Research Note: Effect of age on hematological parameter and reference intervals for commercial Lohmann silver layer

Ding Hong,1,* Chang Liyun,1,* L. I. Fuwei,† Yue Qiaoxian,* Wang Dehe,* Zhou Rongyan,*,2 and Chen Hui*

*College of Animal Science and Technology, Hebei Agricultural University, Baoding 071000, China; and †Poultry Institute, Shandong Academy of Agricultural Sciences, Jinan 25000, China

ABSTRACT Hematological parameters play a key role in assessing the disease states and physiological status of birds, however the reliable information of hematological reference values for Lohmann silver in the pre- and post-laying periods is limited. Thus, the aim of this study was to research the effect of age on hematological parameters and provide reliable reference intervals for commercial Lohmann silver layer. Blood samples were obtained from 231 laying hens at different ages (5−37 wk) with 4-wk sampling intervals. A total of 12 hematological parameters including leucocytes (WBC, 10^9/L), red blood cell (RBC, 10^{12}/L), hemoglobin (HGB, g/L), hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/L), red cell distribution width (RDW, %), platelet count (PLT, 10^9/L), mean platelet volume (MPV, fL), platelet volume distribution width (PDW), plateletcrit (PCT, fL) were determined with a reference value advisor. One-ANOVA and Tukey test were utilized to test for significant differences of each parameter between ages. Our findings showed that these parameters were significantly affected by age, except for HCT in the pre-laying period. And reference intervals for all parameters except MCH, MCHC, MCV, MPV, and PDW in the pre-laying period were higher than that in the post-laying period. The results of this study may serve as indispensable information when diagnosing the health of Lohmann silver layer.

Key words: Lohmann silver layer, reference interval, hematological parameter

INTRODUCTION Nowadays, increasing attention has been paid to hematological characteristics in avian since hematological parameters play a key role in assessing the health as well as the physiological status of birds (Etim et al., 2014). The factors such as age, breed, sex, management systems, and season affect the value of hematological parameters (Addass et al., 2012; Irvboje et al., 2020). Although normal hematological values of some chickens have been determined based on different factors, there is little information about the reference values of hematology for Lohmann silver (Nanbol et al., 2016; Al-Nedawi, 2018). Lohmann silver is a predominately white feathering layer for production of uniform brown eggs with small egg size. Providing an accurate reference interval for Lohmann silver may facilitate the development of genetic selection if correlated with production parameters. Besides, providing reference intervals for selected hematological parameters may have some assistance in identifying disease states and general physiologic abnormalities. Recently, the reference value advisor was utilized to determine hematological and biochemical reference intervals for broilers (Al-Nedawi, 2018). Unfortunately, limited studies on reference hematology existed for commercial Lohmann silver layer in the pre- and post-laying periods. In this study, we investigated the effect of age on hematological parameters and provided reference values for Lohmann silver in the pre- and post-laying periods, and the hematology values in this paper may serve as crucial information when trying to diagnosis the health of Lohmann silver.

MATERIALS AND METHODS

Chickens and Procedures

In this study, a total of 231 commercial Lohmann silver laying hens were used for collection of blood samples, which included 80 pre-laying chickens at 5 to 17 wk and
151 post-laying chickens at 21 to 37 wk. Chickens at 5 to 9 wk were raised in solid hatching cages and they were transferred to 3-tiered cages with 3 birds in each cage after 9 wk. Chickens were fed pelleted before 6 wk and chickens at 6 to 15 wk were kept under common powder diet formulation and management systems used in the production company. A total of 231 commercial Lohmann silver layers were computed in Microsoft Excel 2013 using Reference Value Advisor V2.1. A statistical package (SPSS version 11.0 for Windows) was utilized to perform statistical ANOVA and Tukey test for each of the 12 applicable hematological parameters to test for significant differences between ages.

### RESULTS AND DISCUSSIONS

Descriptive statistics (mean, SD, minimum, maximum, 90% CI for lower and upper limit) and hematological reference intervals for commercial Lohmann silver layer during the pre-laying and post-laying periods are included in Table 1. Eighty samples were available in the pre-laying period and 151 samples were available in the post-laying period. Reference intervals for all parameters except MCH, MCHC, MCV, MPV, and PDW in the pre-laying period were higher than that in the post-laying period. The comparisons of blood hematological parameters among weeks during the pre-laying and post-laying periods are in Table 2. The hematology of Lohmann silver layers showed significant differences concerning age, except for HCT in the pre-laying period. HCT was within the range from 25 to 45%.

In the pre-laying period, there was a highly statistically significant increase among ages regarding the WBC, HGB, MCH, and MCHC. The increasing trend of WBC with the advancement of age was reported (Addass et al., 2012). However, a negative relationship between WBC and age was found (Onyishi et al., 2017). The increased value of HGB with age in the pre-laying period might because of the advancement in oxygen consumption for higher activity when the hens matured. HGB decreased close to the peak laying period and then rebounded. This could explain why the blood parameters related to oxygen transport are easy to change during laying period and the reproduction status influenced some hematological parameters. MCH is dependent on some hematological parameters. MCHC in our study differs from hand, the results of MCHC in our study differ from HGB decreased close to the peak laying period and then then rebounded. This could explain why the blood parameters related to oxygen transport are easy to change during laying period and the reproduction status influenced some hematological parameters. MCH is dependent on

### Hematological Testing

Blood (1 mL–3 mL) was collected from the brachial vein into a disposable 5 mL tube with anticoagulant heparin sodium. The hematological parameters were obtained with an automated hematology analyzer (mindray BC-2800Vet). Leucocytes (WBC, 109/L), red blood cell (RBC, 1012/L), hemoglobin (HGB, g/L), Hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/L), red cell distribution width (RDW, %), platelet count (PLT, 109/L) and the following platelet parameters: mean platelet volume (MPV, fL), platelet volume distribution width (PDW), plateletcrit (PCT, fL) were determined in this study.

This study was carried out in keeping with the institutional and national guidelines and were supported by the Animal Use and Ethics Committee of the Agricultural University of Hebei (University Identification Number: HB/2019/03).

### Statistical Analysis

Descriptive statistics consisting of sample size, mean, median, standard deviation (SD), minimum, and maximum values, confidence intervals (CI), and
| Age at wk | 5 | 9 | 13 | 17 | 21 | 29 | 33 | 37 |
|----------|---|---|----|----|----|----|----|----|
| WBC 10^9/L | 2.66 | 2.74 | 2.83 | 2.83 | 2.83 | 2.95 | 2.95 | 3.05 |
| RBC 10^12/L | 21.93 | 21.93 | 21.93 | 21.93 | 21.93 | 21.93 | 21.93 | 21.93 |
| HGB g/L | 131.70 | 131.70 | 131.70 | 131.70 | 131.70 | 131.70 | 131.70 | 131.70 |
| HCT % | 33.84 | 33.84 | 33.84 | 33.84 | 33.84 | 33.84 | 33.84 | 33.84 |
| MCV fL | 127.60 | 127.60 | 127.60 | 127.60 | 127.60 | 127.60 | 127.60 | 127.60 |
| MCH pg | 49.57 | 49.57 | 49.57 | 49.57 | 49.57 | 49.57 | 49.57 | 49.57 |
| MCHC g/L | 388.70 | 388.70 | 388.70 | 388.70 | 388.70 | 388.70 | 388.70 | 388.70 |
| RDW % | 7.96 | 7.96 | 7.96 | 7.96 | 7.96 | 7.96 | 7.96 | 7.96 |
| PLT 10^9/L | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 |
| MPV fL | 6.34 | 6.34 | 6.34 | 6.34 | 6.34 | 6.34 | 6.34 | 6.34 |
| PDW | 19.45 | 19.45 | 19.45 | 19.45 | 19.45 | 19.45 | 19.45 | 19.45 |
| PCT % | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |

Onyishi et al. (2017) who reported that age did not affect MCHC.

A significant increase was recorded between 5 wk and 13 wk in RBC. Addass et al. (2012) found that there were lower RBC values at a younger age, which was also demonstrated in our study. Adversely, these data differ from that obtained by Gryzinska et al. (2013) who found that the level of RBC was higher at a younger age. In the post-laying period, our findings suggest that erythrocyte values increased near the peak of egg production compared with that at the beginning of laying, and then the values decreased. MCV refers to the average volume of one red blood cell. The decreased values of MCV whether in the pre- or the post-laying period shown in this study agree with Rasheed and Olusegun (2017). This might be as a result of increased values of RBC. Besides the increased level of RDW in the pre-laying period is also similar to that study as above described, although an inverse trend of RDW existed in the post-laying period. The erythrocytic values are within the scope of the previous study (Talebi et al., 2005).

Although the function of thrombocyte in bird is not completely explained, the hemostatic effect is clear (Jones, 2015). Therefore, an in-depth understanding of the changes in blood parameters related to the coagulation system can help to better explain the physiological or pathological conditions of laying hens. In the pre-laying period, PLT and PCT presented an increasing trend, which is also confirmed by Gryzinska et al. (2013). PDW reflects the dispersion of platelet volume size, and reduced PDW indicates high platelet homogeneity. Unlike PLT and PCT, PDW decreased with the age of hens during the rearing period. The results of this study indicate that the homogeneity of platelets in hens is high. The range of values associated with blood clottings such as PLT, PCT, MPV, and PDW in this study is similar to the previous report (Gryzinska et al., 2013), whereas the values of PDW was higher than that obtained in this study.

In conclusion, ages affect hematological parameters, both in the pre- and post-laying periods. Moreover, reference values based on the factor of age in this study are likely to provide key information to assess the health status and to diagnosis the disease of Lohmann silver. Additional studies are needed to confirm whether these reference intervals are influenced by other underlying factors.

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**DISCLOSURES**

The authors declare no competing financial interest.
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