Research Notes

Egg quality and productive performance of laying hens fed different levels of skimmed milk powder added to a diet containing *Lactobacillus acidophilus*

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ABSTRACT The current trial was carried out on a commercial poultry farm to study the effect of skim milk powder (SMP) added to a diet containing *Lactobacillus acidophilus* on performance and egg quality of laying hens from 20 to 49 wk of age. A total of 2,400 Hy-Line W-36 laying hens were housed in 600 unenriched cages (4 hens each) located over 4 tier levels. Animals were assigned to 1 of 3 experimental treatments (0, 3, and 4). The laying hens assigned to treatments 3 and 4 received a diet enriched respectively with 3 and 4% SMP, whereas the animals in treatment 0 were fed a diet without SMP. All diets, moreover, were supplemented with *L. acidophilus* D2/CSL. Hen performance was determined throughout the experimental period and egg quality was measured on 30 eggs per treatment every week. Results showed that productive performance in terms of egg production, egg weight, and feed conversion ratio was not influenced by SMP at 3 or 4% of the diet. Egg quality was significantly affected by SMP included at 3 or 4% of the diet. Eggs from treatments 3 and 4, in fact, displayed higher shell thickness than those from treatment 0 ($P < 0.0001$). Likewise, specific gravity, Haugh unit, and shell percentage were significantly affected by the addition of SMP. In conclusion, in our study, SMP added to a diet containing *L. acidophilus* had no significant effects on the productive parameters of hens during the laying period, whereas significant improvements were found in certain egg quality characteristics.

Key words: skim milk powder, laying hen, performance, egg quality, *Lactobacillus acidophilus*

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INTRODUCTION

Prebiotics are feed ingredients that are not digested by the host. They may reach the large intestine and can be used as substances to promote the growth of beneficial microbes (Kontula et al., 1999). Due to the low lactase activity of the chicken intestine (Chotinsky et al., 2001), lactose, the main carbohydrate of milk, has been suggested as a prebiotic in poultry. Lactose, in fact, is not completely absorbed by the intestinal tract of poultry, but passes unchanged into the lower segments of the intestine and ceca (Van Immerseel et al., 2002).

Among the rich lactose-fermenting bacterial flora present in the intestinal tract and crop of healthy chickens, lactobacilli play an important role in lactose hydrolysis. Fermentation of lactose results in the production of lactic acid or short-chain fatty acids that reduce cecal pH and contribute to producing a barrier effect against *Salmonella* and other intestinal pathogens (Corrier et al., 1990; Takeda et al., 1995; Tellez et al. 1993) and enhance mineral absorption (Roberfroid et al., 2010). Prebiotic additions, such as oligofructose, inulin, galacto-oligosaccharides, resistant starches, and lactulose, in particular, seem to have a positive influence on calcium metabolism (Scholz-Ahrens et al., 2001; Teitelbaum and Walker, 2002).

In poultry production, the use of lactose as a prebiotic was studied by some authors to determine its effect on broiler and turkey performance. In turkeys, Simoyi et al. (2006) found a higher BW and lower total body

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fat content in animals fed a diet containing 4% lactose. Douglas et al. (2003), in contrast, reported decreased feed efficiency from 0 to 20 d in broilers fed 6% lactose and looser, poorly formed excreta after administration of 4 and 6% lactose at 7 and 14 d of age. Rutter et al. (1953), however, reported that the inclusion of 20% lactose in the ration could induce diarrhea in poultry, whereas a lower level of lactose (10%) had no negative effects on growth performance. Some studies on laying hens exist exploring the use of lactose, but little data are available regarding the effect of skim milk powder (**SMP**) on hen production. In a recent preliminary trial, Marelli et al. (2008) found an improvement in egg quality after feeding laying hens SMP and *Lactobacillus acidophilus* D2/CSL. In the present study, at a commercial poultry farm, 3 different levels of SMP were added to a laying hen diet containing *L. acidophilus* to evaluate the effect on hen performance and egg quality.

MATERIALS AND METHODS

Birds and Treatments

The trial, lasting 29 wk, was undertaken at a commercial poultry house located in northern Italy. Bird procedures were carried out in accordance with European Union (1999) regulations related to minimum standards for the protection of laying hens. A regimen of 16 h of light per day was reached after a step-up program. Room temperature of 21 ± 2°C was maintained throughout the experimental period, whereas RH was 55% on average.

On arrival, a total of 2,400 Hy-Line W-36 laying hens (aged 18 wk) were weighed per transport container and divided into 3 groups so that the mean BW was similar for each group. Laying hens were housed 4 per cage in 600 unenriched cages (560 cm^2/hen) provided with a manual feeder and nipple drinker. The experiment was conducted as a randomized block design with 40 blocks located over 4 tier levels (10 blocks per tier, each block consisting of 5 cages per treatment; 50 cages per treatment and per tier).

After 2 wk of acclimatization, each group was assigned to 1 of 3 experimental treatments (0, 3, and 4). Birds in treatment 0 were fed ad libitum a diet without the addition of SMP, whereas birds in the other 2 experimental treatments (3 and 4) received ad libitum diets supplemented with 3 and 4% SMP, respectively (34.0% protein, 1.4% fat, 8.5% ash, 54% lactose, 2,796 kcal/kg of ME). Skim milk powder was substituted on an equal weight basis for soybean meal (Table 1). The diets contained the same commercial probiotic (1.0×10^6 cfu/g of feed), *L. acidophilus* D2/CSL (Centro Sperimentale del Latte, Lodi, Italy), an autochthonous intestinal strain isolated from the chicken (Bianchi Salvadori et al., 1985). This probiotic consists of freeze-dried live cells of *L. acidophilus* D2/CSL (50×10^9 cfu/g).

Data Recorded

For each treatment, productive performance (BW, feed consumption, and egg production) was recorded on the set of 5 cages per block (40 observation per treatment). Live weight was registered at the beginning and end of the experimental period, whereas feed intake was determined once a week. Feed conversion ratio was calculated as grams of feed per grams of egg. From wk 20 to 49, the number and weight of eggs laid were recorded daily, and hen-day egg production was calculated as the total number of eggs collected divided by the number of live hens. Egg mass was calculated as hen-day egg production multiplied by the average egg weight. Mortality was recorded daily. In accordance with European Union (2008) regulation, eggs lacking the necessary characteristics to be market as Category A were considered unsaleable, whereas eggs with cracked shells and damaged membranes were classified as broken.

Egg and Diet Analysis

From wk 20 to 49, a total of 120 eggs (3 eggs from the set of 5 cages/block) were randomly collected on a weekly basis from each treatment and individually weighed. To determine egg quality, a sample of 30 eggs per treatment was chosen so as to have a similar average weight to that of the 120 previously weighed eggs. Egg specific gravity was evaluated with an egg pycnometer on 5 repetitions of 5 eggs each. Shell thickness, after removing shell membranes, was determined with a micrometer at 3 points of the equator, and thereafter the eggs were shelled and shell, yolk, and albumen were weighed. Shell plus membrane was weighed after drying (Mabe et al., 2003). The percentage of shell, yolk, and albumen was calculated as weight of egg components divided by egg weight × 100. Albumen height was evaluated by the Albumen Height Gauge AG-10 (R. Brancker Research Ltd., Ottawa, Canada). The Haugh unit value resulted from the mean of 3 measures of the thick albumen according to Haugh (1937).

The concentration of DM (930.15), ash (942.05), CP (984.01), crude fiber (978.10), and ether extract (954.02) of the diets and SMP were measured following AOAC International (2000) recommendations. The SMP lactose levels were determined by enzymatic method based on the ISO (2002) standard. Calcium, phosphorous, Met, Lys, and ME content of the diets and ME of SMP were calculated based on NRC (1994) recommendations. Feed samples for each treatment were analyzed by lactobacillus enumeration (ISO, 2003).

Statistical Analysis

To assess productive performance, the set of 5 cages of each block was considered as the experimental unit for statistical analysis. The effect of dietary treatment on productive performance was statistically analyzed according to a randomized complete block design by ANOVA using the GLM procedures of SAS (SAS In-

Table 1. Ingredients and chemical composition of experimental diets

Item (%, unless otherwise noted)	Diet 0	Diet 3	Diet 4
Ingredient			
Corn	56.6	56.6	56.6
Wheat flour shorts	5.00	5.00	5.00
Soybean meal, 48% CP	21.0	18.0	17.0
Soybean oil	2.45	2.45	2.45
Corn gluten	3.10	3.10	3.10
Skim milk powder	0	3.00	4.00
Calcium carbonate	9.95	9.95	9.95
Calcium phosphate	0.60	0.60	0.60
Sodium chloride	0.18	0.18	0.18
Sodium bicarbonate	0.13	0.13	0.13
Lys	0.09	0.09	0.09
DL-Met	0.08	0.08	0.08
Vitamin-mineral premix[1]	0.73	0.73	0.73
Phytase[2]	0.09	0.09	0.09
Lactobacillus acidophilus (cfu/g)	1.0×10^6	1.0×10^6	1.0×10^6
Chemical composition			
DM	87.4	87.8	87.3
CP	18.7	18.1	18.4
Ether extract	5.77	5.43	5.27
Crude fiber	3.09	3.06	2.89
Ash	10.8	10.4	10.4
Met[3]	0.38	0.39	0.39
Lys[3]	0.91	0.90	0.90
Ca (calculated)[3]	4.00	4.03	4.05
Ca (analyzed)	4.27	4.30	4.25
P (available)[3]	0.21	0.24	0.25
L. acidophilus (cfu/g)	1.23×10^6	1.18×10^6	1.21×10^6
ME[3] (kcal/kg)	2,861	2,872	2,875

[1]Vitamin and mineral premix supplied the following amounts per kilogram of premix: vitamin A, 1,800,000 IU; vitamin D_3, 200,000 IU; vitamin E, 4,000 mg; vitamin K_3, 300 mg; vitamin B_1, 120 mg; vitamin B_2, 180 mg; vitamin B_{12}, 2 mg; biotin, 20 mg; choline 30,000 mg; pantothenic acid, 500 mg; folic acid 100 mg; vitamin C 5,000 mg; Zn, 5,000 mg; Cu, 300 mg; Mn, 6,000 mg; Fe, 4,000 mg; I, 100 mg; Se, 20 mg; Co, 20 mg; DL-Methionine, 10,000 g.

[2]Phyzyme xp500 (Danisco Animal Nutrition, Marlborough, UK).

[3]Metabolizable energy, Ca, P, Met, and Lys content of diets were calculated from NRC (1994).

stitute, 2001). The effect of dietary treatment on egg quality was analyzed using a one factor per treatment ANOVA model. Differences among treatment means were determined by Tukey test. Significance was set at $P < 0.05$. Mortality data were analyzed by the CATMOD procedure of SAS.

RESULTS AND DISCUSSION

Results for the performance of laying hens are summarized in Table 2. Supplementation with SMP in partial substitution of soybean meal did not affect the BW of the laying hens. Mortality was also found to be

Table 2. Effect of skim milk powder (SMP) added to a diet containing *Lactobacillus acidophilus* in terms of performance, mortality, and productive parameters of hens during the laying period

Item	Treatment[1] 0	3	4	SEM	*P*-value
Initial live weight, 20 wk of age[2] (g)	1,376	1,371	1,382	16.2	0.853
Final live weight, 49 wk of age[2] (g)	1,613	1,581	1,624	23.8	0.847
Hen-day egg production[3] (%)	84.9	83.7	84.5	2.97	0.957
Average egg weight[4] (g)	59.1	59.3	59.4	1.17	0.985
Egg mass[3] (g/d per hen)	51.2	50.3	50.3	2.31	0.949
Feed intake[3] (g/d)	94.1	93.8	94.6	1.23	0.859
Feed conversion ratio[3] (g of feed/g of eggs)	1.84	1.86	1.87	0.03	0.928
Unsaleable eggs (%)	3.22	3.53	3.57	0.39	0.783
Broken eggs (%)	0.42	0.39	0.36	0.27	0.961

[1]0 = diet containing 0% SMP and *L. acidophilus* D2/CSL; 3 = diet containing 3% SMP and *L. acidophilus* D2/CSL; 4 = diet containing 4% SMP and *L. acidophilus* D2/CSL.

[2]Each value is the mean of 40 observations.

[3]Each value is the mean of 1,160 observations (40 blocks of 5 cages, 29 wk).

[4]Each value is the mean of 8,120 observations. Eggs were taken daily from age 20 to 49 wk.

Table 3. Egg quality of laying hens fed a diet containing *Lactobacillus acidophilus* and different levels of skim milk powder (SMP) from age 20 to 49 wk (n = 870; 30 eggs, 29 wk)

	Treatment[1]				
Item	0	3	4	SEM	*P*-value
Shell thickness (mm)	0.331[c]	0.345[b]	0.351[a]	0.0004	<0.0001
Specific gravity (g/cm^3)	1.084[b]	1.085[a]	1.086[a]	0.0002	<0.0001
Haugh unit	92.1[b]	93.4[a]	93.3[a]	0.39	0.002
Shell (%)	10.3[b]	10.4[a]	10.6[a]	0.03	<0.0001
Yolk (%)	25.0	25.1	25.0	0.12	0.752
Albumen (%)	64.7	64.5	64.4	0.12	0.312

[a–c]Values within a row with different superscripts are significantly different ($P < 0.05$).

[1]0 = diet containing 0% SMP and *L. acidophilus* D2/CSL; 3 = diet containing 3% SMP and *L. acidophilus* D2/CSL; 4 = diet containing 4% SMP and *L. acidophilus* D2/CSL.

similar among the treatments and remained low over the complete period (1%, on average; data not shown).

In accordance with Gleaves and Salim (1982), who found similar results in terms of egg production, egg weight, and feed intake using different sources of lactose (pure lactose, dried skim milk, and dried whey), in our study the productive performance of laying hens was not affected by the experimental treatments. In particular, egg mass and feed conversion ratio were similar among groups receiving SMP with respect to treatment 0. Conversely, an improvement in egg production and feed conversion ratio was reported by Aghaei et al. (2010) using 2 or 4% dried whey powder added to the diet of laying hens. Data from the present study are in contrast with those of a preliminary trial by Marelli et al. (2008), who reported a higher egg weight in laying hens fed a diet containing 3 and 4% SMP.

The effect of treatment on egg quality was examined over the whole experimental period and results are shown in Table 3. From age 20 to 49 wk, the eggs from hens fed 3 and 4% SMP had a significantly higher shell thickness than those from hens fed 0% SMP. No significant influence of lactose sources was found by Gleaves and Salim (1982) on shell quality, whereas an improvement in egg shell-breaking strength was reported by the same authors in layers fed a diet supplemented with 1% pure lactose.

The better shell thickness found in our study may be due to the metabolic activities of lactobacilli, which could be higher in the treatments receiving SMP. The supplementation of lactobacilli as probiotic in all experimental groups, moreover, could have enhanced the effect of supplemental SMP, promoting lactose utilization. Lactose, in fact, is fermented by the intestinal microflora, mostly lactobacilli, and produces short-chain fatty acid and other organic acids. These products reduce luminal pH associated with an increased concentration and solubility of calcium, determining an increase in passive diffusion (Cashman, 2003; Roberfroid et al., 2010).

Specific gravity was positively affected by SMP, showing significantly higher values in comparison with treatment 0. Improved shell characteristics, however, were not accompanied by a lower percentage of broken eggs ($P = 0.961$). These results were similar to those reported by Marelli et al. (2008) using 3% SMP, even if no effect in terms of shell thickness and specific gravity were recorded by those authors in laying hens receiving 4% SMP.

The addition of SMP to the hen diet showed an increase in Haugh unit values throughout the experimental period. This result was in contrast with that found previously by Marelli et al. (2008), where no significant differences were reported between hens fed a diet enriched with 0, 3, and 4% SMP. The experimental treatments had no significant effects on egg quality traits (Table 3) in terms of yolk and albumen percentage from 20 to 49 wk of age, whereas shell percentage was influenced ($P < 0.0001$) by the addition of SMP.

In conclusion, in our study, SMP added to a diet containing *L. acidophilus* had no significant effects on the productive parameters of hens during the laying period. Conversely, significant improvements in egg quality characteristics, in terms of specific gravity, shell thickness, Haugh unit, and percentage of shell were reported in animals fed a diet supplemented with SMP. Improved physical shell characteristics are an important aspect for egg producers, mostly at the end of the production period, and further studies should be undertaken to better understand the relationship between milk products and lactobacilli in egg production.

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