



Effects of lipoproteins on yolk microstructure in duck, quail, goose, pigeon, and chicken eggs

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Abstract

Poultry eggs are among the most important protein and nutrient sources in human diets. There are considerable differences in the evolutionary processes of different species. Therefore, we evaluated the differences and correlations among the microstructure, low-density lipoprotein (LDL) level, and high-density lipoprotein (HDL) level in duck, quail, goose, pigeon, dwarf chicken, ShanDong chicken, Rhode Island Red, and HeTian DaHei chicken eggs. The egg microstructures were polygonal, compact, and granular and showed no evidence of cross-linking. There was a significant variation among the eight poultry egg types in terms of yolk particle size (3829.34-10373.25 μm^2), LDL level (0.023-0.048 mmol/l), and HDL level (3.39-7.98 mg/g). There were also significant differences among the chicken species in terms of yolk particle size (4070.87-8139.48 μm^2), LDL level (0.028-0.047 mmol/l), and HDL level (3.57-7.98 mg/g). The yolk granules of local chicken breeds were smaller than those of typical egg-laying chickens. The LDL level weakly correlated with the size of egg yolk ($r = 0.24$) and there was a very weak correlation between HDL level and yolk particle size ($r = 0.008$). This study provides a theoretical basis for variations in the taste of eggs from different poultry species and sub-species.

Keywords: egg; yolk microstructure; low-density lipoprotein; high-density lipoprotein.

Practical Application: In this study, goose eggs, duck eggs, quail eggs, pigeon eggs and five varieties of eggs were selected to determine their structure, LDL content and HDL content, and to conduct correlation analysis. It is the first time to explore the differences between different poultry eggs from a structural point of view. The correlation between lipoprotein content and structure is analyzed, and the structure is further analyzed, which provides a theoretical basis for the application and development of poultry eggs in food.

1 Introduction

Poultry eggs are an important dietary component worldwide owing to their high nutritional value and ease of digestion (Réhault-Godbert et al., 2019). Hard-boiled eggs not only maintain their nutritional value but also have high digestibility that allows the effective absorption of proteins (Partmann & Wedler, 1979). Cooked egg yolks have a fragile and powdery texture (Partmann & Wedler, 1979). Heating of eggs creates a gel layer that provides a medium for the transfer of nutrients and flavor, along with a unique texture to the food (Sun & Hayakawa, 2002). Several factors affect the gel layer formation in the egg yolk; for example, the addition of NaCl can reduce the relative thickness of the electric double layer and shield the surface charge of the protein, thereby promoting gel formation (Li et al., 2018). However, the amount of yolk protein contributing to the gel varies under different pressure treatments (Yan et al., 2010). Although numerous studies have evaluated the egg yolk gel (Ngarize et al., 2004; Sikorski, 2001), the microstructure of boiled eggs from different species has not been analyzed.

Cardiovascular diseases are primarily caused by atherosclerosis, a major factor of which is increased blood cholesterol level (Blesso & Fernandez, 2018). Low-density lipoprotein (LDL) particles can

promote atherosclerosis, whereas high-density lipoprotein (HDL) particles help in the prevention of atherosclerosis (Law et al., 2003). The cholesterol level in human blood is considerably affected by diet, and egg yolk is a predominant source of exogenous cholesterol (Greene et al., 2006). Egg yolk is composed of plasma and granules (Anton, 2013), wherein the plasma comprises 85% LDL and 15% vitellin (Martin et al., 1964), whereas the granules contain 70% HDL, 16% phospholipids, and 12% LDL (Burley & Cook, 1961). The egg yolk-LDL has good gel characteristics, which influence yolk processing and its derivatives and affect their texture (Laca et al., 2014). During the heating process, the protein component of the egg yolk-LDL is denatured and unfolded and the internal functional groups are exposed and rearranged through hydrophobic interactions and crosslinking, resulting in gel formation (Anton, 2013; Kiosseoglou, 2003). However, to date, no relationship has been reported between the LDL and HDL levels and yolk gel microstructure.

In the current study, the yolk microstructure and the LDL and HDL levels after boiling were investigated using eggs of duck, quail, goose, pigeon, dwarf chicken (DF), ShanDong chicken (SD), Rhode Island Reds (RIR), and HeTian DaHei chickens

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(HTDH). Further analyses were conducted to determine the relationship between yolk particles and lipoprotein levels and provide a theoretical basis for food processing and taste.

2 Materials and methods

2.1 Sample collection

Duck, quail, pigeon, and goose eggs were obtained from a supermarket in Beijing and freshly delivered on the day of the experiments. DF, SD, RIR, and HTDH eggs were obtained from a small farm in China Agricultural University (Beijing, China), Rongde Chicken Farm in Hebei Province (China), and Shandong Poultry Farm (Shandong, China). All eggs were laid on the same day and transported to the laboratory within 2 days.

2.2 Egg yolk preparation

Eight eggs of each poultry species were selected for testing. Water (4 L) was brought to a boil in a pot, and after 1 min, the eggs were added. The duration for which the eggs of each type were boiled was different depending on their size: quail eggs, 2 min; pigeon eggs, 2 min; chicken eggs, 5 min; duck eggs, 7 min; and goose eggs, 9 min. All eggs were removed 2 min after the heat source was turned off. The eggshells and egg whites were removed, and the yolks were used in further analyses.

2.3 Microstructure visualization by scanning electron microscopy (SEM)

The microstructure of egg yolks from each of the eight poultry species and sub-species was observed by SEM, as described by Cordobes et al. (2004). Each yolk sample was placed in a test tube containing 2.5% glutaraldehyde solution and 0.2 M phosphate-buffered saline (PBS; pH 7.0) solution. The tube was filled with distilled water to ensure that the sample was completely immersed in the stationary solution and then stored at 4 °C. Thereafter, the fixative was discarded and the sample was rinsed three times with 0.1 M PBS (pH 7.0) for 15 min each. The sample was then treated with 1% osmic acid solution for 1–2 h and the used osmic acid solution was carefully discarded. The samples were rinsed three times with 0.1 M PBS (pH 7.0) for 15 min each. Subsequently, the samples were dehydrated with gradient ethanol concentrations (30%, 50%, 70%, 80%, 90%, and 95%) for 15 min and then treated with 100% ethanol twice for 20 min each. Thereafter, the samples were treated with a mixture of ethanol and isoamyl acetate (v/v = 1/1) for 30 min, and then washed with pure isoamyl acetate for 1 h or overnight. The samples were dried in a critical point dryer (Leica EM CPD300, Hitachi, Tokyo, Japan), and then placed on a carbon conductive adhesive sample tab before being coated on a MC1000 Ion Sputter Coater (Hitachi). The treated samples were observed using a Hitachi SU8010 scanning electron microscope (Tokyo, Japan).

2.4 LDL and HDL determination

An enzyme-linked immunosorbent assay was performed to determine the LDL and HDL levels in the egg yolks. Eight replicates were carried out per species or sub-species. The egg

yolk sample (70 mg) was weighed, and PBS (pH 7.4) was added to attain a ratio of 1:9; subsequently, the samples were homogenized and centrifuged (2000–3000 rpm, 20 min). The supernatant was collected for quantitative analysis. The blank and standard controls (50 µL standard solution) were included for comparison. First, 40 µL of sample diluent was added to the sample wells, and then 10 µL of sample was added. Next, 100 µL of enzyme-labeled reagent was added to each well, and the plate was sealed with sealing film and incubated at 37 °C for 60 min. After incubation, the film was carefully removed, and excess liquid was discarded. The wells were dried and rinsed with detergent five times for 30 s each. The plates were patted dry, and 50 µL of color developer A was added to each well; thereafter, 50 µL of Reagent B added. After incubating the plates at 37 °C for 15 min in the dark with gentle agitation, stop solution (50 µL) was added to each well to stop the reaction. The absorbance (OD) of sample in each well was measured at 450 nm with a Rayto RT-6100 Microplate Reader (Rayto, Shenzhen, China). The relative absorbance of each sample was calculated using the straight-line regression equation derived from the standard curve.

2.5 Statistical analysis

The values are presented as mean with standard deviation. ImageJ software (v1.52, National Institutes of Health, Bethesda, MD, USA) was used to evaluate the area of the yolk particles. One-way analysis of variance (ANOVA) between groups was conducted, and significant differences were defined at $P < 0.05$ using Duncan's multiple range test. Analyses were performed using SPSS software (v.20, SPSS, Inc., Chicago, IL, USA) (Alavi et al., 2020) for mapping and correlation analyses.

3 Results and discussion

3.1 Variation in egg yolk microstructure

The structure of the egg yolk allows a loose texture with low elasticity and adhesion (Woodward & Cotterill, 1985). Dissimilarities in egg quality have been observed among the egg of different species and sub-species (Sun et al., 2019). However, scanning electron micrographs of the eight types of poultry eggs (Figure 1) in this study revealed that the yolk microstructure showed minimal changes after boiling, and displayed a polygonal, granular, and closely interlinked structure in all egg types when heated to 100 °C. No cross-linking was observed between the egg yolk particles, and this is consistent with the results of a previous study (Woodward & Cotterill, 1987). As evident from the grid-like structure, the hydrophobic interactions in LDL micelles play a role in gel formation, causing random aggregation and a gel with a non-uniform distribution of particles (Guerrero et al., 2004).

In this study, although the microstructure of all the poultry eggs was granular, their particle sizes were different (Table 1). There was a significant difference in the yolk granule size among the eight egg types ($P < 0.05$). The coefficient of variation (CV) among the poultry eggs was relatively large (22–50%), reflecting the absence of directional selection (Guerrero et al., 2004). Among the five domestic species, duck eggs contain the highest amount of fat (Hocking et al., 2003). During boiling,

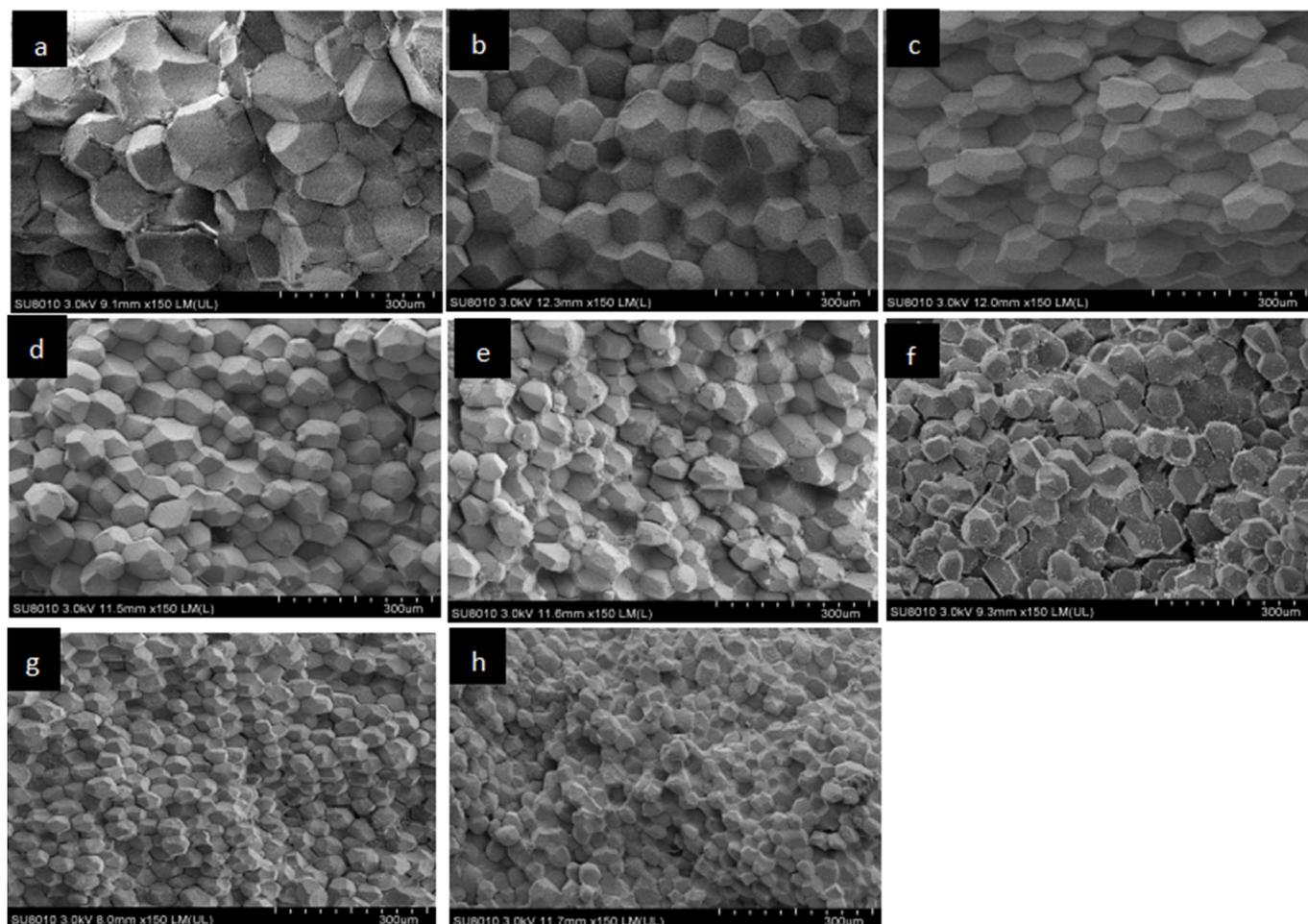


Figure 1. Scanning electron microscopy images of eggs from eight different species and sub-species. The yolk particles were arranged in the order of small to large. The eight images represent: (a) goose eggs; (b) dwarf chicken eggs; (c) Rhode Island Red eggs; (d) ShanDong chicken eggs; (e) quail eggs; (f) pigeon eggs; (g) HeTian DaHei chicken eggs; and (h) duck eggs.

Table 1. Comparison of egg yolk particle size among eggs from different bird species and sub-species.

Species	N	Particle size (μm^2)		CV (%)	Significance
		Mean \pm SD			
Duck	8	3829.34 \pm 895.74		23.00	c
Quail	8	5211.76 \pm 1342.48		26.00	b
Goose	8	10373.25 \pm 5207.80		50.00	a
Pigeon	8	4376.94 \pm 1277.71		29.00	bc
DF	8	8139.48 \pm 1799.35		22.00	a
SD	8	5229.88 \pm 1396.64		27.00	b
RIR	8	5923.44 \pm 2052.49		35.00	ab
HTDH	8	4070.87 \pm 1174.23		29.00	bc

the structure of lipoproteins is destroyed, decreasing the lipid-binding capacity of the proteins and separating lipid molecules from protein molecules. The increased number of lipid molecules hinders the movement and arrangement of protein molecules and reduces the crystallinity of proteins (Xiang et al., 2020), allowing the adjacent proteins to bind to form smaller egg yolk particles. This may explain why the yolk particles of duck eggs are the smallest.

Data are shown as mean \pm S.D. a-c: significant differences between mean values ($P < 0.05$). The same letter means no significant difference. DF: dwarf chicken; SD: ShanDong chicken; RIR: Rhode Island Red; HTDH: HeTian DaHei chicken.

Furthermore, the high-quality poultry breeds have undergone a complex and rigorous selection process for various important production traits such as egg weight, eggshell strength, and body weight (Moula et al., 2009). However, high-intensity breeding may also influence the taste of the yolk (Emmerson, 1997). Consistent with this fact, significant differences in the particle size were observed between the typical egg-laying types of chicken and the local domestic chicken breeds. This is also the reason that the taste of local chicken eggs is preferred by consumers over that of commercial laying hen eggs. This study provides valuable insights for understanding the effect of processing and utilization of egg yolk on its taste.

3.2 LDL level

The content of LDL in eggs has received more and more attention. People use the most eggs for heat treatment. Therefore, we measured the LDL content of eight kinds of eggs after heat

treatment. The average LDL level of the egg yolks after boiling was 0.023-0.048 mmol/L (Table 2), and it varied significantly among the eight egg types ($P < 0.05$). The LDL level of the duck and goose eggs was the highest (0.048 mmol/L), whereas that of the quail eggs was the lowest (0.023 mmol/L). Evolutionary divergence has led to the emergence of varying growth conditions and dietary habits among different species of birds, most likely resulting in different LDL levels in their egg yolks (Zhou et al., 2021). LDL in birds comes from exogenous absorption and endogenous synthesis. Exogenous absorption mainly comes from feed. There are two ways for endogenous synthesis, one is converted from very low density lipoprotein (VLDL), and the second is synthesized by liver. VLDL and LDL are transported to the ovary through the blood, and are transported to the follicle through endocytosis through the receptor on the ovary, forming LDL in the egg yolk (Jolivet et al., 2006; Schneider, 2016; Wang et al., 2014). Genetics, feed types, and the environment are other important factors that influence various components of the egg yolk. Different birds use different feeds, which may lead to different LDL levels after thermal processing in different birds. At the same time, LDL is a major source of nutrition. LDL is composed of 12% protein and 87% lipid, and lipid contains about 71% triacylglycerol, 25% phospholipid, and 4% cholesterol (Anton et al., 2003). LDL also provides essential fatty acids, such as Arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid (Wang et al., 2014). Due to biological evolution, there are huge differences in various parts of different birds, and differences in liver and ovary size. There are differences in the absorption and synthesis of LDL.

LDL is involved in the formation of egg yolk particles, therefore, the content of LDL may affect the size of egg yolk particles. The correlation analysis between the particle size and LDL level of the yolks (Figure 2) indicated a weak correlation ($r = 0.24$, $P < 0.05$). The mechanism of gel formation in the egg yolk mainly involves active plasma proteins and LDL (Hayakawa et al., 1992), which interact with each other upon

boiling to form a gel network (Kiosseoglou & Paraskevopoulou, 2005). Furthermore, the formation of covalent disulfide bonds between the denatured protein molecules in LDLs may play a crucial role in forming the gel structure (Blume et al., 2015), indicating that LDL is involved in gel formation in the egg yolk (Anton, 2013). In this study, a weak correlation between the LDL level and the egg yolk gel particles conformed to the expectation.

3.3 HDL level

Eggs are also rich in HDL, and HDL has attracted widespread attention because it can resist arteriosclerosis. In this study, the average HDL level of the eight types of egg yolks after boiling was 3.39-7.98 mg/g (Table 2), with significant differences among the egg types ($P < 0.05$). The HDL level in HTDHD eggs was the highest (7.98 mg/g) and that in the pigeon eggs was the lowest (3.39 mg/g). HDL is a macromolecular complex composed of proteins and lipids, also known as lecithin, which is a spherical lipoprotein. HDL is composed of 75%-80% protein and 20%-25%

Table 2. Comparison of LDL level and HDL level among eggs from different bird species and sub-species

Species	LDL (mmol/l)			Significance	HDL (mg/g)		
	N	Mean	SD		Mean	SD	Significance
Duck	8	0.048	0.0048	c	6.05	0.74	bc
Quail	8	0.023	0.0049	a	5.17	0.94	c
Goose	8	0.048	0.0057	c	5.67	1.36	bc
Pigeon	8	0.032	0.0086	ab	3.39	0.54	d
DF	8	0.047	0.0071	c	6.40	1.67	abc
SD	8	0.028	0.0067	a	3.57	0.36	d
RIR	8	0.043	0.0059	bc	7.29	1.64	ab
HTDHD	8	0.038	0.0094	abc	7.98	0.87	a

a-c: significant differences between mean values ($P < 0.05$). The same letter means no significant difference. DF: dwarf chicken; SD: ShanDong chicken; RIR: Rhode Island Red; HTDHD: HeTian DaHei chicken

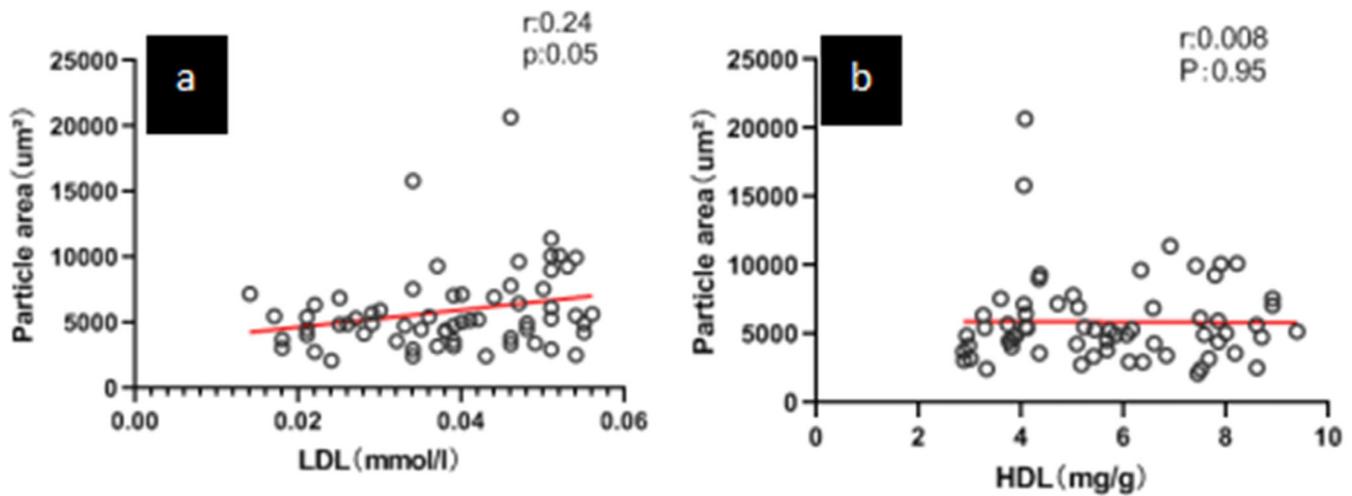


Figure 2. (a) Correlation analysis of low-density lipoprotein (LDL) level and yolk particle size in different eggs. The red line represents the correlation fitting curve; (b) Correlation analysis of high-density lipoprotein (HDL) level and yolk particle size in different eggs. The red line represents the correlation fitting curve.

lipid, and its density is close to that of protein, about 1.120 g/mL (Strixner et al., 2014). In the lipid composition, the content of phospholipids was the highest, with lecithin and cephalin in the majority, accounting for about 18% of the total lipids, and their saturation was high. In addition to phospholipids, it also contains 8% triglycerides and 1% cholesterol (Lee et al., 2005). There are significant differences in protein and lipid synthesis in the body of birds due to differences in species and breeds, which may lead to differences in the HDL content of different birds. HDLs can directly alleviate atherosclerosis (Millar et al., 2017). The intake of HDL in the diet increases the HDL level in the blood, resulting in clarification of the blood vessels and optimum blood flow (Tardy et al., 2014). Therefore, it is important to determine the HDL level in different poultry eggs to provide a basis for nutritional recommendations regarding egg consumption.

HDLs also influence gel formation in egg yolks (Kiosseoglou, 2003). The correlation analysis between the particle size and HDL level (Figure 2) revealed a very weak correlation (0.008) between the yolk particle size and HDL level. It is widely accepted that plasma components play a dominant role in the formation of the egg yolk gel (Anton et al., 2001). Although HDL may also participate in the gel network formation, it is not heat-sensitive and thus does not have a substantial effect (Anton et al., 2001).

4 Conclusions

Overall, the yolk microstructure of eggs from different bird species and sub-species is similar, showing a polygonal, granular structure. However, the particle size differed significantly among various species and sub-species. The local domestic breeds had smaller yolk particles than the highly selected lines of egg-laying hens in terms of microstructure. Moreover, the relationship between LDL and yolk microscopic particles provides valuable insights for research on gel formation.

Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and material

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Author contributions

R.Z. performed the study, drafted the manuscript, and contributed most of the experiments. X.L. software. C.F. resources. Z.N. conceived the study, evaluated the test details, and revised

the manuscript. All authors have read and approved the final manuscript for submission.

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