Research Note: Heterosis for egg production and oviposition pattern in reciprocal crossbreeds of indigenous and elite laying chickens

Yuanmei Wang, Yanyan Sun, Aixin Ni, Yunlei Li, Jingwei Yuan, Hui Ma, Panlin Wang, Lei Shi, Yunhe Zong, Jimmeng Zhao, Shixiong Bian, and Jilan Chen

Key Laboratory of Animal (Poultry) Genetics Breeding and Reproduction, Ministry of Agriculture and Rural Affairs, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China

ABSTRACT Heterosis has been widely utilized in chicken breeding to improve economically important traits. However, few studies focused on revealing the factors contributing to egg production heterosis. In this study, White Leghorn and Beijing-You chickens were used as parental breeds to generate purebreds (WW, YY) and reciprocal crossbreeds (WY, YW) to characterize heterosis for egg production traits including age at first egg (AFE), clutch traits, oviposition pattern, and egg quality traits. Results showed that egg number until 35 wk of age (EN35) was higher in crossbreeds than the average of purebreds (P < 0.05) and exhibited positive heterosis of 4.03% and 2.84% in WY and YY respectively. Crossbreeds commenced laying earlier than the average of purebreds (P < 0.05) with negative heterosis of −1.24% and −0.92% for WY and YY respectively. Moreover, EN35 had negative correlation with AFE (r = −0.85) and positive correlation with average clutch length (ACL) (r = 0.48) and maximum clutch length (MCL) (r = 0.66). However, negative heterosis for ACL (−19.62%, −16.51%) and MCL (−22.88%, −18.97%) were obtained in WY and YY, respectively. This may be due to the positive heterosis for number of pauses, which was highly correlated with ACL (r = −0.68) and MCL (r = −0.74). The crossbreeding improved the oviposition pattern. Percent egg laying that occurs between 7:00 and 14:00 was 91.50% (WW), 68.28% (YY), 76.87% (WY), and 79.68% (YW) in the experimental populations. On the other hand, oviposition interval (OI) had negative heterosis in crossbreeds and was negatively correlated with EN35 (r = −0.60). Positive heterosis for egg weight of 2.63% and 3.94% and yolk weight of 4.74% and 6.07% were observed in WY and YY, respectively. Taken together, egg production related traits did not contribute equally to EN heterosis. The AFE and OI exhibited significant correlation with EN indicating that they would be important drivers for EN heterosis.

Key words: heterosis, egg number, oviposition pattern, clutch traits, egg quality

INTRODUCTION

Heterosis or hybrid vigor is a biological phenomenon in which hybrid progeny exhibit superior growth, fertility or performance to the average of the parental lines. Crossbreeding improves egg production in indigenous chickens by the non-additive genetic effects (Hristakieva et al., 2014). Egg number (EN) in chickens is the overall reflection of the hen’s reproductive potential. However, egg laying is a complex process, in which age at first egg (AFE), oviposition time, oviposition interval, and clutch length are inter-related to each other in an intricate way. The AFE indicates the sexual maturity and different strains vary in optimal ages at which they reach sexual maturity to produce maximum egg mass (Das et al., 2016). In addition, egg production has been genetically positively correlated with average clutch length (ACL) and maximum clutch length (MCL) (Akil and Zakaria, 2015). Moreover, individual oviposition pattern exerts variation related with egg production (Roy et al., 2014). Previous studies proposed AFE, ACL, and MCL as alternative selection criterion for egg production improvement in purebreds. However, heterosis for these egg production traits in crossbreeding Beijing-You and White Leghorn chickens have not been characterized.

White Leghorn (WW) is a purebred layer breed and widely used in the production of commercial layer hybrid strains. Beijing-You (YY) is a Chinese indigenous breed of dual purpose chicken, which produced much less egg mass compared to the White Leghorn. In the current study, these two distinct chicken breeds were used as parents to generate purebreds and reciprocal crossbreeds.
The EN, clutch traits, egg laying pattern, and egg quality of purebreds and crossbreeds were evaluated, aiming at illustrating the heterosis of these traits and their respective contribution to heterosis for EN.

**MATERIALS AND METHODS**

**Experimental Populations**

The White Leghorn and Beijing-You chickens from the experimental farm of IAS-CAAS were used as parent breeds to generate purebreds (WW and YY) and reciprocal crossbreeds: WW × YY (WY) and YY × WW (YW). In total, 30 cocks and 150 hens each from WW and YY chickens were selected based on the following criteria: 1) cocks in each line with similar BW and good semen quality and 2) hens in each line with similar BW and actively laying until 43 wk of age. Semen from each genetic groups was collected and incubated in the same incubator. At hatching, female chicks were identified by vent sexing. Healthy ones were selected and wing-banded.

**Animal Management**

Each bird was vaccinated and reared following the standard brooding procedures from 1 to 8 wk of age. From 9 to 18 wk of age, 5 chickens from the same genetic group were stocked per rearing cage and reared in the same pen. From 19 wk of age till end of the experiment, 216, 290, 242, and 307 hens in YW, WW, WY, and YY genetic groups were kept in individual cages for proper phenotype recording. The birds were fed diet containing 16% CP, 2,800 kcal/kg ME, 2% Ca, and 0.32% nonphytate P before start of lay. During the laying period, diet containing 16.5% CP, 2,700 kcal/kg ME, 3.5% Ca, and 0.32% nonphytate P was offered ad libitum. The lighting program consisted of a systematic reduction of light from 24 h at day-old to 10 h at 8 wk of age. From 8 to 20 wk of age, light was supplied for 9 h. From 21 to 29 wk of age, lighting period was increased incrementally by one hour weekly. Since 30 wk of age, 16 h constant lighting from 06:00 to 22:00 was maintained.

**Egg Production Recording**

Individual egg production was recorded using RFID card. The average EN up to 35 wk of age was calculated. Clutch length was calculated as the number of eggs laid on successive days (Akil and Zakaria, 2015). ACL and MCL were the average and maximum clutch length of each bird respectively.

**Oviposition Pattern**

During the laying peak, birds were monitored for oviposition time which was recorded manually every 30 min between 7:00 and 19:00 for 21 consecutive days. Accordingly, the distribution of oviposition time in the four genetic groups was calculated. The eggs laid between 19:00 and 07:00 were recorded as a separate category.

**Egg Quality Evaluation**

At 34 wk of age, three eggs consecutively laid by each individual hen were collected and subjected to egg quality evaluation. Egg weight, yolk weight, and shell weight were measured using a digital scale (Huachaoice Electrical Appliances, Shanghai, China). Eggshell thickness and uniformity of calcification on the external surface was measured at the acute, middle, and obtuse poles of each egg using the Eggshell Thickness Gauge (Orka Food Technology Ltd., Herzliya, Israel). Eggshell strength was measured at the obtuse pole of each egg using the Egg Force Reader (Orka Food Technology Ltd., Herzliya, Israel). Eggshell ratio was calculated as the percentage of shell weight from the egg weight. Eggshell color was evaluated by the QCR (TSS, England, Britain), which detected the degree of light reflected from the eggshell and measured the darkness of shell color, with darker shell having a lower score.

**Statistical Analysis**

Percent heterosis (H%) of the traits was calculated according to the following equation:

\[ H\% = \frac{F_1 - (P_M + P_F)/2}{(P_M + P_F)/2} \times 100\% \]

Where \( F_1 \), \( P_M \), and \( P_F \) are mean phenotype of crossbreeds, maternal, and paternal lines, respectively. In addition, Student’s t-value was used to calculate the significance of H% based on the formula of Wu and Zhang (1983):

\[ t = \frac{H\%}{\sqrt{\frac{\sum (F_i - F_{1i})^2}{N-1}/[(P_M + P_F) \times \sqrt{N}]}}, \]

where \( F_{1i} \) is the phenotype of individual i in crossbreeds; N is the number of crossbred chickens. H% was considered significant when \( P < 0.05 \).

Oviposition time of each individual in four groups was plotted to illustrate their distribution pattern using Microsoft office excel 2006. Mean value was compared by Student-Newman-Keuls multiple-range tests when significant difference was detected. Significance was designated as \( P < 0.05 \). All values were presented as mean ± standard deviation (SD).

**RESULTS AND DISCUSSION**

**Egg Production**

EN up to 35 wk of age of purebreds and reciprocal cross-breeds was presented in Table 1. Significant differences were observed for EN between purebreds and crossbreeds (\( P < 0.05 \)) and positive heterosis for EN was observed in reciprocal crossbreeds. These results corroborated the study of Amin (2014), who reported heterosis of 5.26% for EN in
crossbred chickens generated by mating White Leghorn dams to Fayoumi (an Egyptian local chicken breed). Admittedly, EN is a complex metric trait affected by AFE and ACL. Attaining sexual maturity at an early age was reported to increase total egg production as long as the development of body frame and reproduction organs was not compromised (Das et al., 2016). It was documented that hybrids attained sexual maturity earlier than the average of purebreds (Sutherland et al., 2018). Here, WW and reciprocal crossbreds commenced laying eggs 21 and 8 d earlier than YY, respectively. Negative heterosis was observed for AFE in crossbreds, consistent with our previous study (Isa et al., 2020). Additionally, AFE exhibited highly negative correlation with EN ($r = -0.85$). These results implied that AFE may be a critical trait contributing to heterosis for EN. The chickens’ reproductive system is regulated by an intricate interplay between hypothalamus, pituitary, and gonads. The light signal could be sensed by hypothalamus and retina, and then affects reproduction activities by regulating the secretion of GnRH, FSH, and LH (Bédécarrats et al., 2016). Moreover, detectors of photoperiod are critical in vertebrates, particularly for timing the onset of reproduction. The crossbreeding may alter AFE of reciprocal crossbreds by improving light perception (Wang et al., 2022).

Compared with YY, superior performance in EN and larger ACL were observed in WW. The ACL and MCL in crossbreds were larger than that of YY. However, significant negative heterosis was observed. This may be due to the positive heterosis of number of pauses, which exhibited high correlation with ACL ($r = -0.68$) and MCL ($r = -0.74$). This result indicated that selection for clutch traits in purebreds would be more effective for egg production improvement than crossbreeding.

**The Egg Quality Traits**

As shown in Table 1, significant difference between purebreds was observed for all measured egg quality traits except for eggshell thickness ($P < 0.05$). Egg weight, yolk weight, eggshell weight, shell ratio, eggshell thickness, eggshell strength, and eggshell color differed between reciprocal crossbreds ($P < 0.05$). The yolk weight and shell thickness in reciprocal crossbreds were higher than purebreds ($P < 0.05$). Positive heterosis was found for all measured egg quality traits, which was below 5% for egg weight, eggshell weight, yolk ratio, shell ratio, and shell thickness. The BW exhibited positive heterosis in reciprocal crossbreds, which may be important factor driving the formation of heterosis for egg weight. Therefore, the crossbreeding between indigenous and elite layer lines results in overall egg qualities.

**Oviposition Pattern**

Egg production in a flock is determined by the individual laying patterns. About 91.50% of WW birds laid eggs between 7:00 and 14:00; the crossbreds laid eggs earlier than YY in a way that 68.28% of YY, 76.87% of WW, and 79.68 of %YW hens laid in this timeslot. To compare the oviposition time of different population, the individuals in purebreds and crossbreds were further divided into four groups: the first group with EN 18 to 20, the second group 15 to 17, the third group 11 to 14, and the fourth group <10 (Figure 1). Notably, WW hens in the first group commonly laid eggs in a very limited timeslot and relatively stable across the monitored 21-d period. In agreement with our study, Roy et al. (2014) reported mean oviposition time in WW to be 5 hours after the onset of light period. Many hens with similar pattern of oviposition were also found in reciprocal crossbreds, but very few in YY. Moreover, in one clutch, the first oviposition generally occurred in the morning, but the last oviposition tended to occur in the early evening in the four genotypes. Specially, the oviposition time of most chickens in YY varied greatly, and was delayed gradually until the last day within one
clutch. However, delay between successive oviposition within one clutch was shorter in reciprocal crossbreeds. It is believed that chickens generally produce a clutch when the time of ovulation is delayed by more than eight or ten hours from the first ovulation within a clutch. Taken together, the low laying rate in YY may be due to shorter sequences (Akil and Zakaria, 2015). Heterosis for EN in reciprocal crossbred hens may be driven by consistency in oviposition time.

In addition, we calculated the oviposition interval of each chicken in crossbreeds and purebreds. The oviposition interval in WW was the shortest, similar to 23.4 h reported by a previous study (Roy et al., 2014). The oviposition interval in reciprocal crossbreeds was more than 25 h and exhibited negative heterosis (Table 1). Moreover, the oviposition interval exhibited strong correlation with EN ($r = -0.60$). It is therefore speculated that crossbreeding may be an effective way to improve EN in YY through shortening the oviposition interval. It is believed that follicle maturation and ovulatory cycling are partially controlled by a circadian rhythm. Hence, it was inferred that the circadian rhythm of reciprocal crossbreeds could drive the formation of oviposition interval heterosis. To our knowledge, this is the

Figure 1. Heatmap of oviposition time of each chicken via hierarchical cluster analysis. Different rows correspond to different individuals. The red and blue strips represent early and late laying time. The 0 indicates a pause day. Abbreviations: WW, purebred offspring of White Leghorns; WY, offspring of White Leghorn sires crossed with Beijing-You chicken dams; YW, offspring of Beijing-You chicken sires crossed with White Leghorn dams; YY, purebred offspring of Beijing-You chickens.
first study that characterizes the oviposition interval of purebreds and crossbreeds, while the molecular mechanism still needs further exploration.

In summary, this study characterized the heterosis for EN, AFE, oviposition pattern, ACL, MCL, number of pauses in reciprocal crossbreeds between indigenous Beijing-You and elite White Leghorn chickens, and found that AFE and oviposition interval may be important drivers for heterosis in EN.

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DISCLOSURES

The authors have no conflicts of interest to report.

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