Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X May - Jun 2016 / v.18 / n.2 / 261-268
http://dx.doi.org/10.1590/1806-9061-2015-0070

Effects of Kluyveromyces marxianus Isolated from Tibetan Mushrooms on the Plasma Lipids, Egg Cholesterol Level, Egg Quality and Intestinal Health of Laying Hens

■Author(s)

Zhong S[I,II]
Liu H[I]
Zhang H[I]
Han T[I]
Jia H[I]
Xie Y[I]

[I] Beijing Key Laboratory of Agricultural Product Detection and Control of Spoilage Organisms and Pesticide Residue, Beijing Laboratory of Food Quality and Safety, Faculty of Food Science and Engineering, Beijing University of Agriculture, Beijing, China 102206
[II] College of Food Science & Technology, Huazhong Agricultural University, Wuhan, China 430070

■Mail Address

Corresponding author e-mail address
Yuanhong Xie
No. 7 Bei Nong Road, ChangPing District, Beijing 102206, China.
Phone: +86(10)80799174
Fax: +86(10)82272963
Email: xieyuanh@163.com

■Keywords

Blood parameters, cholesterol, egg production, poultry nutrition, serum.

Submitted: May/2015
Approved: July/2015

ABSTRACT

The effects of the *Kluyveromyces marxianus* M3 strain, isolated from Tibetan mushrooms, on plasma lipids, egg cholesterol level, egg quality, and intestinal health of laying hens were evaluated. In total, 160 Beijing fatty laying hens (43 weeks old) were divided into four groups and fed a basal diet supplemented with 0%, 0.1%, 0.3%, or 0.5% freeze-dried *K. marxianus* M3 powder for four weeks. The results showed that yeast supplementation reduced serum total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), and very low density lipoprotein-cholesterol (VLDL-C) levels ($p<0.01$), and increased serum high density lipoprotein-cholesterol (HDL-C) level ($p<0.05$). Moreover, regardless of *K. marxianus* M3 dietary addition level, the cholesterol content of the eggs decreased by more than 26%. When0.3% yeast was supplemented, significant differences were found in the egg weights, shell strength, albumen height, Haugh unit and nutrient content of the eggs ($p<0.01$). Finally, 0.3% yeast supplementation improved the intestinal flora conditions of the hens by decreasing the *Salmonella* and *Staphylococcus aureus* counts ($p<0.01$) and increasing the *Bifidobacterium* count ($p<0.01$). The results in this work demonstrated that yeast culture supplementation to the diets deceased the serum and egg yolk cholesterol, and increased egg quality.

INTRODUCTION

Yeast is often used as a protein source in animal diets. Yeast and yeast products may also serve as alternatives to antibiotics to promote growth and disease resistance in poultry (Yalçin *et al*., 2010). In recent years, there has been an increasing number of reports documenting the use of various yeast strains and yeast products, such as inactive dried yeast, yeast culture, whey yeast, selenium yeast, chromium yeast and yeast cell walls, in the diets of laying hens. The inclusion of yeast in layer diets was shown to change egg production (Davis & Anderson, 2002), cholesterol content of the egg yolk (Yousefi & Karkoodi, 2007; Yalçin *et al*., 2008a), intestinal health of the hens (Yalçin *et al*., 2010; Lensing *et al*., 2012), egg quality (Utterback *et al*., 2005; Yalçin *et al*., 2008a, b; Yalçin *et al*., 2009; Yalçin *et al*., 2010), and egg fat content (Savage *et al*., 1985; Hosseini, 2011), among others.

The yogurt prepared from Tibetan mushrooms and milk has an extraordinary taste and provides excellent nutrition. Tibetan kefir has a granular structure due to the symbiotic microorganisms present, such as *Lactobacillus* and yeast (Simova *et al*., 2002). This complex microbial flora forms a long-term symbiotic relationship and creates a special mucopolysaccharide coat on the surface of the grain. Therefore, Tibetan kefir can protect itself against attack by other microbes and, due toits stable formulation, can be preserved for several years. Hence, Tibetan kefir is an economical and easily preserved material.

In recent years, some researchers have found that kefir-fermented milk can decrease the plasma cholesterol levels and promote cancer resistance. Furthermore, it has antioxidant properties and play a role in immune regulation, and may aid the protection against pathogenic bacteria and spoilage organisms, as well as assist in the conservation of the predominant gastrointestinal probiotic flora (Akalin *et al*., 1997; Abd El-Gawad *et al*., 2005; Nguyen *et al*., 2007; Mathara *et al*., 2008; Zheng et al., 2013). The objective of this study was to evaluate the effects of different doses of *Kluyveromyces marxianus* M3 yeast isolated from Tibetan mushrooms on plasma and egg yolkcholesterol contents, egg quality, and intestinal flora of the laying hens.

MATERIALS AND METHODS

Preparation of yeast strains

Kluyveromyces marxianus M3 was isolated from Tibetan mushrooms and was cultured by a resident of Baicheng, Jilin province, China (Liu *et al*. 2005). M3 strains (1-2%) were inoculated into 10 mL potato lactose liquid medium and grown at 28°C for 24 hours (h); by this time, the number of living microbes reached 10^7 cfu/mL in the fermentation liquor. An aliquot (3%) of the fermentation liquor was transferred to 1 L of potato lactose liquid medium and fermented for 24 h in a shaker at 32°C. The number of living microbes was counted using a standard plate count (SPC) procedure. The fermentation liquor was then centrifuged for 10 min at 4°C (8000 r/min), and the sediment was saved as the M3 yeast mire. The yeast mire was added to a freeze-drying bottle and then mixed with 1.5% sodium glutamate and 4.5% skim milk powder. This mixture was then frozen at -40ºC for 13 h and freeze-dried at -55ºC for 48 h until completely dried. The yeast powder of *K. marxianus* M3 was used in the following experiments.

Birds, Diets, and Management

A total of 160 Beijing-You laying hens (Beijing Yufa farm), a commercial white egg strain, with 43 weeks in age, were randomly allocated into a control group and three treatment groups of 40 hens each. Each group was divided into five replicates, comprising of eight hens each. They were housed in cages (30 cm × 50 cm × 50 cm) in a windowed poultry house (four cages per replicate with two birds per cage) under a 16/8 h light/dark regime. Feed, in the mash form, and water were provided *ad libitum* during the 4-week experimental period.

The control group (C) hens received a corn-soybean basal diet (BD, Beijing Huadu Yukou Poultry Industry Co. Ltd) with no additives (Table 1). The other 3 groups, depending on the probiotic addition, were fed the basal diet supplemented with 0.1% (m/m) M3 live yeast powder (low dosage group, LD), 0.3 % (m/m) M3 live yeast powder (medium dosage groups, MD), or 0.5% (m/m) M3 live yeast powder (high dosage group, HD; over 10^9 cfu/g).

Table 1 – Ingredients and chemical composition of the basal diet.

Ingredient	Inclusion level (g/kg, as-fed basis)
Corn	620
Soybean meal	250
Limestone	60
Dicalcium phosphate	15
Lysine HCl	0.5
DL-Methionine	2.5
Salt	2
Vitamin and minerals premix[a]	50

[a] Contained kilogram of premix: 142,000 IU vitamin A (retinyl acetate), 40,000 IU vitamin D_3 (cholecalciferol), 330 mg vitamin E (alpha-tocopherol), 26.5 mg vitamin K_3 (menadione), 16 mg vitamin B_1 (thiamine), 60 mg vitamin B_2 (riboflavin), 13 mg vitamin B_6 (pyridoxine), 0.5 mg vitamin B_{12} (cyanocobalamin), 2 mg D-biotin, 446 mg nicotinic acid, 260 mg pantothenic acid, 9.0 mg folic acid, 1.0 g manganese (MnSO4), 0.8 g zinc (ZnSO4), 1.0 g iron (FeSO4), 8.0 mg iodine ($Ca(IO_3)_2$), and 3.5 mg Se.

Plasma Analysis

At the end of the four-week experiment, three chickens per replicate were randomly selected, and their venous blood was collected when their stomachs were empty. The samples were allowed to stand for 10 min and were then centrifuged at 3500 r/min for 15 min; the sediment was discarded. The TC (total cholesterol), TG (triglyceride), HDL-C (high density lipoprotein-cholesterol), and LDL-C (low density lipoprotein-cholesterol) levels were analyzed using kits (Bio-technology and Science Incorporation) and a fully automatic biochemical analyzer (Hitachi, Japan). The VLDL-C (very low density lipoprotein-cholesterol) was calculated using the following equation: VLDL-C =TC – HDL-C –LDL-C

Assay of egg quality

At the end of the experimental period, four eggs per replicate were randomly collected. Eggs were weighed and hard-cooked by immersion in boiling water for 5 min. Yolks were individually weighed and pooled by blending four yolks per sample, and then stored in a freezer at -20°C.

The yolk cholesterol content was examined using UV spectrophotometry at 550 nm and the *o*-phthalaldehyde method (Rudel & Morris, 1973). The

rate of cholesterol reduction was calculated according to the standard curve of absorbance (y = 0.0919x - 0.002, R^2 = 0.9995). The best dose of the M3 live yeast powder was selected for further testing.

The mineral content of the yolk was measured using an inductively coupled plasma atomic emission spectroscopy (ICP-AES) instrument (Spectro Arcos, Kleve, Germany). The total protein concentration was assayed using a Nanjing Jiancheng Bioengineering Institute assay kit (Nanjing, China). The dry matter content was determined using lyophilization, and the crude fat content was examined using the Soxhlet method. The vitamin A and vitamin E content were assayed using high performance liquid chromatography (HPLC), according to the method described by Rubolini *et al*. (2011).

After four weeks of administration, egg weight (g), yolk weight (g), and eggshell weight (g) of the intermediate yeast dose group and the control group were examined. Eggshell thickness (mm) was measured using a micrometer. Eggshell strength (kg/cm^2) was investigated using an Egg Analyzer (Orka Food Technology, Israel). Albumen height (mm), yolk color, and Haugh unit were automatically analyzed using an EMT-5200 Multi-functional egg quality analyzer (Japan). The Haugh unit (HU) was calculated using the equation: HU =100 log (H – 1.7× W0.37+ 7.6), where H is the observed albumen height (mm), and W is egg weight (g).

Analysis of intestinal flora

Viable *Bifidobacterium*, *Salmonella*, and *Staphylococcus aureus* present in the excreta were enumerated. This experiment was carried out during the rearing test for the laying hens, and three chickens were randomly selected from replicate of control and 0.3 % *K. maxianus* M3 supplementation. Every week, 1 g of feces from each hen was collected and stored in weighed, sterilized test tubes. Due to the presence of the anaerobic *Bifidobacterium* in the feces, the test tubes containing the feces were stored in a Bio-bag containing a de-oxidant. One gram of excreta was transferred into a test tube containing 9 mL sterile normal saline. The samples were mixed well by vortexing, and serial dilutions from 10^{-1} cfu/mL to 10^{-8} cfu/mL were prepared. According to the results of the trial test, three dilutions were chosen, and 0.1 mL of this dilution was inoculated into the corresponding medium. Every dilution was run in duplicate. The process of cultivating anaerobic bacteria was completed in 15 min. *Bifidobacterium* was cultured on MUP-MRS agar plates in Bio-bags at 36ºC±1ºC for 48 h. *Salmonella* was cultured on bismuth sulfite agar plates at 36ºC ±1ºC for 24 h, and *S. aureus* was cultured on Baird-Parker agar plates at 36ºC ±1ºC for 18-24 h. Only the typical bacterial colonies were counted, and the total counts for the three types of bacteria were examined.

Date analysis

Statistical analyses were performed using SPSS program (SPSS INC., Chicago, IL, USA) and were expressed as mean ± sd. One-way analysis of variance was used to evaluate the effects of different concentrations of *Kluyveromyces marxianus* M3 on serum lipids, egg traits, and intestinal flora. Mean differences among groups were compared by the test of Duncan. The level of statistical significance was set at $p<0.01$.

RESULTS

Plasma lipids

The effects of *K. marxianus* M3 live yeast supplementation on the serum lipid levels of laying hens are presented in Table 2. After the 4th week, TC, TG, LDL-C, and VLDL-C levels of the three experimental groups were significantly lower than those of the control group ($p<0.01$). Conversely, the HDL-C levels were higher in the M3-supplemented hens than in the control hens ($p<0.01$). The greatest reductions in serumlevels of TC, TG, LDL-C, and VLDL-C were observed in the MD group, of 24.30%, 25.79%, 45.90%, and 36.05%, respectively, whereas their serumHDL-C level increased by 39.06% relative to the control group. In the LD group, serum TC, TG, LDL-C and VLDL-C levels dropped by 20.39%, 21.41%,

Table 2 – Effect of the M3 live yeast preparation on the serum lipid levels of laying hens ($\bar{x}$ ±*s*, *n*=15)

Group	TC(mmol/L)	TG(mmol/L)	HDL-C(mmol/L)	LDL-C(mmol/L)	VLDL-C(mmol/L)
Control	3.58±0.07^b	17.33±0.09^c	0.64±0.02^a	0.61±0.13^b	2.33±0.12^b
Low-dose	2.85±0.05^a	13.62±0.11^a	0.85±0.05^b	0.37±0.05^a	1.63±0.06^a
Medium-dose	2.71±0.04^a	12.86±0.08^a	0.89±0.06^b	0.33±0.04^a	1.49±0.05^a
High-dose	3.08±0.05^a	14.74±0.07^b	0.78±0.10^b	0.39±0.05^a	1.91±0.06^a

a-b: Means within a column followed by the same superscript are not significantly different ($p<0.01$).

39.34% and 30.04%, respectively, whereas the HDL-C level increased by 32.81% compared with the control group. Moreover, the HD group presented the least reductions of TC, TG, LDL-C and VLDL-C serum levels, which decreased only by 13.97%, 14.95%, 36.07% and 18.03%, respectively, and the HDL-C level increased by 21.88% relative to the control group. Therefore, we concluded that supplementing the basal diet of the laying hens with *K. marxianus* M3 live yeast clearly decreased TC, TG, LDL-C, VLDL-C serum levels and increased HDL-C serum level.

Egg Quality

During the four weeks of administration, the cholesterol content of the eggs was examined every week. As shown in Table 3, the cholesterol contents of the eggs from the yeast-fed groups were significantly lower ($p<0.01$) than those from the control group. Additionally, the cholesterol content of the three yeast-fed groups declined gradually during the four-week administration period. At the end of the fourth week, the cholesterol content of the eggs of the MD, LD and HD groups were 229.88 mg/100 g, 313.758 mg/100 g, and 358.17 mg/100 g, respectively, corresponding to 52.8%, 35.28% and 26.46%, respectively, of the cholesterol content of the eggs of the control group. We determined that a highly significant reduction in the cholesterol content of the eggs could be achieved by adding 0.3% M3 freeze-dried powder to the basal diet over a 4-week period, resulting in a reduction of 52.80%.

We further examined the egg quality of the MD group. As shown in Table 4, egg, yolk, and eggshell weights of the MD group were significantly higher ($p<0.01$) relative to the control group. However, eggshell strength of the eggs laid by yeast-fed hens was lower than that of the control group. Additionally, albumen height and Haugh unit of MD eggs were dramatically higher ($p<0.01$) than the control group, which suggests better quality of the eggs of the experimental group compared with the control group.

Table 4 – Effect of dietary *K. marxianus* M3 live yeast preparation powder on egg quality ($\overline{x} \pm s$, n=20)

Factor	Control group	Medium-dose group
Egg weight (g)	44.90±1.02^a	58.50±1.51^b
Yolk weight (g)	17.71±0.35^a	19.55±0.42^b
Shell weight (g)	6.06±0.10^a	6.91±0.28^b
Egg shell Strength (kg/cm^2)	4.09±0.35^a	3.44±0.23^a
Egg shell thickness (mm)	0.42±0.07^a	0.41±0.05^a
Albumen height (mm)	4.7±0.11^a	6.9±0.17^b
Haugh unit (HU)	69.9±1.2^a	87.9±2.6^b
Yolk color	7	7

a-b: Means within a row with no common superscript differ significantly ($p<0.01$)

However, we did not observe any significant difference in the index production between the yeast-fed and the control hens.

As shown in Table 5, over the course of four weeks, the addition of the 0.3% M3 live yeast preparation to the basal diet not only reduced egg cholesterol content below the international reference value (USDA National Nutrient Database for Standard Reference Release 28), but all the nutrient and chemical values were also significantly higher ($p<0.01$) than those of the control eggs, especially vitamin A, crude fat, vitamin E, calcium, zinc, selenium, and dry matter.

Table 5 – Effect of the M3 live yeast preparation powder on the nutrient and chemical indices of the eggs ($\overline{x} \pm s$, n=20)

Factor	Control group	Medium-Dose group
Protein (g/100 g)	10.20±0.11^a	12.50±0.12^b
Dry matter (g/100 g)	18.56±0.19^a	23.64±0.24^b
Vit A (mg/100 g)	0.172±0.009^a	0.332±0.021^b
Crude fat (g/100 g)	5.52±0.17^a	7.94±0.26^b
Vit E (mg/100 g)	1.99±0.08^a	3.43±0.13^b
Ca (mg/100 g)	31.8±1.97^a	49.44±2.32^b
Fe (mg/100 g)	1.08±0.09^a	2.26±0.17^b
Zn (mg/100 g)	7.73±0.78^a	19.00±1.53^b
Se (mg/100 g)	0.193±0.018^a	0.337±0.035^b

a-b: Means within a row with no common superscript differ significantly ($p<0.01$)

Table 3 – Effect of the *K. marxianus* M3 freeze-dried powder on the cholesterol content of the eggs ($\overline{x} \pm s$, n=20).

Group	Cholesterol content of eggs (mg/100 g)							
	1st week	D (%)	2nd week	D (%)	3rd week	D (%)	4th week	D (%)
Control group	484.65 ± 0.96^a		483.73 ± 1.06^a		485.83 ± 1.30^a		487.02 ± 1.10^a	
Low-dose group	452.84 ± 0.75^b	6.56	426.07 ± 0.95^b	11.92	336.45 ± 1.26^b	30.75	313.75 ± 0.76^b	35.58
Medium-dose group	438.28 ± 0.70^c	9.57	410.82 ± 1.25^c	15.07	252.46 ± 1.21^c	48.04	229.88 ± 0.62^c	52.8
High-dose group	458.26 ± 0.60^b	5.45	438.81 ± 1.03^d	9.29	378.10 ± 1.32^d	22.17	358.17 ± 1.09^d	26.46

a-d: Means within a column followed by the same superscript are not significantly different ($p<0.01$), "D" represents the percent decrease in the cholesterol content of the eggs from the former week in each group.

Intestinal flora

During the experimental period, we examined the changes in the intestinal flora of the hens fed the 0.3% *K. marxianus* M3-supplemented diet every week. As shown in Table 6, during the first two weeks of the experiment, there were no marked differences in *Bifidobacterium*, *Salmonella,* or *S. aureus* counts between the MD group and the control group. After the third week, the *Bifidobacterium* count in the MD-group hens was significantly higher ($p<0.01$) than that of the control-group hens. After the fourth week, the *Bifidobacterium* count of the MD group was 9.72 log cfu/g feces, while the control group was only 7.44 log cfu/g feces. Furthermore, the *Salmonella* and *S. aureus* counts in the feces of both the MD-group hens and the control hens were similar during the first two weeks of the experiment and then began to dramatically decrease during the third week. After the fourth week, the *Salmonella* and *S. aureus* counts in the MD group were 4.47 log cfu/g feces and 5.56 log cfu/g feces, respectively, which were significantly lower ($p<0.01$) than those of the control group (Table 6).

DISCUSSION

Eggs are eaten almost daily and contain abundant nutrients, particularly high-quality protein. However, the large amount of cholesterol in the yolk can lead to high blood cholesterol levels in humans, an important factor contributing to atherosclerosis and coronary heart disease (Mohan *et al*., 1995). Therefore, how to decrease the cholesterol content of eggs is a problem that urgently needs to be solved. Recently, diet manipulation has emerged as a new strategy to improve the nutritional composition of animal products. Supplementing layer feeds with probiotic supplements has been shown to be an efficient and versatile way to lower egg cholesterol level (Mahdavi *et al*., 2005).

Although the mechanism by which probiotics reduce egg cholesterol content has not yet been studied, we infer that the mechanism is similar to those indicated by some recent studies. It is thought that some lactic acid bacteria may secrete high-activity bile salt hydrolase (BSH) during metabolism, and this secretion could considerably affect cholesterol levels (Pereira & Gibson, 2002; Nguyen *et al*., 2007). Furthermore, BSH degrades cholates into amino acids and low-solubility free cholate in the enterohepatic circulation. The free cholate and cholesterol combine into sediment composites, which are then expelled along with the feces. Subsequently, the free cholate in the liver enterohepatic circulation decreases, thus increasing the biosynthesis of cholic acid; this increase in cholic-acid synthesis accelerates the catabolism of cholesterol, which is the precursor ofcholic acid. Thus, total cholesterol plasma level decreases (Mann & Spoerry, 1974; Liong, 2005; Begley *et al*., 2006).

According to our results, supplementing the basal diet of laying hens with *K. marxianus* M3 live yeast clearly decreased the serum TC, TG, LDL-C, VLDL-C levels and increased the serum HDL-C level (Table 2). VLDL is the main storage area or carrier of plasma cholesterol, which combines both cholesterol and the yolk cholesterol. Therefore, a lower VLDL-C value means there is less cholesterol in the egg. Furthermore, the cholesterol content of the egg yolks of the group fed the *K. marxianus* M3-supplemented diet was significantly lower than that of the control group ($p<0.01$). After the fourth week, the egg cholesterol content of the LD, MD and HD groups was reduced by 35.58%, 52.80% and 26.46%, respectively (Table 3). Our results indicate that there was a relationship between the synthesis and reduction of egg cholesterol and the metabolism of cholesterol in the plasma. Recently, considerable attention has been given to the potential of probiotics in altering lipid metabolism. This interest stems from the growing evidence that probiotics reduce cholesterol concentration in the egg yolk (Abdulrahim *et al*. 1996; Haddadin *et al*., 1996; Panda *et al*., 2003; Kalavathy *et al*., 2009) and in the serum (Kurtoglu *et al*., 2004; Alkhalf *et al*., 2010).

In this study, we further demonstrated that feeding laying hens a diet containing *K. marxianus* M3 live yeast increased yolk weight, eggshell weight, albumen height, and Haugh unit (Table 4); however,

Table 6 – Effects of diet probiotic on excreta microflora in laying hens (log10 cfu/g feces, $\overline{x} \pm s$, n=15)

Item	Week 1		Week 2		Week 3		Week 4	
	Control Group	Probiotic Group	Control Group	Probiotic Group	Control Group	Probiotic Group	Control Group	Probiotic Group
Lactobacillus	7.47±0.24^{a}	7.50±0.22^{a}	7.5±0.31^{a}	7.59±0.25^{a}	7.39±0.28^{a}	8.41±0.29^{b}	7.44±0.29^{a}	9.72±0.33^{c}
Salmonella	6.20±0.21^{c}	6.19±0.29^{c}	6.16±0.17^{c}	6.05±0.22^{c}	6.26±0.18^{c}	5.25±0.26^{b}	6.39±0.28^{c}	4.17±0.23^{a}
S. aureus	7.80±0.24^{c}	7.81±0.13^{c}	7.82±0.16^{c}	7.71±0.19^{c}	7.85±0.22^{c}	6.11±0.23^{b}	7.79±0.21^{c}	5.56±0.19^{a}

a-c: Means within a row with no common superscript differ significantly ($p<0.01$)

we did not observe any significant differences in egg production between the yeast-fed and the control hens. Moreover, the egg concentrations of vitamin A, crude fat, vitamin E, calcium, zinc, selenium, and dry matter also dramatically improved in the eggs of yeast-fed hens (Table 5). Similar studies reported a significant improvement of egg production in hens fed a mixed culture of probiotics, such as *Lactobacillus acidophilus* and *Lactobacillus casei* (Tortuero & Fernandez, 1995), *L. acidophilus* alone (Haddadin *et al*., 1996), *Pediococcus acidilactici* (Mikulski *et al*., 2012), *Bacillus subtilis* (Xu *et al*. 2006; Abdelqader *et al*. 2013), *Propionibacterium jensenii* (Luo *et al*., 2010), and *Saccharomyces cerevisiae* (Yalçin *et al*., 2010). Significant improvements in egg weight and eggshell quality were also obtained in hens fed diets supplemented with a mixture of *Lactobacillus* cultures (Davis & Anderson, 2002; Kalavathy *et al*., 2009).

The intestinal microflora plays a major protective role in maintaining the integrity of the intestinal mucosa. The addition of beneficial bacteria to a diet can recover the intestinal integrity, thereby increasing nutrient bioavailability and absorption (Burel & Valat, 2009; Deng *et al*., 2012). Probiotics also improved gut health, which directly improved the birds' health and performance (Burel & Valat, 2009). In this study, we examined the presence of three typical intestinal flora components in the feces of laying hens. Our results indicated that throughout the entire experimental period, the numbers of *Bifidobacterium* present in the feces of the control group steadily grew; however, the *Bifidobacterium* counts in the MD groups were higher than those in the control group after the third week (Table 6). Moreover, the *Salmonella* and *S. aureus* counts in MD group decreased compared to the control group (Table 6). Therefore, we concluded the M3 live yeast modulates the intestinal flora and protects the intestinal tract of laying hens.

ACKNOWLEDGEMENTS

This research was funded by the Comprehensive Reforming Project to promote talents training of BUA, Importation and Development of High-Caliber Talents Project of Beijing Municipal Institutions (CIT&TCD20140315) and the Key Construction Discipline Program of Beijing Municipal Commission of Education (PXM2014-014207-0000029).

DISCLOSURE

There is no conflict of interest with other works.

REFERENCES

Abd El-Gawad IA, El-Sayed E, Hafez S, El-Zeini H, Saleh F. The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. International Dairy Journal 2005;15(1):37-44.

Abdulrahim S, Haddadin M, Hashlamoun E, Robinson R. The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. British Poultry Science 1996;37(2):341-346.

Abdelqader A, Irshaid R, Al-Fataftah AR. Effects of dietary probiotic inclusion on performance, eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production. Tropical Animal Health and Production 2013;45(4):1017-1024.

Akalin AS, Gönç S, Düzel S. Influence of yogurt and acidophilus yogurt on serum cholesterol levels in mice. Journal of Dairy Science 1997;80(11):2721-2725.

Alkhalf A, Alhaj M, Al-Homidan I. Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. Saudi Journal of Biological Sciences 2010;17(3):219-225.

Begley M, Hill C, Gahan CGM. Bile salt hydrolase activity in probiotics. Applied and Environmental Microbiology 2006;72(3):1729-1738.

Burel C, Valat C. The effect of the feed on the host–microflora interactions in poultry, an overview. In: Aland A, Madec F, editors. Sustainable animal production. Wageningen: Wageningen Academic Publishers; 2009.

Davis GS, Anderson KE. The effects of feeding the direct-fed microbial, primalac, on growth parameters and egg production in single comb white leghorn hens. Poultry Science 2002;81(6):755-759.

Deng W, Dong XF, Tong JM, Zhang Q. The probiotic *Bacillus licheniformis* ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. Poultry Science 2012;91(3):575-582.

Haddadin MS, Abdulrahim SM, Hashlamoun EA, Robinson RK. The effect of *Lactobacillus acidophilus* on the production and chemical composition of hen's eggs. Poultry Science 1996;75(4):491-494.

Hosseini S. The effect of utilization of different levels of *Saccharomysec cerevisiae* on broiler chicken's performance. Global Veterinaria 2011;6(3):233-236.

Kalavathy R, Abdullah N, Jalaludin S, Wong CMVL, Ho YW. Effects of *Lactobacillus* cultures on performance of laying hens, and total cholesterol, lipid and fatty acid composition of egg yolk. Journal of the Science of Food and Agriculture 2009;89(3):482-486.

Kurtoglu V, Kurtoglu F, Seker E, Coskun B, Balevi T, Polat ES. Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. British Poultry Science 2004;21(9):817-823.

Lensing M, van der Klis JD, Yoon I, Moore DT. Efficacy of *Saccharomyces cerevisiae* fermentation product on intestinal health and productivity of coccidian-challenged laying hens. Poultry Science 2012;91(7):1590-1597.

Liong M. Bile salt deconjugation ability, bile salt hydrolase activity and cholesterol co-precipitation ability of *lactobacilli* strains. International Dairy Journal 2005;15(4):391-398.

Liu H, Dong R, Pan C, Gao X, Zhang H. Optimization of fermentation conditions of *Kluyveromyces marxianus* bile salt hydrolase. Food Science 2005;26(7):97-100.

Luo J, King S, Adams MC. Effect of probiotic *Propionibacterium jensenii* supplementation on layer chicken performance. Beneficial Microbes 2010;1(1):53-60.

Mahdavi A, Rahmani H, Pourreza J. Effect of probiotic supplements on egg quality and laying hens performance. International Journal of Poultry Sciences 2005;4(7):488-492.

Mann GV, Spoerry A. Studies of a surfactant and cholesteremia in the Maasai. The American Journal of Clinical Nutrition 1974;27(5):464-469.

Mathara JM, Schillinger U, Guigas C, Franz C, Kutima PM, Mbugua SK, Shin HK, Holzapfel WH. Functional characteristics of *Lactobacillus* spp. from traditional Maasai fermented milk products in Kenya. International Journal of Food Microbiology 2008;126(1-2):57-64.

Mikulski D, Jankowski J, Naczmanski J, Mikulska M, Demey V. Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. Poultry Science 2012;91(10):2691-700.

Mohan B, Kadirvel R, Bhaskaran M, Natarajan A. Effect of probiotic supplementation on serum/yolk cholesterol and on eggshell thickness in layers. British Poultry Science 1995;36(5):799–803.

Nguyen T, Kang J, Lee M. Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering effects. International Journal of Food Microbiology 2007;113(3):358-361.

Panda AK, Reddy MR, Rama Rao SV, Praharaj NX. Production performance, serum/yolk cholesterol and immune competence of white leghorn layers as influence by dietary supplementation with probiotic. Tropical Animal Health and Production 2003;35(1):85–94.

Pereira DIA, Gibson GR. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. Applied and Environmental Microbiology 2002;68(9):4689-4693.

Rubolini D, Romano M, Navara KJ, Karadas F, Ambrosini R, Caprioli M, Saino N. Maternal effects mediated by egg quality in the Yellow-legged Gull Larus michahellis in relation to laying order and embryo sex. Frontiers in Zoology 2011;8(1):24-38.

Rudel L, Morris M. Determination of cholesterol using o-phthalaldehyde. Jounal of Lipid Research 1973;14(3):364-366.

Savage TF, Nakaue HS, Holmes ZA. Effects of feeding a live yeast culture on market turkey performance and cooked meat characteristics. Nutrition Reports International 1985;31(3):695-703.

Simova E, Beshkova D, Angelov A, Hristozova T, Frengova G, Spasov Z. Lactic acid bacteria and yeasts in kefir grains and kefir made from them. Journal of Industrial Microbiology & Biotechnology 2002;28(1):1–6.

Tortuero F, Fernandez E. Effect of inclusion of microbial culture in barley-based diets fed to laying hens. Animal Feed Science and Technology 1995;53(3-4):255-265.

Utterback PL, Parsons CM, Yoon I, Butler J. Effect of supplementing selenium yeast in diets of laying hens on egg selenium content. Poultry Science 2005;84(12):1900-1901.

Li L, Xu CL, Ji C, Ma Q, Hao K, Jin ZY, Li K. Effects of a dried *Bacillus subtilis* culture on egg quality. Poultry Science 2006;85(2):364–368.

Yalçin S, Özsoy B, Erol B, Yalçin S. Yeast culture supplementation to laying hen diets containing soybean meal or sunflower seed meal and its effect on performance, egg quality traits and blood chemistry. Journal of Applied Poultry Research 2008a;17(2):229-236.

Yalçin, Erol H, Ozsoy B, Onbaşılar I, Yalçin S. Effects of the usage of dried brewing yeast in the diets on the performance, egg traits and blood parameters in quails. Animal 2008b;2(12):1780-1785.

Yalçin S, Oğuz F, Güçlü B, Yalçin S. Effects of dietary dried baker's yeast on the performance, egg traits and blood parameters in laying quails. Tropical Animal Health and Production 2009;41(1):5-10.

Yalçin S, Yalçin S, Cakin K, Eltan O, Dağaşan L. Effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) on performance, egg traits, egg cholesterol content, egg yolk fatty acid composition and humoral immune response of laying hens. Journal of the Science of Food and Agriculture 2010;90(10):1695-1701.

Yousefi M, Karkoodi K. Effect of probiotic Thepax and *Saccharomyces cerevisiae* supplementation on performance and egg quality of laying hens. International Journal of Poultry Science 2007;6(1):52-54.

Zheng YC, Lu YL, Wang JF, Yang LF, Pan CY, Huang Y. Probiotic properties of Lactobacillus strains isolated from Tibetan Kefir grains. PLoS One 2013;8(7):e69868.

ABBREVIATIONS

SPC: standard plate count
BD: basal diet
TC: total cholesterol
TG: triglyceride
HDL-C: high density lipoprotein-cholesterol
LDL-C: low density lipoprotein-cholesterol
VLDL-C: very low density lipoprotein-cholesterol
HPLC: high performance liquid chromatography
BSH: bile salt hydrolase