

Effect of diet composition on egg white heat-induced gel properties of Roman laying hens: from phenomenon, substance, structure to mechanism

Xiangxin Liao¹, Yanting Yang¹, Ying Gao¹, Sharina Qi², Zhaowei Cui², Shijian Dong³, Jijun Wu⁴, Shugang Li¹ ✉

¹Engineering Research Center of Bio-process, Ministry of Education, Key Laboratory for Agricultural Products Processing of Anhui Province, Key Laboratory of Animal Source of Anhui Province, School of Food and Biological Engineering, Hefei University of Technology, Hefei 230601, China

²Sichuan Sundaily Farm Ecological Food Co., Ltd., Mianyang 621000, China

³Anhui Rongda Food Co., Ltd., Xuancheng 242200, China

⁴Anhui Rongda Poultry Farming Co., Ltd., Xuancheng 242200, China

✉Address correspondence to Shugang Li, lishugang2020@hfut.edu.cn

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Abstract: Multidimensional impact of diet composition on egg quality and function has become a research hotspot in the laying hen industry. However, there were relatively few reports on the effect of diet composition on the composition of egg white and its heat-induced gel behavior. Based on this, the system compared the differences in Roman egg white heat-induced gel behavior, protein composition, molecular conformation and molecular interaction under the dietary models of corn distillers' grains, corn and soybean meal and flaxseed, revealing the potential influence mechanism of dietary composition on heat-induced gel behavior. The results indicated that the egg white protein gel under the corn and soybean meal diet pattern had the best gel properties, with hardness and water holding capacity significantly increased by 23.1% and 2.0% respectively compared to the corn distillers' grains diet pattern. The improvement in the gel behavior might be attributed to the changes in the material composition of egg white proteins (increased protein and amino acid contents), protein aggregation state (reduced particle size and weakened aggregation), molecular structure (increased β -sheet relative content and decreased thermal denaturation temperature), and intermolecular interactions (enhanced hydrophobic interactions and disulfide bonds). These findings will provide a scientific basis for regulating the performance of egg heat-induced gel from the nutritional source of laying hens and for the development of customized egg products.

Keywords: diet composition; gel properties; molecular structure; mechanism research

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1 Introduction

Eggs are rich in high-quality protein, essential amino acids, vitamins (such as vitamins A, D, and B), and minerals (such as iron, zinc, and selenium), they also contain various bioactive substances (such as lecithin, immunoglobulin, and lysozyme (LYZ)), making it an indispensable and important component of human dietary structure^[1]. In addition, eggs exhibited excellent functional properties in the field of food processing, such as emulsifying, foaming and gelation abilities, and were widely used in baking, meat products, dairy products, and other fields^[2-3]. Egg whites, as the main component of eggs (accounting for approximately 60% of the total egg protein), have become an important functional ingredient in food industry due to their high biological potency, low cost and accessibility, and rich nutritional content^[4-5]. Egg white can form a stable gel network under heat treatment, acid-base or mechanical shearing, thereby imparting specific texture and sensory characteristics to food products^[6]. The gel properties of egg whites are one of the key factors determining their application value in the food industry^[7]. In the food industry, the strength, springiness, stability, and other indicators of egg white gel products directly

affect the quality and market competitiveness of the products. For instance, in ham, meat paste products, and plant-based meat substitutes, the hardness and water holding capacity (WHC) of egg white gel determined the juiciness, slice ability, and printability of the products^[8-10]; in baked cake goods, the stability of egg white gel affected the volume and texture of cakes^[11]; in pudding production, gel strength and stability influenced the shaping effect of the product^[12]. Therefore, how to precisely control the texture characteristics of egg white gel has become one of the research hotspots in the field of food science.

Currently, the methods for regulating the properties of egg white gel mainly included physical modification (such as heat treatment, high-pressure treatment, and ultrasonic homogenization), chemical modification (such as acid-base adjustment, addition of salts or phosphates), and enzymatic modification (such as transglutaminase cross-linking)^[13]. Although these methods could optimize the gel properties of egg whites to some extent, they were mostly limited to processing steps and might introduce additional additives or increase production costs. In comparison, directly regulating the composition and structure of egg white proteins through nutritional intervention in laying hens' diets can promise for

optimizing the gel properties of egg whites from the source. This strategy is based on the regulatory pathway of “nutrition-metabolism-protein expression”, meaning that nutrients in the feed (such as amino acids, fatty acids, and polysaccharides) can alter the composition and intermolecular interactions of egg white proteins by influencing the physiological metabolism and protein synthesis of laying hens, thereby affecting their processing characteristics. For example, the addition of quercetin to feed could promote the transformation of conformation and changes in protein abundance in egg white proteins, enhancing gel strength^[14]. Similarly, the addition of 0.5 mg/kg selenium yeast to laying hen feed improved the heat-induced gel properties of egg white; moreover, egg white proteins in feed supplemented with selenium yeast produced more low molecular weight peptides and free amino acids after gastrointestinal digestion^[15]. This “from farm to processing” regulatory model not only avoided the complexity of later processing, but also enhanced the added value of eggs through natural nutrition strategies, aligning with the development trends of clean labeling and sustainable food production.

However, most current researches only focused on the impact of feed on the nutritional quality of eggs (such as fatty acid composition and cholesterol content), while systematic studies on its effect on egg white processing performance (especially gel properties) were still scarce. In addition, the molecular mechanism by which different feeds regulate egg white gel properties was not yet clear, limiting the precise application of this strategy in the food industry. Therefore, using egg white from hens fed three dietary regimens (corn distillers' grains, corn and soybean meal, and flaxseed) as a model, this study systematically analyzes the regulatory mechanism of dietary intervention on the characteristics of egg heat-induced gel. Through the analysis of heat-induced gel properties, microscopic structure observation, multi-spectral characterization, protein structure analysis and molecular interaction research, the regulatory rules of dietary differences on the aggregation state of egg whites, molecular conformation and the formation process of gel networks were revealed. By conducting correlation analysis, we clarified the key material basis and structural characteristics of the formation of heat-induced gel. We then constructed a systematic research framework of “macroscopic appearance-material composition-conformational differences”, and deeply expounded the molecular mechanism of nutrient regulation of egg white thermogel formation. More importantly, it has proposed an innovative strategy of “regulating processing quality from the source”: through nutritional intervention to produce specialized eggs that do not require exogenous additives (such as carrageenan, xanthan gum, etc.), specifically meeting the specific demands of meat products (high gel property) or baked goods (high water retention), providing theoretical basis and technical support for the food industry to achieve clean labels and raw material standardization.

2 Materials and methods

2.1 Materials

Eggs under different dietary patterns (Table 1) were provided by Sichuan Sundaily Farm Ecological Food Co., Ltd. (Mianyang, China); urea, sodium chloride, and 8-anilino-1-naphthalenesulfonic acid (ANS) were purchased from Shanghai Macklin Biochemical

Co., Ltd. (Shanghai, China). Other chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Table 1 The composition of the basal diet.

Item	EW1	EW2	EW3
Corn and soybean meal	81.00	86.00	81.00
Flaxseed meal			5.00
Corn distillers' grains	5.00		
Premix	14.00	14.00	14.00

2.2 Preparation of egg white gel

Fresh eggs pass through an egg white and yolk separator to separate the egg white from the yolk. After separation, the egg whites were stirred using a magnetic stirrer at a speed of 500 r/min for 30 min, and filtered through double-layer sterile gauze to obtain fresh egg whites. The egg white sample (10 mL) was aliquoted into a 30 mL glass bottle and heated in a 90 °C water bath for 15 min. After heating, it was immediately taken out and cooled in an ice-water bath, and stored in a 4 °C refrigerator for 12 h before being used in subsequent experiments.

2.3 Determination of texture properties

The texture analyzer (TA.XT Plus, Stable Micro Systems Co., Oxford, UK) equipped with a P/3.6 aluminum probe was used to conduct texture analysis tests on egg white gel. The test parameters were as follows: the pre-test speed was set as 2.0 mm/s, the test speed was at 1.0 mm/s, the post-test speed was at 2.0 mm/s; the test distance was 5.0 mm, the recovery height was 40 mm, the recovery speed was at 10 mm/s, and the contact force was 5.0 g.

2.4 Determination of WHC

Weighed approximately 1.5 g of gel sample into a 10 mL centrifuge tube, centrifuged for 15 min (4 °C, 10 000 × g), and then used absorbent paper to wipe away the moisture on the gel surface. Recorded the mass of the centrifuge tube as m_0 (g), the total mass of the centrifuge tube and gel sample as m_1 (g), and the total mass of the centrifuge tube and gel sample after centrifuging as m_2 (g). The WHC of the gel sample was calculated by the following formula:

$$\text{WHC (\%)} = \frac{m_2 - m_0}{m_1 - m_0} \times 100$$

2.5 Determination of material composition

2.5.1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE analysis of egg white protein samples was evaluated using a separating gel (10%) and a stacking gel (5%). Briefly, the sample solution (1 mg/mL) was mixed with loading buffer (5×) at a ratio of 5:1 (V/V), and then heated in a 100 °C boiling water bath for 5 min. The 10 μL cooled sample solution was loaded onto the SDS-PAGE gel. The gel electrophoresis experiment was conducted using an electrophoresis apparatus (DYCZ-24DN Liuyi Co., Ltd., Beijing, China) at 80 V for 15 min and 120 V for 45 min. After electrophoresis, the samples were stained with 0.25% Coomassie brilliant blue R-250 solution for 30 min and then destained with acetic acid-ethanol (1:9, V/V) solution.

2.5.2 Amino acid determination

Weighed egg white powder sample with the accuracy to 0.0001 g to ensure the protein content in the sample was within the range of 10–20 mg, and placed the sample into a hydrolysis tube. Based on the protein content of the sample, added 5 mL of hydrochloric acid solution (6 mol/L) into the hydrolysis tube, then filled the tube with nitrogen and sealed it. Placed the tube under the temperature of approximately 130 °C and hydrolyzed it for 12 h. After hydrolysis, removed the sample, cooled it to room temperature, filtered it, and transferred it to a 100 mL volumetric flask. Rinsed the hydrolysis tube with distilled water, then diluted it to the mark and shook well. Accurately pipetted 1.0 mL of the filtrate into a 10 mL centrifuge tube for lyophilization. Added 1 mL of hydrochloric acid solution (0.02 mol/L, chromatographically pure) to the centrifuge tube to dissolve the lyophilized sample, let it stand for 10 min, passed it through a 0.22 µm filter membrane, and transferred it to a sample vial as the sample measurement solution. Automatic amino acid analyzer (S-433D, SYKAM Co., Munich, Germany) was used to determine the amino acid composition of the sample. The concentration of amino acids in the sample measurement solution was calculated by peak area using the external standard method. Chromatographic column: SYKAM LCA K06/Na (4.6 mm × 150 mm, 6 µm); detection wavelengths: 570 and 440 nm.

2.6 Determination of protein aggregation state

2.6.1 Particle size and zeta potential

Took 1 mL of egg white protein solution sample and diluted it with 9 mL of deionized water; then centrifuged at $10\,000 \times g$ for 15 min at 4 °C and collected the supernatant. The particle size and zeta potential of the sample were measured using a nano laser particle size analyzer (Zetasizer Nano-ZSE, Malvern Co., Malvern, UK) under the condition of a refractive index of 1.45.

2.6.2 Soluble protein content

The 1 mL of egg white protein solution was diluted 10 times with deionized water and was centrifuged at $10\,000 \times g$ for 15 min at 4 °C to collect the supernatant. The protein solubility of the sample was determined using the bicinchoninic acid (BCA) rapid protein concentration detection kit (Beyotime Biotechnology Co., Ltd., Shanghai, China).

2.6.3 Confocal laser scanning microscope (CLSM)

The 1 mL of egg white protein solution sample was taken and diluted 50 times with deionized water. Took 5 mL of the diluted protein solution and incubated it with 50 µL of rhodamine B (0.3 mg/mL) fluorescent dye in a shaker for 30 min in the dark. Then, the 10 µL stained sample solution was placed on a single concave glass slide. Covered the slide and sealed the edges with silicone oil to prevent water loss. The CLSM (FV1000, Olympus Co., Tokyo, Japan) was used with an excitation wavelength of 540 nm and a magnification of 200 times to observe the aggregation morphology of protein molecules.

2.7 Determination of protein molecular structure

2.7.1 Fourier transform infrared (FTIR) spectroscopy

The freeze-dried sample of egg white gel was ground, mixed with potassium bromide (1:100), and compressed into a thin film. FTIR spectrometer (Vertex 70, Bruker, Ettlingen, Germany) was used to

scan the spectrum in the range of $500\text{--}4\,000\text{ cm}^{-1}$, perform background correction, and baseline correction of the spectral curve using the program provided with the instrument. Subsequently, PeakFit v4.12 software was used to perform multi-peak fitting on the amide I band ($1\,600\text{--}1\,700\text{ cm}^{-1}$) in the infrared spectrum, and calculate the secondary structure of the protein in the sample.

2.7.2 Raman spectroscopy

The 5 mg of gel sample lyophilized powder was evenly placed on a glass slide and tested using a microscopic confocal laser Raman spectrometer (LabRAM HR Evolution, Horiba Jobin Yvon Co., Paris, France). The specific parameters for Raman spectroscopy scanning were as follows: the laser wavelength was of 785 nm, the objective lens was of 50×, the acquisition time of 2.0 s, the accumulation time was of 6, and the spectral range was from 400 to $3\,500\text{ cm}^{-1}$.

2.7.3 Thermal stability

The thermal stability test on the samples was performed using a thermogravimetric analyzer (TGA8000, TA Instruments, Maplewood, NJ, USA), with the freeze-dried gel samples being heated from 20 to 600 °C at a rate of 50 °C/min.

2.7.4 Gel network structure

After freeze-drying, the gel sample was treated with gold sputtering coating for 30 s, and then the gel network structure was observed using scanning electron microscopy (S-4800, Hitachi, Tokyo, Japan).

2.8 Molecular interaction measurement

2.8.1 Surface hydrophobicity measurement

The 1 mL of egg white solution sample was diluted with 9 mL of deionized water, then centrifuged at 4 °C and $10\,000 \times g$ for 15 min to collect the supernatant. Measured the protein concentration of the supernatant using the BCA method, and adjusted the protein concentration of all samples to 0.1 mg/mL with deionized water. Mixed (0.5 mol/L) as a fluorescent probe with the sample solution and allowed it to react in the dark for 20 min. Fluorescence spectrophotometer (FL6500, PerkinElmer, Waltham, MA, USA) was used to detect the surface hydrophobicity of the protein, with an excitation wavelength of 380 nm, excitation slit of 10 nm, emission wavelength range of 350–450 nm, and emission slit of 10 nm.

2.8.2 Free sulfhydryl content measurement

The free sulfhydryl content of the sample was detected using a 5,5'-dithiobis(2-nitrobenzoic acid) free sulfhydryl content detection kit (Solarbio Science & Technology Co., Ltd., Beijing, China).

2.8.3 Intermolecular forces measurement

According to the method of Wang et al.^[16], the intermolecular forces involved in the determination of gel samples were treated with denaturants and slightly modified. First, four mixed solutions were prepared using deionized water: reagent A: 0.6 mol/L sodium chloride solution; reagent B: 0.6 mol/L sodium chloride + 1.5 mol/L urea; reagent C: 0.6 mol/L sodium chloride + 8.0 mol/L urea; and reagent D: 0.6 mol/L NaCl + 8 mol/L urea + 0.5 mol/L β-mercaptoethanol. After being mixed with the four chemical reagents, these mixed solutions were homogenized for 1 min at

8 000 r/min using a disperser, and then left to stand at 4 °C for 1 h. The supernatant was collected by centrifuging at 4 °C and $10\,000 \times g$ for 30 min using a centrifuge, and the protein content was measured using a BCA rapid protein concentration detection kit (Beyotime Biotechnology Co., Ltd., Shanghai, China). The intermolecular interactions were represented by differences in protein concentration.

2.9 Data statistics and analysis methods

The correlation between indicators was modeled and analyzed using Pearson correlation analysis, and the correlation analysis graph was plotted using Origin 2021 software. All experiments were repeated three or more times, using the mean $\pm$ standard deviation to calculate. One-way analysis of variance and Tukey's test were chosen for significance analysis using Statistix 8.0 software, and Origin 2021 was used for plotting. Different letters in the figures and tables represented statistical differences in significance between groups ($P < 0.05$).

3 Results and discussion

3.1 Effect of dietary composition on the heat-induced gel properties of egg white

3.1.1 Texture properties

The texture properties of a gel were one of the important quality indicators for evaluating gel products, and were closely related to the taste and texture of the gel products. Hardness referred to the ability of gel to resist deformation when subjected to external forces. A gel with high hardness required greater force to deform, usually indicating more compact structure and higher degree of cross-linking. Springiness showed the ability of gel to return to its original shape after being deformed by external forces. Cohesiveness reflected, to some extent, the binding force between components within the gel system. Gumminess indicated the adhesive ability of gel to external objects (such as the surface of the

mouth, teeth, tongue, etc.). Chewiness demonstrated the comprehensive texture and taste of the gel during chewing. The texture properties of egg white gels under different dietary conditions were shown in Figures 1A–E. The EW2 gel with increased corn and soybean meal content in the diet was significantly superior to the egg white protein gels with corn distillers' grains (EW1) and flaxseed (EW3) added to the diet, respectively, in terms of hardness, springiness, chewiness, cohesiveness, and gumminess ($P < 0.05$). Compared to EW1 and EW3, the protein gel hardness in EW2 increased by 23.1% and 19.9%, respectively; springiness increased by 7.6% and 5.3%, respectively; chewiness increased by 25.0% and 16.0%, respectively; cohesiveness increased by 20.0% and 1.4%, respectively; and gumminess increased by 26.3% and 14.5%, respectively. The results indicated that the increase of the content of corn and soybean meal in the diet of laying hens could effectively enhance the texture properties of egg white gels. The texture properties of protein gels were closely related to the degree of cross-linking between protein molecules and the tightness of the ordered arrangement of structures in the gel, which might be a potential reason for the improved texture properties of EW2 and EW3 protein gels^[17–18]. Meanwhile, egg white was a mixed gel system rich in various proteins (ovalbumin (OVA), ovotransferrin (OVT), ovomucin (OVM), etc.), so the improvement in texture properties under different dietary conditions might also be due to differences in the composition of egg white proteins^[5].

3.1.2 WHC

The WHC of gel directly reflects its ability to retain water molecules. A good WHC endows the gel product with a juicy texture. As shown in Figure 1F, under the three dietary conditions, compared to EW1, EW2 and EW3 gels exhibited superior water retention capabilities, with an increase rate of 2.0% and 2.6%, respectively. EW3 had the best WHC, but there was no significant difference between EW2 and EW3. It was reported that the WHC of protein gels was closely related to its ability of forming uniform and stable three-dimensional gel network. A good gel network contained dense pore units that provided excellent storage space for

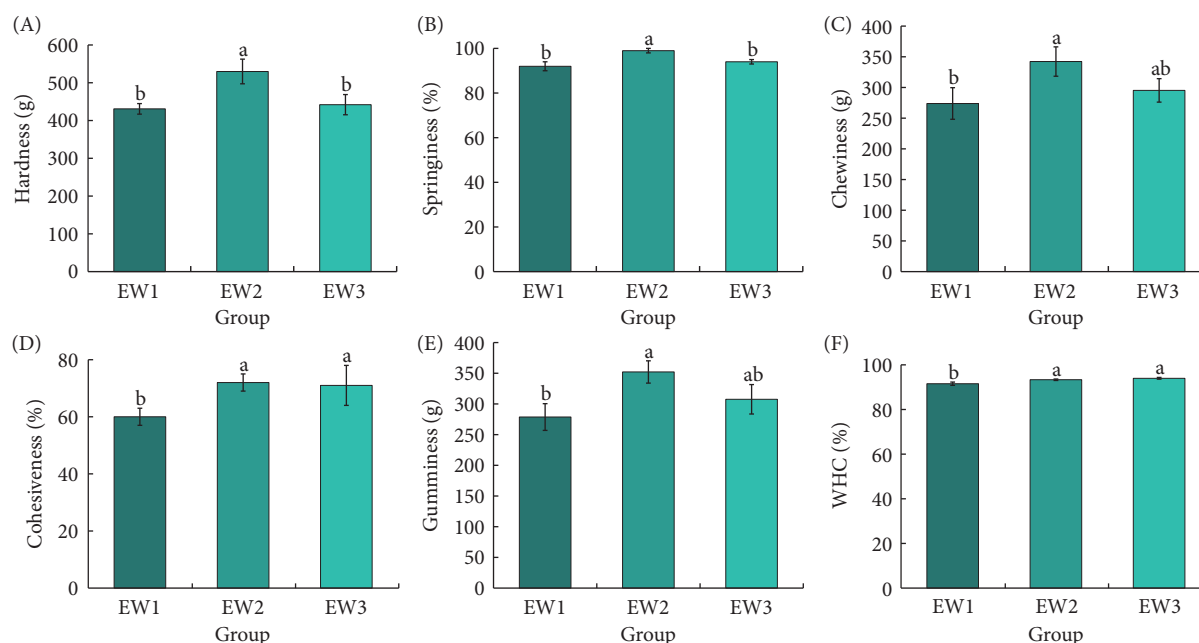


Figure 1 Gel properties of egg white protein gel from different diets. (A) Hardness, (B) springiness, (C) chewiness, (D) cohesiveness, (E) gumminess and (F) WHC. Different lowercase letters indicated significant differences between groups ($P < 0.05$).

water molecules within the gel system^[19]. Therefore, the improvement of WHC in EW2 and EW3 gels might be due to the formation of a denser network structure induced by heat. On the other hand, differences in the composition of egg white protein under different dietary conditions might also affect the WHC of egg white gel. It was also reported that hydrophilic amino acids in the gel system firmly bond water molecules within the gel system by enhancing hydrogen bonding interactions with water, thereby improving the protein gel's ability to retain water^[7]. Therefore, it was possible that EW2 and EW3 protein gel systems contained more hydrophilic amino acids, providing more binding sites for water molecules, thus enhancing the WHC of egg white gel. In summary, the improvement of WHC in EW2 and EW3 protein gels might be determined by multiple factors.

3.2 Effect of dietary composition on the composition of egg white protein

3.2.1 Protein composition

The material composition of proteins has decisive influence on their functional properties. Egg white protein is a complex mixed system, primarily composed of high-abundance proteins such as OVA, OVT, OVM, and LYZ^[1]. The SDS-PAGE pattern of the three egg white proteins showed that the main components of the three egg white proteins remained unchanged. The composition of the electrophoretic bands was basically consistent, including β -OVM at approximately 400 kDa, α -OVM at 220 kDa, OVT at 78 kDa, OVA at 45 kDa, and LYZ at 14.3 kDa (Figure 2). It was noteworthy that the color intensity of the bands in the samples had changed. Compared to EW1, the bands of α -OVM, β -OVM, OVT, and OVA in EW2 were darker, indicating that the content of the three proteins in EW2 was higher than that in EW1. Compared to EW3, the band of OVA in EW2 was darker. In summary, the SDS-PAGE bands analysis results indicated that different diets could lead to significant changes in the content of OVA, OVT, β -OVM, and α -OVM in egg whites. In recent years, numerous studies have shown a close correlation between OVM and OVT and the gel properties of egg whites. It was reported that OVM and OVT played a core role in the formation of egg white protein gel, promoting the formation of egg white protein gel^[20–22]. Therefore, the addition of corn and soybean meal and flaxseed altered the apparent gel properties of egg white gel by affecting the material composition of egg white protein.

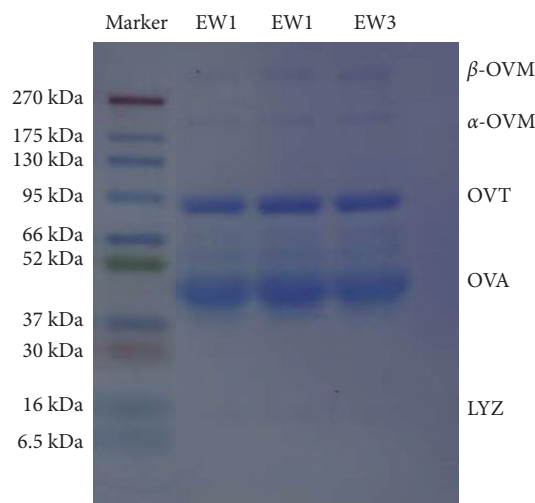


Figure 2 SDS-PAGE pattern of egg white proteins from different diets.

3.2.2 Amino acid composition

Amino acids directly constitute the primary structure of proteins and serve as the structural basis for the higher-level structural domains that determine the functional properties of proteins. Therefore, it is important to investigate whether different diets affect the gel behavior of egg white proteins by influencing their amino acid composition. The results (Table 2) showed that 16 amino acids were identified in egg white protein. The total amino acid (TAA) and essential amino acid (EAA) contents in EW2 and EW3 were significantly higher than that in EW1 ($P < 0.05$), indicating that dietary composition affected the amino acid composition in egg white protein. It was noteworthy that the content of hydrophilic amino acids such as Asp, Glu, Thr and Ser in egg whites of EW2 and EW3 was significantly higher than that in EW1. Compared with EW1, the total amount of hydrophilic amino acids in EW2 and EW3 increased by 36.70% and 20.20% respectively, which was consistent with the results of WHC. The higher content of hydrophilic amino acids in EW2 and EW3 might be an important factor contributing to their improved WHC. Moreover, existing researches have shown that increasing the content of amino acids in proteins could enhance the gel properties of protein gels. It was reported that basic amino acids (Arg, Lys, His) modified the gel properties of whey protein isolate by altering the surface charge and molecular structure of whey protein in an amino acid-dependent manner^[23]. Therefore, the increase in the content of key basic amino acids in EW2 egg white protein might be an important factor contributing to the improvement of EW2 gel properties. Compared with EW1 and EW3, the basic amino acids (Arg, Lys, His) in EW2 increased by 46.30% and 13.67% respectively. Therefore, the increase in the content of key amino acids in EW2 egg white protein might be an important factor contributing to the improvement of EW2 gel properties.

Table 2 The amino acid composition of egg white protein under different dietary compositions.

Amino acid	Content (g/100 g)		
	EW1	EW2	EW3
Asp	0.198 ± 0.010 ^c	0.233 ± 0.010 ^a	0.205 ± 0.010 ^b
Thr	0.028 ± 0.010 ^b	0.046 ± 0.010 ^a	0.040 ± 0.010 ^a
Ser	0.045 ± 0.020 ^a	0.079 ± 0.010 ^a	0.070 ± 0.010 ^a
Glu	0.093 ± 0.040 ^c	0.138 ± 0.010 ^a	0.123 ± 0.010 ^b
Gly	0.023 ± 0.010 ^a	0.050 ± 0.010 ^a	0.043 ± 0.010 ^a
Ala	0.037 ± 0.010 ^b	0.073 ± 0.020 ^a	0.066 ± 0.020 ^b
Cys	0.005 ± 0.010 ^b	0.009 ± 0.010 ^a	0.008 ± 0.010 ^b
Val	0.033 ± 0.020 ^b	0.056 ± 0.010 ^a	0.049 ± 0.010 ^b
Met	0.024 ± 0.010 ^a	0.035 ± 0.010 ^a	0.032 ± 0.010 ^b
Ile	0.025 ± 0.020 ^b	0.040 ± 0.010 ^a	0.035 ± 0.010 ^b
Leu	0.048 ± 0.020 ^b	0.076 ± 0.010 ^a	0.067 ± 0.010 ^{ab}
Tyr	0.022 ± 0.010 ^a	0.030 ± 0.010 ^a	0.026 ± 0.010 ^a
Phe	0.035 ± 0.020 ^a	0.048 ± 0.010 ^a	0.044 ± 0.010 ^a
His	0.033 ± 0.010 ^a	0.049 ± 0.010 ^a	0.043 ± 0.010 ^a
Lys	0.040 ± 0.010 ^a	0.060 ± 0.010 ^a	0.053 ± 0.010 ^a
Arg	0.035 ± 0.020 ^b	0.049 ± 0.010 ^a	0.043 ± 0.010 ^b
EAA	0.025 ± 0.010 ^c	0.044 ± 0.010 ^a	0.038 ± 0.010 ^b
TAA	0.750 ± 0.260 ^c	1.114 ± 0.020 ^a	0.983 ± 0.010 ^b
EAA/TAA	0.210 ± 0.100 ^c	0.326 ± 0.060 ^a	0.287 ± 0.010 ^b

Note: Different lowercase letters in the same row indicate significant differences between treatments ($P < 0.05$).

3.3 Effect of dietary composition on the aggregation state of egg white protein

3.3.1 Particle size

Particle size can reflect the aggregation behavior of protein molecules in solution to a certain extent. As shown in Figure 3A, the particle size distribution curves under different dietary conditions indicated that EW1, EW2, and EW3 all had two distinct peaks, indicating the varying degrees of aggregation in the three protein solution systems. The particle size distribution curve of the EW2 protein solution was primarily concentrated within a smaller particle size range (below 400 nm), indicating the lowest degree of aggregation of protein particles in the EW2 protein solution. Furthermore, the polydispersity index (PDI) of EW2 was lower than that of the other two groups (Figure 3B), indicating that the EW2 protein solution system was more uniform and stable, with optimal protein particle dispersion. In addition, EW2 exhibited the lowest average particle size value (Figure 3C), with reductions of 51.33% and 26.16% compared to EW1 and EW3, respectively, further demonstrated the stability and superior dispersibility of the EW2 solution system. In previous studies, Gao et al.^[24] found that changes in protein particle size might significantly affect the gelation process of proteins. Moreover, smaller particle size allowed protein molecules to have a larger specific surface area during gel formation, which facilitated crosslinking and reaction between adjacent protein molecules, promoting the formation of a denser gel network structure.

3.3.2 Zeta potential

The zeta potential of protein solution indicates the amount of surface electrostatic charge on protein particles, reflecting the degree of aggregation of these particles. As was seen from Figures 3D and E, EW2 had the highest absolute value of zeta potential (26.96 mV), indicating that the surface charge on EW2 protein particles was the highest, resulting in the largest electrostatic repulsive charge barrier.

This limited the self-aggregation effect of EW2 protein particles, promoting their uniform dispersion within the solution system. Research indicated that there was close correlation between protein particle size and surface net charge^[25]. The increase in surface net charge of EW2 might be a significant factor contributing to the reduction in its protein particle size.

3.3.3 Soluble protein content

The solubility of proteins reflects the interaction between protein molecules and water molecules. As shown in Figure 3F, the soluble protein content of EW1, EW2, and EW3 reached 117.8, 132.3, and 120.6 mg/mL, respectively. Under the three dietary conditions, EW2 exhibited the best solubility, which was 12.3% and 9.75% higher compared to that in EW1 and EW3, respectively. Generally speaking, the higher the solubility of proteins, the more likely the molecular collisions within the system, which was conducive to intermolecular and intramolecular interactions between protein molecules and between protein molecules and water molecules. This enhanced the molecular interactions within the system and promoted the formation of protein gels^[26]. Furthermore, the enhanced molecular interactions in the gel system strengthened the compactness between molecules, which could exhibit better gel properties such as hardness and springiness under external stress conditions.

3.3.4 CLSM images

CLSM is widely used to observe the microscopic shape and aggregation state of protein particles. The red area represented the protein phase stained with the fluorescent dye rhodamine B. As shown in Figure 4, in the EW1 protein solution, protein molecules exhibited more spherical and chain-like aggregate particles with larger diameters and varying sizes. In the EW2 protein solution, the protein particle size was significantly reduced and distributed, predominantly presenting a spherical aggregate particle shape. In the EW3 protein solution, both spherical and chain-like aggregates

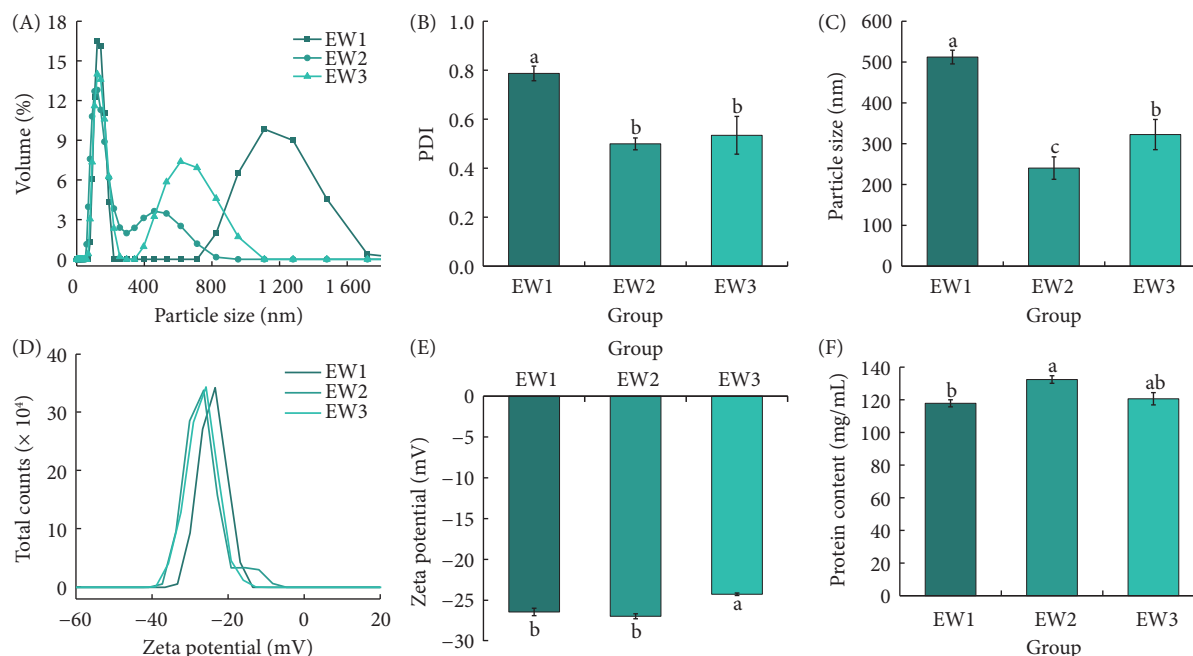


Figure 3 Aggregation state of egg white solutions from different diets. (A) Particle size distribution, (B) PDI, (C) particle size, (D) potential distribution, (E) zeta potential and (F) soluble protein content. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

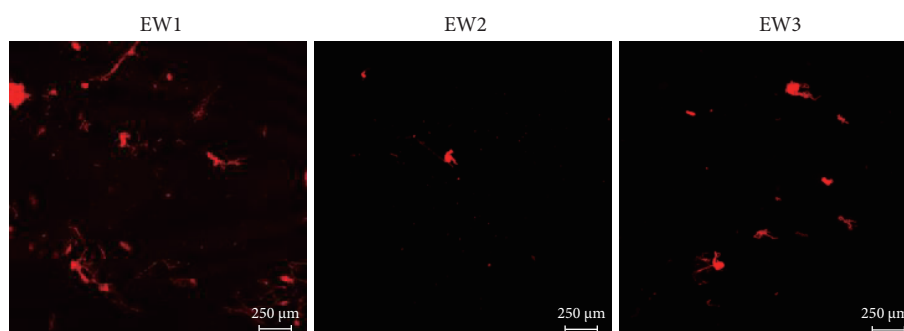


Figure 4 CLSM images of egg white solutions from different diets.

of protein particles coexisted, similar to EW1, but with significantly reduced degree of aggregation compared to EW1. The size and aggregation degree of protein particles affected the formation of gels to some extent. Moderate aggregation and uniformly sized protein particles in the system contributed to the formation of a more stable three-dimensional gel network structure^[27]. The above results further validated the test results of solution dispersibility (particle size distribution and zeta potential), indicating that EW2 had the best solution dispersion stability.

3.4 Effect of dietary composition on the molecular structure of egg heat-induced gel protein

3.4.1 Secondary structure

FTIR spectroscopy is widely used to analyze information related to protein secondary structure, and by fitting the peaks in the amide I band spectrum located at 1 600–1 700 cm^{-1} , the relative content of protein secondary structure can be calculated. As shown in Figure 5A, the infrared spectra of egg white proteins from different diets exhibited significant differences in the 1 600–1 700 cm^{-1} region, indicating that different diets altered the structural composition of egg white proteins. The results on the relative content of protein secondary structure obtained by fitting the amide I

band (Table 3) showed that EW1 had the highest relative content of random coil structure and the lowest relative content of β -sheet and β -turn structures. EW2 had the lowest relative content of random coil and the highest relative content of β -sheet structure. Generally speaking, the molecular structure of proteins determined their functional properties. It was reported that a lower content of random coil structure induced the formation of protein gel and exhibited stronger gel properties^[28], which might be one of the important reasons for the superior gel performance of EW2 compared to EW1. Interestingly, the sum of relative content of β -sheet and β -turn structures in EW3 was close to that in EW2, but its gel performance was inferior to that of EW2. The reason might be that the relative content of β -sheet in egg white protein had a higher decisive proportion in determining the gel properties of egg whites^[29–30]. Compared to EW3, EW2 had a higher relative content of β -sheet, an increase of 5.20%. In addition, the higher α -helix relative content in EW3 might also be an important reason for the deterioration of EW3 gel. According to previous studies, a higher α -helix content often led to a decrease in gel properties^[24].

3.4.2 Tertiary structure

Raman spectroscopy was widely used to study the molecular structural information of protein gels, including the characteristic

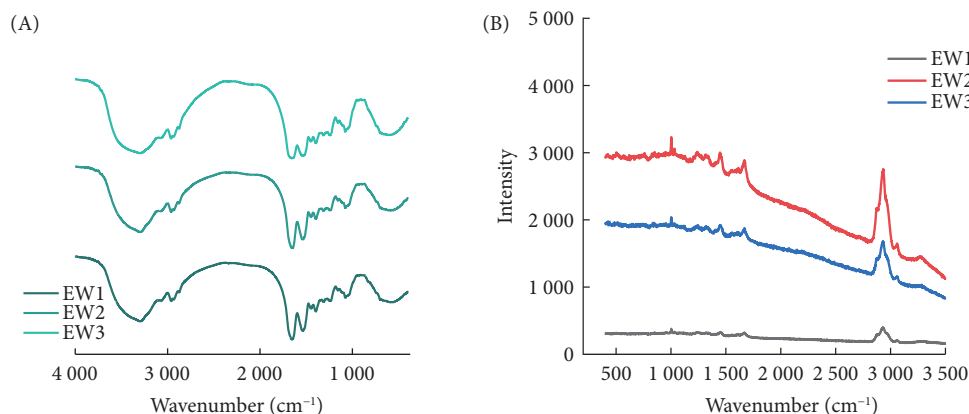


Figure 5 Molecular structure of egg white gel from different diets. (A) FTIR spectroscopy and (B) Raman spectroscopy.

Table 3 Relative contents (%) of protein secondary structure in egg white gel.

Secondary structure	EW1	EW2	EW3
α -Helix	18.66 ± 0.10^b	18.82 ± 0.03^b	19.32 ± 0.03^a
β -Sheet	27.54 ± 0.49^c	33.16 ± 0.08^a	31.52 ± 0.15^b
β -Turn	34.94 ± 0.35^b	35.25 ± 0.03^b	36.15 ± 0.11^a
Random coil	18.86 ± 0.06^a	12.77 ± 0.02^c	13.01 ± 0.01^b

Note: Different lowercase letters in the same row indicate significant differences between treatments ($P < 0.05$).

vibrations of chemical bonds and functional groups. Generally speaking, the absorption peak near the wavelength of 760 cm^{-1} was generally caused by changes in the microenvironment surrounding tryptophan residues, the absorption peaks located near the wavelengths between and 850 cm^{-1} were characteristic absorption peaks of tyrosine residues, and the absorption peak located near the wavelength range of $1\,453\text{ cm}^{-1}$ was characteristic of aliphatic amino acid residues. By normalizing the absorption peaks near the wavelengths of 760 cm^{-1} ($I_{760}/I_{1\,003}$), 850 cm^{-1} (I_{850}/I_{830}), and $1\,453\text{ cm}^{-1}$ ($I_{1\,453}/I_{1\,003}$), it was possible to determine whether tryptophan residues, tyrosine residues, and aliphatic amino acid residues were specifically in a polar or non-polar environment, and to ascertain whether tyrosine residues in protein molecules were exposed or buried. Based on this, the structural unfolding and contraction of protein molecules could also be judged^[31]. As could be seen from Figure 5B, the curves of the Raman spectra of egg white gels from different diets exhibited similar contours, and the peak positions of the Raman absorption peaks did not change significantly. The main differences in the Raman spectra were concentrated in the intensity of the absorption peaks, suggesting that different diets have caused changes in the molecular conformation of egg white protein to some extent (contraction or expansion). To further investigate the changes in the molecular conformation of egg white protein caused by different diets, the characteristic absorption peaks of three types

of egg white protein were normalized (Table 4). The results showed that there were no significant changes in the normalized doublet band ratios among I_{850}/I_{830} , EW1, EW2, and EW3. Interestingly, the ratios of $I_{760}/I_{1\,003}$ and $I_{1\,453}/I_{1\,003}$ in the Raman spectra of EW2 and EW3 were both higher than that of EW1, indicating that the molecular conformation of egg white protein in diets EW2 and EW3 was expanded, and hydrophobic amino acids such as tryptophan and aliphatic amino acids within the protein molecule were exposed, transitioning from a non-polar microenvironment to a polar microenvironment, which was beneficial for enhancing interactions with water molecules.

3.4.3 Conformational stability

Thermogravimetric analysis was widely used to evaluate the conformational stability of protein molecules under extreme conditions. The thermogravimetric analysis (TGA) and first derivative of thermogravimetric (DTG) analysis curves were shown in Figures 6A and B, respectively. The TGA curve indicated that the thermal degradation of egg white gel from different diets occurred in stages, with mass loss mainly concentrated around 300 °C which was similar to previous studies and was mainly caused by the thermal degradation of the protein molecular structural backbone^[7]. It could be observed from the TGA curve that the patterns of the TGA curve among the three samples were similar. To better

Table 4 Raman spectral band normalized intensity of EW gel.

Parameters	EW1	EW2	EW3
I_{850}/I_{830}	1.01 ± 0.01^a	1.01 ± 0.01^a	1.01 ± 0.02^a
$I_{760}/I_{1\,003}$	0.87 ± 0.01^b	0.92 ± 0.02^a	0.94 ± 0.01^a
$I_{1\,453}/I_{1\,003}$	0.83 ± 0.02^b	0.93 ± 0.02^a	0.95 ± 0.01^a

Note: Different lowercase letters in the same row indicate significant differences between treatments ($P < 0.05$).

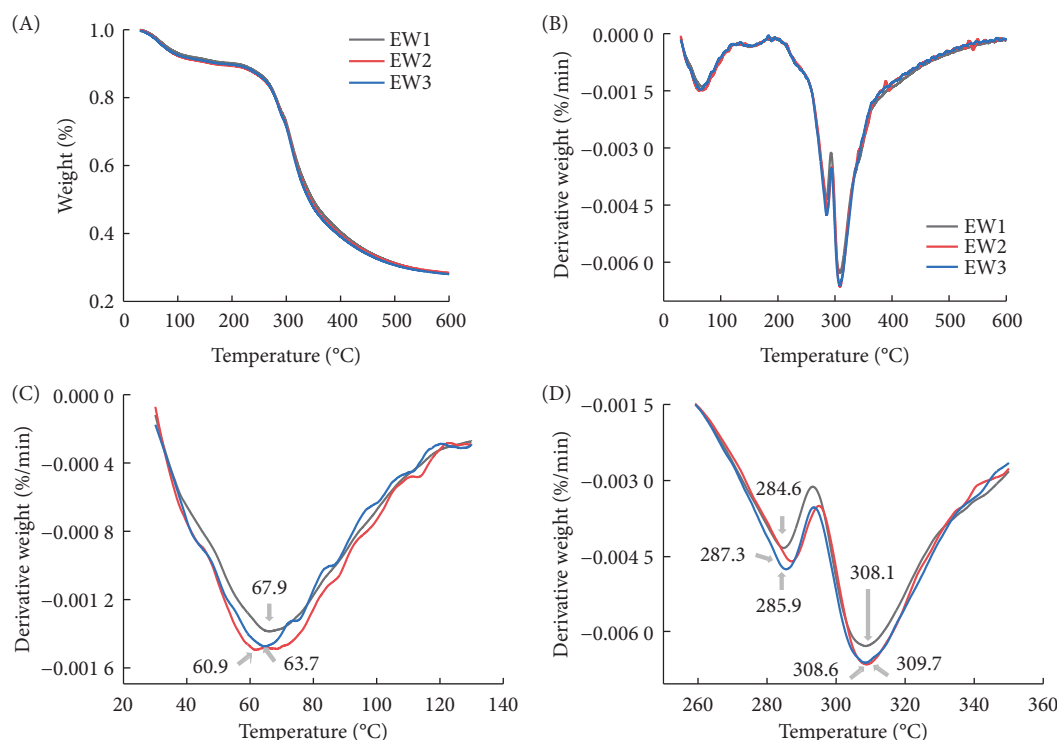


Figure 6 Thermal stability of egg white gel from different diets. (A) TGA spectrum, (B) DTG spectrum, (C) thermal denaturation temperature and (D) degradation temperature.

analyze the conformational stability of the samples, the first derivative of the TGA curve was performed. According to the DTG curve, the degradation of the gel samples could be divided into three stages. The first stage of thermal degradation was concentrated around 65 °C (Figure 6C), mainly characterizing the denaturation temperature of protein molecules^[24]. The denaturation temperature for the gel samples EW1, EW2, and EW3 was set at 67.9, 60.9, and 63.7 °C, respectively. It was reported that proteins with a lower denaturation temperature facilitated faster gelation, providing sufficient time for the formation of the gel network, thereby achieving better gel properties^[21]. EW2 had the lowest thermal denaturation temperature, which was consistent with the test results of texture properties, indicating a possible high correlation between the two. The thermal degradation at the second stage was concentrated around 286 °C (Figure 6D). Interestingly, after this stage, the thermal degradation rate of the sample slowed down, which might be due to the resistance of the protein molecular backbone structure to thermal degradation, and also suggested that the protein backbone structure began to undergo preliminary collapse at this stage. The degradation temperature for EW1, EW2, and EW3 gel samples at this stage was 284.6, 287.3, and 285.9 °C, respectively. After experiencing the collapse of the protein backbone structure at the second stage, the thermal degradation rate of the three samples reached its peak at the third stage, with the maximum degradation temperature for EW1, EW2, and EW3 gel samples being 308.1, 309.7, and 308.6 °C, respectively. Based on the above results, the EW2 protein gel exhibited the best environmental resistance in resisting thermal degradation of the protein structure, with the highest structure degradation temperature and maximum degradation temperature, indicating that the EW2 protein gel might possess the most stable molecular conformation to resist thermal degradation. The improvement might be attributed to the smaller particle size in the EW2 protein gel, which facilitated uniform filled into the gel network structure, enhancing the stability of the gel's three-dimensional network structure, thereby improving the thermal stability of the gel^[32].

3.4.4 Microstructure

The ability of protein gels to form three-dimensional gel network largely determined the performance of the protein gel^[32]. As shown in Figure 7, the microstructures of three types of egg white gels under different dietary conditions exhibited significant differences. The electron microscopy images of EW1 gel revealed large cavities in gel structure and relatively weak cross-linking between them, which might be due to insufficient unfolding of protein molecular structure during the formation of EW1 gel, resulting in low cross-linking with adjacent protein molecules. In contrast, the microstructure of EW2 gel showed better cross-linking and uniform pores. The closed pores formed by protein molecular cross-linking provided physical space for the retention of water molecules. Meanwhile, the relatively uniform dense pore structure

was also the structural basis for good gel performance. The formation of a continuous and dense gel network in EW2 might be attributed to differences in the composition and conformation of egg white proteins caused by variations in dietary rations. Studies have reported that a lower denaturation temperature and an increase in the content of OVM and OVT in egg white protein could induce the formation of a more stable gel network structure, thereby enhancing the EW gel properties^[21]. The microstructure of EW3 gel was relatively compact, but the cross-linking between them was weak, exhibiting a discontinuous structure. Furthermore, due to excessive aggregation of protein molecules during gel formation, some agglomeration phenomena appear in the electron microscopy images. A three-dimensional gel network structure with large cavities, agglomeration, discontinuity, and weak cross-linking was prone to collapse when resisting external stress. Conversely, a three-dimensional gel network structure with uniform pores and high cross-linking could maintain the integrity of the gel when resisting external stress. Therefore, under different diet conditions, the differences in gel properties might be caused by the changes in gel network under multiple factors.

3.5 Effect of dietary composition on the molecular interaction of egg heat-induced gel protein

3.5.1 Surface hydrophobicity

The surface hydrophobicity of protein was an important indicator for evaluating the hydrophobic interactions between protein molecules, which was the main driving force behind the formation of protein gels. This could be judged by the fluorescence intensity after binding with ANS. As shown in Figure 8A, the peak values of the fluorescence curves for the three egg white proteins all appeared near 390 nm, and the peak fluorescence intensities of EW3, EW2, and EW1 decreased in that order. It was worth noting that the peak intensities of EW3 and EW2 were much higher than that of EW1, indicating that EW2 and EW3 had higher surface hydrophobicity compared to EW1. It was reported that the unfolding of protein structure led to the exposure of hydrophobic groups from within the molecule, which might also be an important reason for the increased hydrophobicity of the surfaces of EW2 and EW3^[24,33]. Furthermore, in previous studies, it was found that there was a positive correlation between the surface hydrophobicity of protein molecules and the performance of protein gels^[24]. Therefore, the improvement of egg white protein gel quality might be closely related to the increase of surface hydrophobicity. It was noteworthy that EW3 had higher surface hydrophobicity, but its gel quality was lower than that of EW2. This might be because although EW3 (fluorescence intensity 6.24×10^4) has the highest surface hydrophobicity, the difference between it and EW2 (fluorescence intensity 6.18×10^4) was relatively small. Therefore, EW3 cannot fully offset the advantages brought by the stable molecular structure (secondary structure and thermal stability) and small and uniform particle size of EW2.

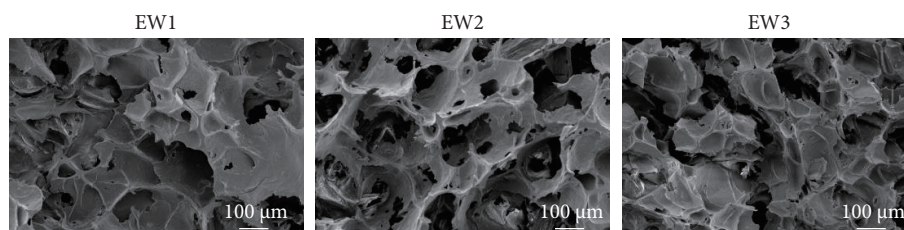


Figure 7 Gel network structure of egg white gel from different diets.

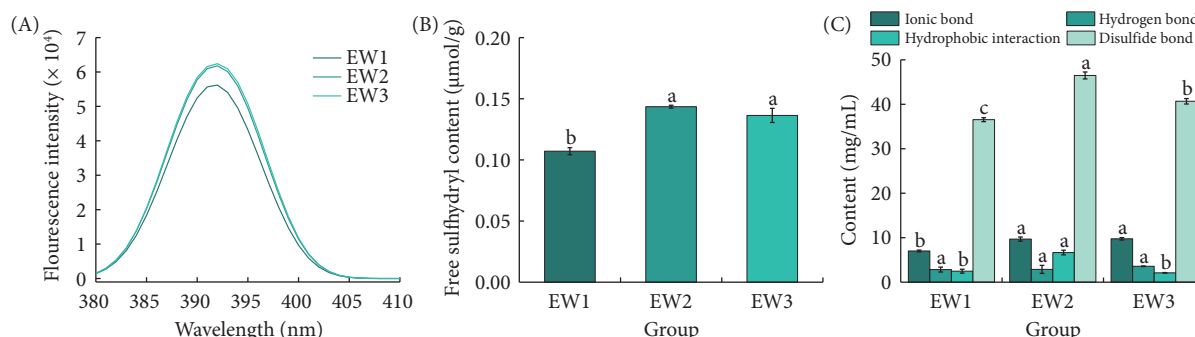


Figure 8 Molecular interaction of egg white gel from different diets. (A) Surface hydrophobicity, (B) free sulfhydryl content and (C) intermolecular forces. Different lowercase letters indicated significant differences between groups ($P < 0.05$).

3.5.2 Free sulfhydryl content

During the process of protein denaturation and gel formation upon heating, exchange reactions between sulfhydryl groups and disulfide bonds were prone to occur, thereby enhancing the interactions between proteins and significantly affecting the gelation behavior of protein gels. As shown in Figure 8B, the free sulfhydryl content of EW1, EW2, and EW3 reached 0.107, 0.143, and 0.136 $\mu\text{mol/g}$, respectively. EW2 had the highest free sulfhydryl content, which was 33.99% and 5.28% higher than that of EW1 and EW3, respectively. Researches indicated that the higher the content of sulfhydryl groups in the system, the more active sites participated in the crosslinking reaction, and the tighter the crosslinking would become between proteins, thereby affecting the mechanical properties and stability of the gel. In the silk fibroin-hyaluronic acid composite hydrogel system, as the content of sulfhydryl groups increased, the degree of crosslinking increased, and the strength and toughness of the gel was also improved^[34]. Similar results were also observed in the gel system of isolated soybean amyloid fibrils mediated by cellulose nanocrystals. The higher sulfhydryl content induced the isolated soybean amyloid fibril gel to exhibit superior gel properties and water retention^[25]. The abundant sulfhydryl groups in EW2 provided active sites for adjacent protein molecules to tightly crosslink with each other. During the deformation, unfolding, and rearrangement of the thermal gel, the interaction between protein molecules was strengthened, promoting the formation of the EW2. Furthermore, sulfhydryl groups were prone to undergo oxidation reactions at high temperatures, generating disulfide bonds. A higher content of sulfhydryl groups generally indicated the formation of more intermolecular or intramolecular disulfide bonds in the system, enhancing the overall interaction within the protein gel system and facilitating the formation of a strongly interacting three-dimensional gel network. In summary, the abundant total sulfhydryl content in EW2 might be an important material basis for the superior gel properties of EW2 compared to the other two groups. This might be attributed to the high proportion of corn-soybean meal in EW2 diet, which led to an increase in the content of sulfur-containing amino acids (Cys and Met) in egg whites through metabolism. This was also consistent with the results of amino acid content analysis.

3.5.3 Molecular interaction forces

The interaction forces between protein molecules (ionic bonds, hydrogen bonds, hydrophobic interactions and disulfide bonds) were important driving forces for the formation of protein gels^[35]. The levels of intermolecular forces in three egg white protein gels were obtained through denaturant treatment. As shown in Figure 8C, different diets led to differences in the molecular forces

among these three egg white protein gels. The hydrophobic interaction and disulfide bond of EW2 protein gel were significantly higher than that of EW1 and EW3, and these two forces were the main forces driving the formation of protein gel^[36–37], which were closely related to the behavior of protein gel. The differences in egg white gels among different diets might be mediated by different molecular interactions. Furthermore, the results of molecular forces were also consistent with the data on sulfhydryl groups and surface hydrophobicity. The differences in total sulfhydryl content and surface hydrophobicity of egg white gels provided a material basis for the differences in molecular forces. The higher sulfhydryl content in EW2 laid the foundation for the formation of more disulfide bonds, while the abundant surface hydrophobic groups enhanced the hydrophobic interactions in EW2. Therefore, the molecular interactions in EW2 were stronger than that in EW1 and EW3, facilitating the formation of a rigid gel network structure and maintaining the stability of molecular conformation.

3.6 Correlation analysis between heat-induced gel properties and other indicators of egg quality

Pearson correlation analysis was employed to further investigate the correlation between the gel properties of egg white protein (texture properties and WHC) and other indicators. As shown in Figure 9, the correlation among the texture properties of the gels (hardness, springiness, cohesiveness, gumminess, and chewiness) and other indicators exhibited a relatively consistent trend. Specifically, hardness was positively correlated with protein solubility ($r = 0.53$; $P \leq 0.05$), β -sheet relative content ($r = 0.71$; $P \leq 0.001$), free sulfhydryl content ($r = 0.61$; $P \leq 0.01$), ionic bond content ($r = 0.48$; $P \leq 0.001$), hydrophobic interaction content ($r = 0.90$; $P \leq 0.001$), and disulfide bond content ($r = 0.84$; $P \leq 0.001$); it was significantly and negatively correlated with particle size ($r = -0.72$; $P \leq 0.001$), PDI ($r = -0.55$; $P \leq 0.05$), zeta potential ($r = -0.53$; $P \leq 0.05$) and random coil relative content ($r = -0.55$; $P \leq 0.05$); and there was no correlation between hydrogen bond content, surface hydrophobicity, β -turn and α -helix relative contents.

The WHC of the gel was significantly and positively correlated with zeta potential ($r = 0.56$; $P \leq 0.05$), protein solubility ($r = 0.83$; $P \leq 0.001$), β -turn relative content ($r = 0.67$; $P \leq 0.01$), β -sheet relative content ($r = 0.81$; $P \leq 0.001$), α -helix relative content ($r = 0.75$; $P \leq 0.001$), free sulfhydryl content ($r = 0.81$; $P \leq 0.001$), surface hydrophobicity ($r = 0.93$; $P \leq 0.001$), ionic bond content ($r = 0.92$; $P \leq 0.001$), and disulfide bond content ($r = 0.60$; $P \leq 0.01$); it was significantly and negatively correlated with particle size ($r = -0.73$; $P \leq 0.001$), PDI ($r = -0.82$; $P \leq 0.001$), and random coil relative content ($r = -0.89$; $P \leq 0.001$); there was no correlation between hydrophobic interaction and hydrogen bond contents.

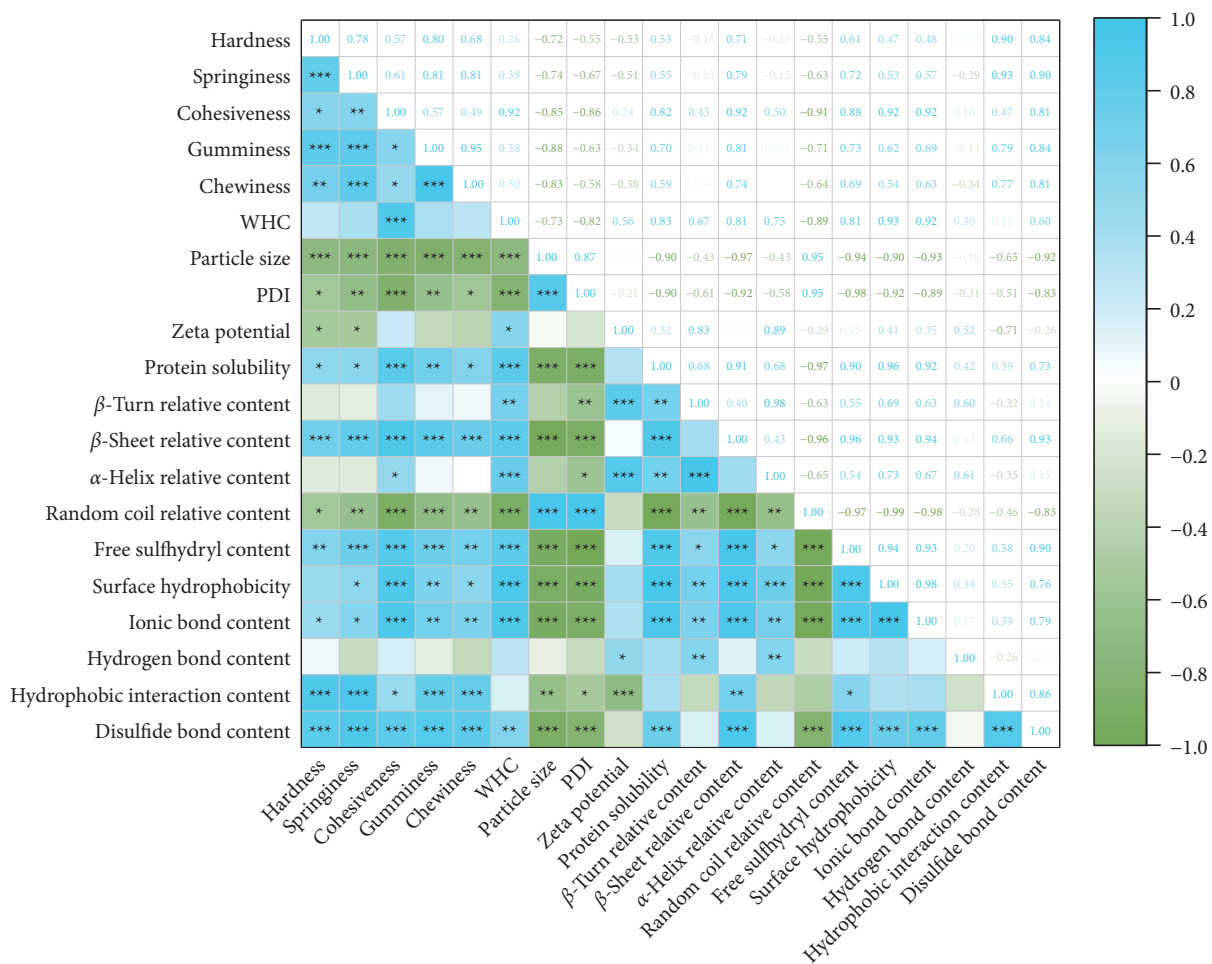


Figure 9 Correlation diagram of egg white gel index. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Based on the above results, the gel properties (hardness and WHC) of egg white protein gels were closely related to the aggregation state, molecular structure, and molecular interactions of proteins. The differences in gel properties mediated by different diets were the result of the combined effects of various factors, including protein aggregation state, molecular structure, and molecular interactions.

3.7 Potential mechanism of dietary composition affecting egg heat-induced gel properties

In this study, the addition of different diets affected the formation and properties of egg white protein gel. Based on the changes in the material composition, aggregation state, molecular structure, and molecular interactions of egg white protein, a schematic diagram of the mechanism by which different diets regulated the properties of egg white protein gel was proposed (Figure 10).

The skeleton network structure of heat induced protein gel was mainly formed by aggregation, cross-linking and molecular rearrangement of protein molecules after heat induced denaturation. The structural rigidity of network structure directly determined the gel performance of protein gel. EW1 (corn distillers' grains) might be due to its high denaturation temperature failing to crosslink sufficiently with adjacent protein molecules in the process of thermal denaturation, and the degree of molecular cross linking and order was low, so its gel network structure was chaotic and contained large cavities. In addition, the content of free amino acids

occurring with water molecules in EW1 and the content of OVM and OVT that constituted the gel skeleton were lower than that in EW2 and EW3. Therefore, EW1 protein gel had lower performance than EW2 and EW3. The addition of corn and soybean meal (EW2) and flaxseed (EW3) in the diet significantly improved the egg white protein gel properties. The lower thermal denaturation temperature of EW2 and EW3 could promote the gel of protein molecules in advance, providing sufficient time for the cross-linking between protein molecules, thus forming a tightly cross-linked and orderly gel network structure, providing a structural basis for the improvement of gel performance (texture performance and WHC). On the other hand, when corn and soybean meal (EW2) and flaxseed (EW3) were added to the diet, the contents of free amino acids, OVM and OVT were significantly higher than that of EW1, which provided a material basis for the improvement of gel properties. The correlation analysis showed that the egg white gel performance was closely related to the protein aggregation state, molecular structure and intermolecular interaction. From the perspective of protein aggregation status, adding corn and soybean meal (EW2) and flaxseed (EW3) to the diet increased the surface charge of egg white protein particles, weakened the aggregation phenomenon between protein particles, and led to a decrease in protein particle size, uniform distribution in the system, and improved stability of egg white protein in the system. From the perspective of protein molecular structure, the flexible and random coil in the secondary structure of egg white protein gel was

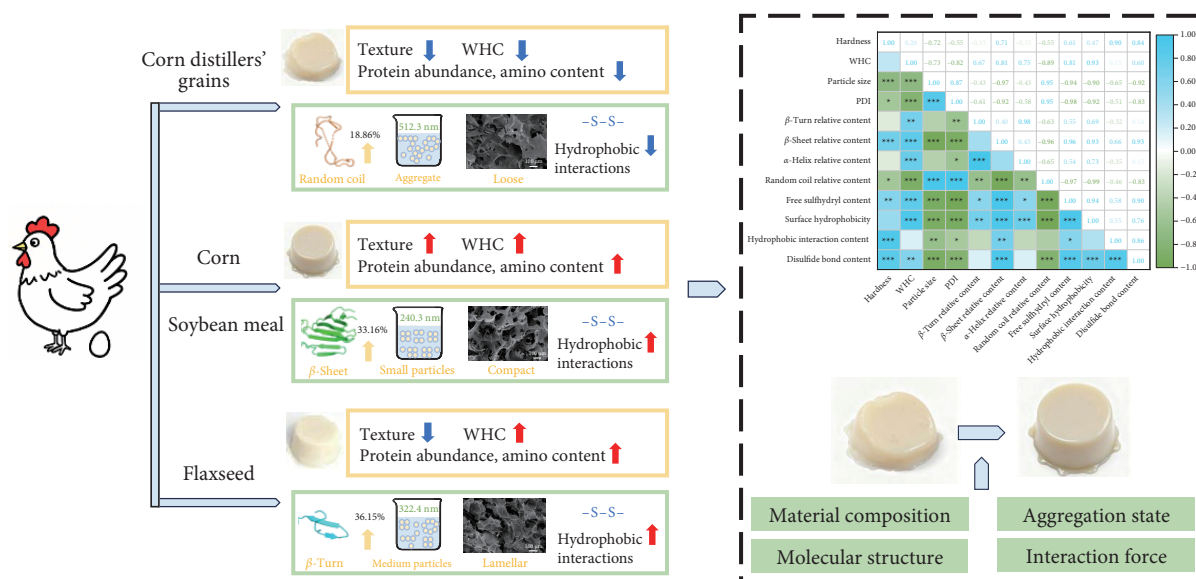


Figure 10 Potential mechanism diagram of different diets regulating egg white gel performance.

transformed into more stable β -sheet and β -turn, which improved the structural stability of egg white protein gel, promoted the expansion of protein molecular structure, and exposed tryptophan residues and aliphatic amino acid residues embedded in the molecule. From the perspective of protein molecular interaction, after the addition of corn and soybean meal and flaxseed in the diet, the surface hydrophobicity and sulfhydryl content of egg white protein increased, which directly led to the enhancement of disulfide bond and hydrophobic interaction driving the formation of gel in EW2 and EW3. Therefore, the increase of corn and soybean meal and flaxseed in the diet for laying hens increased the content of key amino acids and proteins in egg white protein, promoted the uniform distribution of protein particles, improved the dispersion stability of egg white protein, exposed the hydrophilic amino acid residues, sulfhydryl groups and other active groups originally embedded inside, strengthened the orderly cross-linking of protein molecules, enhanced the intermolecular interaction between egg white protein molecules, protein molecules and water molecules in the gel network, and formed a stable gel system. Under the combined effect of the above improvements, EW2 and EW3 egg white protein gel formed a uniform and dense three-dimensional gel network structure, providing more favorable conditions for resisting external pressure and retaining water molecules.

4 Conclusion

This study revealed that different dietary interventions had a significant impact on the performance of egg white gel. Adding corn and soybean meal and flaxseed to the diet could regulate the composition and structure of egg white protein, thereby enhancing the texture performance and WHC of egg white gel. Moreover, considering the texture performance and WHC, the increase of the grain contents such as corn and soybean meal in the diet had a more significantly positive effect on egg white protein gels. This regulatory effect was mainly achieved through the following mechanisms. The Alterations in the composition of proteins and amino acids: dietary intervention led to an increase in the content of major proteins such as OVA, OVT, and OVM in egg whites,

while also raised the content of hydrophilic amino acids and key amino acids (such as arginine and lysine), and enhanced the gel properties of proteins. The improvement of protein aggregation state: diet intervention enhanced the protein surface charge, reduced protein particle size, improved the dispersibility and stability of the protein solution. The molecular structure and intermolecular forces optimization: diet intervention changed egg white protein secondary structure, reduced random coil structure, increased the β -sheet and β -turn structures, at the same time enhanced the hydrophobic interactions and the formation of disulfide bond, strengthened the stability and thermal stability of the gel network. To sum up, from source by laying hens diet nutrition intervention can optimize gel properties of egg white, to the egg whites gel performance of precise control and develop new eggs gel products provides a new train of thought.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Xiangxin Liao: Investigation, methodology, writing-original draft. Yanting Yang: Investigation, data curation. Ying Gao: Formal analysis, writing-reviewing and editing. Sharina Qi: Software, data curation. Zhaowei Cui: Resources. Shijian Dong: Methodology,

resources; funding acquisition. Jijun Wu: Funding acquisition, validation. Shugang Li: Project administration, supervision, conceptualization, funding acquisition, writing-review and editing.

Data availability

Data will be made available on request.

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