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Effects of Bacteriocin-Like Substance on Performances and Egg Quality of Laying Hens

Yaoqi Guo, Guang Jia, Zhanqiao Yu and Rijun Zhang
Feed Biotechnology Laboratory, Key Laboratory of Animal Nutrition,
College of Animal Science and Technology, China Agricultural University, 100193 Beijing, China

Abstract: Feed supplements had been widely studied for improvement of egg quality. The effects of Bacteriocin-Like Substance (BLS), an antimicrobial peptide produced by a new *Bacillus licheniformis* strain, on performances and egg quality of laying hens were evaluated here. About 150 Hy-Line Brown laying hens which were 32 weeks old were randomly divided into five groups with 5 replicates (6 birds per replicate). Four groups were fed different concentrations of bacteriocin-like substances (500, 1500, 2500 and 3500 AU kg^{-1} basic dietary, respectively) whereas the group fed basic diet was served as control. After experiment, groups fed BLS had a higher egg production without significance comparing to control. There were also no significant differences between control and trial groups, in terms of feed to egg ratio, egg weight, egg shell thickness, egg shell strength, egg shape index and Haugh unit. However, groups supplemented with BLS exhibited significant higher yolk color ($p<0.01$) and lower ratio of blood spots eggs.

Key words: Bacteriocin-like substance, performance of laying hens, egg quality, antimicrobial peptide, blood spots eggs

INTRODUCTION

Egg quality is the characteristics that effect acceptability of eggs to consumers (Monira *et al*., 2003). These characteristics include egg size, shell integrity, shell quality, interior quality and nutrition (Holt *et al*., 2011). Federal quality standard has been established in US to ensure high quality eggs on market (http://www.access.gpo.gov/nara/cfr/waisidx_08/7cfr56_08.html). In European Union, eggs are classed into two groups, A and B and only group A can be directly retailed or consumed by human. There are many factors that influence egg quality, namely, feed supplements, disease, environment, age and breed of bird (Rabie *et al*., 1997). These factors had been extensively studied and efforts are being made to improve egg quality in industry based on these factors (Scott and Silversides, 2000; Bell, 2003; Pappas *et al*., 2005).

Bacteriocins are ribosomally synthesized antimicrobial peptides by bacteria (Borrero *et al*., 2011). Susceptible pathogens to bacteriocins include *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus* sp. and *Campylobacter jejuni* and application of bacteriocins for treatment of mastitis or other bacterial disease had been evaluated (Rodriguez *et al*., 2002; Stern *et al*., 2005; Fernandez *et al*., 2008). Beside treatment of diseases, the effects of bacteriocins on chicken performance were also documented. Diversin was reported to significantly increase body weight gain and albusin improves nutrients absorption and enhances fecal Lactobacillus count in broiler chickens (Grilli *et al*., 2009; Jozefiak *et al*., 2011). Bacteriocin also improves performance of broilers which are challenged with *Clostridium perfringens* (Grilli *et al*., 2009). However, there are no studies about the influence of bacteriocins on egg quality. During a screening for Bacillus strains with potent antimicrobial activity, a new strain was isolated and identified as *B. licheniformis* in the laboratory. The new isolate showed broad antimicrobial activity against gram-positive and negative pathogens and the antimicrobial agent was partially characterized. In the present study, the effects of bacteriocin-like substance on performance and egg quality of laying hens were investigated.

MATERIALS AND METHODS

Preparation of bacteriocin-like substance: Fermentation and preparation of BLS and definition of activity were described elsewhere (Guo, 2008). The activity of BLS used in this study was 160 AU mL^{-1}.

Experimental design and animals: One hundred and fifty 32 weeks old Hy-Line Brown laying hens were randomly divided into five groups with 5 replicates and 6 laying hens per replicate. These hens were housed in individual cages over an experimental period of 34 days. Basal layer

Corresponding Author: Rijun Zhang, College of Animal Science and Technology, China Agricultural University, No. 2 Yuanmingyuan West Road, 100193 Beijing, China

diet was formulated to be adequate in all nutrients for this strain (Table 1). The birds were fed on the basal diet supplemented with five levels of BLS (0, 500, 1500, 2500 or 3500 AU kg^{-1} diet) before the experiment was terminated. Diets were presented in mash form and feed and water were given *ad libitum* during experiment. The number of eggs, egg weights and abnormal eggs were recorded on a daily basis. At the end of experiment, ten eggs were randomly picked from each replicate and analyzed for shell strength, shell thickness, Haugh unit, shape index and yolk color. Haugh unit was calculated using Haugh formula (Salma *et al*., 2007). The yolk color was measured by a colorimetric fan (Roche) and color was scored according to their density. The experiment was conducted in Hebeikaite poultry farm.

Statistics: Data were subjected to one-way ANOVA analysis using SPSS. Significant differences between treatment were analyzed by Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Supplementation of BLS slightly increased egg production. However, the egg production, feed to egg ratio and average egg weight between treatments were not significantly different (Table 2). For egg quality, intake of BLS did not significantly change the shell strength, shell thickness, shape index and Haugh unit (Table 3). Groups supplemented with BLS had a consistently higher yolk color with significance and a much lower rate of eggs with blood spots (Table 3).

The BLS has no effects on performance of laying hens and egg quality except yolk color and rate of eggs with blood spots. Although, it does not mean a higher nutritional quality, most consumers associate yolk color with freshness, tastiness, safety and nutritive value (Hasin *et al*., 2006). Besides, yolk color is important in manufacture of egg products, e.g., liquid frozen and dried whole egg and separated egg components (Johnson *et al*., 1980). The color of yolk is determined by carptenoid pigments and can be manipulated by addition of natural or synthetic ingredient containing carotenoid into the diet. Breed, housing conditions and individual capacity may also influence the ability of hens to absorb and deposit the pigments (Ponsano *et al*., 2004). It has been reported that probiotics may enhance the concentration of caronoid in layer serum (Li *et al*., 2006). This was probably the same case for BLS. Supplementation of BLS improved the absorption of pigments in gastro-intestine and resulted in a higher deposition of carotenoids in egg yolk.

Blood spots are typically generated from the ovary during the ovulatory process or from the upper oviduct (Shirley, 1965). The incidence of eggs with blood spots is effected by capillary fragility, blood clotting time and blood pressure (Fey *et al*., 1968). Other than genetic factors, nutrients and disease also influence the occurrence of eggs with blood spots. It has been reported that deficiency of vitamin K can result in a higher ratio of blood spot, as vitamin K plays an important role in blood clotting (Chukwuka *et al*., 2011). A low level of vitamin A also increases the incidence of blood spots (Roberts,

Table 1: Composition and nutrients of basal diet

Ingredients	Percentage	Nutrient[2]	Values
Corn	62.49	ME (MJ kg^{-1})	12.60
Soybean meal	14.98	Crude protein (%)	14.99
Cottonseed meal	3.99	Crude fat (%)	3.09
Wheat bran	4.49	Calcium (%)	3.46
Corn DGGS	3.00	Total P (%)	0.53
Limestone powder	8.79	Nonphytate P (%)	0.31
Dicalcium phosphate	1.00	Lysine (%)	0.72
Salt	0.15	Methionine (%)	0.33
Phylase	0.01	Methionine + cysteine (%)	0.57
DL-methionine	0.10	-	-
1% premix[1]	1.00	-	-

[1]Provided per kg of diet: Cu, 8 mg; Fe, 40 mg; Zn, 80 mg; Mn, 40 mg; I, 1.0 mg; Se, 0.3 mg; vitamin A, 6000 IU; vitamin D, 1000 IU; vitamin E, 15 IU; riboflavin, 4.8 mg; vitamin B_6, 3 mg; vitamin B_{12}, 0.02 mg; vitamin K, 2 mg; nicotinic acid, 20 mg; calcium pantothenate, 10 mg; folic acid, 0.6 mg; biotin, 0.2 mg; thiamin, 1.6 mg. [2]Obtained by calculation

Table 2: Effects of Bacteriocin-Like Substance (BLS) on performance of laying hens[1]

Groups	Egg production (% eggs/hen/day)	Feed to egg ratio (kg of feed/kg of egg)	Average egg weight (g)
Basal Diet (BD)	86.80±4.47	2.06±0.06	61.97±1.80
BD + 500 AU kg^{-1} BLS	89.27±4.08	2.13±0.09	62.35±1.64
BD +1500 AU kg^{-1} BLS	90.89±3.68	2.14±0.09	60.63±1.42
BD + 2500 AU kg^{-1} BLS	87.08±2.67	2.08±0.09	61.98±2.61
BD + 3500 AU kg^{-1} BLS	91.53±1.66	2.03±0.02	62.75±0.38

[1]Values were expressed as means±SEM

Table 3: Effects of Bacteriocin-Like Substance (BLS) on egg quality

Groups	Shell strength (kg cm^{-2})	Shell thickness (μm)	Shape index	Haugh unit	Yolk color	Ratio of blood spots egg (%)
Basal Diet (BD)	3.27±0.07	0.341±0.006	1.34±0.03	62.57±3.59	7.82±0.14[a]	26
BD + 500 AU kg^{-1} BLS	3.10±0.24	0.334±0.013	1.34±0.04	60.12±9.17	8.38±0.24[b]	24
BD +1500 AU kg^{-1} BLS	3.12±0.17	0.329±0.004	1.32±0.04	61.48±3.44	8.88±0.18[b]	14
BD + 2500 AU kg^{-1} BLS	3.51±0.11	0.340±0.014	1.34±0.02	61.63±8.12	8.88±0.07[b]	10
BD + 3500 AU kg^{-1} BLS	3.45±0.20	0.346±0.007	1.34±0.02	62.94±3.04	8.80±0.09[b]	22

[a-b]Means±SEM with different superscripts are significantly different ($p<0.05$)

2004). Probiotics are known to improve hens performance and egg quality and prevent disease, by production of antimicrobial agents and immune modulatory activity (Kurtoglu *et al*., 2004; Cutting, 2011).

CONCLUSION

The BLS used in this study antagonizes, *in vitro*, against several bacterial strains which are the same species of causative pathogens for diarrhea and necrotic enteritis in animals (Asai *et al*., 2002; Fairbrother *et al*., 2005; Guo, 2008). Although the application in treatment of disease for chickens remains investigation, the BLS may play a role in maintenance of better intestinal microbial balance, induction of immune response and uptake of nutrients and therefore, resulted in a lower incidence of eggs with blood spots.

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