IJAS Iranian Journal of Applied Animal Science

The Impact of Bentonite Feed Additives on Laying Hens Performance and Egg Quality: A Meta Analysis

Meta Analysis

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Received on: 22 Mar 2022
Revised on: 2 May 2022
Accepted on: 27 May 2022
Online Published on: Dec 2022

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Online version is available on: www.ijas.ir

ABSTRACT

This study aimed to evaluate the effect of bentonite as a feed additive on the performance and egg quality of laying hens through a meta-analysis approach. The keywords “bentonite”, “clay”, “montmorillonite” and “laying hens” were used to search scientific articles in Science Direct, PubMed, Google Scholar, and Scopus. A total of 14 articles were included in the database and were analyzed for a meta-analysis using a mixed model method. Different studies were considered as random effects, while bentonite levels were considered as fixed effects. The statistical models used were P-value, root means square error and Akaike information criterion. Results showed that bentonite level quadratically (P<0.05) increased egg production and decreased feed intake. The optimum supplementation of bentonite levels for egg production and feed intake were 2.19% and 1.63%, respectively. Egg weight, eggshell strength, and percentage of eggshell weight represented a linear increase (P<0.05) with increasing bentonite levels. Yolk's color score showed a linear decrease (P<0.05). Meanwhile, feed conversion ratio, egg mass, egg crack, eggshell thickness, and Haugh unit were not affected by dietary bentonite. It can be concluded that dietary supplementation of bentonite at the appropriate level has a positive impact on egg production, feed intake, egg weight, eggshell strength, and percentage of eggshell weight, but has no significant effect on feed conversion ratio, egg mass, egg crack, eggshell thickness, and Haugh unit. Increasing the level of bentonite can reduce yolk color. The optimum dietary bentonite level for egg production is 2.19% which is close to the EFSA recommendation.

KEY WORDS bentonite, eggshell, egg production, feed additives, laying hens.

INTRODUCTION

The prohibition on the application of antibiotics as growth promoters in poultry is a serious problem for the poultry industry. Since it is inseparable from the efficacy of antibiotics in increasing productivity, and reducing mortality by boosting the immune system (Khanedar *et al.* 2015; Mehdi *et al.* 2018). The prohibition is based on the presence of antibiotic residues and microbial resistance in meat and eggs, which can lead to allergic reactions, intestinal microbiota imbalance, and antibiotic resistance in humans (Kumar *et al.* 2020). According to De Kraker *et al.* (2016), antibiotic resistance become a serious problem, since global human deaths due to resistant infections are predicted to reach 10 million by 2050. Therefore, European countries have banned antibiotic growth promoters since 2006 (Choi, 2018). Consequently, industry and farmers have to look for an alternative compound to replace antibiotics. On the other hand, the prohibition of antibiotics application potentially has several negative effects on the animals, such as lower growth and egg production, as well as higher illness, and death rates. Furthermore, lower egg production and egg qualities lead to significant economic losses. As a result, it is necessary to find an effective feed additive to replace antibiotics, one of which is the supplementation of bentonite in laying hens diet.

Several studies have reported that bentonite has the potential to replace antibiotic growth promoters at appropriate levels due to its ability to bind to pathogenic bacteria, improve gut health, and increase digestive enzyme secretion (Safaeikatouli *et al.* 2012; Chen *et al.* 2020; Mgbeahuruike *et al.* 2021). Bentonite is a high-water absorption natural clay formed by the devitrification of volcanic ash (Moosavi, 2017). The use of bentonite as a poultry feed additive to enhance production performances, nutrient digestibility, and poultry health has been widely studied. Supplementation of 0.50 g/kg bentonite in the laying hen diet improved gut health and contributed to an increase in egg production (Gul *et al.* 2016; Chen *et al.* 2020). The inclusion of aluminosilicate-based clay in the diet improves the digestibility of calcium and phosphorus in broilers and the tibia ash content in laying hens (Juzaitis-Boelter *et al.* 2021). Moreover, dietary bentonite can bind aflatoxins and eliminate their toxicity (Mgbeahuruike *et al.* 2021). Also, dietary 0.50% bentonite can eliminate the negative effects of an ochratoxin-contaminated diet and increase body weight growth (Ghazalah *et al.* 2021). On the other hand, there was an inconsistent result where the dietary bentonite up to 1.20% did not affect the laying hen's productivity, egg qualities, and immune response (Qu *et al.* 2018) as well as decreased feed intake (Ezzat *et al.* 2016). Accordingly, the inconsistent results were expected to be mediated by using a meta-analysis approach aimed at describing a quantitative summary of the effect of bentonite on the laying hens' performance and egg qualities.

MATERIALS AND METHODS

Database development

A database was developed based on online scientific publications that were searched using several search tools such as Science Direct, Scopus, PubMed, and Google Scholar. Keywords used were “bentonite”, “clay“, “montmorillonite“ and “laying hens”. Following a search using these keywords, 108 articles were found and identified in accordance with this study's aim. The next step was an assessment and evaluation of the title and abstract which were 30 articles remaining to be content evaluated. Finally, we used data from 14 eligible studies that were entered into the database. The detail of the selection process for the database is provided in Figure 1.

The criteria of content assessment were articles conducting *in vivo* trials in laying hens with a control group and bentonite treatment group, reporting laying hen's performances and egg qualities, and reporting bentonite levels without mixing with other compounds. The parameters included in the database were egg production, egg weight, and mass, feed intake, feed conversion ratio (FCR), eggshell weight, eggshell strength, eggshell thickness, egg crack, Haugh unit (HU), and yolk color score. The database and descriptive statistics used in the meta-analysis are presented in Table 1 and Table 2, respectively. The database was published between 2000 and 2020 with a total of 3421 laying hen chickens using sodium bentonite or montmorillonite. Levels of bentonite added were expressed as a percentage (%). The conversion of bentonite units into percentages was conducted when the study reported other units such as g/kg. Bentonite levels ranged from 0 (control) to 4% with an average study duration of 11 weeks where the maximum and minimum durations were 22 and 4 weeks, respectively. Before statistical analysis, all data in the database was converted into similar units of measurement, and where possible, incomplete data was calculated from the recorded data.

Statistical analysis

The data were analyzed by meta-analysis using the mixed model procedure (St-Pierre, 2001), where the different studies were random effects and bentonite levels were fixed effects. The mixed model procedure was:

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

Where:

Y_{ij}: dependent variable.

B_0: overall intercepted across all experiments.

B_1: linear regression coefficient of Y on X.

B_2: quadratic regression coefficient of Y on X.

X_{ij}: value of the predictor variable (bentonite level).

s_i: random effect of experiment i.

b_1: random effect of experiment *i* on regression coefficient of Y on X.

e_{ij}: unexplained residual error.

The P-value < 0.05 was used to determine the statistical significance of the bentonite effect for each variable. SAS® on Demand for Academics was used to conduct the statistical analysis (SAS, 2004).

RESULTS AND DISCUSSION

Table 3 shows the regression equation for the effect of bentonite level on performance and egg qualities of laying hens.

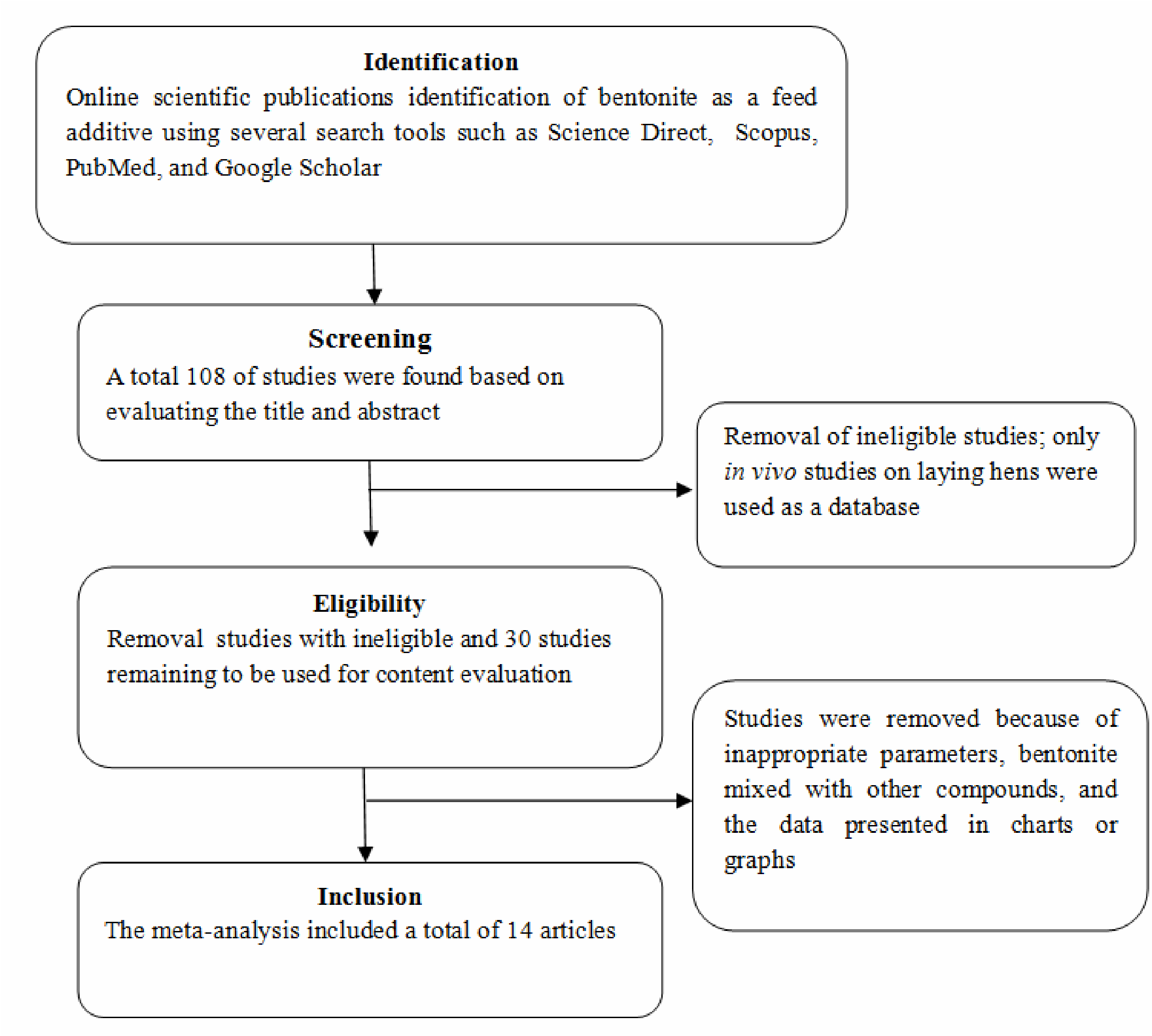

Figure 1 Flowcharts for meta-analysis eligible studies

Supplementation of bentonite in laying hens diet quadratically (P<0.05) increased egg production and decreased feed intake. The quadratic equation for egg production was $y= -0.5527x^2 + 2.4173x + 72.67$, while for feed intake was $y= 0.6798x^2 - 2.2211x + 111.25$ where the optimum level of bentonite supplementation was 2.19% and 1.63%, respectively (Figure 2 and Figure 3).

The positive effect of dietary bentonite has also resulted in egg qualities. Egg weight, eggshell strength, and percentage of eggshell weight represented a linear increase (P<0.05) with improving bentonite level. Yolk's color score showed a linear decrease (P<0.05) by increasing bentonite levels in diets. Meanwhile, feed conversion ratio, egg mass, egg crack, eggshell thickness, and Haugh unit were not affected by dietary bentonite (P>0.05).

Bentonite clay is included in the smectite group consisting of crystalline minerals with the main composition of montmorillonite (Olegario and Gili, 2021). The two main types of bentonite are sodium montmorillonite and calcium montmorillonite, which have distinctive characteristics. Sodium montmorillonite has a higher swellability than calcium montmorillonite due to its ability to absorb a larger volume of water and increase its original volume (Park *et al.* 2016). Bentonite as a poultry feed additive is commonly intended to improve nutrient digestibility and performance. Through this meta-analysis study, the use of bentonite at the proper dose reduced feed intake while increasing egg production, and egg weight which was consistent with the findings of Yenice *et al.* (2015), Prasai *et al.* (2017), and Chen *et al.* (2020).

Table 1 Description of studies in the database

No	References	Birds (n)	Period (week)	Strain	Type of bentonite	Dosage (%)
1.	Ambula *et al.* (2003)	108	32-38	Isa brown	Bentonite	0-0.5
2.	Azazi *et al.* (2011)	210	24-31	Inshas	Sodium bentonite	0-0.5
3.	Chen *et al.* (2020)	360	30-40	Lohmann pink	Bentonite (montmorillonite)	0-0.5
4.	Choi (2018)	300	74-78	Hyline brown	Sodium bentonite	0-0.5
5	Gilani *et al.* (2013)	300	51-63	Hy-line W-36	Sodium bentonite	0-2
6.	Gul *et al.* (2016)	45	34-38	White leghorn	Bentonite	0-2.5
7.	Hashemipour *et al.* (2010)	256	36-47	Hy-line W-36	Sodium bentonite	0-3
8.	Inal *et al.* (2000)	320	32-42	Babcock B-380	Sodium bentonite	0-3.5
9.	Manafi *et al.* (2012)	192	28-36	Unknown	Sodium bentonite	0-1
10.	Prasai *et al.* (2016)	200	26-37	Bond brown	Bentonite	0-4
11.	Ezzat *et al.* (2016)	270	28-40	Matrouh local	Sodium bentonite	0-1
12.	Prasai *et al.* (2017)	200	21-43	Bond brown	Sodium bentonite	0-4
13.	Qu *et al.* (2018)	480	30-40	Lohmann brown	Bentonite (montmorillonite)	0-1.2
14.	Yenice *et al.* (2015)	180	26-38	Barred rock	Sodium bentonite	0-1

Table 2 Descriptive statistics of laying hens performances and egg quality parameters in the database

Parameters	Unit	n	Mean	SD	Max	Min
Performance parameters						
Egg production	%	51	76.87	10.57	96.00	46.61
Egg weight	g/egg	50	60.03	5.49	67.83	41.46
Feed intake	g/bird/day	47	114.07	9.50	131.99	88.16
FCR	g feed/egg mass	42	2.50	0.41	3.85	2.05
Egg mass	g/bird/day	34	47.31	7.91	58.40	24.08
Egg quality parameters						
Egg crack	%	14	1.34	0.57	2.19	0.49
Eggshell strength	Kgf	14	3.88	0.38	4.60	3.23
Eggshell thickness	mm	39	0.37	0.06	0.54	0.31
Eggshell weight	%	22	8.84	2.87	13.57	5.20
Haugh unit	-	28	75.27	6.94	85.60	54.90
Yolk color	-	29	8.24	2.44	13.96	5.50

n: number of data; FCR: feed conversion ratio; SD: standard deviation; Max: maximum and Min: minimum.

Table 3 Regression equations on the influence of bentonite dosage on laying hens performance and egg quality

Parameters	Unit	n	Parameter estimates				Statistics model			Interpretation trend	Model
			Intercept	SE intercept	Slope	SE slope	P-value	RMSE	AIC		
Performance parameters											
Egg production	%	51	72.67	2.999	2.4173	0.8169				Positive	Q
					-0.5527	0.235	0.025	22.81	28.70		
Egg weight	g/egg	50	56.66	1.78	0.2322	0.1140	0.049	1.60	203.70	Positive	L
Feed intake	g/bird/day	47	111.25	2.719	-2.2211	1.1899				Negative	Q
					0.6798	0.3276	0.046	20.84	279.90		
FCR	g feed/egg mass	42	1.987	0.046	-0.01142	0.014	0.405	0.18	-44.00	-	L
Egg mass	g/bird/day	34	46.74	2.883	-0.019	0.618	0.976	8.49	205.30	-	L
Egg quality parameters											
Egg crack	%	14	1.37	0.29	-0.037	0.058	0.541	0.54	17.70	-	L
Eggshell strength	Kgf	14	3.81	0.20	0.199	0.049	0.003	0.34	5.30	Positive	L
Eggshell thickness	mm	39	0.37	0.014	0.006	0.005	0.184	0.07	-144.50	-	L
Eggshell weight	%	22	9.20	1.06	0.110	0.050	0.049	0.61	45.20	Positive	L
Haugh unit		28	75.85	2.65	0.530	0.636	0.244	4.89	154.70	-	L
Yolk color		29	8.48	0.82	-0.270	0.074	0.002	0.99	71.20	Negative	L

n: number of data; FCR: feed conversion ratio; RMSE: residual mean square error; SE: standard error; AIC: akaike information criterion and L: linear.

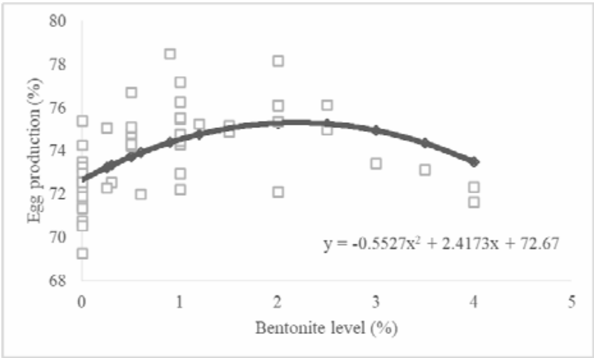

Figure 2 Relationships between dietary bentonite (%) and egg production (%) of laying hens

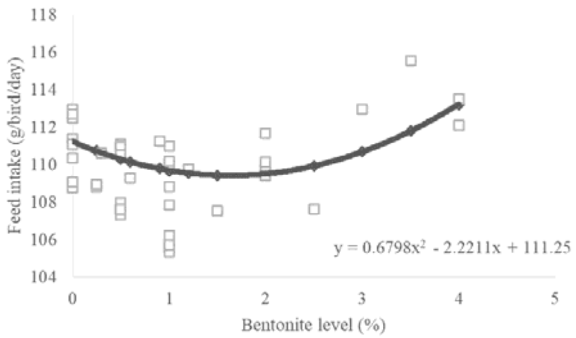

Figure 3 Relationships between dietary bentonite (%) and feed intake (g/bird/day) of laying hens

It suggested that the bentonite addition in laying hen's diet might promote more efficient nutrient utilization in supporting productivity. Such a positive effect could be attributed to the dietary sodium bentonite that can efficiently increases water absorption, nutrient digestibility, and digestive enzymes in the gastrointestinal tract (Khalifeh *et al.* 2012). Nadziakiewicz *et al.* (2021) reported that dietary aluminosilicate clay reduced water consumption, which might result in slower digesta flow in the bird's digestive tract and better digestion. With the absorption and swelling capacity of bentonite, it was also effective in binding the fungal and bacterial toxins and ameliorating the negative effect in the digestive tract (Mgbeahuruike *et al.* 2021) through electrostatic adsorption and excreted through the gut tract (Qu *et al.* 2018). Furthermore, bentonite effectively absorbs aflatoxins B1 in the gastrointestinal tract, where aflatoxin was able to reduce body weight gain and feed efficiency, increase liver damage, and change immune responses (Dos Anjos *et al.* 2015; Tinelli *et al.* 2019). According to Wang *et al.* (2018), bentonite binds to mycotoxins through several mechanisms including electron donor, selective chemisorption, ion interactions, cation exchange, and carbonyl groups.

In addition, the enhancement in egg production can be associated with some mineral content of bentonite that helps egg production (Gul *et al.* 2016). Furthermore, the improvement in egg production and egg weight in the presence of sodium bentonite may be due to a decrease in feed transit rate along the digestive tract, thereby allowing more time for nutrient absorption (Saçakli *et al.* 2015) such as protein, energy, and linoleic acid that were recognized as the most nutrient needed for egg production and egg weight (Godbert *et al.* 2019). In addition, bentonite was reported to improve the gut health of chickens as indicated by an increase in the jejunum villi height (Attar *et al.* 2017; Chen *et al.* 2020), which may indicate a greater luminal absorptive surface area for nutrients and contribute to the in the improvement of a hen laying productivity. Safaeikatouli *et al.* (2012) also confirmed an increase in maltase, alkaline phosphatase, and aminopeptidase enzyme activities on the small intestine brush border membrane indicating a positive effect on intestinal epithelial function with bentonite addition in the diet. The increase of maltase and aminopeptidase can elevate the degradation of carbohydrates and protein to maintain poultry performance (Leigh *et al.* 2017). However, based on this meta-analysis the dietary bentonite in high concentrations contributed to the deterioration of egg production. Bentonite is an absorbent that easily undergoes ion exchange due to the low pH in the digestive tract, which affects the availability of minerals (Lukman *et al.* 2013).

Furthermore, adsorbents with high cation exchange capacity can absorb microminerals and vitamins from animal feed, which can lead to these nutrient deficiencies (Elliott *et al.* 2019). On the other hand, micronutrients and vitamins are required for the functioning of hormones and enzymes to maintain growth and egg production (Smith *et al.* 2018). The current meta-analysis study confirmed that dietary bentonite improved eggshell strength and percentage of eggshell weight (Table 3). Previous studies reported that increasing eggshell quality by the inclusion of sodium bentonite may be due to high mineral content in bentonite, ion exchange capacity, and calcium affinity (Elliott *et al.* 2019). This reason is supported by Choi (2018) and Gul *et al.* (2016) that the most important mineral content in sodium bentonite is calcium which promotes calcium absorption in hens to help in the improvement of various eggshell traits. Furthermore, dietary bentonite increased alkaline phosphatase that has responsible for bone mineralization and phytate degradation in the small intestine (Kriseldi *et al.* 2021). Thus it may be can improve calcium and phosphorus availability for eggshell formation. Calcium intake is the main factor in determining eggshell qualities since the main component of eggshells is calcium with the composition of calcium carbonate (98.2%), magnesium (0.9%), and phosphorus (0.9%) (Shwetha *et al.* 2018). The meta-analysis approach revealed that yolk color represented a linear decrease with increasing the level of bentonite which was in line with the stated by Gilani *et al.* (2013) and Elliott *et al.* (2019). This result may be due to the binding of pigments in the diet by sodium bentonite and excreted rather than absorbed (Kermanshahi *et al*. 2011). According to Omri *et al*. (2019) yolk color was closely related to carotenoid content in the diet and it is not able to be synthesized by hens *de novo*.

Bentonite has non-specific absorbance properties, therefore it is necessary to strictly monitor the dosage used. Moreover, the European Feed Safety Authority (2012) recommends a maximum dose of 2% bentonite in animal feed due to its ability to reduce the efficacy of oral veterinary drugs.

CONCLUSION

This meta-analysis confirms that dietary supplementation of bentonite at the appropriate level has a positive impact on egg production, feed intake, egg weight, eggshell strength, and percentage of eggshell weight, but has no significant effect on feed conversion ratio, egg mass, egg crack, eggshell thickness, and Haugh unit. Increasing the level of bentonite can reduce yolk color. The optimum dietary bentonite level for egg production is 2.19% which is close to the EFSA recommendation. Furthermore, bentonite appears to be suitable as a feed additive in laying hens.

ACKNOWLEDGEMENT

Authors gratefully acknowledge all staff of Animal Feed Nutrition Division, Department of Animal Science, Ondokuz Mayis University and members of Animal Feed and Nutrition Modelling Research Group, Faculty of Animal Science, IPB University of their assistance in completing this study.

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