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Effects of probiotic supplementation in different energy and nutrient density diets on performance, egg quality, excreta microflora, excreta noxious gas emission, and serum cholesterol concentrations in laying hens

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ABSTRACT: This 6-wk study was conducted to determine the effects of probiotic (*Enterococcus faecium* DSM 7134) supplementation of different energy and nutrient density diets on performance, egg quality, excreta microflora, excreta noxious gas emission, and serum cholesterol concentrations in laying hens. A total of 432 Hy-Line brown layers (40 wk old) were allotted into 4 dietary treatments with 2 levels of probiotic supplementation (0 or 0.01%) and 2 levels of energy (2,700 or 2,800 kcal ME/kg) and nutrient density. Weekly feed intake, egg quality, and daily egg production were determined. Eighteen layers per treatment (2 layers/replication) were bled to determine serum cholesterol concentrations at wk 3 and 6. Excreta microbial shedding of *Lactobacillus*, *Escherichia coli*, and *Salmonella* and noxious gas emission were determined at the end of the experiment. Hens fed the high-energy and high-nutrient-density diets had less ($P < 0.01$) ADFI than those fed the low-energy and low-nutrient-density diets throughout the experimental period. During wk 4 to 6 and overall, hens fed the diets supplemented with the probiotic had greater ($P < 0.01$) egg production, egg weight, and eggshell thickness than hens fed the diets without the probiotic. Dietary supplementation of the probiotic increased ($P = 0.01$) excreta *Lactobacillus* counts and decreased ($P = 0.02$) *Escherichia coli* counts compared with hens fed the diets without the probiotic. The excreta ammonia emission was decreased ($P = 0.02$) in hens fed the probiotic diets compared with hens fed the diets without the probiotic. Serum total cholesterol concentration was decreased ($P < 0.01$) by feeding hens with the probiotic at wk 3 and 6. Layers fed the probiotic-incorporated diets had greater ($P < 0.01$) high-density lipoprotein (HDL) cholesterol and lower ($P = 0.03$) low-density lipoprotein (LDL) cholesterol concentrations than hens fed the nonsupplemented diets at wk 6. Interactive effects ($P < 0.05$) of energy and nutrient density and the probiotic on excreta *Lactobacillus* counts and serum HDL cholesterol concentration were observed at wk 6. In conclusion, dietary supplementation of 0.01% probiotic improved egg production and egg quality and decreased excreta ammonia emission. The use of a probiotic in the high-energy and high-nutrient-density diets may be more favorable than the low-energy and low-nutrient-density diets in laying hens.

Key words: energy and nutrient density, excreta, laying hens, performance, probiotic

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INTRODUCTION

It has been well documented that probiotics can serve as alternatives to antibiotics for increasing performance and disease resistance in poultry (Patterson and Burkholder, 2003). Many studies have reported that the addition of probiotics could improve egg production (Nahashon et al., 1994; Tortuero and Fernández, 1995; Abdulrahim et al., 1996), egg weight (Nahashon et al., 1996; Davis and Anderson, 2002), and egg quality (Mohan et al., 1995; Ramasamy et al., 2009) in laying hens. In contrast, other studies did not find beneficial effects of probiotics on these criteria (Balevi et al., 2001; Yoruk et al., 2004). These discrepancies may be caused by the microbial species, the administration dose, the breed and age of hens, or environmental stress factors (Mikulski et al., 2012). In addition, reduced serum cholesterol concentration (Haddadin et al., 1996; Kurtoglu

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et al., 2004; Mahdavi et al., 2005), favorable intestinal microbial ecosystem (Yu et al., 2008; Choe et al., 2012), and enhanced immunity (Koenen et al., 2004; Zhang et la., 2012) were also reported by application of probiotics.

Previous studies reported a decrease in feed intake in laying hens as dietary ME increased (Jackson and Waldrup, 1988; Grobas et al., 1999; Harms et al., 2000), which may indicate that the intake of a probiotic may be affected by the energy density of the diet. Moreover, our previous studies have confirmed that the efficacy of probiotics in pigs was influenced by dietary energy and nutrient density (Meng et al., 2010; Yan and Kim, 2013). Therefore, we hypothesized that this effect may also exist in laying hens. The objective of this study was to evaluate the effects of a probiotic in different energy and nutrient density diets on performance, egg quality, excreta microflora, excreta noxious gas emission, and serum cholesterol concentrations in laying hens.

MATERIALS AND METHODS

The experimental protocols were approved by the Animal Care and Use Committee of Dankook University in Cheonan, Choongnam, South Korea.

Source of Probiotic

The probiotic preparation used in the current experiment was provided by a commercial company (Bonvital, Schaumann Agri International GmbH, Pinneberg, Germany). This product was composed of spray-dried spore-forming *Enterococcus faecium*, which was guaranteed to contain at least 1.0×10^{10} viable spores/g of *Enterococcus faecium* DSM 7134.

Animals and Diets

A total of 432 Hy-Line brown laying hens (40 wk of age) were raised in a windowless and environmentally controlled room that was maintained at 23°C for 6 wk. Sixteen hours (0500 to 2100 h) of artificial lighting with a light intensity of 5.2 lx at bird level were provided daily. Layers were kept individually, and 12 pens (20 cm width × 50 cm length × 40 cm height) were regarded as a replication. Hens were assigned to 4 dietary treatments with 2 levels of probiotic (0 or 0.01%) and 2 levels of energy (2,700 or 2,800 kcal/kg ME) and nutrient density with 9 replications per treatment. Experimental diets were formulated to meet or exceed the NRC (1994) recommendations with the exception of the ME of the low-energy-density diet for Hy-Line brown layers (Table 1), and were provided in mash form. The high-nutrient-density diet was analyzed to contain 18.12% CP, 4.33% ether extract, 0.89% Lys, 0.75% Met + Cys, 3.57% Ca, and 0.65% total P, whereas the low-nutrient-density diet was analyzed to contain 17.04% CP, 3.98% ether extract, 0.75% Lys, 0.63% Met + Cys, 3.45% Ca, and 0.61% total P.

Table 1. Basal diet composition (as-fed basis)

Item	High density	Low density
Ingredient, %		
Corn	50.40	56.28
Soybean meal, 46% CP	18.70	15.53
Wheat grain	10.00	10.00
Corn gluten meal	2.00	2.00
Wheat bran	5.00	5.00
Tallow	4.40	1.70
Limestone	7.50	7.52
Dicalcium phosphate, 18% P	1.40	1.37
Salt	0.30	0.30
DL-Met, 50%	0.10	0.10
Vitamin premix[1]	0.10	0.10
Trace mineral premix[2]	0.10	0.10
Calculated energy content		
ME,[3] kcal/kg	2,800	2,700
Analyzed nutrient content, %		
CP	18.12	17.04
Ether extract[4]	4.33	3.98
Lys	0.89	0.78
Met + Cys	0.75	0.63
Ca	3.57	3.45
Total P	0.65	0.61

[1]Provided per kilogram of diet: 12,500 IU vitamin A, 2,500 IU vitamin D_3, 13 IU vitamin E, 2 mg vitamin K_3, 1 mg vitamin B_1, 5 mg vitamin B_2, 1 mg vitamin B_6, 0.04 mg vitamin B_{12}, 0.9 mg folic acid, 55 mg niacin, 14 mg Ca-pantothenate, and 0.1 mg D-BIOTIN.

[2]Provided per kilogram of diet: 8 mg Mn (as MnO_2), 60 mg Zn (as $ZnSO_4$), 5 mg Cu (as $CuSO_4 \cdot 5H_2O$), 40 mg Fe (as $FeSO_4 \cdot 7H_2O$), 0.3 mg Co (as $CoSO_4 \cdot 5H_2O$), 1.5 mg I (as KI), and 0.15 mg Se (as $Na_2SeO_3 \cdot 5H_2O$).

[3]Values for ingredients used in diet formulation were based on laying hen requirements in NRC (1994).

[4]Ether extract represents total fat content in the diet.

Chemical Analysis

Feed samples were ground to pass through a 1-mm screen, after which they were analyzed for DM (method 934.01; AOAC, 2000), N (method 968.06; AOAC, 2000), ether extract (method 920.39; AOAC, 2000), Ca (method 984.01; AOAC, 1995), and P (method 965.17; AOAC, 1995). Individual AA composition was measured using an AA analyzer (Beckman 6300; Beckman Coulter Inc., Fullerton, CA) after a 24-h hydrolysis in HCl (Spackman et al., 1958). For the determination of Cys and Met, the samples were oxidized with performic acid overnight at 0°C. Performic acid is an oxidizing reagent that converts Cys quantitatively to cysteic acid and Met to Met sulfone (Moore, 1963). Nitrogen was determined (Kjectec 2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes, Sweden), and CP was calculated as N × 6.25.

Feed Intake and Egg Production

Feed intake was recorded weekly on a replication basis (12 laying hens). Hens were allowed ad libitum access to feed and water throughout the experimental period. Eggs were collected on a daily basis, and egg production was calculated as an average hen-day production.

Egg Quality

A total of 30 eggs with the exception of soft and broken eggs were randomly collected at 1700 h from each treatment on a weekly basis and were used to determine the egg quality at 2000 h the same day. Eggshell breaking strength (kg/cm^2) was evaluated (Eggshell Force Gauge Model II; Robotmation Co., Tokyo, Japan). Eggshell thickness was measured at the large end, the equatorial region, and the small end (Dial Pipe Gauge; Ozaki MFG. Co., Tokyo, Japan). Finally, the egg weight, yolk color, and Haugh unit were evaluated (Egg Multi-Tester; Touhoku Rhythm Co., Tokyo, Japan).

Excreta Microflora

At the end of the experiment, excreta samples were collected from 6 layers randomly selected from each replicate, then pooled and placed on ice for transportation to the laboratory, where analysis was immediately performed by the method of Wang and Kim (2011). One gram of the composite excreta sample from each replication was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Rutherford, NJ) and homogenized. Viable counts of bacteria in the excreta samples were then determined by plating serial 10-fold dilutions (in 10 g/L peptone solution) onto MacConkey agar, Lactobacilli MRS agar, and *Salmonella Shigella* agar plates to verify the *Escherichia coli, Lactobacillus*, and *Salmonella*, respectively. The Lactobacilli MRS agar plates were incubated for 48 h at 39°C, and the MacConkey agar and *Salmonella Shigella* agar plates were incubated for 24 h at 37°C under anaerobic conditions. The bacteria colonies were counted immediately after removal from the incubator. A single colony was removed from selective media plates and cultivated in peptone yeast glucose broth. Subsequently, the bacteria were characterized to genus level on the basis of colonial appearance, gram reaction, spore production, cell morphology, and fermentation end product formation.

Excreta Noxious Gas Emission

At the end of the experiment, fresh excreta samples were collected from each replication (12 hens per replication) for the analysis of noxious gas emission according to the method described by Cho et al. (2008). A total of 300 g of excreta were stored in 2.6-L sealed plastic boxes in duplicate. The samples were permitted to ferment for a period of 30 h at 32°C. After the fermentation period, an instrument (Gas Detector, GV-100S; Gastec Corp., Kanagawa, Japan) was used for gas detection. In these measurements, the plastic boxes were punctured, and headspace air was sampled approximately 2.0 cm above the samples at a rate of 100 mL/min. Levels of ammonia, hydrogen sulfide, and total mercaptans (Gastec Detector Tube No. 3La, No. 4LL, and No.70L, respectively; Gastec Corp.) were measured.

Serum Cholesterol Concentrations

At the end of the third and sixth weeks of the experiment, the same 2 layers per replicate (18 layers per treatment) were selected, and 5 mL of blood were collected from their left jugular veins using a sterilized needle. Blood samples were centrifuged at 3,000 × *g* for 20 min at 4°C, and serum samples were stored at –4°C. The total cholesterol, high-density lipoprotein (**HDL**) cholesterol, and low-density lipoprotein (**LDL**) cholesterol in the serum samples were analyzed with an autoanalyzer (Automatic Biochemical Analyzer, RA-1000; Bayer Corp., Tarrytown, NY) using colorimetric methods.

Statistical Analyses

All data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) for a randomized complete block design with a 2 × 2 factorial arrangement. The data were tested for the main effects of dietary energy and nutrient density, the probiotic, and their interaction. The significance level was set at $P < 0.05$, whereas $P < 0.10$ was considered a tendency.

RESULTS

Interaction effects between energy and nutrient density and the probiotic were only observed for excreta *Lactobacillus* counts ($P = 0.04$) and serum HDL cholesterol concentration ($P = 0.02$). Therefore, only the main effects of energy and nutrient density and the probiotic are presented in the tables.

Performance and Egg Quality

Laying hens fed the low-energy and low-nutrient-density diets had greater ($P < 0.01$) ADFI than hens fed the high-energy and high-nutrient-density diets during the experiment (Table 2). During wk 4 to 6 and overall, hens fed the diets supplemented with the probiotic had greater ($P < 0.01$) egg production than those fed the diets with no supplementation.

Table 2. Main effects of probiotic in different energy and nutrient density diets on feed intake and egg production in laying hens[1]

	Nutrient density		Probiotic (Pro)			*P*-value	
Item	High	Low	+	–	SE	Density	Pro
ADFI, g							
wk 0 to 3	102.5	112.6	107.5	107.6	2.1	<0.01	0.34
wk 4 to 6	102.8	111.6	106.5	107.9	2.4	<0.01	0.89
wk 0 to 6	102.6	111.8	106.7	107.7	1.8	<0.01	0.46
Egg production, %							
wk 0 to 3	89.5	89.5	89.7	89.3	0.3	0.92	0.11
wk 4 to 6	91.3	91.2	91.8	90.7	0.3	0.61	<0.01
wk 0 to 6	90.5	90.4	90.8	90.0	0.2	0.69	<0.01

[1]Each mean represents 9 replications with 12 hens/replication. High = high-energy and high-nutrient-density diet, Low = low-energy and low-nutrient-density diet, and + and – = supplemented with and without 0.01% Pro.

Egg quality was not affected by dietary energy and nutrient density (Table 3). Layers fed the diets supplemented with the probiotic had greater ($P < 0.05$) eggshell thickness compared with those fed the diets with no probiotic throughout the experimental period. During wk 4 to 6 and overall, egg weight in layers fed the diets containing the probiotic increased ($P < 0.01$) compared with those fed the diets with no probiotic. An increasing trend ($P = 0.07$) in eggshell strength was observed in hens fed the diets supplemented with the probiotic during wk 0 to 6.

Excreta Microflora

Excreta *Lactobacillus*, *E. coli*, and *Salmonella* concentrations were unaffected by dietary energy and nutrient density (Table 4). Hens with the probiotic treatments showed greater ($P = 0.01$) excreta *Lactobacillus* counts and lower ($P = 0.02$) *E. coli* counts compared with layers fed the diets without supplementation. Excreta *Salmonella* counts were not affected by the dietary probiotic.

Excreta Noxious Gas Emission

There was no difference in excreta noxious ammonia, total mercaptans, and hydrogen sulfide emission among treatments with different energies and nutrient densities (Table 5). Laying hens fed the diets with the probiotic had less ($P = 0.02$) ammonia emission than those fed the diets with no probiotic.

Serum Cholesterol Concentrations

Serum total cholesterol concentration decreased in hens fed the probiotic-enriched diets at wk 3 ($P = 0.04$) and 6 ($P < 0.01$; Table 6). Serum HDL cholesterol concentration increased ($P < 0.01$) by feeding either high-energy and high-nutrient-density diets or probiotic diets at wk 6. In addition, layers fed the diet with the probiotic had lower ($P = 0.03$) LDL cholesterol concentration than those fed the no-probiotic diets at wk 6.

DISCUSSION

In our study, we increased the dietary ME (100 kcal/kg of diet) and nutrient density (1.08% CP, 0.11% Lys, and 0.12% Met + Cys) by substitution of corn with soybean meal and tallow. The overall ADFI was increased

Table 3. Main effects of probiotic in different energy and nutrient density diets on egg quality in laying hens[1]

	Nutrient density		Probiotic (Pro)			*P*-value	
Item	High	Low	+	–	SE	Density	Pro
wk 0 to 3							
Egg weight, g	60.47	60.36	60.72	60.11	0.38	0.77	0.11
Yolk color	8.68	8.72	8.74	8.66	0.06	0.49	0.22
Haugh unit	94.12	94.14	94.24	94.02	0.46	0.96	0.64
Eggshell strength, kg/cm^2	3.674	3.699	3.723	3.650	0.061	0.69	0.24
Eggshell thickness, 10^{-2} mm	40.09	40.07	40.25	39.91	0.16	0.87	0.04
wk 4 to 6							
Egg weight, g	60.92	60.78	61.33	60.37	0.36	0.68	0.01
Yolk color	8.85	8.80	8.85	8.80	0.05	0.31	0.31
Haugh unit	94.61	94.42	94.62	94.41	0.39	0.63	0.59
Eggshell strength, kg/cm^2	3.721	3.728	3.772	3.677	0.070	0.92	0.17
Eggshell thickness, 10^{-2} mm	40.70	40.70	40.93	40.48	0.16	0.99	0.01
wk 0 to 6							
Egg weight, g	60.70	60.57	61.03	60.24	0.26	0.62	<0.01
Yolk color	8.76	8.76	8.79	8.73	0.04	0.93	0.12
Haugh unit	94.37	94.28	94.43	94.22	0.30	0.79	0.48
Eggshell strength, kg/cm^2	3.698	3.713	3.747	3.664	0.046	0.74	0.07
Eggshell thickness, 10^{-2} mm	40.39	40.38	40.58	40.19	0.12	0.91	<0.01

[1]Each mean represents 30 randomly collected eggs/treatment on a weekly basis. High = high-energy and high-nutrient-density diet, Low = low-energy and low-nutrient-density diet, and + and – = supplemented with and without 0.01% Pro.

Table 4. Main effects of probiotic in different energy and nutrient density diets on excreta microflora in laying hens (log_{10} cfu/g)[1]

	Nutrient density		Probiotic (Pro)			*P*-value	
Item	High	Low	+	–	SE	Density	Pro
Lactobacillus	7.79	7.60	7.88	7.51	0.10	0.79	0.01
Escherichia coli	6.33	6.48	6.28	6.53	0.09	0.14	0.02
Salmonella	2.57	2.52	2.49	2.60	0.08	0.58	0.21

[1]Each mean represents 9 replications with 6 hens/replication. High = high-energy and high-nutrient-density diet, Low = low-energy and low-nutrient-density diet, and + and – = supplemented with and without 0.01% Pro.

Table 5. Main effects of probiotic in different energy and nutrient density diets on noxious gas emission in excreta of laying hens (ppm)[1]

	Nutrient density		Probiotic (Pro)			*P*-value	
Item	High	Low	+	–	SE	Density	Pro
Ammonia	40.1	42.6	32.3	50.4	4.7	0.60	0.02
Total mercaptans	6.9	6.7	5.8	7.8	1.7	0.86	0.28
Hydrogen sulfide	6.7	6.4	5.7	7.4	1.8	0.87	0.35

[1]Each mean represents 9 replications with 12 hens/replication. High = high-energy and high-nutrient-density diet, Low = low-energy and low-nutrient-density diet, and + and – = supplemented with and without 0.01% Pro.

by 9.0% in laying hens fed the low-energy and low-nutrient-density diets compared with hens fed the high-energy and high-nutrient-density diets, which is in agreement with Harms et al. (2000), who reported that hens fed the low-energy diet (2,519 kcal ME/kg) consumed 8.5% more feed than hens fed the control diet (2,798 kcal ME/kg). Similar results that showed a decrease in feed intake as ME levels increased were reported by Carew et al. (1980) and Jackson and Waldrup (1988). In contrast, Jalal et al. (2006) reported no effects of dietary ME level on feed intake in laying hens. In their study, the ME levels of diets were 3,097 and 2,979 kcal/kg, which were much greater than the values in our experiment (2,700 and 2,800 kcal/kg). It has been suggested that hens are more sensitive to decreasing the energy than increasing the energy in the diet, which may explain the discrepancy between studies (Harms et al., 2000).

Patterson and Burkholder (2003) suggested that a daily intake of 10^8 to 10^9 microorganisms could exert beneficial effects in animals. In the current study, the egg production, egg weight, and eggshell thickness were increased by inclusion of a probiotic in the diet. In agreement with our results, previous studies reported that a probiotic, such as lactobacilli and *Bacillus subtilis,* has a positive impact on daily egg production and egg quality (Nahashon et al., 1994; Abdulrahim et al., 1996; Zhang et al., 2012). In other studies, no positive effects of probiotics were observed on egg production (Davis and Anderson, 2002; Mikulski et al., 2012) and egg quality (Balevi et al., 2001). The main source of inconsistencies may be the breed and age of layers, microbial species, and supplemental dose (Mikulski et al., 2012). Although egg weight is a relatively high heritability trait, the increased egg weight and eggshell thickness in our study were probably due to the improved intestinal microbial balance, which may benefit the utilization of nutrients (Nahashon et al., 1994; Mohan et al., 1995; Nahashon et al., 1996). In addition, a growing trend toward an increase in eggshell strength was observed in hens fed the probiotic diet. A similar effect of a probiotic was also observed by Panda et al. (2003, 2008).

A probiotic exerts beneficial effects by suppressing intestinal harmful microorganisms and favoring beneficial microorganisms, ultimately enhancing the gut health (Fuller, 1989). In the present study, the excreta counts of *Lactobacillus* were increased, and *E. coli* count was decreased by application of the probiotic. In agreement with our results, Hassanein and Soliman (2010) reported that the number of ileal lactobacilli was increased and *E. coli* count was decreased in laying hens fed 0.4% to 1.6% *Saccharomyces cerevisiae*. Moreover, a greater *Lactobacillus* count was observed in response to the probiotic in high-nutrient-density diets than in a low-nutrient-density diet. Our previous studies indicated that administration of a probiotic in a high-nutrient-density diet could be more effective than in a low-nutrient-density diet on the gastrointestinal environment and subsequent nutrient utilization in pigs (Meng et al., 2010; Yan and Kim, 2013). Patterson and Burkholder (2003) also suggested that the efficacy of a probiotic depends on the overall diet composition. In the current study, an acidic environment created by the increased *Lactobacillus* may have enhanced the absorption of minerals such as Ca (Haddadin et al., 1996; Kabir et al., 2004; Panda et al., 2005), which may in turn have led to an improvement of eggshell quality.

Table 6. Main effects of probiotic in different energy and nutrient density diets on serum cholesterol concentrations in laying hens (mg/dL)[1]

	Nutrient density		Probiotic (Pro)			*P*-value	
Item[2]	High	Low	+	–	SE	Density	Pro
wk 3							
LDL cholesterol	109.1	108.9	109.9	108.1	3.4	0.83	0.22
HDL cholesterol	42.0	37.0	42.4	36.5	4.3	0.67	0.77
Total cholesterol	183.7	180.8	172.9	191.6	8.5	0.73	0.04
wk 6							
LDL cholesterol	106.3	105.9	102.5	109.7	2.1	0.95	0.03
HDL cholesterol	48.6	38.1	47.3	39.4	3.4	<0.01	<0.01
Total cholesterol	170.8	170.4	158.5	182.7	5.3	0.94	<0.01

[1]Each mean represents 9 replications with 2 hens/replication. High = high-energy and high-nutrient-density diet, Low = low-energy and low-nutrient-density diet, and + and – = supplemented with and without 0.01% Pro.

[2]HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol.

In the present study, the shift of excreta fecal microbial composition was further followed by a decrease in the excreta ammonia emission. It has been suggested that fecal ammonia emission is related to nutrient utilization and the intestinal microbial ecosystem (Ferket et al., 2002). The nitrogen excretion in laying hen manure consists mainly of uric acid and bacterial protein with some ammonia and endogenous nitrogen (Song et al., 2012). Canh et al. (1998) reported that the bacterial proteins in the feces are more stable than uric acid, which led to less ammonia volatilization. Therefore, in the current study, the decreased ammonia emission was, perhaps, due to the improvement of the intestinal microbial balance. Although beyond the scope of this work, a nitrogen balance experiment is warranted to confirm the beneficial effect.

Recently, much attention has been paid to targeting cholesterol-lowering effects in animals. In this study, the serum total cholesterol concentration was decreased by administration of a probiotic. Similar to our results, Kurtoglu et al. (2004) and Mahdavi et al. (2005) reported that a probiotic reduced serum cholesterol concentrations in laying hens. St-Onge et al. (2000) suggested that the production of enzymes by lactic acid bacteria decreased the bile acid recycling, which resulted in a reduction of serum cholesterol concentration because cholesterol is used for bile acid synthesis (Haddadin et al., 1996). Interestingly, we found that serum HDL cholesterol increased more dramatically in layers fed the probiotic in the high-energy and high-nutrient-density diets in this study. Khan et al. (2011) found that serum HDL cholesterol was increased by probiotic supplementation to a layer diet compared with the control treatment. However, Adlouni et al. (1997) reported that fasting increased the serum HDL cholesterol by 14.3% in humans. It appears that the enormous difference in energy intake led to the variation compared with our results.

In conclusion, the probiotic improved egg production, and egg quality and decreased excreta ammonia emission. The use of a probiotic in the high-energy and high-nutrient-density diets may be more favorable than in the low-energy and low-nutrient-density diets in laying hens. However, more research is needed to determine the mechanisms of probiotics in enhancing intestinal function and cholesterol metabolism in laying hens.

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