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Basic nutritional investigation

Quantitative proteomic analysis reveals the role of tea polyphenol EGCG in egg whites in response to vanadium stress

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ABSTRACT

Objectives: Tea polyphenol (TP) epigallo-catechin-3-gallate (EGCG) can alleviate vanadium (V) stress in laying hens; however, our understanding of the molecular mechanisms and proteomic changes occurring in the egg albumen remains limited. The aim of the present study is to better understand the response in layers under V challenge and mechanism of EGCG detoxification.
Method: We divided 120 layers into four treatments in the absence and presence of 130 mg/kg EGCG, supplemented with either 0 or 5 mg/kg V.
Results: The Haugh unit (HU) was decreased and the apoptosis rate of magnum and V residual in egg was increased by the effect of vanadium and EGCG alleviated the detrimental effect in HU and apoptosis rate induced by vanadium (interactive effect, $P < 0.05$). In all, 379 proteins were identified and 28 differential proteins were observed with and without EGCG and V. Eight proteins, which respond to stress stimuli (five immune response proteins [F1P3B2, P21760, A2N881, F2Z4L6, and P02789], and one cell redox homeostasis protein [Q5F472] were presented in the albumen of laying hens with EGCG administration. Proteins involved in heavy metal binding (E1C5J4) and cell proliferation (F1NX05 and E1BT2) also were changed in EGCG-treated albumen.
Conclusion: The detoxification mechanism of EGCG under V stress may act through regulating metal-binding mediation, cell proliferation, and immune function–related proteins.

Introduction

The chicken egg white (albumen) represents a substantial part of the essential nutrients for the developing embryo and also protects the embryo against invading bacteria. The albumen is an aqueous solution (88% water) of protein (90% dry matter), mineral (6% dry matter), and free glucose (3.5% dry matter) [1,2]. In all, 227 proteins have been identified in egg albumen [3–5]. Unlike the yolk constituents, all of the albumen proteins are synthesized and secreted locally by the magnum. The Haugh unit (HU) is a measure of egg protein quality based on the height of albumen and is an important industry measure of egg quality [6]. Many factors, such as heavy metals, virus, and other stressors can adversely affect albumen quality. Vanadium (V) in feed mainly comes from phosphate sources. Although the concentration of V in phosphate may vary, it has been reported to be as high as 6000 mg/kg in some deposits [7]. Due to the growing use of heavy metals contaminated phosphate sources or other feed ingredients in the layers' feed, levels of V contamination have been observed to range from 0 to 5.2 mg/kg in laying peak feeds of the China market [8]. V toxicity in animals includes the induction of reactive oxygen species (ROS) and the disruption of cellular processes/structures. It decreases albumen height (less viscous), HU, and quality by disrupting the magnum's cellular integrity and function [7,9–13], which may cause V residual in eggs and a large economical lost in industry. The green tea polyphenols (TPs) include (-)-epigallo-catechin-3-gallate (EGCG), (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epicatechin [14,15]. Of these polyphenolic green tea components, EGCG is the major constituent (≥40%) and is also the component with the highest levels of antioxidant properties

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[16–19]. In a previous study, dietary supplementation with EGCG improved the inferior albumen quality (indicated by HU and albumen height), which was induced by V [12]. However, the mechanism is still unknown. Additionally, there is very limited proteomic research regarding the quality and nutritional features in chicken albumen. The viscosity of egg white may decrease as the composition of the protein changes. Ovalbumin, as the most abundant protein in egg white with free sulfhydryl and high hydrophobicity, may affect the liquidation of thick egg white proteins [20]. At the same time, the ovomucin's α- and β-subunit contents and composition in albumen may be responsible for the HU [21,22]. With thinning-induced disaggregation, less of the highly glycosylated β-subunit is found in the thick albumen gel over time, depleting the carbohydrate proportion of the water-insoluble ovomucin [23–25].

In the present study, we assessed the proteomic changes in the egg white protein from the addition of EGCG and the V-challenged layer using the isobaric tags for relative and absolute quantitation (iTRAQ) technique, a reliable technique that provides more sensitivity and accurate quantification levels. These findings provide a better understanding of the response in layers under V challenge and of detoxification.

Materials and methods

Ethics statement

This study was undertaken in strict accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals of the State Council of the People's Republic of China. The experimental protocol used in the study was approved by the Animal Care and Use Committee of Sichuan Agricultural University.

Birds, experimental design, and management

We randomly divided 120 Lohman laying hens (67 wk of age) into four treatment groups. The dietary treatments were as follows:

1. –V/–EGCG (control), fed a basal diet;
2. +V/–EGCG, control + 5 mg/kg V;
3. –V/+EGCG: control + 130 mg/kg EGCG; and
4. +V/+EGCG, 5 mg/kg V + 130 mg/kg EGCG.

V (added in the form of ammonium metavanadate) and TPs (EGCG) with 98% purity were purchased from Sigma (St. Louis, MO, USA). There were 30 replicates with one bird per replicate. The birds were housed individually in wooden cages (38.1 cm width × 50 length × 40 height) and room temperature was controlled with 22°C by a daily lighting schedule of 16 h light and 8 h dark. Hens were allowed ad libitum feeding for 35 d.

Egg quality analysis

The HU of egg was evaluated using an egg multitester (EMT-7300, Robotmation Co., Ltd., Tokyo, Japan). The pH value of egg white was evaluated by pH meter (Sartorius PB-10, Ltd, German).

Apoptosis of magnum cell assay

On day 35, 24 chicks were sacrificed by cervical dislocation; magnum sites were quickly removed and observed with TUNEL method (in situ cell death detection kit-POD, Roche Group, Switzerland).

Egg albumen protein extraction and peptides preparation

Twenty-four eggs were collected from each treatment, and the egg whites were carefully separated from yolk and gently homogenized with a magnetic stirrer for 15 min to reduce the viscosity and stored at −80°C. Total protein mining was performed by grounding the frozen albumen samples into powders in a mortar with liquid nitrogen and homogenized in extraction SDT buffer (4% sodium dodecyl sulphate, 1 mM dithiothreitol [DTT], 150 mM Tris-HCl, pH 8.0), vortexed briefly, and heated in a boiling hot water bath for 5 min. After ultrasonicating 10 times (80 w, 10 s ultrasonicating with 15 s pause per time), the sample was heated 5 min in boiling water bath again, and then cleared by centrifuging at 14 000*g* for 45 min at 25°C. The supernatant were extracted and stored at −80°C for further analysis. The final protein concentration was measured by the bicinchoninic acid method. Protein digestion was performed by filter-aided sample preparation method. Briefly, protein suspension was digested with 4 μg trypsin (Promega, Madison, WI, USA) in 40 μL DS buffer for 18 h at 37°C, resulting peptides were collected as a filtrate and the peptide concentration was analyzed at OD280 nm.

iTRAQ labeling and strong cation-exchange chromatography separation

Peptide mixture was labeled with an iTRAQ isobaric tagging reagent according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Briefly, mixture of extracted proteins of the 12 samples (four treatments with three biological replicate samples, 100 μg of each) was used as reference (REF). DTT (10 mM) was added into 400 μg of REF and each protein group, respectively. The sample were labeled using 8-plex iTRAQ kit with the egg albumen peptide from "−V/−EGCG,", "+V/−EGCG," "−V/+EGCG," and "+V/+EGCG" labeled with reagent 113, 114, 116, and 117, respectively. The iTRAQ-labeled peptides were pooled and fractionated by AKTA Purifier 100 (GE Healthcare, Chicago, IL, USA) with strong cation-exchange chromatography (SCX) followed by polysulfoethyl column (4.6 × 100 mm, 5 μm, 200 Å) (PolyLC Inc., Columbia, SC, USA) and analyzed in a continuous gradient wash. SCX buffer A: 10 mM KH_2PO_4 pH 3.0, 25% (v/v) acetonitrile; SCX buffer B: 10 mM KH_2PO_4 pH 3.0, 500 mM KCl, 25% (v/v) acetonitrile. The solvents were applied using 0 to 45% B in 0 to 52 min, 100% B in 52 to 60 min, 0% B in 60 to 75 min, with flow rate of 1 mL/min. These samples were combined into 20 fractions and then desalted on C18 cartridges (66872-U, Sigma).

Capillary high-performance liquid chromatography separation and MS/MS analysis

Chromatographic separation of the experimental samples was carried out with an EASY-nLC (Thermo Fisher Scientific, Waltham, MA, USA) that was coupled with Q Extractive mass spectrometer (MS; Thermo Finnigan, San Jose, CA, USA). The SCX fractions were thawed and dissolved in buffer C (0.1% [v/v] formic acid in Milli-Q water). Five mg of each peptide mixture sample were separated using the Thermo Scientific EASY protecting column (2 cm × 100 μm 5 μm-C18) and Thermo Scientific EASY column (75 μm × 100 mm 3 μm-C18). Sample injection was followed by an initial wash step with 0.1% formic acid (buffer A) and eluted with a linear gradient of 84% acetonitrile and 0.1% formic acid (buffer B) at a flow rate of 300 nL/min with a segmented gradient from 0 to 50% (v/v) buffer D in 0 to 55 min, from 50 to 100% (v/v) in 55 to 57 min, and then at 100% (v/v) in 57 to 60 min. The column eluate was directed into the Q Exactive MS, and the instrument was run with positive ion mode. MS data was acquired using a data-dependent top 10 method; a time-of-flight MS survey was acquired (300 e1800 m/z, 0.5 s) for high-energy collisional dissociation fragmentation. Determination of the target value is based on predictive automatic gain control. Dynamic exclusion set to 40 s. Survey scans were acquired at a resolution of 70 000 at m/z 200 and resolution for high-energy collisional dissociation spectra was set to 17 500 at m/z 200. Normalized collision energy was 30 eV and the under fill ratio was defined as 0.1%, which specifies the minimum percentage of the target value likely to be reached at maximum fill time.

Proteomic data analysis

Protein identification and quantification were performed with the Protein Discoverer 1.0 software (Thermo Fisher) using Mascot 2.2 (Matrix Science, England, United Kingdom) as the search engine. The following search parameters were set: monoisotopic mass, fragment MS tolerance at 0.1 Da and peptide mass tolerance at ±20 ppm, trypsin as the enzyme and allowing up to two missed cleavages, variable modifications with oxidation (M), uniprot_Gallus_gallus_24072_0605.fasta database. Carbamidomethyl (C), iTRAQ 8 plex (N-term) and iTRAQ 8 plex (K) were specified as fixed modifications, and oxidation (M) as the variable modification. Decoy database search for false discovery rate analysis was set at ≤1% by applying corresponding filters. For protein quantification, a protein contained at least two unique peptides. The quantitative protein ratios were weighted and normalized by the median ratio in Mascot. Differences were indicated when a P value was <0.05, and only fold changes >1.2 were considered.

Function analysis

To better understand the annotation and distribution of protein functions, we used the Blast2 GO program to obtain gene ontology (GO) annotations. GO provides a set of dynamic updating controlled vocabulary to describe genes and gene product attributes in the organism and is an international standardization of gene function classification system. GO has three ontologies that can describe