

The effects of probiotics on the performance, egg quality and blood parameters of laying hens: A meta-analysis

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ABSTRACT. A meta-analysis was conducted to determine the effects of probiotics on the performance, egg quality and blood parameters in laying hens. A database was designed based on published papers reporting the use of probiotics in laying hens. Articles were rigorously selected according to the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) protocols. The final database consisted of 47 *in vivo* studies with 190 treatment units. The statistical meta-analysis was performed according to the linear mixed models by using R software version 3.6.3. It was shown that dietary addition of probiotics linearly increased ($P < 0.001$) egg production and concomitantly decreased ($P < 0.01$) feed egg ratio (FER) with a linear pattern. Egg mass and feed intake were not associated with the probiotic treatment. Concerning egg quality parameters, probiotics did not affect egg weight but increased eggshell thickness ($P < 0.001$), eggshell weight ($P < 0.01$) and yolk colour ($P < 0.01$). Probiotics reduced ($P < 0.05$) cholesterol and low-density lipoprotein cholesterol while elevated ($P < 0.05$) high-density lipoprotein cholesterol blood concentrations. In conclusion, poultry products with health-promoting properties can be obtained with the use of probiotics which positively affect production performance, egg quality and blood metabolites parameters in laying hens.

Introduction

Microorganisms with beneficial properties, as *Lactobacillus* strain, were firstly used in animal feeding in the early 1900s in the Caucasus Mountains (Markowiak and Śliżewska, 2018). During extensive investigations they were further named probiotics and their multiple positive effects primarily in maintaining intestinal integrity and gut health, improving nutrient digestibility and production performance in most animal species were

observed. Genera of microorganisms commonly used as probiotics in animals include *Bifidobacterium*, *Lactococcus*, *Lactobacillus*, *Bacillus*, *Streptococcus* and yeasts. The development of probiotics use commenced when sub-therapeutic levels of antibiotics began to be banned for livestock in 1996 in Germany and Denmark (Maron et al., 2013). The European Union introduced probiotics as an alternative to antibiotics and this has subsequently become an area of great interest for researchers worldwide. In 1997, antibiotic growth promoters (AGPs)

continued to be banned including the use of tylosin, spiramycin, bacitracin, virginiamycin, carbadox and olaquinox in the Netherlands. In 2005, Taiwan announced a ban on the use of such drugs in the live-stock (Maron et al., 2013).

The ban for using AGPs has been extended to developing countries, with Indonesia being the last of them (ban introduced in 2018). The most recent Indonesia regulation states that it is no longer acceptable to use AGPs in animal production including laying hens. Research on the use of probiotics in laying hens has been widely conducted worldwide and published in various scientific journals. The number of publications in Scopus on this topic increased from less than 50 in 1995 to more than 250 in 2015 (Park et al., 2016a). However, this increase in publications number was not matched by consistent trial results. Yörük et al. (2004) reported that probiotics had no consistent effects on egg quality parameters. In other studies (Kurtoglu et al., 2004; Forte et al., 2016; Abd El-Hack et al., 2017; Mikulski et al., 2020) it was reported that probiotics consistently increased egg quality parameters.

Such results inconsistency generated from different studies may be mediated by employing a meta-analysis method. Meta-analysis is a term that refers to a quantitative and systematic approach which forms a continuous analysis of existing research (Hidayat et al., 2020). Meta-analysis may also be applied to confirm quantitatively the nature of results within a body of research (Hooge and Conolly, 2011). Accordingly, the aim of the current study was to determine the effects of probiotics on the performance, egg quality and blood parameters of laying hens by using the meta-analysis of previously published articles.

Material and methods

Development of database

A database was constructed based on peer-reviewed and published research articles which reported the use of probiotics in laying hens diet. The probiotics here are specifically for lactic acid bacteria, yeast and their combination. Articles were selected based on the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) (de Vries et al., 2015) and Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) (Liberati et al., 2009) protocols. Articles were retrieved from PubMed, Web of Science, Scopus, Google Scholar and Science Direct databases as well as individual journals such as World Poultry Journal Science, British Poultry Science and

International Journal of Poultry Science using the key words: ‘probiotic’, ‘laying hens’, ‘performance’, ‘egg quality’ and/or ‘blood serum’. Details for the selection process are provided in Figure 1.

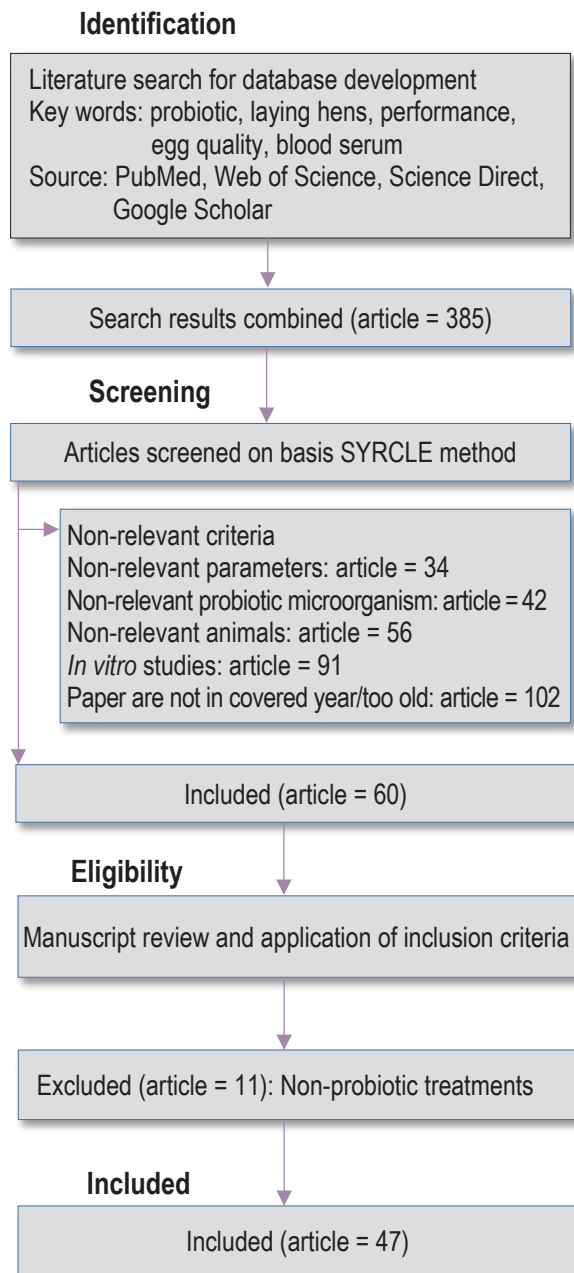


Figure 1. Diagram flow of article selection in the meta-analysis using Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) method

Criteria for an article to be included in the database were as follows: (a) the article is published in English in a peer-reviewed journal published between 2003 and 2020, (b) the experiment was performed in a controlled-trial environment, (c) the experiment was performed directly on laying hens *in vivo* as the experimental animals, (d) the

concentrations of probiotic both in powder and liquid forms are provided in the methods section, allowing for calculation and transformation into a logarithmic unit, (e) in the experiment the information on the experimental period and specific ages of animals is provided, and (f) dosages of probiotics constituted 0–5 g/kg of the formula. The final database consisted of 47 *in vivo* studies with

190 treatments. General information, details of treatment and variable outputs from these articles were summarized in a spreadsheet prior to analyses. When data were presented in graphical forms, the data were extracted by using WebPlotDigitizer in order to obtain the exact values (Drevon et al., 2017). The details of the studies included in the meta-analysis are summarized in Table 1.

Table 1. Studies included in the meta-analyses of the effect of probiotics on the performance, egg quality and blood parameters in laying hens

References	Kind of probiotic	Form	Dosage, g/kg	Periods, week
Zhu et al. (2015)	lactic acid bacteria	powder	0–0.1	15–20
Panda et al. (2008)	lactic acid bacteria	powder	0–0.15	25–40
Afsari et al. (2014)	yeast	powder	0–0.06	56–64
Mohebbifar et al. (2013)	lactic acid bacteria	powder	0–0.1	74–82
Panda et al. (2003)	lactic acid bacteria	powder	0–0.2	24–64
Sobczak and Kozłowski (2015)	lactic acid bacteria	powder	0–0.14	18–44
Li et al. (2011)	lactic acid bacteria	powder	0–0.1	56–64
Zhang and Kim (2013)	lactic acid bacteria	powder	0–0.01	15–40
Baghban-Kanani et al. (2019)	lactic acid bacteria	powder	0–0.1	15–32
Fathi et al. (2018)	lactic acid bacteria	powder	0–0.4	15–36
Khan et al. (2011)	yeast	powder	0–0.5	20–40
Hayirli et al. (2005)	lactic acid bacteria	powder	0–0.3	46
Kashani et al. (2013)	yeast	powder	0–0.05	80–87
Asli et al. (2007)	combination	powder	0–1	40–62
Pan et al. (2011)	lactic acid bacteria	powder	0–0.15	58
Kurtoglu et al. (2004)	lactic acid bacteria	powder	0–0.75	1–90
Zhang et al. (2012)	lactic acid bacteria	powder	0–0.06	24
Arpášová et al. (2016)	lactic acid bacteria	powder	0–0.5	21
Mahdavi et al. (2005)	lactic acid bacteria	powder	0–0.12	28–39
Forte et al. (2016)	lactic acid bacteria	powder	0–0.05	20
Mikulski et al. (2012)	lactic acid bacteria	powder	0–0.05	23–46
Tang et al. (2015)	lactic acid bacteria	powder	0–0.01	20–36
Tang et al. (2017)	lactic acid bacteria	powder	0–1.01	20–52
Elnagar (2013)	yeast	powder	0–0.6	26
Abd Elhalim et al. (2007)	lactic acid bacteria	powder	0–0.1	39–47
Hassan et al. (2019)	lactic acid bacteria	powder	0–0.1	29–50
Abdel-Wareth (2016)	lactic acid bacteria	powder	0–0.1	24–36
Fujiwara et al. (2008)	lactic acid bacteria	powder	0–0.1	15–29
Loh et al. (2014)	lactic acid bacteria	liquid	0–0.6	23
Saleh et al. (2017)	lactic acid bacteria	powder	0–0.05	28–34
Bonsu et al. (2014)	lactic acid bacteria	powder	0–0.15	22
Yalçın et al. (2012)	yeast	powder	0–2	18–23
Behnamifar et al. (2015)	lactic acid bacteria	liquid	0–1	85
Lei et al. (2013)	lactic acid bacteria	powder	0–0.09	28
Yalçın et al. (2015)	yeast	powder	0–0.5	54
Aghaie et al. (2010)	lactic acid bacteria	powder	0–0.2	41–49
Anwar and Rahman (2016)	lactic acid bacteria	liquid	0–0.85	70
Xiang et al. (2019)	yeast	powder	0–0.15	15–30
Yalçın et al. (2014)	yeast	powder	0–4	26
Desoky and Kamel (2018)	yeast	powder	0–0.125	32–43
Lee et al. (2019)	lactic acid bacteria	liquid	0–0.1	40
Mikulski et al. (2020)	lactic acid bacteria	powder	0–0.1	32–47
Zhan et al. (2019)	lactic acid bacteria	powder	0–0.2	48–58
Yalçın et al. (2010)	yeast	powder	0–4	22
Yalçın et al. (2008)	yeast	powder	0–2.85	16–21
Al-Harhi (2015)	yeast	powder	0–0.4	48–56
Sun et al. (2015)	yeast	powder	0–5	40–48

Data analysis

Analysis of the database was carried out according to the mixed-model methodology (St-Pierre, 2001; Sauvant et al., 2008; Patra, 2013), performed by using the R software version 3.6.30 with library 'nlme' (Pinheiro et al., 2020; R Core Team, 2020). The experiments were considered as the random effects while the probiotics concentrations were taken as the fixed effects, using the following mathematical model:

$$Y_{ij} = \beta_0 + \beta_1 \text{Level}_{ij} + \text{Experiment}_i + \text{Experiment}_i \times \text{Level}_{ij} + e_{ij},$$

where: Y_{ij} – dependent variable, β_0 – value when level intersects the Y axis for all random effect combinations, β_1 – coefficient level of order 1, Level_{ij} – level addition of the probiotics (fixed effects), Experiment_i – number of trial-i (random effects), e_{ij} – model error. Initially, the formula used was a quadratic model, but it was modified to the corresponding linear model as above since the quadratic model was insignificant.

Results

Dietary addition of probiotics increased ($P < 0.001$) egg production and decreased ($P < 0.01$) feed egg ratio (FER) (Table 2). Egg mass and feed intake were not affected by the addition of probiotics. With regard to egg quality parameters, probiotics did not affect egg weight but increased eggshell thickness ($P < 0.001$), eggshell weight ($P < 0.01$) and yolk colour ($P < 0.01$). Haugh unit tended to increase ($P < 0.1$) whereas the egg index tended to decrease ($P < 0.1$) by probiotic addition. Probiotics reduced ($P < 0.05$) blood cholesterol and low-density

lipoprotein cholesterol (LDL-C) while elevated ($P < 0.05$) blood high-density lipoprotein cholesterol (HDL-C) concentrations in laying hens.

Discussion

The present study confirmed that the application of probiotics improved laying hens' productive performance as evidenced by the increasing egg production and feed efficiency. A number of scientific reports in the last few years have provided strong pieces of evidence explaining the role of probiotics in enhancing poultry production including broiler chickens and laying hens which could be connected with the current finding. For instance, Mikulski et al. (2020) reported that the use of *Pediococcus acidilactici* probiotics increased laying rate and feed efficiency by approximately 2.8%. They also demonstrated that probiotics could successfully compensate low apparent metabolizable energy (AME) diet by maintaining productive performance. Studies on other probiotics strains such as *Bacillus subtilis*, *Enterococcus faecium*, *Lactobacillus* and yeasts also demonstrated similar amelioration in production traits of laying hens (Mikulski et al., 2012; Zhang and Kim, 2013; Park et al., 2016a; Wang et al., 2020).

These improvements are mainly associated with increasing nutrient use efficiency as a result of the role of probiotics in many biological pathways. There is a general convention that probiotics can effectively enhance the morphology of intestinal epithelial cells and their barrier system, digestive enzyme secretion and favourable microorganisms (Ding et al., 2020). From this point, further beneficial effects are explained such as immune system

Table 2. Regression linear model of the effect of probiotics on the laying hen performance, egg quality and blood parameters

Indices	Unit	M	N	Parameter estimates				Model estimates			Interpretation trend
				intercept	SE intercept	slope	SE slope	P-value	RMSE	AIC	
Egg production	%	L	190	84.63	1.263	0.156	0.0363	<0.001	1.914	975	positive
Egg mass	g/hen/day	L	190	53.05	0.972	0.033	0.0664	0.612	4.246	1.112	positive
FER	g feed/g egg	L	190	2.14	0.051	-0.005	0.0018	0.008	2.249	-170.46	negative
Feed intake	g/hen/day	L	190	118.7	11.36	0.520	1.746	0.766	5.97	2.236	positive
Egg weight	g	L	190	60.11	0.732	0.018	0.0327	0.570	3.64	891.25	positive
Egg shell thickness	mm	L	190	0.37	0.015	0.0012	0.0004	<0.001	3.50	-738.80	positive
Egg shell weight	g	L	190	5.01	0.245	0.0133	0.0044	0.003	2.80	229.00	positive
Yolk colour	roche	L	190	7.22	0.276	0.015	0.0056	0.007	2.13	308.49	positive
Haugh unit	no unit	L	190	80.00	1.600	0.010	0.050	0.067	1.94	1.082	positive
Egg index	no unit	L	190	0.92	0.04	-0.002	0.0012	0.076	4.21	-297	negative
Cholesterol	mmol/l	L	189	1.70	0.115	-0.011	0.005	0.030	3.70	192.37	negative
HDL-C	mmol/l	L	189	40.31	2.044	0.1277	0.062	0.042	3.00	1.165	positive
LDL-C	mmol/l	L	189	130.00	4.86	-0.400	0.202	0.050	2.07	1.574	negative

M – model; N – number of data; SE – standard error; RMSE – root mean square errors; AIC – akaike information criterion; FER – feed egg ratio; L – linear; HDL – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol

improvement (Deng et al., 2012; Rehman et al., 2020). Specifically, some mechanisms of nutrient absorption in laying hens during probiotics supplementation will be described.

First, it can be attributed to the higher enzyme secretion that is positively associated with increasing digestion and nutrient absorption. This was in line with studies by Zhang and Kim (2013) and Park et al. (2016b) who found that probiotics increased nitrogen and energy utilization. Increasing nitrogen digestibility is beneficial to lesser fermentable substrates available for pathogens in the intestine which also contributed to improve microbial balance and gut health as well as to reduce ammonia secretion to the environment (Zhang and Kim, 2013).

Secondly, probiotics have been reported to increase bone mineralization by increasing the calcium (Ca) and phosphorus (P) absorption (Yan et al., 2019). It makes sense when eggshell thickness and eggshell weight increased in the present meta-analysis because probiotics are able to promote an acidic pH in the intestinal tract due to antibacterial, organic acids and volatile fatty acids production (Al-Khalifa et al., 2019). Probiotics are not only effective to increase minerals absorption but also inhibit pathogenic growth (Ding et al., 2020). In their fermentation pathway, probiotics produce organic acids such as butyric acid as a major end-product. Butyrate is an important source of energy for intestinal epithelial cells that can inhibit inflammation, enhance the barrier function for pathogenic defence, and reduce oxidative stress (Guo et al., 2020; Tang et al., 2020). Animal well-being is an important physiological condition to support optimal metabolism and production (Sjöfjan et al., 2021).

Furthermore, we have also notice an increase in yolk colour of the egg. Similar results were reported by Sobczak and Kozłowski (2015) and Neijat et al. (2019) who found an improvement in the interior quality of eggs such as yolk colour, Haugh unit, and weights of yolk and albumin in laying hens receiving *Bacillus subtilis* at the age of 18–42 weeks. Increasing nitrogen utilization and improving the gut environment might possibly explain the reason of beneficial effect of probiotics. Thinning of albumen as a result of increased protein transfer rate is associated with the increase of Haugh unit (Lei et al., 2013). In addition, decreasing intestinal pH and improving caution solubility which favour gut environment to increase mineral absorption are also connected with the enhancement of interior and exterior egg quality parameters (Behnamifar et al., 2015; Neijat et al., 2019).

In regard to eggshell thickness and weight, it was reported that probiotics increased eggshell thickness and weight when fed to laying hens at the age of 28–32 and 32–36 weeks as well as at late production phase (72 to 79 weeks of age), respectively (Fujiwara et al., 2008; Behnamifar et al., 2015; Wang et al., 2020). Fujiwara et al. (2008) suggested that eggshell parameters were equally influenced by the metabolic activity of beneficial bacterial colonies, which could positively influence the absorption rate of calcium (Ca^{2+}) and magnesium (Mg^{2+}). This was also beneficial to increase the egg weight and its interior quality (Lei et al., 2013). However, it should be noted that the microbial strains used as probiotics may have different effects. For example, Loh et al. (2014) reported that *Lactobacillus plantarum* probiotics supplementation had no effect on egg weight.

It was stated that in laying hens probiotics reduced cholesterol and LDL-C while elevated HDL-C blood concentrations. Zhang et al. (2012) suggested that it might be related to the activity of the microorganisms in recycling lipids in the intestine of laying hens. Some LAB, such as *Bacillus subtilis*, were reported to prevent bile salts re-absorption and to increase their extraction with faeces. Simultaneously the probiotic-derived cholesterol blood concentration reduction can be connected with inhibited synthesis of enzymes participating in the cholesterol synthesis, increased cholesterol excretion with the faeces and increased utilization of circulation cholesterol for the synthesis of the bacterial cell wall (Loh et al., 2014).

Conclusions

The present meta-analysis confirms that probiotics supplementation increases egg production of laying hens and alters eggs interior and exterior qualities such as Haugh unit, yolk colour, eggshell thickness and eggshell weight. Probiotics are also effective to decrease low-density lipoprotein cholesterol while increasing high-density lipoprotein cholesterol blood concentrations, so can increase health-promoting properties of poultry products. However, bacterial strains may result differently and therefore future studies in this area are needed.

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Conflict of interest

The authors declare that there is no conflict of interest.

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