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Postfertilization Changes in Nutritional Composition and Protein Conformation of Hen Egg

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ABSTRACT: Fertilized hen egg is traditionally considered as a dietary supplement in many Asian countries. This work aimed to obtain information on the effect of fertilization on total nutritional composition of egg contents and protein conformation. Chemical analysis showed that the lipid level in fertilized egg began to decrease after day 9. Fertilized egg before day 9 was higher in essential free amino acids (EFAA) and monounsaturated fatty acids (MUFA) contents but lower in cholesterol and polyunsaturated fatty acids (PUFA) than unfertilized counterparts. Fertilized egg proteins were characterized by an increase in hydrophobicity and a decrease in electrostatic interaction. Circular dichroism analysis showed that β-sheet decreased with increasing incubation time, whereas unordered structure increased. The findings observed in this work provide a crucial basis for understanding nutritional composition and protein conformation of fertilized egg, with the potential of being utilized as an EFAA/MUFA-rich, low-cholesterol dietary supplement.

KEYWORDS: *fertilized egg, chemical composition, dietary supplement, protein conformation*

INTRODUCTION

Hen egg has been recognized as an excellent source of nutrients and foods for centuries.[1] There are two distinct types of hen egg, unfertilized and fertilized eggs. The eggs in the market are mostly those that are unfertilized.[2] In fact, fertilized egg is traditionally considered as a natural dietary supplement in some Asian countries. The *Compendium of Materia Medica*, the most famous Chinese pharmacopoeia, has recorded that fertilized egg contributes to appetite increase and immune function enhancement, especially for pregnant women and the elderly.[3]

There have been some reports describing changes in chemical composition during incubation period, such as developmental relationships between yolk and embryo body composition,[4] changes in amount of carbohydrates in yolk,[5] and utilization of docosahexaenoic acid (DHA) and some amino acids.[6,7] These research studies[4−8] paid more attention to mobilization and utilization of biochemical composition by the chick embryo and to egg white and yolk alterations from the perspective of physiology. However, information regarding the change in total nutritional composition of egg contents from the perspective of food is still limited.

Hen eggs are also used as a vital ingredient in several foods, especially for their exceptional functional properties, which mainly depend on the physicochemical properties of egg proteins.[9] These proteins might be expected to undergo conformational and structural changes during embryonic development. An understanding of these proteins' structural characteristics provides the fundamental basis for investigation of their applications.[10] To our knowledge, this is the first study to examine changes in the conformation and structure of egg proteins during incubation.

The objectives of the present study were to (1) investigate the change in total nutritional composition of egg contents and (2) determine the protein conformation during the incubation period, thereby providing information for application of fertilized egg.

MATERIALS AND METHODS

Materials. Unfertilized and fertilized hen eggs were obtained from a local producer (Zudai Poultry Co., Wuxi, Jiangsu, China). The eggs were incubated at 37.8 °C and 60% relative humidity in a benchtop incubator (Brinsea Products, Banwell, UK). Eggs were turned automatically through an angle of 90° every hour. Fertilized eggs were obtained on days 3, 6, 9, 12, and 15 of the incubation period. On each day, representing the development stage studied, 10 eggs were obtained and the entire contents were homogenized in a DS-1 high-speed tissue triturator (Specimen Model Factory, Shanghai, China). The obtained samples were then lyophilized for analysis. The process was repeated three times for each development stage. All animal protocols in this study adhered to the *Guide for the Care and Use of Laboratory Animals* (Ministry of Science and Technology of China, 2006) and were approved by the animal ethics committee of Jiangnan University. We made all efforts to minimize animal use and suffering in these experiments. Wide molecular weight markers (NEB, P7702) (New England Biolabs Ltd., Beijing, China) were used to identify proteins in SDS-PAGE. The protein and phospholipid (PL) standards for studying the molecular weight (MW) distribution and PL composition were purchased from Sigma (St. Louis, MO, USA). All other reagents used were of analytical grade (Shanghai Chemical Reagents Co., Shanghai, China).

Analysis of Protein Composition. *Protein Content.* The crude protein content of unfertilized and fertilized egg was determined by AOAC's Kjeldahl method conducted according to the procedure of Tkachuk,[11] and a nitrogen conversion factor of 6.25 was used to quantify the crude protein content.

Protein Extraction. The unfertilized and fertilized samples were defatted by means of washing with at least 20 volumes (w/v) of cold

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