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Effect of dietary supplementation with fermented *Ginkgo*-leaves on performance, egg quality, lipid metabolism and egg-yolk fatty acids composition in laying hens

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ABSTRACT

The present study was conducted to investigate the effect of 3 types of fermented *Ginkgo*-leaves (FGL) on layer performance, egg quality, lipid metabolism, microbial populations, and egg-yolk fatty acids composition in laying hens. A total of 432 Lohmann Brown laying hens, 49 weeks of age, were randomly allocated to 4 dietary treatments with 6 replications of 18 birds each. Layers were fed basal diets (Control) or basal diets supplemented with 0.5% *Ginkgo*-leaves fermented with *Candida utilis* (CF group), *Aspergillus niger* (AF group), or their combined fermentation (CAF group), respectively, for an 8-week feeding trial. Compared with the control group, AF and CAF supplementation improved ($P<0.05$) laying rate and feed conversion ratio. Birds fed FGL (CF, AF and CAF) supplemented diet had a decrease ($P<0.05$) in cracked-egg rate, egg-yolk cholesterol, serum total cholesterol, triglyceride and low density lipoprotein cholesterol levels, while serum high density lipoprotein cholesterol concentrations of birds from CF and AF groups were increased ($P<0.05$) compared with that of the control. The concentrations of C18:1 (n-9), C18:2 (n-6), total polyunsaturated fatty acids (PUFA), ratio of PUFA/saturated fatty acids (SFA) and n-6: n-3 was increased ($P<0.05$ or $P<0.01$) with FGL supplementation. While FGL supplementation led to a decrease in total saturated fatty acids ($P=0.01$) concentrations. In addition, ileal and cecum *Lactobacilli* and *Bifidobacteria* populations of birds fed FGL were increased ($P<0.05$) compared with the control group, while ileal *Escherichia coli*, *Salmonellas*, and cecal *Salmonellas* were decreased in birds fed FGL. In conclusion, dietary supplementation of FGL, especially AF and CAF, in layer diets may be a feasible means of producing eggs with lower cholesterol and higher PUFA contents for health conscious consumers.

1. Introduction

During the last decades, the phytogenic compounds have been incorporated into the diet to improve animal productivity by enhancing the production performance and the quality of food derived from those animals (Yan et al., 2011). Therefore, a number of medicinal herbs have been considered most acceptable because of their positive

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effects on growth performance and lipid metabolism (Park and Yoo, 1999). Chicken eggs are well established as an excellent source of all essential nutrients for persons of all ages. However, to avoid elevations in blood cholesterol and reducing the risk of coronary heart disease (CHD), consumption of no more than 300 mg of cholesterol per day has been recommended (Weggemans et al., 2001). Therefore, approaches of nutritional interventions with natural products have been investigated to reduce the cholesterol content in egg-yolk (Ting et al., 2011).

As a traditional natural medicinal herb in China, *Ginkgo biloba* L. (Family: Ginkgoaceae) is one of the oldest living plant species (Jacobs and Browner, 2000). Leaves of *G. biloba* are well known for its high content of flavonoids. Chemically, the active components of *G. biloba* leaf are flavonoids (flavonol and flavone glycosides, primarily composed of quercetin), polysaccharides (polymers of glucose, rhamnose, arabinose, mannose, galactose and xylose) and terpenoids (ginkolides and bilobalides) (Li et al., 2012; van Beek and Montoro, 2009). China has a large-scale production of *Ginkgo* leaves for years, about 40,000 tons every year. In the last few years, large-scale cultivation of *Ginkgo* has been initiated. Therefore, it is important to find out a way to utilize this herbal resource as feed ingredient and unveil its potential economic value in feed industry.

Probiotics strains are commonly used to preserve feed stuffs or ferment feeds for many years with beneficial effects (Boguhn et al., 2006; Cumby, 1986). Previous study showed that the use of solid-state fermentation may improve the nutritive value of botanical resource when it is fed to poultry (Chen et al., 2009). Presently *Aspergillus niger* and *Candida utilis* are the major probiotic strains applied in broilers (Chen et al., 2009). *A. niger* is a fungus that has the capacity to produce enzymes such as hemicellulases, hydrolases, pectinases, protease, amylase, lipases, and tannases (Mathivanan et al., 2006; Pinto et al., 2001), and is one of the major probiotic strains applied in broilers (Dei et al., 2008; Tannock, 2001). *C. utilis* belongs to *Saccharomyces,* and can secrete protease, amylase, and lipase (Koh et al., 2002; Santoso et al., 2001). Chen et al. (2009) reported that the beneficial effects of broilers fed fermented feed was probably not through single-strain fermentation, but due to the 2-stage combined fermentation process using *Bacillus subtilis* and *Saccharomyces cerevisiae* strains. To promote processing of *Ginkgo* leaves, we have developed a process for *A. niger* fermentation wherein the functionality of this resource is preserved and enhanced. Our previous researches confirmed that the use of *A. niger*-fermented-*G. biloba* leaves (FGL) had a positive influence on growth performance and lipid metabolism in broiler chicks (Cao et al., 2012; Zhang et al., 2012). Dietary total flavonoids and polysaccharides were most likely the key compounds responsible for the health-improving effect of the fermentation products (Zhang et al., 2012). Despite these findings, there has been a dearth of information on the possible beneficial effect of FGL on layer hens. The aim of the present study was to compare the effects of *Ginkgo* leaves processed with single strain (*C. utilis or A. niger*) or combined (*C. utilis+A. niger*) fermentation on layer performance, egg quality, lipid metabolism, and microbial populations of laying hens.

2. Materials and methods

2.1. Culturing of C. utilis and A. niger

The *C. utilis* (NFU-Y-186) and *A. niger* NL-1 used in this study was a laboratory strain isolate obtained from the College of Chemical Engineering, Nanjing Forest University, Nanjing, Jiangsu, PR China. The seed culture of *C. utilis*, containing (g/l) glucose 20, peptone 10, and yeast extract 10 at pH 5.0, was prepared in a flask on a reciprocal shaker at 200 rpm at 30 °C for 24 h. Medium for shake flask culture contains (g/l): glucose 30, ammonium sulfate 8, KH_2PO_4 3 and anhydrous $MgSO_4$ 0.25. The final amount of *C. utilis* was $6–9 \times 10^9$ CFU/ml. *A. niger* was cultured by an agar plating technique using sabouraud dextrose agar (Oxoid Ltd., Basingstoke, UK) and incubated at 24 °C for 7 days. *A. niger* spores were harvested by tapping the top of the plate when turned upside down. Spore counts were determined using a haematocytometer according to the Fuchs-Rosenthal technique to be approximately 4.0×10^6 spores/ml, which were equivalent to 0.25 g.

2.2. Preparation of fermented Ginkgo leaves sample

Comminuted (2.0-mm sieve) dried *Ginkgo* leaves picked during the last third September (*Ginkgo* garden for leaf use, Nanjing Forestry University, Jiangsu Province, PR China) were used for this study. They were divided into 3 lots after autoclave sterilization, and were fermented using yeast *C. utilis, A. niger* or their combination, respectively.

The solid-state fermentation medium contained 10 g solid medium (*Ginkgo* leaves:wheat bran:corncob=8:1.5 0.5) and 16 ml nutritive salt (glucose:urea:$(NH_4)_2SO_4$:peptone:KH_2PO_4:$MgSO_4 \cdot 7H_2O$=4:2:6:1:4:1) and was inoculated with 10% (ml/ml) of the *C. utilis* and *A. niger* seed for an aerobiotic fermentation. In total, 0.1% of the *C.* utilis and *A. niger* seed was inoculated into the fermentation medium for an aerobiotic fermentation. Both the samples were fermented for 48 h at 28–30 °C. For the combined fermentation, medium was inoculated with 1 ml *C. utilis* seed at 28–30 °C for a 24 h aerobic fermentation in the first stage and then 2 ml *A. niger* seed *was* inoculated for a 84 h aerobic fermentation in the second stage.

Six fermented samples were taken randomly, and were spread on a polythene sheet in a room at 30–40 °C, dried for 6 days up to about 900 g/kg of the dry matter, and ground to pass through a 0.15-mm sieve. The changes of the ingredients before and after the fermentation are shown in Table 1. Repetitious examination showed that the proportion of components in polysavone was constant within a minute range.

2.3. Husbandry, diets and experimental design

A total of 432 Lohmann Brown laying hens 49 weeks of age, having an average body weight of 1.858 ± 7 g were used in this experiment. Birds were fed on the maize-based basal diet for 4 weeks before the experiment to allow them to adapt and reach a standard rate of egg production. After a one-week adaptation period to cages (70 cm length × 30 cm width × 40 cm height), the birds