The Effect of Dietary Probiotic Supplementation on Egg Weight in Laying Hens: A Meta–Analysis Study

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ABSTRACT

The aim of this study was to determine the effect of probiotic supplementation on egg weight in laying hens by using meta–analysis. The limitations of the eight studies –indexed in SCI– taken into the meta–analysis were 18 – 42 week old brown and white laying hens; 10⁷ – 10¹⁰ CFU/g Bacillus subtilis was used as a probiotic and the dose ranged between 400 – 1000 g/ton. The meta–analysis was carried out using (experimental–control) means for continuous data. As a result of the research, it was determined that all studies did not share a single common effect (heterogeneous). In addition, the overall effect size using the random effect model was calculated as 0.223. Based on z and p values, the hypothesis of the study was accepted (z = 2.90; p < 0.05). In other words, "the probiotic has a significant effect on egg weight in laying hens".

Keywords: Probiotic, Bacillus subtilis, egg weight, laying hens, meta-analysis

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INTRODUCTION

Due to the long-term genetic selection to improve economically important production characteristics such as meat and eggs, the birds' tolerance for negative environmental conditions decreased while yields increased, animal health and well-being were adversely affected. Probiotics have been used instead of therapeutic agents such as antibiotics to struggle with stress (Fathi et al. 2018) and diseases (Forte et al. 2016a) caused by intensive production methods (Kopp-Hollihan 2001). More commonly, the probiotics, containing beneficial and viable microorganisms that balance the gut microbiota are used as commercial feed additives in poultry feeds to improve growth rate and laying performance by increasing the utilization of feed (Fuller 1989). *Bacillus subtilis* is the most commonly used probiotic species in animal feeding (Simon et al. 2001). It was reported that the addition of *B. subtilis* to the laying hen's feed improve the balance of gut microbiota and intestinal absorption capacity (Abdelgader et al. 2013), enhance immune response (Zhang et al. 2012), decrease the cholesterol content in egg yolk (Sobczak and Kozlowski 2015) and promote bird growth (Lee et al. 2015). Although there are reports that the supplementation of probiotics in poultry feed has positive effects on egg weight, internal and external egg quality traits, these reports conflict with each other (Fathi et al. 2018) and do not demonstrate clearly the beneficial effect of probiotics. In such cases where different results can be found in different studies, meta-analysis provides a more general and excellent solution.

Meta-analysis is a statistical method that helps to combine qualitative and quantitative research results carried out on the same subject in different places, times and centers to reach a general conclusion (Boissel et al. 1989). As it looks at special aspects rather than relies solely on judgment, meta-analysis uses quantitative methods and this differentiates it from the classic reviews in literature (Mosteller and Colditz 1996). According to Yach (1990), meta-analysis is part of the re-examination process. Additionally, it deals with data analysis that draws results from the main study and uses quantitative methods to explain the heterogeneity of the results, calculating the combined overall impact. In brief, meta-analysis is a method of evaluating previous studies (Dawson et al. 1994).

The aim of meta-analysis is to achieve the most accurate quantitative results by combining the studies carried out via small samples and increasing the total sample range. Thus, a more reliable estimation of parameters is ensured and the inconsistencies that emerge in scientific literature can be evaluated.

Furthermore, this method, in which the size of the common (overall) effect is determined, turns small-scale omitted reports into effective and useful materials (Fitz-Gibbon 1985, Cohen and Manion 2001).

In the light of this information, the aim of this study was to determine whether the probiotic supplementation (*B. subtilis*) was effective on egg weight in laying hens by meta-analysis.

MATERIALS and METHODS

The following criteria were taken into consideration for eligibility and the studies selection in meta-analysis process, respectively.

1. Genotype 2. Hen age 3. Probiotic type 4. Probiotic dose 5. Database 6. Publication year range.

Within the framework of the study protocol, the limitations of the studies included into meta-analysis conducted in this study are as follows: Brown and white laying hens were in the age range of 18 – 42 – weeks; The probiotic type was *B. subtilis*; the dosage of *B. subtilis* was in the range of 400 – 1000 g/ton (400 – 1000 g per 1 ton feed) as $10^7$ – $10^{10}$ CFU/g. In meta-analysis studies, a database should be determined for the selection of the researches (Boissel et al. 1989, Yach 1990, Mosteller and Colditz 1996, Dawson et al. 1994). In this study, the Web of Science was determined as database. Studies have been published in the last 15 years in journals indexed at least SCI-exp. Reason for selecting articles indexed at SCI-exp was to set a certain restriction for search, and it was decided that there would be journals above a certain level of impact factor. The keywords searched according to the study protocol were laying hens, probiotic, *B. Subtilis* and egg weight. In this study, 37 studies were examined and 8 studies (Mahdavi et al. 2005, Xu et al. 2006, Zhang et al. 2012, Amani et al. 2013, Sobczak and Kozlowski 2015, Forte et al. 2016b, Mazanko et al. 2017, Hosseindoust et al. 2018) that met the above conditions were selected and subjected to meta-analysis. In these studies, the difference between experimental and control groups was examined and none of them were statistically significant. In this framework, the hypothesis tested by meta-analysis of the study is presented below: Hypothesis: The dietary probiotic supplementation (*B. subtilis*) has a significant effect on egg weight in laying hens.

In the study, there were no a confounding variables whose presence affects the variables being studied (or the confounding effects which may related to the basic hypothesis). This study is a meta-analysis study and it was carried out by calculating the effect size.
using means calculated for experimental–control groups in continuous data. Hedges g effect sizes for each study were calculated by equation 1, Cohen’s d effect sizes by equation 2 and Jacobian correction coefficient j by equation 3. Begg rank correlation, Egger regression methods and funnel plot were used to determine bias of studies. To determine the heterogeneity between studies, Cochran’s Q statistics (equation 4) were used and the heterogeneity criteria H, T2 and F were calculated (Cochran 1954, Hedges 1981, Cohen 1988, Begg and Mazumdar 1994, Egger et al. 1997, Higgins and Thompson 2002, Borenstein et al. 2009).

$$g = J \times d$$  \hspace{1cm} (1)

$$d = \frac{\bar{X}_1 - \bar{X}_2}{S_{within}}$$  \hspace{1cm} (2)

$$J = 1 - \frac{3}{4(n_1 + n_2 - 2) - 1}$$  \hspace{1cm} (3)

$$Q = \sum_{i=1}^{k} W_i ES_i^2 - \left( \frac{\sum_{i=1}^{k} W_i ES_i}{\Sigma_{i=1}^{k} W_i} \right)^2$$  \hspace{1cm} (4)

Since the heterogeneity between the studies was determined, the overall effect size was calculated by the random effect model. Because the variance between studies is also taken into account in the random effect model, variance and standard error corrections were made. The data were analyzed by Microsoft Excel and Comprehensive Meta-Analysis Trial Software.

**RESULTS and DISCUSSION**

In this study which examined the effect of *B. subtilis* on egg weight in laying hens by meta-analysis study, the effect size was calculated for each study and the obtained findings were given in Table 1. According to these results, it has been determined that the study of Sobczak and Kozłowski (2015) the smallest effect size (Hedges g = 0.040), while the study of Ammani et al. (2013) has the biggest effect size (Hedges g = 0.683). The effect sizes of other studies ranged around 0.20 as "low", 0.50 as "medium" and 0.80 as "high".

According to the total values shown in Table 1, Cochran’s Q statistics were calculated as 46.635 – (110.879) 2 / 502.216 = 22.155. This value was given in Table 2. The findings for the determination of heterogeneity among the studies were given in Table 2. According to Table 2, the heterogeneity among studies was determined with respect to 3 criteria (in the Cochran Q test, Q = 22.155 > 14.07 and p < 0.05; H = 2.881 – the H statistic does not contain confidence intervals 1--; T2 > 0). Moreover, F statistics showed that there is medium level heterogeneity with 65.285%. On the other hand, the results of Begg’s rank correlation method (z = 0.521; p = 0.602) and Egger’s regression test (t = 0.149; p = 0.886) showed that there was no publication bias for studies. Also, funnel plot regarding publication bias was given in Figure 1.

As the heterogeneity between studies was determined from the findings in Table 2, the random effect model was used instead of the fixed effect model in the general effect size calculations. In the random effect model, the total variance ($V_{ES}$) of the studies is the sum of the variance within the studies and the variance between the studies. Weight is $W = 1 / V_{ES}$. Total values used for calculation of the overall effect size in the random effect model were given in Table 3.

The overall effect size using the random effect model was calculated as $M = 32.69 / 146.26 = 0.223$ from the formula $M = \Sigma(W*ES) / \Sigma W$. The findings of the overall effect size were given in Table 4. Here, the estimated variance and standard error of the overall effect size was found from the formulas $V_M = 1 / \Sigma W$ and $V_M = (SE_{50})^2$. According to the results in Table 4, the overall effect size was found as 0.223. Based on z and p values, the hypothesis of the study was accepted (z = 2.90; p < 0.05). In other words, "the probiotic has a significant effect on egg weight in laying hens". This effect can be defined as the "low impact" in the effect size classification of Cohen (1988). Because, Cohen (1988) made classified effect size around 0.20 as "low", 0.50 as "medium" and 0.80 as "high".

There are some studies reporting that probiotics do or do not affect (Panda et al. 2003, Zarei et al. 2011, Sheoran et al. 2018) egg weight (Mahdavi et al. 2005, Kalavathy et al. 2009). A similar inconsistent status is also available for internal and external quality traits of eggs (Zhang et al. 2012, Youssef et al. 2013). While Mahdavi et al. (2005) showed the effect on albumin quality, Xu et al. (2006) proved increasing egg weight. On the other hand, Sobczak and Kozłowski (2015) and Hosseindoust et al. (2018) did not find the results statistically significant. These results show that using probiotics is effective on egg weight and other traits but this could not be clearly demonstrated.

In each of the 8 studies that were included into this study, the number of laying hens used ranged from 20 to 300 and the differences between experimental and control groups were not statistically significant for all studies. So, there were 1154 laying hens in total for each group in the meta-analysis. The average rate of
increase in egg weight was about 2% in 8 studies. However, this increase was not statistically significant when considered for a single study because the sample size was not sufficient for statistical significance. In other words, statistical significance is affected by the sample size. This increase ratio (2%) becomes statistically significant as more samples are used, since the studies are combined with meta-analysis.

A meta-analysis diagram of the random effect model was shown in Figure 2. The effect sizes and relative weights of each study, overall effect size and Forest graph were given in Figure 2. The square shape "■" in the forest graph indicates the effect size, the size of the squares of the relative weight of the studies, the width of lines in the 95% confidence interval while diamond shape "♦" indicates the overall effect size of each study. The relative weight is the percentage of the study weight and the highest relative weight (17.97%) was calculated for the study done by Forte et al. (2016b).

Figure 1. Funnel plot regarding publication bias

| Studies             | ES    | RW %  |
|---------------------|-------|-------|
| Mahdavi et al.      | 0.177 | 5.35  |
| Xu et al.           | 0.256 | 16.39 |
| Zhang et al.        | -0.067| 16.59 |
| Ammari et al.       | 0.683 | 7.69  |
| Sobczak and Kozlowski | 0.040 | 15.17 |
| Forte et al.        | 0.420 | 17.97 |
| Mozuno et al.       | 0.393 | 11.38 |
| Hosseindoust et al. | 0.047 | 9.46  |
| Overall             | 0.223 |       |

RW: Relative weight

Figure 2. A meta-analysis diagram of the random effect model
### Table 1. The effect size for each study

| Studies                     | Cohen's d | J     | Hedges g | W      | W*ES  | W*ES² |
|-----------------------------|-----------|-------|----------|--------|-------|-------|
| Mahdavi et al. (2005)       | 0.180     | 0.980 | 0.177    | 10.367 | 1.834 | 0.324 |
| Xu et al. (2006)            | 0.256     | 0.998 | 0.256    | 95.593 | 24.452| 6.254 |
| Zhang et al. (2012)         | -0.067    | 0.998 | -0.067   | 100.321| -6.747| 0.454 |
| Ammani et al. (2013)        | 0.690     | 0.989 | 0.683    | 17.359 | 11.855| 8.096 |
| Sobczak and Kozłowski (2015)| 0.040     | 0.997 | 0.040    | 72.365 | 2.880 | 0.115 |
| Forte et al. (2016a/2016b)  | 0.421     | 0.999 | 0.420    | 147.123| 61.812| 25.970|
| Mazanko et al. (2017)       | 0.395     | 0.995 | 0.393    | 34.706 | 13.650| 5.368 |
| Hosseindoust et al. (2018)  | 0.047     | 0.992 | 0.047    | 24.382 | 1.143 | 0.054 |
| **Total**                   |           |       |          | 502.216| 110.879| 46.635|

ES: Effect Size; W: Weight

### Table 2. The findings for the determination of heterogeneity or homogeneity among studies

| Methods                  | Parameters | df | Chi square table value | P        |
|--------------------------|------------|----|------------------------|----------|
| Cochran's Q test         | 22.155     | 7  | 14.07                  | < 0.05   |

95% Confidence Interval

| Method | Min. | Max. |
|--------|------|------|
| H      | 2.881| 2.456|
| $T^2$  | 0.031| 0.018|
| $I^2$  | 65.285| 52.189|

Begg’s rank correlation $z = 0.521$; $P = 0.602$ and Egger's regression test $t = 0.149$; $P = 0.886$ for publication bias

### Table 3. Total values used for the overall effect size in the random effect model

| Studies                      | Hedges g (ES) | SEES | VES | W  | W*ES |
|------------------------------|---------------|------|-----|----|------|
| Mahdavi et al. (2005)        | 0.177         | 0.357| 0.128| 7.830| 1.385 |
| Xu et al. (2006)             | 0.256         | 0.204| 0.042| 23.971| 6.132 |
| Zhang et al. (2012)          | -0.067        | 0.203| 0.041| 24.258| -1.632|
| Ammani et al. (2013)         | 0.683         | 0.298| 0.089| 11.253| 7.685 |
| Sobczak and Kozłowski (2015) | 0.040         | 0.212| 0.045| 22.185| 0.883 |
| Forte et al. (2016a/2016b)   | 0.420         | 0.195| 0.038| 26.279| 11.041|
| Mazanko et al. (2017)        | 0.393         | 0.245| 0.060| 16.647| 6.547 |
| Hosseindoust et al. (2018)   | 0.047         | 0.269| 0.072| 13.837| 0.649 |
| **Total**                    |               |     |     | 146.260| 32.690|

### Table 4. The findings of overall effect size

| Model                  | Overall effect size | V_M | SE_M | 95% Confidence Interval | Min. | Max. | z value | P     |
|------------------------|---------------------|-----|------|-------------------------|------|------|---------|-------|
| Random Effects Model   | 0.223               | 0.006| 0.077|                         | 0.073| 0.374| 2.90    | < 0.05|
CONCLUSION

Eight studies were investigated by meta-analysis and the sample size was a total of 1154 laying hens for each group (experimental and control groups) in the study. It was detected that using probiotics had an effect on egg weight and thus, a more general result was obtained. As a matter of fact, it was possible to encounter different findings in the literature. These results emphasized once again the importance of working with larger samples gives a more realistic result.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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