Journal of Animal and Feed Sciences, 31, 3, 2022, 241–248 https://doi.org/10.22358/jafs/149388/2022
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Efficacy of the probiotic *Bacillus licheniformis* DSM 28710 in laying hens fed barley-sunflower meal-based diets on performance egg quality and excreta composition

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KEY WORDS: *Bacillus licheniformis*, egg production, egg quality, laying hens, probiotic

Received: 11 February 2022
Revised: 30 March 2022
Accepted: 22 April 2022

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ABSTRACT. The present study was conducted to determine and confirm the effect of dietary supplementation of *Bacillus licheniformis* DSM 28710 spores on productive performance, egg quality and nutrient excretion in laying hens. A total of 375 Lohmann Brown laying hens (initial body weight 1.70 kg), aged 25 to 44 weeks, were used in this study. Hens were randomly allotted to 1 of 3 dietary treatments: CON (basal diet), BL0.5 (basal diet plus 0.5 g/kg *B. licheniformis* DSM 28710) and BL1.0 (basal diet plus 1.0 g/kg *B. licheniformis* DSM 28710) consisting of 25 replicate cages, 5 hens each. Overall, the results of the present study demonstrated that supplementation of 0.5 g/kg *B. licheniformis* DSM 28710 significantly improved the feed conversion ratio and egg mass ($P < 0.05$) compared to control and 1.0 g/kg *B. licheniformis* DSM 28710. However, no significant differences in other performance parameters were observed between treatments ($P > 0.05$). Supplementation with different levels of *B. licheniformis* DSM 28710 was effective in improving egg quality by increasing shell thickness, Haugh unit ($P < 0.05$) and dirty egg percentage ($P < 0.05$). Moisture and protein contents of excreta were also significantly reduced by 0.5 g/kg *B. licheniformis* DSM 28710 supplementation ($P < 0.05$), while ash content was increased ($P < 0.05$). Overall, supplementation with *B. licheniformis* DSM 28710 at a dose of 0.5 g/kg provided a probiotic effect leading to improved egg mass, feed efficiency and egg quality, as well as lower protein content in excreta in Lohmann brown hens fed a barley and sunflower meal-based diet.

Introduction

In large-scale rearing facilities, poultry is often exposed to stressful conditions such as feed problems, diseases and suboptimal environmental conditions. These challenges can result in significant economic losses, and thus should be either avoided or effectively managed. To this end, multiple in-feed management tools are applied, including probiotics, i.e. viable micro-organisms that benefit the host's health when administered in adequate amounts. These advantages can affect the health, physiology or technical performance of the host and can be achieved, i.a. by improving intestinal structure, enhancing immunity against pathogens, increasing gut microflora stability, suppressing pathogen colonization and/or regulating gut colonisation by symbiotic bacteria (Callaway et al., 2008; Gaggia et al., 2010; Xiang et al., 2019). Several selected probiotics have been previously applied in poultry nutrition, e.g. species of *Lactobacillus*, *Streptococcus*, *Saccharomyces*, *Aspergillus* and *Bacillus*

(Tannock, 2001). Specifically for layers, earlier reports indicated that dietary supplementation with probiotics not only increased egg production, but also improved feed conversion efficiency, hen performance and egg shell quality (Mikulski et al., 2012; 2020).

The use of probiotic bacteria in poultry diets is not a new concept. However, probiotic strains differ in their properties and clinical effects they elicit, even when the strains belong to the same bacterial species. This also necessitates specific research using the probiotic strain of interest, including studies in target animal species. For example, although there are many studies involving *Bacillus licheniformis* in broilers, only limited research is available in laying hens (Upadhaya et al., 2019).

In addition, the experiments involving probiotics and specific diets, especially low quality feed ingredients for laying hens, are scarce. These components interact with the microbiota and chicken productivity, thus examining the efficacy of probiotic application in such diets would be extremely relevant. Feed cost is the largest item in poultry production and accounts for about 60–75% of the total production expenses. The prices of conventional feed ingredients such as corn and soybean meal are increasing and fluctuate depending on the season and production level. Additionally, the increasing competition between animal feed, human food and biofuel production must be taken into account (Singh et al., 2019). Currently, grains such as barley and wheat, as well as agricultural by-products such as sunflower meal (SFM), are used as alternative feedstuffs to reduce the production cost of laying hen feeds. However, these feed ingredients are inherently high in certain anti-nutritional factors, primarily non-starch polysaccharides (NSP). These NSPs contain β-1,4 or β-1,3 glucosidic links, which pose a challenge when present in poultry feeds, as chickens lack endogenous enzymes necessary to break this type of beta links, resulting in improper NSP digestion. Consequently, viscosity increases and nutrients become trapped in the digesta, thereby decreasing feed utilisation and ultimately process efficiency (Tiwari et al., 2018). Higher viscosity also slows down the gastric passage rate, which adversely affects feed intake and microbiological status of birds (Slominski, 2011). Additionally, the viscous nature of the digesta can lead to an increase in sticky droppings and wet litter, which may promote pathogen development, deterioration of animal welfare, increase in the number of dirty eggs and reduced air quality inside the poultry house (Bach Knudsen, 2014).

To date, to our knowledge, limited or no research has been conducted on the use of *B. licheniformis* in laying hens whose diets are based on barley and SFM at high inclusion levels. Therefore, the objective of the current study was to evaluate the effect of supplementing two different levels of a specific *B. licheniformis* strain (DSM 28710) on production performance, egg quality parameters and excreta nutrient composition in Lohmann Brown laying hens compared to the non-supplemented control, under commercial conditions, but with challenging feedstuffs. This work contributes to the available research on the application of specific probiotic strains in laying hens, while paying extra attention to the simultaneous use of challenging feed ingredients.

Material and methods

Birds and housing

The experimental protocol was approved by the Animal Use and Care Committee of Ankara University (2018-15-91).

A total of 375 Lohmann Brown hens from a commercial source (Evrenkaya Egg Company, Afyonkarahisar, Turkey) were subjected to a 7-week pre-experimental period, during which the hens were fed a commercial pre-lay diet containing 2750 kcal ME/kg, 17.0% crude protein, 2.25% calcium and 0.40% available phosphorus (supplied by Evrenkaya Egg, Afyonkarahisar, Turkey) until they reached 5% egg production. The birds had been previously vaccinated against infectious bronchitis and Newcastle disease virus. At 18 weeks of age, hens were relocated to a poultry research facility and placed in cages with a wire-mesh floor under controlled climate conditions (22–27 °C). Before the start of the experiment, all hens were given 3 weeks (22 to 25 weeks of age) to adapt to their new housing environment and fed a regular layer diet. Egg production and body weight (BW) were monitored during the pre-experimental period to be used in the trial set-up (see below). The proper study started at the hens' age of 25 to 44 weeks, during which they had free access to water via automatic nipple drinkers. Treatment diets differed as described below, but were all supplemented in the same way and amount per cage (110 g mash feed per hen per day via a common trough feeder). A photoperiod of 16 h of light and 8 h of darkness was applied (incandescent lighting, 10 lux).

Experimental design and diets

The study was conducted according to a completely randomised block design. At 25 weeks of age, hens were assigned to one of 3 dietary treatments based on their 3 weeks of pre-lay egg production and BW at 25 weeks, with 25 cage replicates, 5 hens each. The 3 groups were divided into one CON and 2 treatment groups. A basal diet based on barley and SFM without probiotic bacteria (Table 1) was assigned to the CON group. Two treatment diets (BL0.5 and BL1.0) were obtained by supplementing the basal diet with *B. licheniformis* DSM 28710 at a level of either 1.6×10^9 colony-forming units (CFU) or 3.2×10^9 CFU per kg feed. The test probiotic bacteria were B-Act®, containing the unique strain *B. licheniformis* DSM 28710 (supplied by HUVEPHARMA N.V.) and applied in either 0.5 (standard) or 1 g (double) B-Act®/kg addition to the diets, respectively. The basal diet was formulated considering the analysed nutrient (AOAC International, 2005) contents of raw materials to meet the peak nutrient requirements of Lohmann Brown hens at a daily intake of 110 g of feed (Table 1). During the 19-week experimental period, the mixed diets were stored for no longer than 10 weeks, hence two batches of the diets were produced throughout the experiment and stored in covered containers in a dry and well-ventilated storage room.

Table 1. Chemical composition of basal and treatment diets

Ingredients, g/kg	Basal diet
Barley	375.00
Corn	216.07
Sunflower meal	150.00
Soybean meal, 46% CP	89.40
Limestone	86.25
Soybean oil	59.93
Dicalcium phospate	12.96
Vitamin-mineral premix[1]	2.50
Salt	2.25
Sodium bicarbonate	1.93
L-lysine HCl, 78%	1.23
DL-methionine	1.07
L-threonine	1.00
Cholin chloride	0.40
Total	1000

Calculated and analysed nutrient composition[2]

Nutrients	Unit	CON	BL0.5	BL1.0
Dry matter	%	90.65 (90.95)	(90.87)	(90.79)
Metabolisable energy	kcal/kg	2775 (2741)	(2734)	(2729)
CP	%	15.5 (15.98)	(15.83)	(15.68)
Crude fat	%	7.86 (7.84)	(7.93)	(7.64)
Crude fiber	%	5.24 (5.66)	(5.49)	(5.41)
Crude ash	%	12.5 (12.34)	(12.49)	(12.77)
Soluble NSP	%	3.35		
Insoluble NSP	%	8.02		
Calcium	%	3.73		
Phosphorus available	%	0.35		
Sodium	%	0.17		
Chloride	%	0.23		
Digestible lysine	%	0.62		
Digestible Met + Cys	%	0.56		
Digestible arginine	%	0.93		
Digestible threonine	%	0.53		
Digestible leucine	%	1.00		
Digestible isoleucine	%	0.53		
Digestible valine	%	0.62		

CON – control, BL0.5 – control + 0.5 g/kg *B. licheniformis*, BL1.0 – control + 1.0 g/kg *B. licheniformis*; CP – crude protein, NSP – non-starch polysaccharides; [1] vitamin-mineral premix per kilogram of the complete diet: IU: vit. A 10 000, vit. D 2 500, vit. E 30; mg: menadione 3, thiamine 1.5, riboflavin 6, pyridoxine 4, vit. B_{12} 0.02, niacin 30, pantothenic acid 10, folic acid 0.6, biotin 0.05, copper 10, iron 30, manganese 100, iodine 0.8, zinc 60, selenium 0.3, calcium carbonate 500, ethoxyquin 0.63, wheat middlings 3 773; [2] paranthesis indicates the results of chemical analysis

Analyses and measurements

Feed samples were collected at the beginning and at the end of the study for microorganism recovery analysis using the manufacturer's proprietary assay (Biovet JSC, Pesthera, Bulgaria). The analysed content of *B. licheniformis* DSM 28710 in B-Act®, batch number 18011717002, was 3.2×10^9 CFU/g and the recovery results of treatment feeds were presented in Table 2.

Table 2. *Bacillus licheniformis* counts in experimental feeds

	CON, CFU/g feed	BL0.5, CFU/g feed	BL1.0, CFU/g feed
Expected	0	1.6×10^6	3.2×10^6
Analysed	$<5 \times 10^2$	1.5×10^6	3.2×10^6

CON – control, BL0.5 – control + 0.5 g/kg *B. licheniformis*, BL1.0 – control + 1.0 g/kg *B. licheniformis*; CFU – colony-forming unit

The main ingredients and experimental diets were analysed for proximate (AOAC International, 2005) amino acid and N-corrected apparent metabolisable energy content using near-infrared reflectance spectroscopy (Evonik Nutrition & Care GmbH, Hanau, Germany; Table 1).

Eggs were collected daily and classified as normal (intact egg with a clean shell and without visual cracks), dirty (intact egg with blood spots or faeces on its shell) and unmarketable (visually cracked or shell-less egg). The egg collection area, the interior of cages and trays under the cages were thoroughly checked for shell-less eggs. Hen-day egg production and dirty egg % were calculated on a weekly basis. The productions of cracked, shell-less and unmarketable eggs were calculated on a weekly and

cumulative basis. Within a cage, the weekly cumulative hen-day production was calculated using the following formula: weekly cumulative hen-day production = (total unmarketable eggs, cracked eggs or shell-less eggs laid up to a given week) / (five hens × total days up to a given week) × 100%. Egg weights (EW) were determined weekly by weighing all laid eggs from one day for each replicate. Feed was provided to each cage by adding 550 g of the appropriate treatment diet into cage feeders every morning from 8.30 to 9.30. Residual feed left in the feeders was measured at 2-week intervals to determine daily feed intake (DFI) per hen. Feed conversion ratio (FCR) was calculated as kg of eggs laid per kg of feed. Egg mass (EM) (EM = egg production percentage × average egg weight) was also calculated to better evaluate the overall hen performance. Hens were weighed individually at 25, 35 and 44 weeks of age. Mortality was recorded daily.

Egg quality parameters were also evaluated. A minimum of 100 eggs per treatment were assessed by collecting eggs from each replicate biweekly (on the same day of the week) and analysing egg quality characteristics within 24 h after collection. Egg shell thickness was measured after peeling off the membrane from under the shell with a Mitutoyo digital micrometre gauge (digital 395 series with a sensitivity of 0.001 mm; Kawasaki, Japan) at three locations from the equatorial region of each egg (broad, equatorial and sharp end), and presented as an average value. Egg shell breaking strength, egg albumen height and Haugh unit were measured using the Futura 3/A egg quality measuring system (Futura, Lohne, Germany). Yellowness of egg yolk was determined using the Roche Colour Fan Scale (DSM Nutritional Products Ltd., Basel, Switzerland).

Excreta samples were collected by hand from at least 10 different locations of the manure belt under each cage and placed in a Whirl-Pak® bag (Koroplast Company, İstanbul, Turkey), ensuring that any feathers or feed particles were removed. Samples were pooled for each replicate and stored at −20 °C until further analysis. The pooled excreta samples were homogenized and 100-g sub-samples were weighed out into aluminium trays. They were subsequently oven dried at 60 °C for 24 h, after which the dried excreta samples were thoroughly ground and analysed for dry matter, ash, and nitrogen contents (AOAC International, 2005).

Statistical analysis

All data generated in the present study were analysed by ANOVA using general linear models (GLM) of MINITAB®18 software (Minitab Ltd., Coventry, UK) in a randomised complete block design, with cage defined as a replicate experimental unit. Mortality data were subjected to a chi-square test. Probability values less than 0.05 were considered significant and differences between treatments were separated by Duncan's multiple range test. The data were expressed as the means ± standard error of the mean (SEM).

Results

Dosing of *B. licheniformis* either in the BL0.5 or BL1.0 diet (equal to 1.6 and 3.2 × 10^9 CFU of *B. licheniformis* DSM 28710/kg feed, respectively) had no significant impact on EP, EW and DFI, as shown in Table 3 ($P > 0.05$). However, BL0.5 treatment significantly improved EM output and FCR compared to the CON diet (59.48 vs 60.70% and 1.847 vs 1.805, respectively) (Table 3; $P < 0.05$).

Table 3. Effects of *Bacillus licheniformis* strain DSM 28710 supplementation in laying hen diets on average performance and egg quality in the peak period from 25 to 45 weeks of age

Parameters	CON	BL0.5	BL1.0	*P*-value
Performance parameters				
EP, %	90.2 ± 0.80	91.9 ± 0.67	90.7 ± 0.78	0.120
EW, g	66.0 ± 0.40	66.1 ± 0.36	65.5 ± 0.33	0.226
EM, g	59.5 ± 0.69^{b}	60.7 ± 0.55^{a}	59.4 ± 0.48^{b}	0.010
DFI, g	109.9 ± 0.14	109.6 ± 0.06	109.8 ± 0.34	0.746
FCR, g:g	1.847 ± 0.023^{a}	1.805 ± 0.017^{b}	1.849 ± 0.016^{a}	0.013
mortality, %	5.60 ± 2.17	4.00 ± 1.63	5.60 ± 2.45	0.538
IBW, kg	1.714 ± 0.013	1.711 ± 0.013	1.703 ± 0.01	0.874
FBW, kg	1.869 ± 0.016	1.855 ± 0.013	1.876 ± 0.017	0.291
BWG, kg	0.154 ± 0.018	0.144 ± 0.014	0.172 ± 0.020	0.361
External egg quality parameters				
CE, %	1.66 ± 0.62	1.29 ± 0.35	2.29 ± 0.68	0.331
ESW, g	6.74 ± 0.04^{ab}	6.83 ± 0.07^{a}	6.60 ± 0.07^{b}	0.048
ESR, %	9.97 ± 0.05	10.14 ± 0.06	9.94 ± 0.12	0.082
ST, mm	0.389 ± 0.002^{b}	0.393 ± 0.003^{a}	0.385 ± 0.003^{b}	0.050
ESBS, N	42.15 ± 0.82	43.18 ± 0.68	41.31 ± 0.86	0.119
Dirty eggs, %	8.97 ± 0.95^{a}	7.44 ± 0.67^{b}	5.02 ± 0.64^{c}	0.000
DYE, %	3.43 ± 0.85	2.79 ± 0.51	1.84 ± 0.50	0.841
Internal egg quality parameters				
Haugh unit	80.6 ± 0.80^{b}	82.0 ± 0.96^{a}	80.4 ± 0.76^{b}	0.044
AH, mm	6.94 ± 0.11	7.11 ± 0.13	6.88 ± 0.10	0.167
YC	11.16 ± 0.06	10.93 ± 0.07	11.01 ± 0.07	0.065

CON – control, BL0.5 – control + 0.5 g/kg *B. licheniformis*, BL1.0 – control + 1.0 g/kg *B. licheniformis*; EP – egg production, EW – egg weight, EM – egg mass, DFI – daily feed intake, FCR – feed conversion ratio, IBW – initial live body weight, FBW – final live body weight, BWG – body weight change, CE – cracked eggs, ESW – egg shell weight, ESR – egg shell ratio, ST – shell thickness, ESBS – egg shell breaking strength, DYE – double yolk eggs, HU – haugh unit, AH – albumen height, YC – yolk colour; number given according to the Roche Yolk Colour Fan; data are expressed as means ± standard error of the mean (SEM), ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

There was no significant effect of dietary supplementation of basal ration with the probiotic on body weight gain (BWG) of hens ($P > 0.05$). All experimental birds subjected to 3 different dietary treatments gained weight during the trial period (Table 3). Regarding egg quality parameters, both the BL0.5 and BL1.0 probiotic treatments significantly reduced the number of dirty eggs ($P < 0.05$) (Table 3), while only BL0.5 significantly increased shell thickness and Haugh unit compared to CON ($P < 0.05$). A significant effect of the *B. licheniformis* probiotic DSM 28710 was neither observed on the total count of cracked or double-yolked eggs nor on egg shell braking strength, albumen height and yolk colour.

Mortality was not significantly affected by the treatments ($P > 0.05$). In total, 7 hens died in the CON group, while 5 and 7 hens died (Table 3) in the BL0.5 and BL1.0 ($P > 0$ 05) groups, respectively. The cause of death was unknown in any of the cases, with no apparent lesions or diseases present.

Finally, both levels of *B. licheniformis* DSM 28710 supplementation significantly reduced excreta moisture content. *B. licheniformis* DSM 28710 at a dose of 0.5 g/kg also significantly decreased protein levels, while the excreta ash content increased significantly ($P < 0.05$) (Table 4).

No significant dose-response effect was observed for these parameters when the probiotic dose was increased. The improved egg mass and FCR obtained with the application of probiotic *B. licheniformis* DSM 28710 were highly consistent with previous studies on the use of probiotics in laying hens, including Mohan et al. (1995), Lei et al. (2013), Park et al. (2016) and Mikulski et al. (2020). These researchers also reported significant improvements in egg production and feed utilisation with the supplementation of *Lactobacillus, B. licheniformis, Enterococcus faecium* and *Pediococcus acidilactici*, respectively. Park et al. (2016) found that probiotic *Enterococcus faecium* improved energy and nitrogen utilisation in ISA Brown laying hens, potentially leading to a significant increase in egg production. Similarly, the consumption of *B. licheniformis* DSM 28710 in the present study may have contributed to enhanced performance by improving nutrient digestibility (Park et al., 2016), maintaining a beneficial gut microbiota and promoting proper intestinal integrity (Xiang et al., 2019). This hypothesis is further supported by the relationship between the probiotic and its effect on NSP fractions of the diet, as detailed below (see excreta moisture content). In contrast to the results of the present work, other studies in the available literature reported no effect of

Table 4. Effects of *Bacillus licheniformis* strain DSM 28710 supplementation in laying hen diets on excreta moisture (fresh sample %), protein and ash content (DM %)

Treatments	Moisture (initial)	Moisture (final)	Moisture reduction from start to end	Manure protein (final)	Manure ash (final)
CON	80.2 ± 0.61	75.6 ± 0.38	4.5 ± 0.76^{b}	24.1 ± 1.34^{a}	21.4 ± 0.83^{b}
BL0.5	80.8 ± 0.55	75.4 ± 0.43	5.3 ± 0.77^{a}	20.5 ± 0.98^{b}	24.4 ± 0.81^{a}
BL1.0	80.7 ± 0.50	74.7 ± 0.48	5.9 ± 0.57^{a}	22.7 ± 1.25^{ab}	22.8 ± 0.75^{ab}
P-value	0.690	0.290	0.047	0.050	0,025

CON – control, BL0.5 – control + 0.5 g/kg *B. licheniformis*, BL1.0 – control + 1.0 g/kg *B. licheniformis*; the data were expressed as the means ± standard error of the mean (SEM), ^{ab} – means within a column with different superscripts are significantly different at $P < 0.05$

Discussion

The analysed nutrient content and *Bacillus* count in the treatment feeds indicated that both the levels of expected crude nutrients and probiotic bacteria were consistent with the study design (Table 1 and 2), demonstrating that the dietary objective was achieved.

With regard to technical performance, the present study showed that supplementing *B. licheniformis* DSM 28710 at a dose of 1.6×10^9 CFU/kg feed significantly improved egg mass production and FCR compared to hens fed only the basal diet. probiotic supplementation on technical performance parameters (Mahdavi et al., 2005; Forte et al., 2016; Upadhaya et al., 2019). The aforementioned studies evaluated genetically different laying hens and different probiotic bacterial strains, including *B. subtilis* and other strains of *B. licheniformis*. Therefore, variation in the results might be explained by differences in study design and/or external factors, including feed composition, age of animals, probiotic inclusion levels and environmental factors (Corduk et al., 2008; Park et al., 2016). This reiterates the importance of conducting strain-specific studies when evaluating a probiotic in a specific target species.

In terms of egg parameters, supplementing *B. licheniformis* DSM 28710 at a dose of 1.6 × 10^9 CFU/kg feed significantly improved egg shell thickness and Haugh unit. When the dose was increased to 3.2 × 10^9 CFU/kg feed, a significant reduction was recorded in the number of reduced dirty eggs. The improvements in egg shell thickness following probiotic supplementation could be attributed to increased utilisation of nutrients, including calcium (Abdelqader et al., 2013). Similarly to our observations, Park et al. (2016) and Fathi et al. (2018) showed that the thickness of egg shell was increased when *E. faecium* and *B. subtilis* were supplemented, respectively. This was also confirmed by Lei et al. (2013), who examined a different probiotic strain of *B. licheniformis* in layers and noted that egg shell thickness and strength significantly improved compared to the non-supplemented control and that this effect was dose-dependent (0.01% up to 0.09% *B. licheniformis*). In the present study, no other significant effects of *B. licheniformis* DSM 28710 supplementation on the evaluated egg parameters were recorded; this was in line with the findings of Guo et al. (2017) regarding albumen height and Zhang and Kim (2013) with respect to yolk colour when laying hens' diet was supplemented with *Bacillus*-based probiotic.

Dietary inclusion of *B. licheniformis* DSM 28710 was found to be effective in significantly reducing the moisture content of manure, showing an overall moisture reduction of almost 30%. This decrease was consistent with the significant decline in the number of dirty eggs after probiotic supplementation, as the amount of wet litter logically affects the count of dirty eggs (Table 4). One of the main objectives of the present study was to investigate the relationship between probiotic supplementation and the digestive capacity of animals with respect to diets rich in water soluble NSP (wsNSP) and insoluble NSP (wiNSP) based on barley and SFM. In the current experiment, trial diets were formulated with wiNSP and wsNSP levels of 8.06 and 3.35%, respectively, which was almost 50% higher compared to standard corn-soybean meal-based diets. The use of cereals rich in wsNSP such as rye, barley and wheat have been associated with litter problems, especially in terms of an increased amount, stickiness and water content of excreta (Francesch and Brufau, 2004). Roberts et al. (1998) compared the effect of different cereals, including sorghum, barley, wheat and triticale on excreta moisture content in laying hens, and found that barley diets caused the wettest litter. This was most likely due to the high wsNSP content in these cereals, which affected viscosity. High molecular weight wsNSP, such as β-glucans, when present in large amounts in barley and sunflower meal are responsible for increased intestinal viscosity, which slows down nutrient migration and absorption. Immerseel et al. (2004) proposed that enteric infections, such as necrotic enteritis, were associated with wsNSP present in rye and barley, which leave undigested nutrients for pathogenic microbial propagation. The above leads to an increased water consumption, and consequently high faecal moisture in birds fed barley, rye or wheat. Previous studies reported that *B. licheniformis* produced a variety of biologically active substances, such as digestive enzymes (Luise et al., 2022), which could possibly be associated with significant reductions in digesta viscosity. Latorre et al. (2015) also found that the inclusion of selected *Bacillus* strains to poultry diets *in vitro* resulted in the presence of extracellular enzymes, depending on the type of the diet (rye, wheat, barley and oat based-diets), and thus a significant reduction in digesta viscosity. The hypothesis that *B. licheniformis* DSM 28710 improved digestion was also supported by the significantly reduced manure protein content observed in the present study (20.5% in the probiotic group compared to 24.1% in control; Table 4). This marked reduction could reflect better protein utilisation in the probiotic group, which may also have contributed to significant improvements in egg mass production and FCR after *B. licheniformis* DSM 28710 supplementation.

The exact underlying mechanism is not yet completely clear and requires further research, but based on the current findings and in line with most peer-reviewed studies on probiotics, the *B. licheniformis* strain DSM 28710 has exerted a clear beneficial effect when supplemented to layers, even in a challenging diet based on barley and SFM. Future work on probiotics will need to include the results on the apparent digestibility of all diets, especially in combination with NSP-rich diets.

Conclusions

In summary, dietary supplementation of at least 1.6 × 10^9 colony forming units of *B. licheniformis* DSM 28710 (0.5 g/kg feed) in Lohmann Brown laying hen diets based on barley and SFM, resulted in a significantly improved egg mass output, FCR and shell thickness, as well as significantly reduced dirty egg count. Therefore, *B. licheniformis* DSM 28710 can be considered a beneficial feed additive in layer feeds, especially when relatively cheap and low quality feed ingredients are preferred.

Acknowledgements

The authors would like to thank Huvepharma N.V for supplying the probiotic B-Act®. We also deeply thank Mr. Wouter Van der Veken, MSc, for his valuable contributions in editing the manuscript in English.

Conflict of interest

The Authors declare that there is no conflict of interest.

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