Effect of Dietary Multi-Strain Probiotic Supplementation on Egg Quality in Laying Hens

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Abstract
This work was designed to investigate whether inclusions of probiotic to drinking water during laying period improves egg quality. Total 100 Lohman brown classic hens were divided to 4 groups: control (n=25) birds were fed a diet without additive), other treatment birds were fed with diets containing probiotic preparation in liquid form at of 8.5 ml (n=25), 10 ml (n=25) and 11.5 ml (n=25) per 20 l of drinking water during laying period. Active ingredients of additive were *Lactococcus lactis* 6.0×10^9 CFU/g, *Lactobacillus casei* 6.0×10^9 CFU/g, *Lactobacillus plantarum* 6.0×10^9 CFU/g, *Carnobacterium divergens* 6.0×10^9 CFU/g and *Saccharomyces cerevisiae* 6.0×10^7 CFU/g. A sample of 60 eggs from each group was collected randomly to determine egg quality every 30 days. There were significant effect of probiotic addition on egg weight, albumen index, Haugh units, yolk weigh, yolk % and yolk colour ($P<0.05$). Eggshell quality for hens supplemented with probiotic was not different but was greater than for control hens ($P>0.05$). Supplementation of probiotic during the laying period decreased albumen % ($P<0.05$). We can conclude that laying hens with higher doses of probiotics in drinking water achieved a higher effect in egg quality.

Key words: egg, egg quality, laying hen, probiotic, yeast.

1. Introduction

Probiotics are defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [1].
Probiotics are an attractive feed additive, and this new technology is addressing the challenges of both cost and efficacy [2].
Probiotics have been shown to improve feed utilization, reduce the prevalence of disease and improve digestion and absorption, reducing the pathogen load, regulating the immune system and generally improving the gastrointestinal tract (GIT) microbiome. Furthermore, being safe and natural, probiotics do not risk the well-being of poultry or consumers with on-going use [3-6].
Different types of bacteria have been included in different brands of probiotics some of which include; *Escherichia*, *Prevotella*, *Streptococcus*, *Clostridium*, *Enterococcus*, *Bacillus* and *Lactobacillus* species [7-9].
The type of bacteria used has an impact on the effect that particular probiotic has on the poultry [10].

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Probiotics increased egg laying performance and quality and improved feed conversion ratio (FCR) and meat quality [11].
Administration of probiotics to laying hens had a positive effect on hen performance, increasing eggshell thickness, eggshell strength, and albumen height [12].
The aim of this study was to determine the effect of inclusion of various doses of multi-strain probiotics in drinking water on external and internal egg quality.

2. Materials and methods

2.1 Animal management and treatments

The hens were allocated to its own covered shelters with straw litter and with access to a grass paddock; feeders and drinkers were available both outdoors and indoors. Hens were placed at stocking density of 8 birds per m^2.
The environmental temperature was around 20 to 22 °C and the relative humidity varied from 65 to 75%. Artificial lighting was carried out in a pattern of 18 hours of light alternating with 6 hours of darkness. The ventilation in the house was natural with additional fans in order to ensure a minimum air exchange per hour.
Total 100 Lohman brown classic hens were divided to 4 groups: control (n=25) birds were fed a diet without additive, other treatment birds were fed with diets containing multi-strain probiotic preparation in liquid form at of 8.5 ml (n=25), 10 ml (n=25) and 11.5 ml (n=25) per 20 l of drinking water during laying period.
The hens in all treatments were fed commercial feed mixture (Bonamix Ltd., Gemerská Panica, Slovak Republic). Nutritional value of diet is shown in Table 1. The hens had available drinking water and feed mixture *ad libitum*.
The multi-strain preparation (JHJ, Poland) contained probiotic strains *Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 with minimum dose of $5x10^8$ colony forming unit (CFU) lactic acid bacteria (LAB)/g and *Saccharomyces cerevisiae* KKP 2059p with minimum dose of $5x10^6$ CFU/g.

2.2 Egg quality parameters and analytical procedures

A sample of 60 eggs from each group of Lohmann Brown classic was collected randomly to determine egg quality every 30 days. The collected eggs were kept under room temperature and egg quality was determined within 24 hours of collection.
The individual egg weight was measured using a electronic scale Owa Labor (VEB Wägetechnik Rapido, Germany) with an accuracy of 0.01 g. The egg length and width were detected using digital calliper (Insize Ltd., China) with an accuracy of 0.01 mm. Based on the determined length (L) and width (W) of the egg, we calculated egg shape index (SI) according to the formula SI=(W/L)*100 [13].
After breaking the egg and removing the yolk and albumen, the eggshell was washed under running tap water to remove any remaining any albumen fragments. Subsequently, eggshell naturally dried for 48 hours. eggshell weight (with membrane) was measured using electronic scale Owa Labor (VEB Wägetechnik Rapido, Germany) with an accuracy of 0.01 g. Eggshell thickness was measured in three locations on the egg (sharp pole, equator part, blunt pole) with a digital Thickness Gauge (Bröring Technology, Germany) and the mean values represented the eggshell thickness [14]. The eggshell strength was determined manually using an Egg Crusher device (VEIT Electronics, Czech Republic).
The albumen weight was calculated as the difference among the egg weight and the yolk and eggshell weights. We determined albumen index (%) by Alkan et al. [15] as the ratio of the thick albumen height (mm) to the average of width (mm) and length (mm) of thick albumen by using a digital calliper (Insize Ltd., China) with an accuracy of 0.01 mm. Haugh units was calculated according to the procedure of Haugh as log (height albumen − 1.70 × egg weight$^{0.37}$ + 7.60)*100 [16].
Yolk weight with 0.01 g accuracy was determined using an electribuc scale Owa Labor (VEB Wägetechnik Rapido, Germany) with an accuracy of 0.01 g and its percentage proportion was calculated. Yolk index (%) was measured as ratio of the yolk height (mm) to the yolk width (mm) by Funk [17] using digital calliper (Insize Ltd., China) with an accuracy of 0.01 mm. Yolk

colour was determined visually using La Roche Yolk Colour Fan (Hoffman–La Roche, Switzerland). with colour scores ranging from 1 (the light yellow) to 15 (the dark yellow) [18].
The proportion of eggshell, yolk and albumen relative to egg weight was expressed as eggshell or albumen or yolk weight/egg weight*100 [19].

2.3 Statistical analysis

Data were evaluated using a one-way analysis of variance (ANOVA) procedure of statistical program JASP 0.8.6 (2018) [20] followed by comparison among means using the Duncan's multiple-range test [21] and differences were considered at P<0.05.

3. Results and discussion

3.1 Egg quality parameters

It was observed that there were significant (P<0.05) differences among experimental groups and control with respect to egg weight (Table 2). Our results are consistent with other authors [22-25] who reported a significant increase in egg weight compared to control when layer diet was supplemented with probiotics.
Additionally, probiotics inclusion in layer diets did not have a significant influence on the egg weight. These contrasting reports may be due to the difference in probiotic dosages administered and the bacterial concentration used in the diet offered [26].
Egg shape index was not affected (P>0.05) by addition of multi-strain probiotic in different doses to layer hen diet (Table 2). Our results are in agreement with those reported by Hrnčár et al. [27] who found insignificant effects of probiotic on egg shape index.

3.2 Eggshell quality parameters

The inclusion of multi-strain probiotic had no significant effect (P>0.05) on eggshell weight (Table 3). Similar to the current study, several authors have reported no effect of probiotic supplementation on eggshell weight [26,28,29-31]. In contrast, Fathi et al. [23] reported an increase in eggshell weight by probiotics was supplemented in layers diet.
The effect of inclusion of multi-strain probiotic on eggshell % is shown in Table 3. There was no significant treatment effect (P>0.05). Fathi et al. [23] reported a significant increase in contribution of eggshell to egg weight when probiotics were incorporated in layers' diet. Milkuski et al. [34] reported a significant higher eggshell % when dietary single strain probiotic was supplemented in layers' diet. In addition, Ray et al. [33] reported a significantly higher eggshell % both single and multi-strain probiotic application. Several studies have reported no effect of probiotic supplementation on eggshell % [28,37,38].
The addition of multi-strain probiotic had no significant effect (P>0.05) on eggshell thickness (Table 3). Bidura et al. [32] reported a significant increase in eggshell thickness when *Saccharomyces* spp. probiotic was incorporated in hens' diet respectively. Ray et al. [33] reported a significant increase in eggshell thickness in both single strain and multi-strain probiotic treated groups compared to control. In addition, Fathi et al. [28] and Mikulski et al. [34] reported a significant increase on eggshell thickness in probiotic supplemented groups. Other studies have reported no effect of probiotic supplementation on eggshell thickness [22,35,36].

3.3 Albumen quality parameters

Results of the present study indicated that the addition of multi-strain probiotic to drinking water affected albumen index and Haugh units (P<0.05) compared to control counterparts. The albumen weight was not fundamentally affected by the addition of the probiotic. Inclusion of multi-strain probiotic prep rate decrease albumen percentage (P<0.05) (Table 4).
Probiotics supplementation in the diet of laying hen increases the Haugh units [30]. Jha et al., [39] conducted a study with commercial probiotics and reported an increase in the albumen height and a greater Haugh units. Multi-strain probiotics improved the protein quality of laying hen eggs by improving the albumin quality [40]. Additionally, Mikulski et al. [34] reported that probiotics inclusion in layer diet increases the crude protein content of the egg albumin which in turn improves it quality. On the contrary, probiotic had no effect on albumen height and Haugh units [12,41].

3.4 Yolk quality parameters

Addition of multi-strain probiotic in different concentrations significantly increased (P<0.05) yolk weight, yolk % and yolk colour (Table 5).

In a study conducted by Aalaei et al. [22] layer eggs yolk scores for both fed probiotic increased. Macit et al. [30] and Antara et al. [42] reported an increase in yolk colour in probiotic supplemented groups compared to control while Aalaei et al. [22]; Fathi et al. [23]; Marwi et al., [31]; Mikulski et al. [34] and Ray et al. [33] reported no effect of probiotic supplementation on yolk colour. Neijat et al. [26] reported a decrease in yolk colour by when probiotic was supplemented in layers' diet which was attributed to the temporal variation in the dose of probiotic used in the study. However, the improvement in the yolk colour may be accrued to the probiotic composition [41,43-45].

Table 1. Nutritional value of complete feed mixture

Nutrient	Unit	Feed mixture
Crude protein	g/kg	Min 153.00
Fat	g/kg	Max. 34.00
Ash matter	g/kg	Max. 125.00
Fibre	g/kg	Max. 60.00
Lysine	g/kg	Min. 7.00
Methionine	g/kg	Min. 3.50
Ca	g/kg	Min 35.00
P	g/kg	Min 5.00
Na	g/kg	Min 1.20
Fe	mg/kg	45.00
Cu	mg/kg	7.00
Mn	mg/kg	80.00
Zn	mg/kg	70.00
Se	mg/kg	0.15
I	mg/kg	0.70
Vitamin A	IU/kg	11000
Vitamin D_3	IU/kg	2500
Vitamin E	mg/kg	20.00
Vitamin K_3	mg/kg	2.00
Vitamin B_1	mg/kg	2.00
Vitamin B_2	mg/kg	5.00
Vitamin B_6	mg/kg	3.00
Vitamin B_{12}	mg/kg	0.015
Niacin amide	mg/kg	20.00
Calcium pantothenate	mg/kg	8.00
Biotin	mg/kg	0.05
Folic acid	mg/kg	0.50
Choline chloride	mg/kg	250.00
Betaine	mg/kg	100.00

Notes: CP=crude protein; Ca=calcium; P=phosphorus; Na=natrium; Cu=cooper; Fe=iron; Mn= manganese; Zn=zinc; Se=selenium; I=iodine; IU=International units

Table 2. Effect of different probiotic concentrations on egg parameters

Parameter	Control	Experimental 1	Experimental 2	Experimental 3
Egg weight (g)	62.87 ± 3.75^b	64.51 ± 3.82^a	64.94 ± 3.88^a	$64,92 \pm 3.89^a$
Egg length (mm)	65.59±3.95	65.68±3.96	65.87±3.99	65.79±3.98
Egg width (mm)	49.14±2.88	49.16±2.86	49.31±2.87	49.28±3.88
Egg shape index (%)	74.91±1.72	74.85±1.76	74.86±1.78	74.90±1.77

Values shown are mean ± SD (standard deviation)

^{a,b} means in a row with different superscript differ significantly (P<0.05)

Table 3. Effect of different probiotic concentrations on eggshell parameters

Parameter	Control	Experimental 1	Experimental 2	Experimental 3
Eggshell weight (g)	6.32±0.59	6.49±0.57	6.57±0.55	6.54±0.58
Eggshell percentage (%)	10.05±1.01	10.06±0.98	10.12±0.99	10.07±0.99
Eggshell strength ($N.m^{-2}$)	38.12±3.06	38.63±3.16	38.87±3.13	38.78±3.15
Eggshell thickness (µm)	438.88±33.17	441.07±35.24	441.76±35.78	440.65±35.11

Values shown are mean ± SD (standard deviation)

Table 4. Effect of different probiotic concentrations on albumen parameters

Parameter	Control	Experimental 1	Experimental 2	Experimental 3
Albumen weight (g)	38.89±3.21	38.72±3.11	38.38±3.09	38.39±3.10
Albumen percentage (%)	61.87±2.12^a	58.98±1.98^b	59.11±2.02^b	59.13±2.09^b
Albumen length (mm)	77.78±4.34	77.99±4.47	78.05±4.55	78.06±4.61
Albumen height (mm)	6.24±0.56	6.77±0.59	6.82±0.61	6.80±0.63
Albumen index (%)	8.02±0.34^b	8.68±0.33^a	8.73±0.29^a	8.71±0.31^a
Haugh unit	86.57±4.11^b	89.54±5.11^a	89.97±5.23^a	89.67±5.18^a

Values shown are mean ± SD (standard deviation)
^{a,b} means in a row with different superscript differ significantly (P<0.05)

Table 5. Effect of different probiotic concentrations on yolk parameters

Parameter	Control	Experimental 1	Experimental 2	Experimental 3
Yolk weight (g)	17.66±1.35^b	19.71±1.44^a	19.99±1.58^a	19,95±1.61^a
Yolk percentage (%)	28.09±1.11^b	30.55±1.19^a	30.79±1.23^a	30.73±1.21^a
Yolk length (mm)	19.25±1.45	19.58±1.61	19.67±1.67	19.66±1.64
Yolk height (mm)	39.78±2.37	39.51±2.34	39.55±2.31	39.54±2.29
Yolk index (%)	48.39±2.28	49.55±2.38	49.73±2.42	49.72±2.44
Yolk colour (°HLR)	8.98±0.88^b	10.21±1.01^a	10.38±1.02^a	10.33±1.01^a

Values shown are mean ± SD (standard deviation)
^{a,b} means in a row with different superscript differ significantly (P<0.05)

4. Conclusions

In conclusion, the finding of the current study has shown that the tested multi-strain probiotic with *Lactococcus lactis, Lactobacillus casei, Lactobacillus plantarum, Carnobacterium divergens* and *Saccharomyces cerevisiae* applied in drinking water was beneficial for some external and internal characteristics of table eggs.

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