Effects of Bacillus coagulans Probiotic Preparation on Anti-lipid Peroxidation Capacity in Beijing Oil Chicken Laying Hens (Postprint)

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Abstract

This study aimed to investigate the effects of Bacillus coagulans probiotic preparation on the anti-lipid peroxidation capacity of laying Beijing oil chickens. A Bacillus coagulans Liu-g1 strain with strong neutral protease-producing ability was isolated from traditional cheese and used to prepare the probiotic preparation through fermentation. One hundred twenty healthy 14-week-old laying Beijing oil chickens were randomly allocated into four groups with three replicates per group and ten chickens per replicate. The blank control group was fed a basal diet, while the experimental groups were fed experimental diets supplemented with 10, 100, or 1,000 mg/kg of Bacillus coagulans Liu-g1 in the basal diet. The experimental period lasted 10 weeks. Serum triglyceride content and antioxidant indices were measured, and after slaughter, liver crude fat content was determined and liver tissue sections were examined. The results showed that compared with the blank control group, serum triglyceride contents in the 10, 100, and 1,000 mg/kg Bacillus coagulans Liu-g1 groups were all significantly decreased ($P<0.05$), and liver crude fat contents were all extremely significantly reduced ($P<0.01$). Oil red O staining of liver tissue sections revealed that with increasing supplementation levels of Bacillus coagulans Liu-g1, the number of hepatic fat droplets decreased markedly, with the 100 mg/kg Bacillus coagulans Liu-g1 group showing the optimal effect. Serum total antioxidant capacity in the 10 and 100 mg/kg Bacillus coagulans Liu-g1 groups was significantly higher than that in the blank control group ($P<0.05$), and serum glutathione peroxidase activity in the 10, 100, and 1,000 mg/kg Bacillus coagulans Liu-g1 groups was significantly higher than that in the blank control group ($P<0.05$), while serum superoxide dismutase activity and malondialdehyde content were increased and decreased to certain extents, respectively ($P>0.05$). It can be concluded that dietary supplementation with Bacillus coagulans Liu-g1 can significantly reduce fat accumulation and inhibit fatty liver formation in laying

chickens, and possesses good anti-lipid peroxidation capacity, with the appropriate supplementation level being 100 mg/kg.

Full Text

Effects of *Bacillus coagulans* Microecological Preparation on Anti-Lipid Peroxidation Capability in Beijing-You Laying Hens

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Abstract: This study investigated the effects of *Bacillus coagulans* microecological preparation on anti-lipid peroxidation capability in Beijing-You laying hens. The *Bacillus coagulans* Liu-g1 strain, previously isolated from traditional cheese and identified as a strong neutral protease producer, was used to prepare the microecological preparation through fermentation. A total of 120 healthy 14-week-old Beijing-You laying hens were randomly divided into four groups with three replicates per group and ten birds per replicate. The blank control group was fed a basal diet, while experimental groups received diets supplemented with 10, 100, or 1,000 mg/kg *Bacillus coagulans* Liu-g1. The trial lasted for ten weeks. Serum triglyceride content and antioxidant indices were measured, and after slaughter, liver crude fat content was determined and liver tissue sections were examined. The results demonstrated that compared with the blank control group, serum triglyceride levels in the 10, 100, and 1,000 mg/kg *Bacillus coagulans* Liu-g1 groups were significantly decreased ($P<0.05$), while liver crude fat content was extremely significantly reduced ($P<0.01$). Oil red O staining of liver sections revealed that the number of hepatic fat droplets decreased markedly with increasing supplementation levels of *Bacillus coagulans* Liu-g1, with the 100 mg/kg group showing the optimal effect. Serum total antioxidant capacity (T-AOC) in the 10 and 100 mg/kg groups was significantly higher than in the blank control group ($P<0.05$), and serum glutathione peroxidase (GSH-Px) activity in all three treatment groups was significantly elevated ($P<0.05$). Serum superoxide dismutase (SOD) activity and malondialdehyde (MDA) content showed respective improvements and reductions, though these changes were not statistically significant ($P>0.05$). These findings indicate that dietary supplementation with *Bacillus coagulans* Liu-g1 can effectively reduce fat accumulation in laying hens, inhibit fatty liver formation, and confer substantial anti-lipid peroxidation capability, with an optimal supplementation level of 100 mg/kg.

Keywords: *Bacillus coagulans*; Beijing-You chickens; fatty liver; lipid peroxidation

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Introduction

Fatty liver syndrome (FLS) is a metabolic disorder characterized by abnormal fat accumulation and metabolic disturbances in the liver, commonly affecting laying hens during peak and late production phases [1-3]. First identified in laying hens in the southwestern United States by Couch [4] in 1954, FLS primarily occurs in caged laying hens, causing significant declines in egg production and increased mortality, thereby imposing substantial economic losses on the poultry industry [5]. Field observations reveal that most hens dying from FLS accumulate excessive liver fat, leading to hepatic rupture and hemorrhage. Since FLS is often detected only after hens fail to reach peak production or after post-mortem examination, microecological preparations represent a promising preventive strategy to enhance antioxidant capacity during the rearing period [6]. Xu [7] demonstrated that *Bacillus licheniformis* TS-01 could prevent FLS in laying hens, while Pan et al. [8] found that dietary probiotic supplementation increased serum GSH-Px activity in laying hens. However, research on the preventive effects of *Bacillus coagulans* on FLS and its anti-lipid peroxidation capability in Beijing-You laying hens remains unreported in domestic and international literature. Therefore, this study utilized Beijing-You chickens as experimental subjects to investigate the effects of dietary supplementation with freeze-dried *Bacillus coagulans* Liu-g1 powder on lipid peroxidation marker MDA, anti-lipid peroxidation indices, and serum triglyceride (TG) content. Through histological examination of oil red O-stained liver sections and determination of hepatic crude fat content, we evaluated the preventive effects against FLS and antioxidant activity of *Bacillus coagulans* Liu-g1, aiming to provide scientific theoretical basis for developing antioxidant microecological preparations.

Materials and Methods

Experimental Strain

Bacillus coagulans Liu-g1, isolated from traditional cheese and identified as a neutral protease-producing strain in our previous research [9].

Microecological Preparation

The *Bacillus coagulans* Liu-g1 was fermented under optimized conditions, then concentrated by centrifugation and vacuum freeze-dried to produce a freeze-dried powder with viable cell count of 7.35×10^{1} CFU/g.

Culture Media

Fermentation medium [9]: soybean meal 1.0%, corn starch 4.0%, calcium carbonate 0.6%.

Plate Count Agar (PCA) medium: tryptone 5.0 g, yeast extract 2.5 g, glucose 1.0 g, agar 17.0 g, distilled water 1,000 mL, pH 7.0.

Instruments and Equipment

BT2202S Electronic Balance (Sartorius, Germany), Vacuum Freeze Dryer (Labconco, USA), TGL-20M High Speed Refrigerated Centrifuge (Shanghai Luxiangyi Centrifuge Instrument Co., Ltd.), 202-001 Electrothermal Constant-Temperature Dry Box (Beijing Kewei Yongxing Instrument Co., Ltd.).

Experimental Design and Diets

One hundred twenty healthy 14-week-old Beijing-You laying hens with non-significant weight differences ($P>0.05$) were randomly allocated into four groups: blank control, low-dose, medium-dose, and high-dose, with three replicates per group and ten birds per replicate. The blank control group received a basal diet, while experimental groups received diets supplemented with 10, 100, or 1,000 mg/kg *Bacillus coagulans* Liu-g1, providing viable bacteria at 10 , 10 , and 10^{1} CFU/kg, respectively. The basal diet formulation followed NRC (1994) standards, with composition and nutrient levels shown in . The *Bacillus coagulans* Liu-g1 microecological preparation was produced in our laboratory. Experimental birds were provided by the Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, and housed in a three-tier cage system with 16 h lighting, temperature maintained at 20–23°C, ad libitum access to feed and water, and routine immunization protocols.

Sample Collection

Serum collection: At week 10, blood samples (2–3 mL) were collected from ten birds per replicate via wing vein using sterile syringes. After clotting at

37°C for approximately 2 h, serum was transferred to 2 mL centrifuge tubes and centrifuged at 4,000 r/min for 10 min. The supernatant was collected and stored at -20°C for subsequent analysis.

Liver tissue: At week 10, five birds per replicate were randomly selected and slaughtered. Liver samples measuring 1.5 cm × 1.5 cm × 1.5 cm were excised and fixed in 4% paraformaldehyde solution for histological examination.

Analytical Methods and Parameters

Liver histological observation: Liver tissue sections were stained with oil red O and examined under microscopy.

Liver crude fat content determination: Fresh liver samples were freeze-dried for 48 h, passed through a 40-mesh sieve, and 2.00–5.00 g portions were extracted using the method described in GB/T 5009.6-2003 "Determination of Fat in Foods" [10].

Serum antioxidant indices: Frozen serum samples were analyzed by Beijing Zhongtong Lanbo Clinical Laboratory Co., Ltd. for triglyceride content, total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity, and malondialdehyde (MDA) content.

Statistical Analysis

Experimental data were analyzed using one-way ANOVA with SPSS 22.0 software, followed by Duncan' s multiple range test. Results were expressed as mean ± standard deviation. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

Results

Effects of *Bacillus coagulans* Liu-g1 on Serum Triglyceride Content in Laying Hens

As shown in [Figure 1: see original paper], serum triglyceride levels in the 10, 100, and 1,000 mg/kg *Bacillus coagulans* Liu-g1 groups were significantly lower than in the blank control group ($P<0.05$), with reductions of 18.91%, 57.55%, and 56.05%, respectively. The 100 and 1,000 mg/kg groups exhibited significantly lower serum triglyceride content than the 10 mg/kg group ($P<0.05$), with the 100 mg/kg group showing the lowest triglyceride levels.

Oil Red O Staining of Liver Tissue Sections

Following oil red O staining, hepatic intracellular fat droplets appeared red while nuclei appeared blue. As illustrated in [Figure 2: see original paper], liver sections from the blank control group displayed numerous red fat droplets, whereas all treatment groups showed markedly fewer red fat droplets. The

100 mg/kg *Bacillus coagulans* Liu-g1 group exhibited virtually no fat droplets, indicating optimal efficacy.

Effects of *Bacillus coagulans* Liu-g1 on Liver Crude Fat Content in Laying Hens

As depicted in [Figure 3: see original paper], liver crude fat content in the 10, 100, and 1,000 mg/kg groups was extremely significantly reduced compared with the blank control group ($P<0.01$), with decreases of 56.45%, 70.91%, and 66.78%, respectively. The 100 mg/kg group showed an extremely significant reduction compared with the 10 mg/kg group ($P<0.01$), with a 33.20% decrease. These findings confirm that hens not receiving *Bacillus coagulans* Liu-g1 exhibited hepatic fat accumulation, while supplementation significantly ameliorated this condition and demonstrated potent fatty liver-inhibiting effects, with 100 mg/kg being the most effective dose. These results align with the oil red O staining observations in [Figure 2: see original paper].

Effects of *Bacillus coagulans* Liu-g1 on Serum Antioxidant Indices in Laying Hens

As presented in , compared with the blank control group, serum T-AOC in the 10 and 100 mg/kg groups increased significantly by 19.81% and 29.62%, respectively ($P<0.05$). Serum GSH-Px activity in the 10, 100, and 1,000 mg/kg groups rose significantly by 39.15%, 48.15%, and 40.21%, respectively ($P<0.05$). Serum SOD activity showed improvements of 1.38%, 6.64%, and 10.45% across the three treatment groups ($P>0.05$), while serum MDA content decreased by 13.58%, 20.79%, and 19.64%, respectively ($P>0.05$). These results demonstrate that *Bacillus coagulans* Liu-g1 possesses substantial lipid antioxidant capacity, with the 100 mg/kg supplementation level yielding optimal effects.

Discussion

Effects of *Bacillus coagulans* Liu-g1 on Serum Triglyceride and Liver Crude Fat Content in Laying Hens

Triglycerides are fat molecules synthesized from fatty acids and glycerol [11]. Elevated blood triglyceride levels are associated with atherosclerosis and heart disease [12-15] and may contribute to fatty liver development. In laying hens, fat synthesis occurs primarily in the liver [16], and the balance between hepatic lipid synthesis and secretion is crucial for regulating fat deposition [17]. During the pre-laying period, the liver synthesizes large quantities of fat to meet yolk lipid deposition requirements. When the rate of fat synthesis exceeds export capacity, excess fat accumulates in the liver, leading to lipid metabolism disorders and potentially fatal hepatic hemorrhage [11]. High serum triglyceride levels can increase hepatic uptake of free fatty acids (FFA), promoting excessive triglyceride synthesis and potentially causing hepatic lipid metabolism disorders

and fatty liver formation [18]. Cao et al. [19] investigated the correlation between hyperlipidemia, FFA, and fatty liver, finding that hyperlipidemic groups exhibited significantly higher incidence of fatty liver and FFA content compared with normal lipid groups, and that fatty liver patients had significantly higher triglyceride and FFA levels than non-fatty liver patients. These findings suggest that elevated triglyceride levels may induce fatty liver formation. In the present study, Beijing-You laying hens were used to examine serum triglyceride content. The blank control group not receiving *Bacillus coagulans* Liu-g1 showed elevated serum triglyceride levels, suggesting potential fatty liver development. Treatment groups exhibited significantly reduced serum triglyceride content, indicating the potential of this bacterium to inhibit fatty liver formation. However, further verification through hepatic fat content measurement and serum antioxidant indices was required to confirm the preventive effects and antioxidant activity of *Bacillus coagulans* Liu-g1. As shown in [Figure 2: see original paper] and [Figure 3: see original paper], determination of liver crude fat content and oil red O staining revealed that the blank control group had elevated hepatic crude fat content and numerous fat droplets in oil red O-stained sections. With increasing dietary *Bacillus coagulans* Liu-g1 supplementation, hepatic crude fat content decreased while oil red O staining showed markedly reduced fat accumulation. These findings demonstrate that *Bacillus coagulans* Liu-g1 can regulate the balance between hepatic lipid synthesis and secretion, significantly ameliorate hepatic fat accumulation, and effectively inhibit fatty liver formation in laying hens. The inhibitory mechanism may be related to the lipid antioxidant activity of *Bacillus coagulans* Liu-g1.

Effects of *Bacillus coagulans* Liu-g1 on Serum Antioxidant Indices in Laying Hens

Day et al. [20] proposed the "two-hit" hypothesis for fatty liver pathogenesis, where the first hit involves hepatic steatosis and the second hit involves lipid peroxidation triggered by oxidative stress. Research indicates that fatty liver formation is associated with antioxidant system impairment and lipid peroxidation [21]. During energy metabolism, incomplete reduction of oxygen during electron transfer processes inevitably generates reactive oxygen species (ROS) [22], which can initiate lipid peroxidation, damage DNA strands, and injure biomembranes and tissues [23]. Under normal conditions, the antioxidant defense system protects against ROS damage, with SOD and GSH-Px serving as two crucial enzymes. SOD effectively converts superoxide anions to hydrogen peroxide, which GSH-Px then degrades to water [24-25]. Excess ROS that evades the antioxidant system attacks unsaturated fatty acids in biomembranes, initiating lipid peroxidation and producing MDA as a hallmark product of oxidative stress [26]. Total antioxidant capacity reflects the ability to scavenge excess ROS and maintain appropriate intracellular levels, representing overall antioxidant capability [27].

The present study demonstrated that compared with the blank control group,

the 100 mg/kg *Bacillus coagulans* Liu-g1 group showed significantly increased serum T-AOC, all three treatment groups exhibited significantly elevated serum GSH-Px activity, and serum SOD activity and MDA content showed respective improvements and reductions. Gong et al. [28] reported that *Bacillus coagulans* significantly increased serum T-AOC in broilers, extremely significantly elevated serum SOD activity, significantly reduced serum MDA content, and significantly increased serum GSH-Px activity, findings consistent with our results. Kodali et al. [29-30] extracted exopolysaccharide (EPS) with notable free radical scavenging and antioxidant activity from *Bacillus coagulans* RK-02 fermentation broth. Yuan et al. [31] isolated a *Bacillus* strain producing EPS with strong antioxidant activity and lipid peroxidation inhibition capacity. Mei et al. [32] demonstrated that lactic acid bacteria EPS significantly increased serum SOD activity and decreased serum MDA content, indicating enhanced antioxidant capacity. Li [33] showed in vitro that EPS significantly increased serum T-AOC and SOD activity while reducing serum MDA content, demonstrating free radical scavenging and antioxidant capacity enhancement. Whether the improved lipid antioxidant capacity observed with *Bacillus coagulans* Liu-g1 in laying hens is mediated by EPS production requires further investigation.

Conclusion

Dietary supplementation with 10, 100, or 1,000 mg/kg *Bacillus coagulans* Liu-g1 reduced serum triglyceride and liver crude fat contents while increasing serum T-AOC, GSH-Px, and SOD activities and decreasing serum MDA content in laying hens. Based on comprehensive evaluation, *Bacillus coagulans* Liu-g1 supplementation at 100 mg/kg yielded optimal results.

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Note: Figure translations are in progress. See original paper for figures.