Relationship Between Egg Productivity and Insulin-Like Growth Factor-I Genotypes in Korean Native Ogol Chickens

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ABSTRACT

Endocrine factors, such as steroid hormones and growth factors, regulate egg productivity in terms of the quantity of egg production, egg weight, sexual maturity, and the number of small yellow follicles (SYF). Insulin-like growth factors (IGF) are involved in the regulation of ovulation rate and ovarian follicular development in chickens, and a relationship between IGF-I genotype and egg weight has been reported. However, the effect of IGF on egg productivity in Korean Native Ogol chickens (KNOC) has been little studied. Therefore, this study was conducted to identify the relationship among endocrine factors (IGF-I, IGF-II, estradiol, and progesterone), IGF-I genotypes, and egg productivity. Frequencies of IGF-I genotypes (AA, AB, BB) were 17.3, 26.9, and 55.8%, respectively, within a population. When compared with the IGF-I genotypes, the AB genotype had the highest serum levels of estradiol and progesterone at 40 and 30 wk of age, respectively; the highest IGF-II concentration in F1 follicles at 60 wk; and was positively associated with the number of SYF at 60 wk. The results showed that the A allele was associated with a higher IGF-II expression in the follicles and stimulated the development of follicles, indicating a positive association of the A allele with egg production and the number of SYF. Therefore, these results suggest that there is a possibility of IGF-I genotypes acting as a genetic marker for egg productivity of KNOC.

(Key words: egg productivity, insulin-like growth factor, insulin-like growth factor-I genotype, steroid hormone, Korean Native Ogol chicken)

INTRODUCTION

Egg productivity is the most important economic trait in laying hens. It involves egg production, egg weight, sexual maturity, and the number of small yellow follicles (SYF), which are regulated by endocrine factors (Johnson and van Tienhoven, 1984; Su et al., 1996). Recently, the effects of insulin-like growth factor (IGF-I) on egg production of laying hens have been studied by McMurtry et al. (1997). In addition, IGF-I has been evaluated in DNA synthesis of granulosa cells (Roberts et al., 1994). IGF-I reportedly regulates follicular growth and differentiation (Onagbesan et al., 1999) and stimulates ovarian progesterone production (Williams et al., 1994). IGF-II also participates in the processes occurring in the theca-interstitial androgen-producing compartment (Giudice, 1992), and 2 variant forms of chicken IGF-II with different amino acid sequences in the second exon of chicken IGF-II would be a major determinant of follicular fate (Armstrong and Hogg, 1996). These reports imply the involvement of IGF along with steroids in the regulation of egg productivity in the chicken.

Analyses of genetic markers in animals could lead to understanding of the genetic architecture of quantitative traits. Recently, IGF-I genotypes were reportedly associated with egg weight (Nagaraja et al., 2000). However, the relationship between IGF-I genotypes and growth factor expression has not been established. Specifically, the effect of growth factors on egg productivity of Korean Native Ogol chickens (KNOC) with different IGF-I genotypes has yet to be studied. Furthermore, KNOC is a dual-purpose (egg and meat) chicken with low egg productivity. Therefore, this study was performed to elucidate the association among IGF-I genotypes, endocrine factors, and egg productivity and to estimate the possibility of selection for improvement of egg productivity by IGF-I genotype in KNOC.

MATERIALS AND METHODS

Birds

Female KNOC (n = 104) were raised at the Korea University Animal Breeding center. The KNOC were vacci-

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TABLE 1. Distribution of IGF-I genotypes and gene frequency determined by PCR-RFLP

<table>
<thead>
<tr>
<th>Chickens (n)</th>
<th>Genotype</th>
<th>Gene frequency</th>
<th>Predicted genotype frequency</th>
</tr>
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<tbody>
<tr>
<td>KNOC²</td>
<td>AA</td>
<td>18 (17.3%)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>28 (26.9%)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>58 (55.8%)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

¹p = frequency of A gene; q = frequency of B gene.
²Korean Native Ogol chicken.

DNA Extraction

The KNOC genomic DNA was extracted from the clotted blood (Seo et al., 1999). In brief, 250 µL of lysis solution (360 µg/mL proteinase K, 150 mM sodium chloride, 50 mM EDTA, and 2% SDS) was mixed to lyse the clotted blood, and the mixture was incubated at 55°C for 3 h. After 5.5 M NaCl and 600 µL of phenol:chloroform (25:24) were added, the mixture was centrifuged for 10 min at 5,000 × g. The supernatant was mixed with absolute ethanol, and the mixture was centrifuged again under the same conditions. The pellet was dried, resuspended with TE solution (10 mM Tris-Cl and 1 mM EDTA), and stored at −20°C.

PCR-RFLP

To elucidate an association between genetic variants and each trait, IGF-I genotypes were analyzed using PCR-RFLP. Primers of IGF-I gene were prepared according to the report by Nagaraja et al. (2000) (forward 5'-GACTATTACAGAAAAGACCCAC-3'; reverse 5'-TATCCTGACTGGCTCAAGT-3'). The PCR reaction was performed with AccuPower Premix-Top.² PCR amplification was carried out for 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 90 s in a GeneAmp PCR System 2400.³ The PCR products were digested overnight at 37°C with PstI and analyzed by electrophoresis on a 10% polyacrylamide gel.

Protein Extraction from Ovaries and the Granulosa Layer from Follicles

The KNOC were killed by cervical dislocation, and the first (F1), second (F2), and third (F3) follicles were removed from the ovary at 60 wk. The vascular and connective tissues surrounding the follicles were removed with forceps, and an incision was made in the follicle to allow most of the yolk to flow out. The granulosa layer was carefully separated from the theca layer as described by Roberts et al. (1994). The ovaries and granulosa layers were washed 3 times in ice-cold Ringer solution (125 mM NaCl, 1 mM CaCl₂, 2H₂O, 5 mM KCl; pH 7.5) to remove any adhering yolk. These samples were homogenized in prechilled RIPA buffer (150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris; pH 8.0) containing protease inhibitors [100 mM phenylmethylsulfonyl fluoride (PMSF), 1.0 mg/mL aprotinin] and incubated at 4°C for 30 min. The samples were centrifuged at 10,000 × g in a prechilled centrifuge at 4°C for 15 min. The supernatant from the ovary lysate was collected, and the protein concentration of the supernatant was measured using an RIA kit. The supernatants were stored at −70°C for IGF-I/-II RIA.

RIA

Serum and tissue concentrations of IGF-I were determined by a heterologous RIA (Furlanetto et al., 1977), which has been validated for chicken plasma (Huybrechts et al., 1985). The concentration of IGF-II was determined and validated as previously described (Buonomo et al., 1988; Buonomo, 1989; Beccavin et al., 1999). The recombinant chicken IGF-I/-II were iodinated by the chloramine-T method (Lee and Henricks, 1990). Ovarian, follicular, and serum IGF-binding proteins were removed using an acid-ethanol method (Daughaday et al., 1980). The assay used rabbit anti-human IGF-I polyclonal antiserum and mouse IGF-II monoclonal antiserum (final dilution of 1:10,000) and anti-mouse IGF-II monoclonal antiserum (final dilution of 1:4,000). The intra- and interassay variations of IGF-I were 8.1 and 13.4%, respectively, and of IGF-II were 7.8 and 14.7%, respectively. Sera estradiol (E₂) and progesterone (P₄) concentrations were measured using an RIA kit.

²BIONEER Co., Seoul, Korea.
³Perkin Elmer Co., Boston.
⁴GroPep, Pty., Ltd., North Adelaide, Australia.
⁵Upstate Biotech., Lake Placid, NY.
⁶Coat-A-Count, DPC, Los Angeles.
Statistical Analysis

The quantity of egg production, egg weight, tissue weight, number of SYF, and IGF-I/-II, E2, and P4 concentrations from the 3 groups were statistically analyzed using the Duncan method of the one-way ANOVA procedure of the SAS software (1995). For each group, the mean and SE for egg productivity, endocrine factors, and follicle weight were calculated. Significance was determined at \( P < 0.05 \).

RESULTS

Analysis of the IGF-I PCR-RFLP in KNOC

For the comparison of egg productivity and IGF-I genotypes in the KNOC, IGF-I genotypes first were analyzed with PCR-RFLP using primers as previously reported (Nagaraja et al., 2000). The PCR-amplified products of the IGF-I gene from 104 hens were identified at 621 bp in 10% polyacrylamide gel. IGF-I genotypes (AA, AB, and BB) were present in 17.3% (18 each), 26.9% (28 each), and 55.8% (58 each), respectively, of the chickens (Figure 1, Table 1).

Association of the IGF-I Genotype with Economic Traits

Figure 2 shows the association between IGF-I genotypes and egg production. Egg production of the AA genotype was greater than that of the BB genotype at 50 wk (\( P < 0.05 \)). Figure 3 shows the association between IGF-I genotypes and egg weight. Egg weight for the AA genotype was not significantly lower at any age than for the other genotypes.

Expression Pattern of IGF by IGF-I Genotype

The least square means of serum IGF-I concentration for the IGF-I genotypes are shown in Figure 4. The serum IGF-I level fluctuated in each group, showing a peak at 40 wk (34.02 ± 0.96 ng/mL, \( P < 0.05 \)) and then decreased (25.32 ± 0.93 to 25.46 ± 0.93 ng/mL). But, a significant difference was not detected among the 3 groups. Figure 5 shows the association between IGF-I genotypes and serum IGF-II concentration. Serum IGF-II concentration peaked at 20 wk (47.59 ± 1.43 ng/mL, \( P < 0.05 \)) and then decreased (35.01 ± 1.33 to 31.58 ± 1.32 ng/mL). However, differences in serum IGF-II concentrations were not detected among the 3 groups.

Expression Pattern of Steroid Hormones by IGF-I Genotype

The least square means of serum E2 concentration for the IGF-I genotypes are shown in Figure 6. Serum E2 concentration increased rapidly after 10 wk (21.16 ± 8.55 to 111.65 ± 7.29, \( P < 0.05 \)). Serum E2 of the AB genotype was more highly expressed than that of the AA genotype.
FIGURE 4. Changes in serum insulin-like growth factor-I (IGF-I) concentration according to IGF-I genotype. All values are expressed as means ± SE. Serum P₄ concentration was rapidly increased after 30 wk and peaked at 40 wk (0.379 ± 0.021 ng/mL, \( P < 0.05 \)) and then decreased (0.220 ± 0.021 to 0.093 ± 0.022 ng/mL, Figure 7). The P₄ expression of the AB genotype was higher than those of the BB genotype at 30 wk (\( P < 0.05 \)).

Expression Pattern of IGF in Reproductive Tissue by IGF-I Genotype

Figure 8 shows the ovarian and follicular IGF-I expressions at 60 wk by IGF-I genotypes. Depending on the follicle class, a different pattern of IGF-I expression was detected with higher IGF-I expression being shown by F1 (8.36 ± 0.499 ng/mg, \( P < 0.05 \)) than that of the other follicles and ovary (6.99 ± 0.432 to 3.66 ± 0.312 ng/mg). This finding suggests that follicular IGF-I regulates the development of follicles, although a significant difference was not detected among the 3 groups. However, follicular IGF-II expressions in F1 and F2 were not different (Figure 9). The AB genotype showed higher IGF-II expression than the BB genotype in F1 (\( P < 0.05 \)), but differences among genotypes were not detected in F2, F3, and the ovary. Profiles of follicle weight, ovary weight, and SYF numbers among the 3 genotypes by IGF-I genotypes are summarized in Table 2. The ovary and follicle weights did not differ among the 3 groups. The number of SYF of AB genotype was higher than that of the AA genotype (\( P < 0.05 \)).

DISCUSSION

The present study elucidated the relationships among IGF-I genotypes, endocrine factors, and egg productivity.
The number of eggs laid by a hen is determined by the number of follicles selected for ovulation and by the capacity of the oviduct. Bluhm et al. (1983) reported that cessation of egg laying induced by stress is associated with low circulating levels of P4 and E2 in Mallard ducks. Low levels of circulating P4 have also been reported for immature and molting chicken hens (Johnson and van Tienhoven, 1981). These reports indicated that E2 and P4 regulate follicular development and have a positive association with egg production. Furthermore, reports have shown that chicken granulosa and thecal cells contain IGF-I receptors, that IGF-I stimulates DNA synthesis in both cell types (Huybrechts et al., 1985; Roberts et al., 1994), and that IGF stimulates production of P4 by granulosa cells (Williams et al., 1994; Onagbesan and Peddie, 1995). These findings imply that IGF regulate egg production by ovary growth and steroid hormone production. However, there have been no reports on the relationship among IGF, hormones, and economic traits. Although a comparison between economic traits and IGF-I genotypes has been reported (Nagaraja et al., 2000), any association between IGF expression and IGF-I genotypes has not been documented.

The chicken IGF-I gene has been shown to be conserved in several vertebrate species (Klein et al., 1996). It is reportedly located in the short arm of chromosome 1 (Klein et al., 1996) and has single PstI polymorphism in the 5' region (Nagaraja et al., 2000). In this study, the PstI-digested PCR product of the IGF-I gene revealed polymorphic fragments of 257, 364, and 621 bp (Figure 1), which was consistent with the previous reports by Nagaraja et al. (2000). The comparison for 2 allele sequences indicated the loss of the PstI restriction enzyme site (CTGCA↓G) by point mutation.

The result of the genotype frequency for the IGF-I genetic locus in this study with the KNOC is somewhat different from that reported for White Leghorns (Nagaraja et al., 2000). The frequency of the A [PstI (+)] allele was 0.30 (Table 1) in this study but was 0.83 in White Leghorns. Such a difference may reflect differences in hereditary characteristics among chicken breeds because the genetic distance is long between the White Leghorn and the KNOC. Nagaraja et al. (2000) speculated that the A allele is associated with a higher IGF-I expression, but Amills et al. (2003) reported that plasma IGF-I levels at 73 d do not show any association with IGF-I genotypes. Similarly, in this study with KNOC, no difference between IGF genotypes and serum IGF levels was observed (Figures 4 and 5). However, the IGF-II expression based on IGF-I genotypes showed a significant difference in F1 (P < 0.05; Figure 9). IGF-I genotype also was associated with the number of SYF (Table 2), which indicated that ovarian IGF-II expression in the A allele group was higher than that of BB genotype and that the high expression of IGF-II regulated follicular development. Also, E2 and P4 expressions in the A allele group were higher than those of BB genotype at 30 and 40 wk (Figures 6 and 7). These, therefore, contributed to the increased egg production in AA genotype (50 wk; Figure 2), which agreed with findings in the report by Williams et al. (1994) that IGF are positively associated with P4 expression. This result also implies that the A allele is associated with a higher IGF-II expression in the ovary. Taken together, IGF-I genotypes seem to be a regulatory factor for ovulation and follicle development in KNOC, suggesting that IGF-I genotypes may be able to act as a genetic marker for egg productivity of KNOC.

ACKNOWLEDGMENTS

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Table 2. Tissue weight means and number of small yellow follicles (SYF) at 60 wk of age by insulin-like growth factor (IGF)-I genotype

<table>
<thead>
<tr>
<th>Factor</th>
<th>IGF-I genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
</tr>
<tr>
<td>Follicle 1 (g)</td>
<td>9.43 ± 1.73</td>
</tr>
<tr>
<td>Follicle 2 (g)</td>
<td>6.85 ± 1.33</td>
</tr>
<tr>
<td>Follicle 3 (g)</td>
<td>3.75 ± 0.95</td>
</tr>
<tr>
<td>Ovary (g)</td>
<td>4.77 ± 0.39</td>
</tr>
<tr>
<td>SYF (each)</td>
<td>7.77 ± 1.16b</td>
</tr>
</tbody>
</table>

*abMeans with different superscript differ (P < 0.05).
*All values are expressed as means ± SE.

References


