

Research Article

Study on the Relationship between Egg O₂ Respiration and Eggshell Ultra Structure

^{1,2}Wang Qiaohua, ¹Zhang Tao, ^{2,3}Ma Meihu and ¹Li Xiaoming

¹College of Engineering,

²National Research and Development Center for Egg Processing,

³College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

Abstract: Respiration is a fundamental physiological phenomenon of egg and egg gas exchange is realized through the shell. This study aims to reveal the relationship between egg respiration intensity and eggshell surface ultra structure. In this study O₂ flux via eggshell surface was selected as a symbol of egg respiration intensity and it was determined real-time dynamically based on Non-invasive Micro-test Technique (NMT). Images of eggshell ultra structure were acquired through High Magnification scanning electron microscopy. After processing the images, the distribution of stomata and roughness of eggshell surface was observed. Studies show that eggshell surface ultra structure morphology affect egg gas exchange, rougher surface and denser stomata promoted egg gas exchange. The greater the O₂ flow is, the rougher the surface and the larger hole density. The linear relationship between respiration and eggshell thickness, egg weight was not significant.

Keywords: Egg, eggshell ultra structure, micro-test technique, O₂ flux, relationship, respiration

INTRODUCTION

Eggshell quality (shell structure, shell thickness, strength, etc.) is an important indicator impacting the quality of eggs. Eggshell quality not only affects egg storage and transportation, but also has an important effect on the hatch of breeder eggs and poultry breeding (Wang *et al.*, 2011a; Hunton, 2005; Sun *et al.*, 2011; Wang *et al.*, 2013). Shell structure, from inside to outside consist of shell layer, mastoid layer, palisade layer, the crystal layer and the epidermis, respectively (Wan *et al.*, 2006; Tyler, 1964; Becking, 1975). The epidermis is the outermost layer of the shell structure, only 2-3% of entire thickness. It is also the thinnest layer. Because the epidermis is the outermost layer, it makes the most intimate contact with the outside. This will definitely affect that the egg's gas exchange with the outside world, so egg's surface structure plays an important role (Zhang, 2012, 2013). Looking for the relationship between egg surface structure and the breathing can provide a theoretical basis for preservation and fresh-keeping of eggs such as how to extend storage time, slow down metabolism and change modes of transport, at the same time it lays a theoretical foundation of improving the hatchability of eggs and adjusting hatch way (Zhou, 2010; Huang *et al.*, 2001; Yao, 2010; Lian *et al.*, 2010; Zou *et al.*, 2006; Li *et al.*, 2009; Chen *et al.*, 2010; Li *et al.*, 2011; Xiong *et al.*, 2013). In recent years, with the application of high resolution scanning electron microscopy, more and

more experts and scholars began to explore the differences in shell ultra structure and composition (Yang *et al.*, 2007; Di *et al.*, 2012; Wang *et al.*, 2011b; De Ketelaere *et al.*, 2002; Rodriguez-Navarro *et al.*, 2002; Roberts, 2004) as well as the change in the different kinds of eggshell during incubation. Yang *et al.* (2008) studied the relationship between color, eggshell quality and eggshell ultra structure and indicated that the shallow color of the eggshell surface pore was more than the deep color. Yu *et al.* (2008) studied the elemental composition and ultra structure of eggshell and the results showed that the density of ultra structure directly impacted on the eggshell quality and the total amount of calcium and eggshell matrix protein deposition was closely related to its density, however it had no significant association between the elemental composition of eggshell and eggshell quality. Li *et al.* (2008) studied the relationship between quality and ultra structure and obtained that the difference of eggshell ultra structure is an important factor for affecting eggshell quality change. Zhang *et al.* (2005) carried out researches on the porous shell to explore the mechanism of the respiratory shell and obtained that the concentration difference of oxygen and other gases both inside and outside of the shell is the power source of the respiratory system and the diffusion is the main factor. Most of these studies are limited to the formation of the structure and composition of matter, however few people explore that the structural differences have any effect on gas exchange of eggs. This study will observe

Corresponding Author: Ma Meihu, College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

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the egg surface ultra structure through High Magnification scanning electron microscopy and studied that different surface structure of eggshell was how to affect respiration intensity.

MATERIALS AND METHODS

Test materials and equipment:

Test material: Test samples were the same species, the same batch and the same day-old fertilized eggs and non-fertilized eggs each 50 (Provided by the Beijing Huadu Poultry Meiyukou Co. Ltd.).

Test instrument: Electronic Balance (Accuracy 0.0001 g, FA2004N, Shanghai Precision and Scientific Instrument Co. Ltd.), ESTG-1 Ultrasonic eggshell thickness detector (Accuracy 0.01 mm, Orka Technology Ltd.), Ion sputtering instrument (JEOL JFC-1600, Japan), JSM-6390LV scanning electron microscopy (Magnification from 5X to 300000X, resolution up to 0.3 nm, JEOL, Japan), Polarographic oxygen electrode non-invasive micro-measurement system (YG-FC-005, Younger USA), constant temperature and humidity box (RXZ-158, Ningbo Jiangnan instrument factory), test fluid, correction fluid, etc.

Test methods and procedures:

O₂ flux measurement: Wang *et al.* (2014) in this experiment, O₂ flux of fertilized egg and non-fertilized egg was determined based on Non-invasive. The blunt end of the egg was selected and one random point marked (Ne Micro-test Technique (NMT) The highest point), the vertical distance from the probe to the

eggshell was set at 50 μm, measurements were taken for 10 min and the average recorded. Fertilized eggs and non-fertilized eggs were tested sequentially. Test principle and equipment could be found in the references (Wang *et al.*, 2014). Test instrument is shown in Fig. 1.

Egg weight and shell thickness measurement: Using the FA2004N electronic balance to measure non-fertilized and fertilized eggs weight; each egg was tested 3 times before the average taken. Two kinds of egg shell thicknesses were measured by using the ultrasonic ESTG-1 eggshell thickness detector. The blunt end of the egg was painted with coupling agents then the Eggshell thickness of marked points were measured, each egg was tested 3 times before taking the average.

Eggshell ultra structure: To better study the relationship between eggshell structure and O₂ flux, high-powered scanning electron microscope was used to observe the ultra structure of eggshell surface. Test steps are as follows: Crack the egg and a collect a piece of each section marked with the blunt tipped marker from each egg sequentially with tweezers, wipe with alcohol after using distilled water to clean up and dry out. Using a good experimental section fixed on the slide, using JEOL JFC-1600 ion sputtering apparatus for metal coating treatment about 20 min. Using JSM-6390LV scanning electron microscopy, under the mode of high vacuum, with LED probes in the case of 2000 and 5000 times to be amplified respectively, scan the sample eggshell surface microstructure and each slice got two pictures to save.

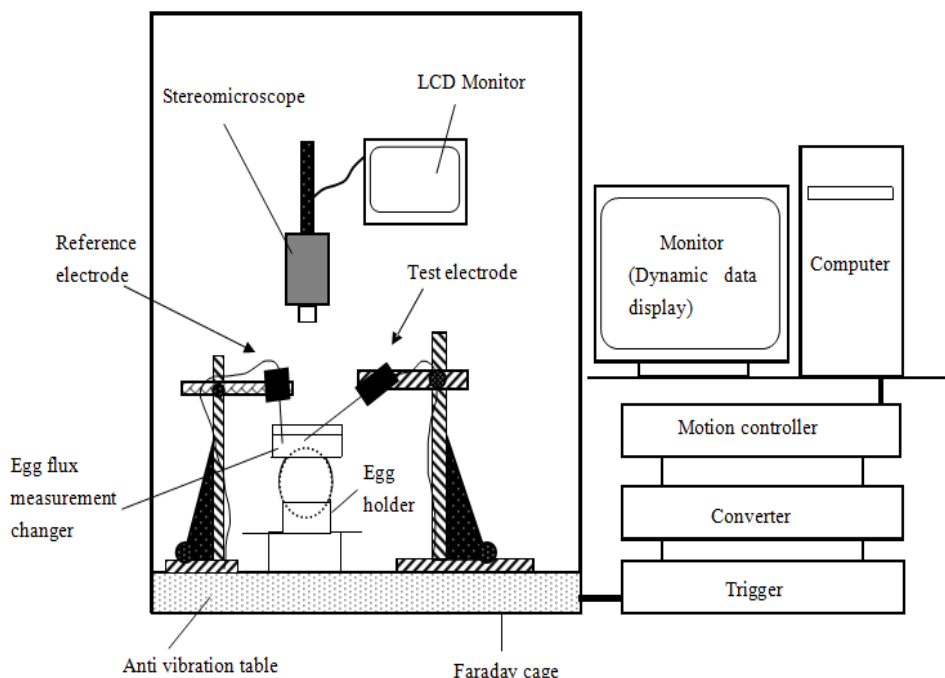
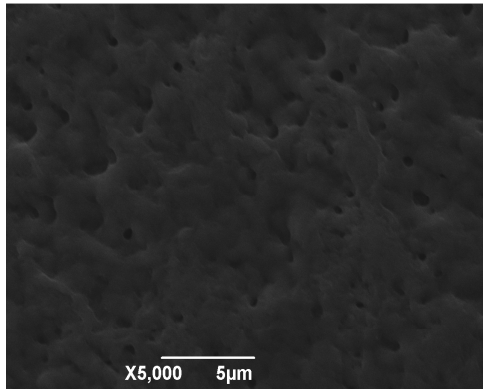
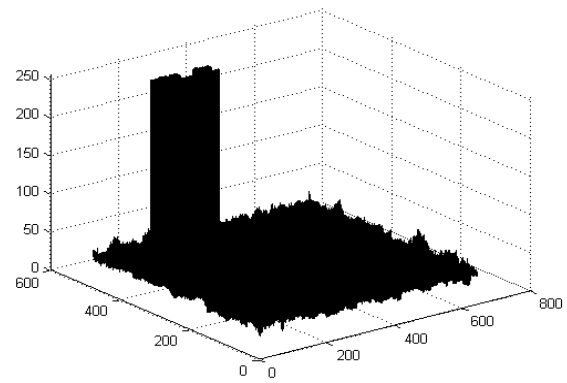


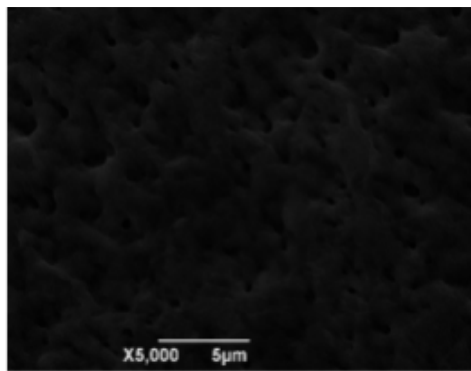
Fig. 1: The setup of NMT-ETS



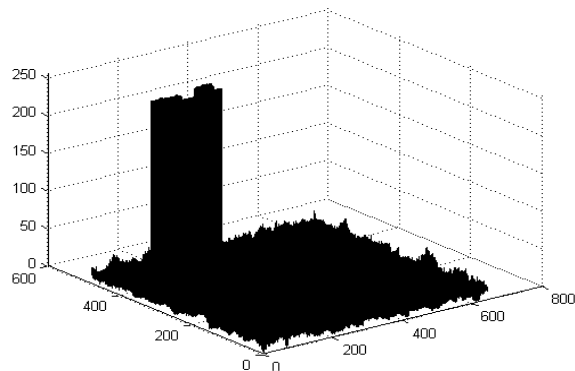
(a) Original image



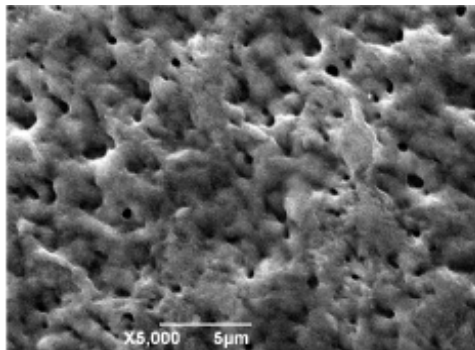
(b) Three dimensional visualization effect chart of original image



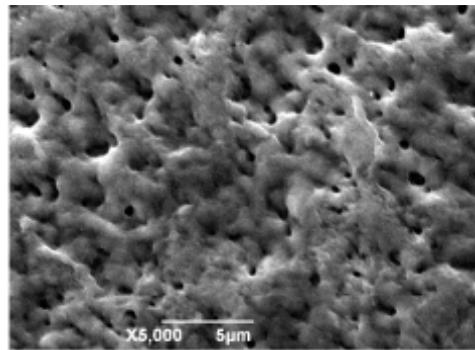
(c) Opening operation and top hat transformation



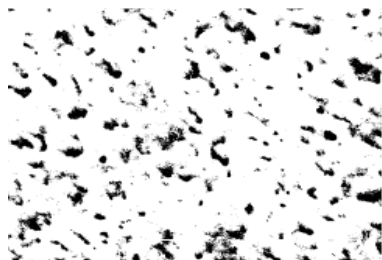
(d) Three dimensional visualization effect chart of image c



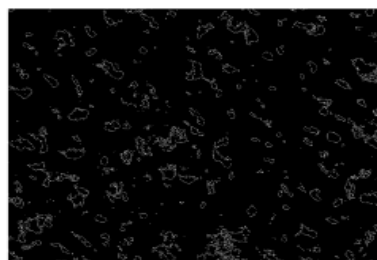
(e) Contrast stretch



(f) Median filtering



(g) Binary image



(h) Edge detection by sobel operator

Fig. 2: Image processing step

Table 1: Statistical analysis of test parameters

Measurement index		Fertilized eggs	Non-fertilized eggs
Weight/g	Max.	54.15	56.94
	Min.	47.79	49.52
	Average	51.56	52.58
	S.D.	1.97	2.93
Thickness/m	Max.	0.52	0.50
	Min.	0.46	0.43
	Average	0.49	0.47
	S.D.	0.02	0.02
O ₂ flux/pmol/cm ² /sec	Max.	45.03	30.51
	Min.	18.52	7.44
	Average	26.98	18.63
	S.D.	8.10	7.37
Hole density/mm ²	Max.	1.60E+05	1.14E+05
	Min.	6.30E+04	8.79E+03
	Average	1.11E+05	5.74E+04
	S.D.	3.38E+04	4.55E+04

The image processing of egg surface ultra structure:

Due to super micro images of shell surface the structure that was captured had many defects, such as noise influence, image blur, edge shadow and distortion caused by uneven illumination and brightness, which caused porosity identification to become difficult, also image contrast had different standards, therefore image processing needed to be conducted. The test was based on the MATLAB below image processing steps, signal as follows (Fig. 2).

After image processing such as opening operation, smoothing hole boundary, eliminating small spikes, top-hat transform, contrast stretch, transform and strengthen contrast, median filter, binary processing, original image of eggshell surface ultra structure is showed as Fig. 2g. Finally, whole edge was extracted by the sobel edge detection operator; closed circle was the shell surface porosity (h). Calculating the number of closed round hole, you knew it is the stomatal number N in picture area S, the egg whole density (P) is calculated as the Following:

$$P = \frac{N}{S} \tag{1}$$

Hole density: The number of stomata per unit area. Units: a/mm².

Data processing: Test statistics of egg weight, shell thickness, the oxygen flow rate and eggshell whole density are shown in Table 1.

RESULT ANALYSIS

O₂ flux discrepancy between fertilized and non-fertilized eggs: O₂ flux of fertilized eggs was significantly higher than non-fertilized eggs (p<0.05), shown in Fig. 3.

Surface ultra structure comparison between fertilized and non-fertilized eggs: Observing the ultra structural morphology of Egg surface by scanning electron microscopy (X2000), we know shape of pores of the eggshell surface, there were two: round holes (Fig. 3a) and crack-like flaws (Fig. 4b).

Analyzing the shell surface shape of non-fertilized eggs and fertilized eggs under scanning electron microscope (X5000) revealed there was a big difference in the two eggs after processing the images by MATLAB. Fertilized egg had a rougher surface, denser stomata, larger pore size and less crack, about 111,000/mm², only a small portion of the surface of fertilized eggs exhibited crack-like (only 12.5% of overall). The surface form of the non-fertilized eggs was relatively smooth, smaller pore size, the small pore density, about 57,400/mm² and 43.8% of non-fertilized eggs showed irregular surface crack-like (Fig. 5: fertilized eggs a b c. non-fertilized egg d e f). Pole density of fertilized egg was significantly greater than the non-fertilized eggs (p<0.05).

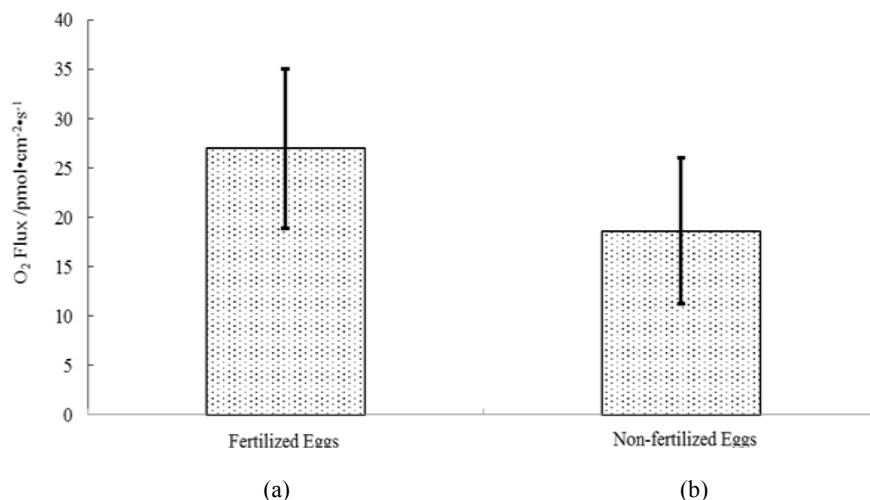


Fig. 3: O₂ flux comparison between fertilized and non-fertilized eggs

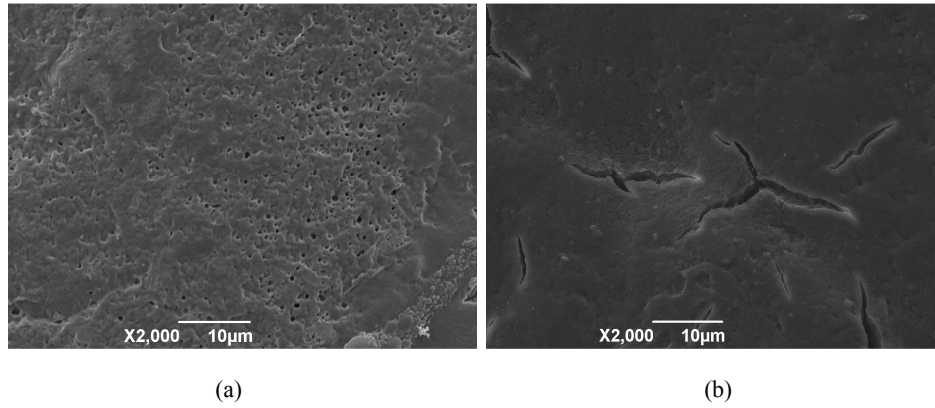


Fig. 4: Shape of pores (a) holes, (b) flaws

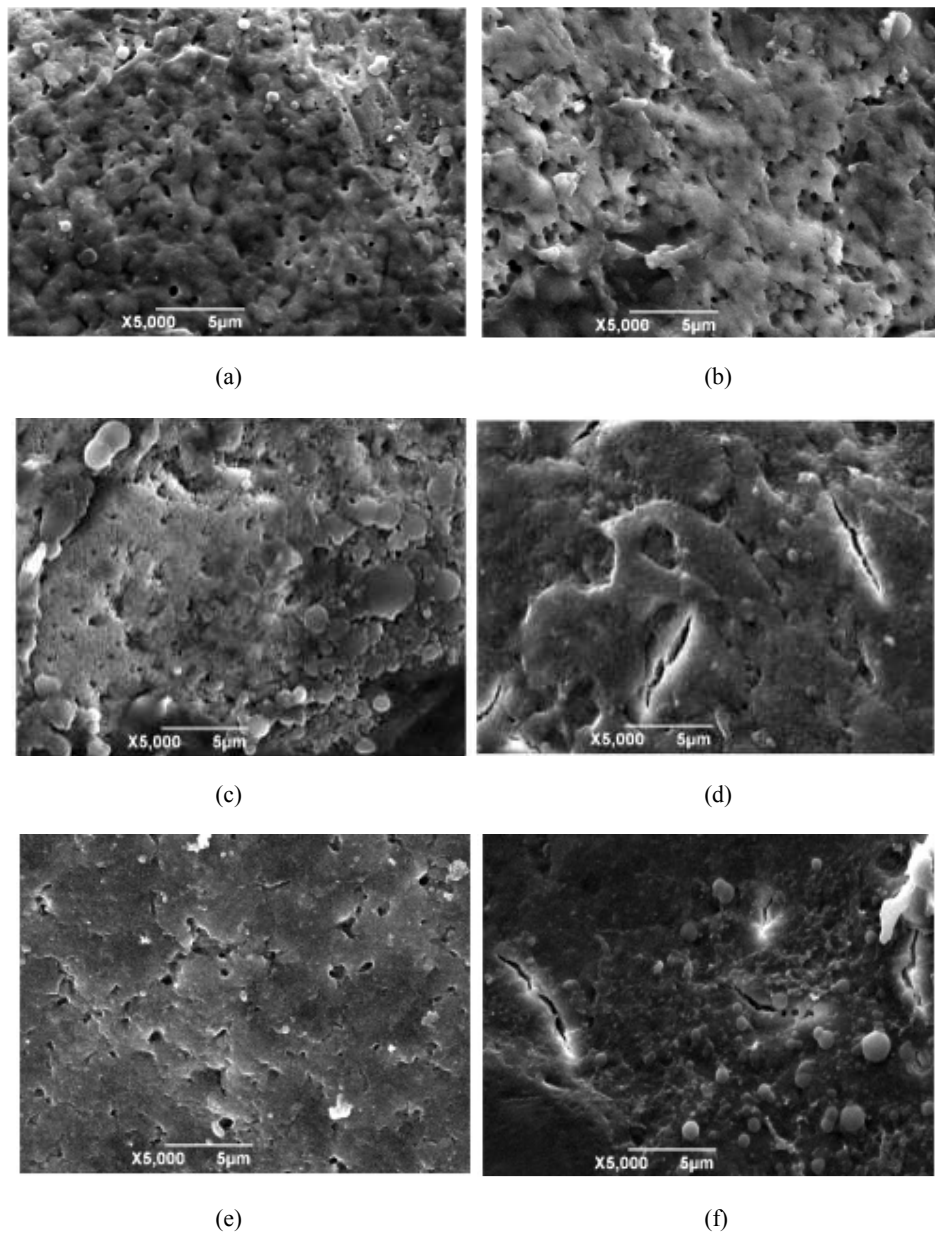


Fig. 5 Surface ultra structure comparison between fertilized and non-fertilized eggs

Relationship between O₂ flux and eggshell surface ultra structure: We found there are close relationship between the shell surface ultra-structure and its O₂ flow. By comparing the eggshell surface shape of non-fertilized eggs and fertilized eggs in Fig. 2, it can be seen that two egg shells were complex and there were different patterns of surface structure, in which this structure is closely related to respiration intensity. The rough surface in Fig. 4 showed that fertilized eggs crack

less, they have dense stomata. Stomata are helpful for its gas exchange with the outside world and it plays a vital role in enhancing the respiration intensity of fertilized eggs. However, the non-fertilized egg surface was relatively smooth, compact structure, stomatal distribution sparse, a small number, small stomatal density, more crack-like and low O₂ flux. Further research showed that: In all individuals (fertilized eggs and non-fertilized eggs), the more rough individuals

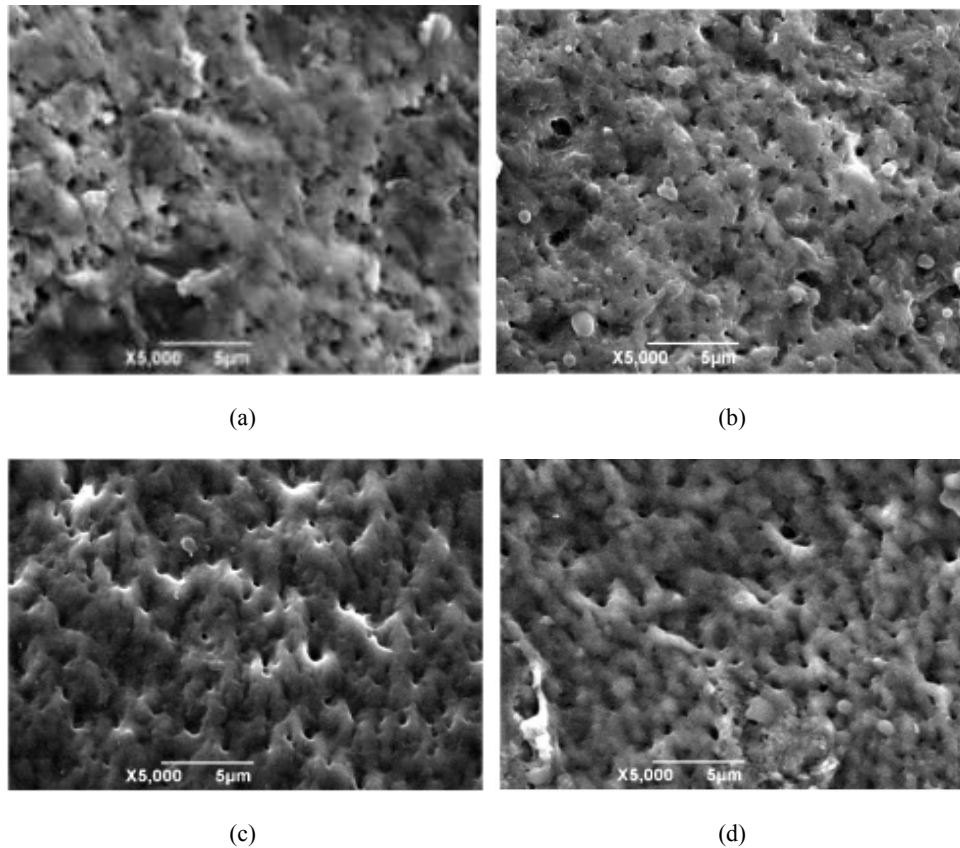
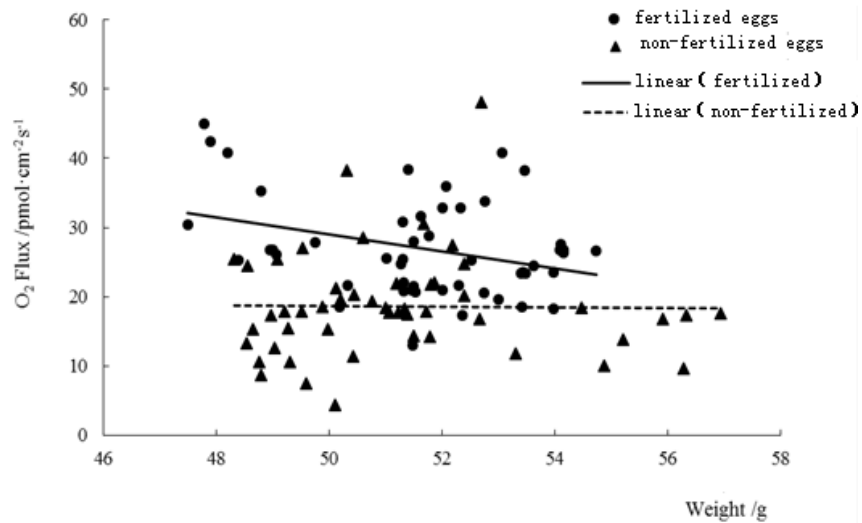


Fig. 6: Eggshell surface ultra structure with different respiration intensity



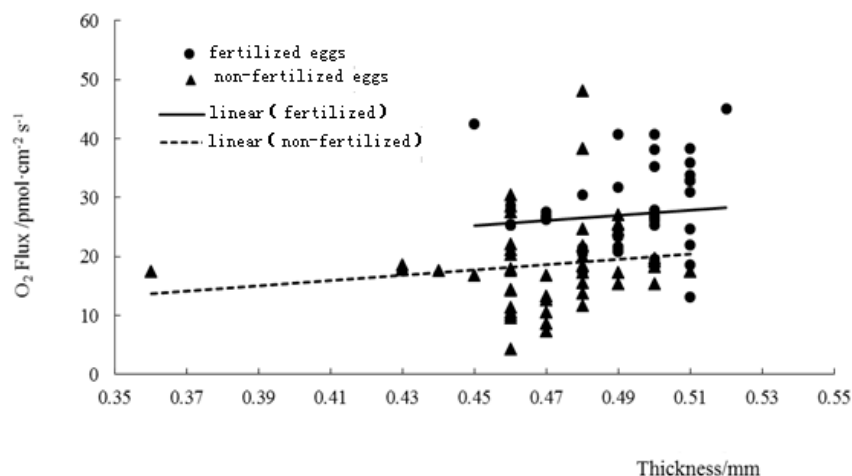


Fig. 7: Relationship between O₂ flux and weight, thickness

eggshell surface was, the greater O₂ flux was (Fig. 6, O₂ flux of a b c d was 45.03, 30.5, 18.52 and 13.07 pmol/cm²/sec, respectively).

Relationship between egg O₂ flux, weight and thickness: From Fig. 7 it can be seen there is no significant linear relationship between O₂ flow and shell thickness, egg weight.

DISCUSSION AND CONCLUSION

- O₂ exchange strength of egg is closely related to eggshell surface ultra structure. The greater the O₂ flow is, the rougher the surface, the larger hole density.
- Compared with non-fertilized eggs, fertilized eggs have a rougher surface, denser stomata, larger pore size and fewer cracks, thicker eggshell. The average O₂ flux of fertilized eggs is higher; it is consistent with conclusion 1).
- The linear relationship between egg O₂ respiration and shell thickness, egg weight is not obvious.

ACKNOWLEDGMENT

This study was supported by National Science Foundation of China (31371771), Corpus of Public Welfare Industry (agriculture) Scientific Research Projects (201303084 and National Science and Technology Support Programme (2015BAD19B05).

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