on the performance parameters of broiler chickens. Other authors also reported beneficial effects of flavonoids administration in poultry, such as increasing performance in quails (Juráni *et al*., 2008) and lowering of cholesterol in ducks (Tugiyanti & Susanti, 2018).

Regarding the effects of flavonoids on laying hens, few studies (increasing growth performance, quality of eggs and enhancing gut morphology and functionality) have been conducted, but there is no study attempting to summarize their results. The meta-analysis is used for statistically combining the results of studies from various literatures in which all influencing factors were calculated (St-Pierre, 2001; Sauvant *et al*. 2008). Therefore, the present study aimed to evaluate the effects of dietary flavonoids administration on production performance, egg quality, liver, and antioxidant enzyme profile in laying hens using a meta-analysis approach.

Materials and Methods

Database development

A database was constructed from published journal papers reporting flavonoids as feed additives in laying hens. The documents were collected using various searchplatforms such as Scopus, Science Direct, and Google Scholar. The parameters integrated in the database were including production parameters (final body weight, laying rate, feed conversion rate [FCR], and feed intake), egg quality (egg weight, shape index, Haugh unit, egg mass, egg thickness, egg strength, egg cholesterol, shell weight, shell percentage, shell strength, albumen mass, albumen index, albumen height, yolk color, yolk mass, egg mass and yolk cholesterol), liver profile (weight, triglyceride and cholesterol concentrations) and antioxidant enzyme profile (superoxide dismutase, catalase and glutathione peroxidase). The following requirements were used for the inclusion of articles into the database: (a) in vivo studies carried on laying hens; (b) flavonoids were as feed additives, and (c) the articles were published in the English language. Based on the title and abstract screenings, 34 articles met the inclusion criteria. After further full-text evaluation, the final database consisted of 21 studies with 153 treatments, as presented in Table 1. The laying hens included in the database were Hessian, Lohmann Brown, Leghorn, Lohmann White, Hy-line White, ISA Brown, White Leghorn, and Hy-line Brown. In tabulating data into the database, data relating to the parameters were converted into the same measurement units to facilitate further analyses.

Table 1. Studies included in the meta-analysis

No	Reference	Layer Type	Period (weeks)	Flavonoid Type	Dosage (g kg^{-1})
1	Galal *et al*. (2008)	Hy-line White	46-50	Quercetin	0.0.15
2	Lien *et al*. (2008)	Leghorn	30-42	Hesperitin	0-5
3	Deng *et al*. (2010)	Hy-line Brown	28-36	Quercetin	0-0.034
4	Ariana *et al*. (2011)	Hy-line White	45-53	Naringenin	0-5
5	Ting *et al*. (2011)	White Leghorn	26-36	Hesperitin & naringenin	0-4
6	Liu *et al*. (2013)	Hessian	39-47	Quercetin	0-0.6
7	Goliomytis *et al*. (2014)	Lohmann White	26-30	Hesperidin	0-3
8	Liu *et al*. (2014)	Hessian	28-36	Quercetin	0-0.0619
9	Lokhande *et al*. (2014)	Hy-line Brown	45-53	Quercetin & hesperdin	0-7.5
10	Simitzis *et al*. (2014)	Lohmann Brown	28-36	Hesperidin & naringenin	0-1.5
21	Ying *et al*. (2015)	Hessian	39-47	Quercetin	0-0.6
11	Damaziak *et al*. (2017)	ISA Brown	16-32	Quercetin	0-0.032
12	El-Tarabany (2018)	Tetra Brown	58-63	Quercetin	0-0.2
13	Iskender *et al*. (2017)	Lohmann White	28-36	Hesperidin, naringenin & quercetin	0-0.5
14	Li *et al*. (2017)	Lohmann Brown	72-79	Hesperidin, naringenin & quercetin	0-5
15	Yang *et al*. (2017)	Hessian	37-45	Quercetin	0-0.6
16	Dogan (2018)	Lohmann Brown	40-48	Quercetin	0-20
17	Goliomytis *et al*. (2018)	Lohmann Brown	54-63	Hesperidin	0-1.5
18	Shahyar *et al*. (2018)	Hy-line Brown	58-70	Hesperitin	0-20
19	Simitzis *et al*. (2018)	Lohmann Brown	70-74	Quercetin	0-0.8
20	Abid & Ahmed (2019)	ISA Brown	40-50	Quercetin	0-1.2

Statistical analysis

The database was analyzed using a mixed-model methodology that has been widely used in meta-analysis research related to animal nutrition (Sauvant *et al*., 2008; St-Pierre, 2001). Different studies were considered as random effects, and the doses of flavonoids administered were considered as fixed effects. The statistical model was as follows:

$$Y_{ij} = B_0 + B_1X_{ij} + B_2X_{ij}{}^2 + s_i + b_iX_{ij} + e_{ij}$$

where Yij = dependent variable, B_0 = overall intercept across all studies (fixed effect), B_1 = linear regression coefficient of Y on X (fixed effect), B_2 = quadratic regression coefficient of Y on X (fixed effect), X_{ij} = value of the continuous predictor variable (flavonoid addition levels), s_i = random effect of study i, b_i = random effect of study i on the regression coefficient of Y on X in study i, and e_{ij} = unexplained residual error. Linear and quadratic regression models were analyzed and considered significant effects for p-values below 5% ($P < 0.05$). The class statement was declared in the variable study because it does not contain any quantitative information. The p-value was used as the statistical model. All statistical analyses were carried out using R Software (version 3.60).

Results

The effects of flavonoids on the production performance of laying hens are presented in Table 2. Dietary addition of flavonoids linearly decreased ($P < 0.001$) feed intake of laying hens. The final body weight of laying hens was affected quadratically ($P < 0.001$) by flavonoids. The laying rate was not affected by the addition of flavonoids, while the FCR tended to decrease linearly ($P < 0.1$). Flavonoids positively affected egg quality profiles of laying hens (Table 3). The addition of flavonoids reduced egg and yolk cholesterol linearly (both at $P< 0.001$), and increased egg mass ($P < 0.001$, linearly), egg strength ($P< 0.05$, linearly) and yolk color ($P<0.001$, linearly).

The effects of flavonoids on laying hens' liver and antioxidant enzyme profiles are presented in Tables 4 and 5, respectively. Triglyceride and cholesterol concentrations in the liver decreased linearly (at $P < 0.001$) after flavonoid supplementation, whereas liver weight was not affected. Superoxide dismutase was influenced quadratically (P < 0.05) following the addition of flavonoids, whereas catalase and glutathione peroxidase enzymes were not affected by dietary flavonoids supplementation.

Discussion

This meta-analysis generally indicates the potential of flavonoids as feed additives for substituting antibiotic growth promoters, which are now banned by most countries. Flavonoids do not impair egg production of laying hens and positively impact egg quality, liver profile, and blood profile. Decreased feed intake due to flavonoids implies the greater feed efficiency in the laying hens. Such a decrease in feed intake has also been reported by Ariana *et al*. (2011), Deng *et al*. (2012) and Freitas *et al*. (2017). This decline is caused by the bitterness of flavonoids and thus reduced feed palatability (Goliomytis *et al*., 2018). Flavonoids contribute to feed intake regulation even when supplemented at low levels (Wenk, 2002). Final body weight loss is associated with a decrease in feed intake and body fat reduction (Jennings *et al*., 2017). The anti-lipogenic effect of flavonoids might be associated with their quercetin and hesperidin contents (Simitzis *et al*., 2014; Zarrouki *et al*., 2010). Quercetin plays a role in inhibiting cell population growth and adipogenesis in 3T3-LI pre-adipocytes, which is a key to increasing fat mass. It seems that, at least in the short term, feed intake is not the primary factor affecting laying rate as reported by Oyedeji *et al*. (2007). In addition, flavonoids help the digestive tract to absorb nutrients by inhibiting pathogens and simultaneously increase beneficial genera such as *Bifidobacterium* and *Lactobacillus* (Pei *et al*., 2020).

The decrease of triglycerides (in the liver) and cholesterol (both in liver and eggs) due to flavonoid supplementation was previously reported in laying hens (Kismiati *et al*., 2020; Zhao *et al*., 2013). Furthermore, flavonoid compounds such as quercetin, luteolin (Nekohashi *et al*., 2014) and kaempferol (Salvamani *et al*., 2014) help to decrease the cholesterol content of whole egg and yolk in laying hens. This may occur because flavonoids can form insoluble complexes with fat or cholesterol originating from feed, thereby inhibiting the absorption of endogenous and exogenous cholesterol (Zhao *et al*., 2013; Kuang *et al*., 2018; Lien *et al*., 2008). Zhao *et al*. (2013) reported that triglycerides greatly influence the cholesterol content of eggs, which is in line with the results of the present study. Concerning reducing triglyceride levels, the antioxidant property of flavonoids stimulates lipoprotein-lipase activity (Roza *et al*., 2007) that can hydrolyze VLDL, which converts triglyceride to fatty acids and glycerol. Flavonoids can increase superoxide dismutase due to the ability of flavonoids to resist oxidative disorders. Ion H^+ in flavonoid has donated an electron to anion superoxide to stabilize it and prevent DNA and lipoprotein oxidation (Wilmsen *et al*., 2005; Aritanoga *et al*., 2019).

The positive effect of flavonoids on egg mass is in line with Juráni *et al*. (2008) report, but in contrast with the study of Ahmad *et al*. (2017). Flavonoids provide a beneficial effect on the eggshell of laying hens. A decrease in FCR without affecting body weight indicates an increase in nutrient absorption, which was also reported by Prihambodo *et al*. (2020), mainly on calcium and phosphorus absorption.

Table 2. Regression equation on the influence of flavonoid (in g kg^{-1}diet as fed) on growth performance of laying hens

Response Parameter	Unit	Model	N	Intercept	SE Intercept	Slope	SE Slope	*P*-value	RMSE	AIC	Trend
Final Body Weight	g	Q	22	1,670	36.4	-5.20	1.26	<0.001	0.981	215	Neg
Laying Rate	%	L	83	85.5	1.81	-0.118	0.094	0.212	1.44	512	Neg
Feed Conversion Rate	g	L	64	2.24	0.091	-0.007	0.004	0.078	1.47	1.75	Neg
Feed Intake	g/g	L	57	112	2.07	-0.369	0.090	<0.001	1.56	345	Neg

SE, standard error; RMSE, root mean square error; AIC, Akaike information criterion; Q, quadratic; L, linear; Min, minimal; Neg, negative.
1)AIC is an estimator of the relative quality of statistical models for a given set of data (smaller is better)

Table 3. Regression equations on the influence of flavonoid (in g kg^{-1} of diet as fed) on egg quality of laying hens

Response Parameter	Unit	Model	N	Intercept	SE Intercept	Slope	SE Slope	*P*-value	RMSE	AIC	Trend
Egg Weight	g	L	52	62.9	0.749	-0.002	0.026	0.941	1.65	198	Neg
Shape Index	%	L	17	75.5	0.413	0.019	0.090	0.945	1.06	52.6	Pos
Haugh Unit	Unit	L	76	82.9	1.24	0.048	0.108	0.657	1.40	392	Pos
Egg Mass	g	L	85	57.7	1.58	0.267	0.062	<0.001	1.52	397	Pos
Egg Thickness	10^{-2} mm	L	73	39.3	1.22	0.091	0.066	0.173	1.91	340	Pos
Egg Strength	N	L	39	33.8	3.09	0.390	0.181	0.041	1.42	214	Pos
Egg Cholesterol	mg	L	29	184	14.6	-5.58	1.36	<0.001	0.97	257	Neg
Shell Weight	g	L	12	5.80	1.00	0.026	0.021	0.264	1.08	33.1	Pos
Shell Percentage	%	L	20	9.81	0.317	-0.001	0.016	0.935	1.19	44.2	Neg
Albumin Mass	g	L	16	18.4	8.96	0.004	0.031	0.894	1.31	70.9	Pos
Albumin Index	%	L	10	8.14	0.160	-0.063	0.246	0.808	0.98	17.8	Neg
Albumin Height	mm	L	10	6.65	0.751	0.002	0.096	0.982	1.08	35.0	Pos
Yolk Color	Unit	L	35	9.62	0.672	0.146	0.034	<0.001	1.21	84.2	Pos
Yolk Mass	g	L	78	15.9	0.468	0.027	0.019	0.169	1.52	176	Pos
Yolk: Egg Mass	g/g	L	65	26.1	0.661	-0.115	0.102	0.266	1.63	211	Neg
Yolk Cholesterol	mg/g	L	80	12.8	0.852	-0.296	0.052	<0.001	2.09	324	Neg

SE, standard error; RMSE, root mean square error; AIC, Akaike information criterion; L, linear; Pos, positive Neg, negative
1)AIC is an estimator of the relative quality of statistical models for a given set of data (smaller is better)

Table 4. Regression equations on the influence of flavonoid (in g kg^{-1} of diet as fed) on liver profile of laying hens

Response Parameter	Unit	Model	N	Intercept	SE Intercept	Slope	SE Slope	*P*-value	RMSE	AIC	Trend
Liver Weight	g/100g	L	16	1.63	0.018	-0.007	0.014	0.606	1.06	-40.7	Neg
Liver Triglyceride	mg/g	L	16	14.8	0.243	-1.29	0.199	<0.001	1.06	32.7	Neg
Liver Cholesterol	mg/g	L	16	3.39	0.050	-0.205	0.038	<0.001	1.08	-12.8	Neg

SE, standard error; RMSE, root mean square error; AIC, Akaike information criterion; L, linear. Neg, negative
[1)]AIC is an estimator of the relative quality of statistical models for a given set of data (smaller is better)

Table 5. Regression equations on the influence of flavonoid (in g kg^{-1} of diet as fed) on antioxidant enzyme profile of laying hens

Response Parameter	Unit	Model	N	Intercept	SE Intercept	Slope	SE Slope	*P*-value	RMSE	AIC	Trend
Superoxide Dismutase	Unit	Q	16	28.2	2.23	1.72	1.39	0.024	1.03	115	Pos
Catalase	Unit	L	16	85.5	2.31	0.82	1.10	0.470	1.06	108	Pos
Glutathione Peroxidase	Unit	L	16	21.7	1.41	0.68	0.684	0.346	1.41	95.4	Pos

SE, standard error; RMSE, root mean square error; AIC, Akaike information criterion; Q, quadratic; L, linear; Max, maximum; Pos, positive
[1)]AIC is an estimator of the relative quality of statistical models for a given set of data (smaller is better)

This also increases egg mass because shells are thicker and stronger. The presence of flavonoids also improved yolk color. As natural coloring agents in plants, flavonoids increase pigment deposition in egg yolks (Freitas *et al*., 2017), making them look more reddish-yellow than ordinary eggs (Omri *et al*., 2019).

Conclusion

Dietary supplementation of flavonoids is able to favourably modulate production performance and egg quality of laying hens, and therefore reveals the potency of flavonoids for substituting antibiotic growth promoters for future production systems of laying hens.

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