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Original research article

# Effect of dietary protein sources and storage temperatures on egg internal quality of stored shell eggs

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ABSTRACT

This study was conducted to evaluate the effects of various protein sources (soybean meal, SBM; cottonseed protein, CSP; double-zero rapeseed meal, DRM) on the internal quality (Haugh unit, yolk index, albumen pH, yolk hardness and yolk springiness) of eggs when stored at either 4 or 28°C for 28 d. A total of 288 laying hens (32 wk of age) were randomly allotted to 6 treatment groups (4 replicates per treatment) and fed diets containing SBM, CSP, or DRM individually or in combination with equal crude protein content (SBM-CSP, SBM-DRM, and CSP-DRM) as the protein ingredient(s). A 6 × 2 factorial arrangement was employed with dietary types and storage temperatures (4 and 28°C) as the main effects. After 12 wk of diet feeding, a total of 216 eggs was collected for egg internal quality determination. The results showed as follows: 1) lower egg quality was observed in the DRM group compared with the other groups when stored at 4 and 28°C for 28 d ($P < 0.05$), while there was no difference in egg internal quality among the other groups. 2) The CSP diet resulted in higher yolk hardness compared with the other diets when eggs were stored at 4°C for 28 d ($P < 0.05$). Lower Haugh unit was observed in the DRM and SBM-DRM groups compared with the other groups when eggs were stored for 28 d at 4°C ($P < 0.05$). 3) Yolk breakage occurred in the DRM group and eggs could not be analyzed for egg internal quality when stored at 28°C for 28 d. The overall results indicated that CSP or DRM as the sole dietary protein source for laying hens may adversely affect the internal quality of stored eggs as compared with the SBM diet, and half replacement of CSP combined with SBM may maintain similar egg quality to SBM diet alone for eggs stored under refrigerated conditions.



## 1. Introduction

Traditionally, soybean meal (SBM) has been used widely in China as a common protein source for poultry feeds, due to its high nutritional value and favorable amino acid profile (Martens et al., 2012). However, the fluctuation of SBM price in the world market limits its supply to the poultry feed industry. Thus, it is of great importance to develop alternative protein sources especially in some regions like China where animal production heavily depends on imported SBM. Eggs are one of the most complete foods for human consumption because they are rich in vitamins, minerals, fatty acids, and proteins that provide several essential amino acids of excellent biological value. Any dietary factors including protein sources that affect the egg quality are of concern to nutritionists.

Since 2008, China has officially promulgated cottonseed protein (CSP) as a potential alternative protein ingredient to replace SBM. The more refined technology, without high-temperature heating, greatly reduces husk and maintains nutrient density to the maximum extent during oil extraction, meanwhile integrated degossypolization in the solvent processes after oil extraction highly decreases free gossypol (FG) levels in this protein ingredient.

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Compared with the nutrient profile of SBM, CSP contains slightly lower levels of Lys, Thr and ME, and higher contents of CP, Met, Cys and Arg by 15.6, 45.8, 60.0 and 79.0% (Feed database in China, 2011). The improvements in rapeseed breeding has developed new varieties of double-zero rapeseed meal (DRM, similar to canola meal). Double-zero rapeseed meal is used as a protein source for poultry feed, and has proved to be a good source with well-balanced amino acid composition, and higher sulfur-containing amino acids in particular (Khajali and Slominski, 2012). However, published studies on DRM are mainly focused on broilers (Jung et al., 2012; Woyengo et al., 2010). Our preliminary trial observed that there was some influence of dietary protein sources on fresh egg quality of Jinghong laying hens during the peak production (Wang et al., 2015a). In addition, environmental factors such as storage temperature are known to affect internal quality of eggs post-lay (Sekeroglu et al., 2008). Storage of egg in a refrigerator (<7°C) can maintain egg internal quality, and retard weight loss compared with storage at room temperature, and refrigerated eggs can maintain a quality grade of AA for at least 4 weeks (Biladeau and Keener, 2009; Jirangrat et al., 2010; Torrico et al., 2014). Akyurek and Okur (2009) reported a significant interaction between hen age and storage temperature for the changes in egg weight loss and albumen quality. However, information concerning the effects of dietary protein sources and storage temperatures on internal quality of stored shell eggs is lacking. The exploration of the interaction of dietary protein sources and storage environment is very important for improving the utilization of diets formulated with these plant protein ingredients.

Therefore, the objective of the present study was to investigate the effects of three protein sources and two storage temperatures on the internal quality of stored shell eggs.

## 2. Materials and methods

### 2.1. Egg preparation

This study was approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences. Three protein sources, SBM (Sanhe Hopefull Grain & Oil Group Co., Ltd.), CSP (Shandong Futai Grain & Oil Group Co., Ltd., free gossypol: 302.54 mg/kg), and DRM (Fuzhou Jijia Oils & Fats Co., Ltd., isothiocyanate: ND; oxazolidine thioketone: 0.34 mg/g) were obtained from commercial sources. Two hundred and eighty-eight Jinghong laying hens at 32 wk of age were randomly allotted to 6 treatment groups that received variations in dietary protein sources, including SBM, CSP, or DRM individually or as combinations of two different protein sources, in which each ingredient provided an equal amount of crude protein in the diet. The specific treatment groups were as follows: SBM, SBM-CSP, CSP, SBM-DRM, DRM, and CSP-DRM. Each treatment consisted of 4 replicates with 3 cages each and 4 hens per cage. The cages were made of galvanized metal wire (approximately 55 cm × 40 cm × 40 cm). Each cage included a nipple waterer, and all hens were provided feed and water ad libitum. The temperature and relative humidity (RH) of the housing were 14 ± 2°C and 50 to 65%, respectively, and the photoperiod was set at 16L:8D throughout the 12-wk feeding period. The hens were fed a mashed diet, and all nutrient levels met or exceeded the NRC requirements (National Research Council, 1994). The recommended ratios of standardized ileal digestibility (SID) Met, Met + Cys, Ile, Thr, Trp, Val, and Arg to SID Lys among all group diets were 50, 91, 80, 70, 21, 88, and 104%, respectively (Lemme, 2009). The dietary composition and nutrient levels and the AA patterns of SID of diets are shown in Table 1.

A total of 216 eggs was collected over two consecutive days when the laying hens were 45 wk of age. The eggs were screened for desirable weight range (close to the average egg weight of the replicate) and no defects (crack and breakage) and were weighed using an electronic balance (ALC-2000.2; Sartorius Group, ACCU-LAB, BJ, Germany).

### 2.2. Experimental design and storage of eggs

A total of 72 fresh eggs was collected and measured for egg quality within 24 h after laying. The other 144 eggs were used in a factorial arrangement with 6 dietary protein sources and 2 storage temperatures as the main effects. Each of the 30 eggs was placed small-end down (Kim et al., 2009) on egg racks and stored 28 d at either 4 or 28°C. The RH was regulated at 50 to 60% for all treatments.

### 2.3. Measurement of weight loss, Haugh unit (HU), yolk index and albumen pH

The weight loss (%) of the whole egg was calculated as follows: 100 × [initial whole egg weight (g) at day 0 − whole egg weight (g) after storage]/[initial whole egg weight (g) at day 0], as reported by Wardy et al. (2013). The HU of each egg was measured using the Egg Analyzer (Orka Food Technology Ltd, Ramat Hasharon, Israel). An egg quality measurement stand (Fuji Ping Industrial Co. Ltd., Tokyo, Japan) and a vernier caliper (General Tools & Instruments, New York, USA) were used to measure the yolk width (mm), and the yolk index was computed as yolk height (mm)/yolk width (mm) (Stadelman, 1995). The albumen pH was measured using a pH/temperature measuring instrument (Testo AG, Lenzkirch, Germany) after thoroughly mixing both the thick and thin albumen. Eight measurements were performed for each treatment. The egg quality in the DRM group when stored for 28 d at 28°C was not measured due to the extremely low albumen quality (HU below 25) and yolk breakage.

### 2.4. Measurement of hardness and springiness of cooked yolk

The eggs were placed in an egg cooker for 10 min, and then the eggshell and egg white were removed, ensuring the integrity of the egg yolk to the greatest extent. The hardness and springiness of the cooked yolk were measured using the Texture Profile Analysis (TPA) of the TMS-Pro Texture Analyzer (Food Technology Corporation, Virginia, USA). The parameters were employed as follows: yolk deformation, 50%; detection speed, 30 mm/min; probe pick up to the sample surface height, 40 mm; input force sensing element, 24 N; and force sensing element diameter, 38.15 mm and height, 20.00 mm. Four measurements were performed for each treatment.

### 2.5. Statistical analysis

All data were analyzed by analysis of univariate using the general linear model (GLM) procedures (SPSS 19.0 for Windows, SPSS Inc., Chicago, IL) as a 6 × 2 factorial arrangement with dietary types and storage temperature as the main effects. The Duncan's multiple range tests were used to separate the mean values. All statements of significance are based on $P < 0.05$ unless otherwise specified.

## 3. Results

### 3.1. Haugh unit and albumen pH of raw eggs

The effect of dietary protein sources and storage temperatures on the HU of raw eggs is shown in Table 2. The HU of fresh eggs (0 d)