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# Gene Effects on Body Weight, Carcass Yield, and Meat Quality of Thai Indigenous Chicken

Amonrat Molee, Petladda Kuadsantia and Porntiwa Kaewnakian

School of Animal Production Technology, Institute of Agricultural Technology,
Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand

The selection of rapidly growing animals in breeding programs has had inadvertent detrimental effects on meat quality. Thus, the aim of the present study was to investigate the relationship between body weight (BW) and meat quality traits, and the effects of genes encoding insulin-like growth factor I (*IGF-I*), insulin-like growth factor II (*IGF-II*), melanocortin-4 receptor (*MC4R*), and calpain 1 (*CAPN1*) on BW, carcass yield, and meat quality of the Thai indigenous chicken, Leung Hang Khao. Five hundred and ten chickens were used for genotyping. PCR-restriction fragment length polymorphism and PCR-single strand conformation polymorphism were used to determine the genotypes of *IGF-I*, *IGF-II*, *MC4R*, and *CAPN1*. BWs were collected from 0−16 weeks of age. The chickens were sacrificed at 16 weeks and individual carcass yields and meat qualities (drip loss, cooking loss, and shear force) were recorded. The correlations between BW and meat qualities were determined. Significant correlation between BW and cooking loss and shear force of breast meat and between BW and drip loss of thigh meat were detected ($P<0.05$); however, the magnitude of the association was low (−0.1−0.1). *IGF-I* was eliminated from the association analysis because genotype *AA* was lost and the frequency of occurrence of the *AC* genotype was low (0.04). Significant associations between *IGF-II*, *CAPN1*, and BW, and *CAPN1* and meat quality were detected, while non-significant association between *MC4R* and BW was observed. The results indicated a low, negative relationship between BW and meat quality, and that the *IGF-II* and *CAPN1* could be used as genetic markers in Leung Hang Khao chickens to improve growth and meat quality through breeding.

**Key words**: body weight, calpain 1, indigenous chicken, insulin-like growth factor I-II, meat quality, melanocortin-4 receptor

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## Introduction

Genetic engineering for improving the performance of indigenous chicken is currently a significant issue for developing countries, particularly in Asia, which is a major source of genetically diverse indigenous chickens. Food security and the accessibility of small holder farmers to good breeding practices are important issues for animal husbandry-based industries.

In 2001, the Thailand Research Fund (TRF) and the Department of Livestock Development (DLD) cooperated to collect four varieties of Thai indigenous chickens from around the country, namely, Leung Hang Khao (LHK), Pradoo Hang Dam, Chee, and Deang. Each variety was maintained at different breeding centers of the DLD; LHK, Pradoo Hang Dam, Chee, and Deang were reared at the Kabinburi Livestock Research and Breeding Center in the eastern region, the Chiang Mai Livestock Research and Breeding Center in the northern region, the Tha Pra Livestock Research and Breeding Center in the northeastern region, and the Surat Thani Livestock Research and Breeding Center in the southern region, respectively.

Desired meat texture and flavor are the main advantages of Thai indigenous breeds (Teltathum and Mekchay, 2010). Moreover, the LHK chicken has yellow skin, which is attractive to consumers. However, their slow growth rate compared to that of commercial breeds is an obvious disadvantage, which increases the cost of production. Therefore, from a commercial point of view, improvement of growth performance via genetic manipulations is necessary while maintaining the existing meat quality.

More than 50 years of genetic selection has lead to the development of commercial broiler chickens with rapid growth rate (attaining 2.5−3.0 kg in 37−40 days) (Zerehdaran *et al*., 2004) and high feed efficiency (Havenstein, 2006).

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Correspondence: Associate Professor Dr. Amonrat Molee, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand. (E-mail: amonrat@sut.ac.th)

However, the flavor and texture of the produced meat has deteriorated because of the rapid growth (Dransfield and Sosnicki, 1999). This problem highlights the need for animal breeders to better understand the relationships among various economically desirable traits when designing a breeding program.

Previous studies have concluded that growth, carcass yield, and meat quality traits are negatively correlated. Dransfield and Sosnicki (1999) reported that an increase in growth rate in chickens might induce morphological abnormalities, larger fiber diameters, higher proportions of glycolytic fibers, and lower proteolytic potential in the muscle, which might lower the meat quality. Their findings were in accordance with the results of Duclos *et al*. (2007), who reported that lean chickens have lower levels of glycogen stores than fat chickens, which consequently reduced exudation and post-mortem acidification of meat. This is a direct consequence of the speed of growth. Moreover, an indirect effect of higher growth rate is increase in stress, which results in histological and biochemical modifications of the muscle tissue, impairing meat quality (Petracci and Cavani, 2011). These studies provided evidence regarding the antagonistic relationship between growth and meat quality traits. Determining this relationship in an unselected population of LHK chickens is necessary to develop a breeding scheme.

Numerous studies on genetic markers have been performed, such as those by Li and Li (2006), Zhang *et al*. (2008), Wang *et al*. (2009), and Promwatee *et al*. (2011). However, their use in selection programs is not well understood, particularly the relationship between genes that control different economically desirable traits, which might be negatively correlated. In the current study, the genes encoding insulin-like growth factor I (*IGF-I*), insulin-like growth factor II (*IGF-II*), melanocortin-4 receptor (*MC4R*), and calpain 1 (*CAPN1*) were used to study the relationship between growth, carcass yield, and meat quality traits.

*IGF-I, IGF-II, MC4R*, and *CAPN1* (accession numbers: M 74176, AY 267181, AY 545056 and NC_006090.1, respectively) are located on chromosome 1 (Kajimoto and Rotwein, 1991; Klein *et al*., 1996), chromosome 5 (Darling and Brickell, 1996; Yokomine *et al*., 2001), chromosome 2 (Takeuchi and Takahashi, 1998), and chromosome 3 (Zhang *et al*., 2008), respectively. IGF-I and IGF-II stimulate proliferation, differentiation and metabolism of myogenic cell lines from different species (Florini *et al*., 1996). IGFs regulate body and muscle growth in chickens (Duclos *et al*., 1999), whereas MC4R controls food intake, energy balance, and body weight (Li and Li, 2006). *MC4R* is significantly associated with carcass and meat quality traits (Wang *et al*., 2009). Regulation of CAPN1 activity is associated with variation in meat tenderness (Geesink and Koohmaraie, 1999), and *CAPN1* has been associated with live weight, carcass weight, breast muscle weight, and leg muscle weight (Zhang *et al*., 2008).

Thus, the objective of this study was to investigate the relationship between body weight and meat quality, as well as between *IGF-I*, *IGF-II*, *MC4R*, and *CAPN1* and body weight, carcass yield, and meat quality in LHK chickens. The results of this study will be useful for designing breeding programs for LHK and other indigenous chickens in Thailand and developing countries that harbor different varieties of indigenous chicken.

## Materials and Methods

### *Animal and Data Collection*

The indigenous chickens used in this study were LHK. They were collected from around the country in 2001, delivered to the DLD and raised in the Kabinburi Livestock Research and Breeding Center. Random mating was used to produce the replacement flock. In 2009, 60 LHK males and 300 LHK females were drawn from the flock and moved to the Suranaree University of Technology. Random mating of this parent stock was used to produce 600 LHK chicks for this study. Each chicken was tagged with an ID for individual data collection.

The chicks were raised conventionally, with free access to a starter diet (21% crude protein) from hatching to 3 weeks of age. Thereafter, they received a grower diet (19% crude protein) from 3 to 6 weeks of age, and a finisher diet (17% crude protein) from 6 weeks of age to slaughter. At 16 weeks of age, their average body weight reached the market size of 1.4−1.5 kg.

At 16 weeks of age, the chickens were fasted for 10 h before being weighed and slaughtered by manual exsanguination. The dressing-out percentage, abdominal fat, breast meat (*pectoralis major*) and thigh meat (*biceps femoris*) were weighed. The dressing-out percentage was calculated as the ratio between the dressing-out weight and live weight after fasting. The percentages of breast meat, leg meat, and abdominal fat were calculated as a percentage of the dressing-out weight.

The percentage drip loss was measured for raw meat samples weighing approximately 4−5 g, cut into pieces with dimensions of approximately 1.0×2.0×0.5 cm (width, length, and height, respectively). The breast and thigh meat samples were trimmed at both ends and weighed before and after storage. The samples were wrapped in absorption pads and placed in polyethylene bags before being hung on hooks in a refrigerator for 24 h and 48 h at 4℃. Drip loss percentages were calculated as:

(Weight before storage−Weight after storage)/
Weight before storage×100

Shear force was measured on cooked breast and thigh meat according to the method of Dawson *et al*. (1991). Both parts of the meat were boiled until the core temperature was 78−80℃ in 10 min, before being cut into pieces with dimensions of of approximately 1.0×2.0×0.5 cm (width, length and height, respectively). A TA-XT2 texture analyzer (Stable Micro System, Godalming, UK) with a Warner–Bratzler shear apparatus was used. The operating parameters consisted of a cross-head speed of 2 mm/s and a 5 kg load cell. The descriptive data for all traits measured in the study are shown in Table 1.

The numbers of samples shown in Table 1 were 510, 500,

Table 1. **Characteristics for body weight, carcass yield traits, and meat quality traits of the Leung Hang Khao chickens used in this study**

| Trait | Number of samples (N) | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| | | Body weight | | | |
| 0 week | N=510 | 32.8 | 3.25 | 23 | 42 |
| 2 weeks | | 86.1 | 16.64 | 32 | 143 |
| 4 weeks | | 208.8 | 41.06 | 65 | 435 |
| 6 weeks | | 385.3 | 73.13 | 170 | 920 |
| 8 weeks | | 588.2 | 116.8 | 260 | 1280 |
| 10 weeks | | 827.2 | 153.70 | 380 | 1750 |
| 12 weeks | | 1065.8 | 205.94 | 460 | 2100 |
| 14 weeks | | 1211.1 | 215.71 | 400 | 1900 |
| 16 weeks | | 1457.6 | 264.3 | 580 | 2260 |
| | | Carcass yield | | | |
| Dressing % | N=500 | 66.34 | 2.66 | 50.85 | 82.86 |
| AbF % | | 0.64 | 0.67 | 0.00 | 5.48 |
| BM % | | 12.35 | 1.40 | 6.73 | 22.61 |
| TM % | | 15.39 | 1.47 | 5.96 | 29.44 |
| ToM % | | 27.74 | 2.29 | 18.93 | 48.57 |
| | | Meat quality | | | |
| 24 h drip % B | N=317 | 2.64 | 0.64 | 1.09 | 4.97 |
| 48 h drip % B | | 2.16 | 0.58 | 0.82 | 4.28 |
| 24 h drip % T | | 2.18 | 0.39 | 1.31 | 4.09 |
| 48 h drip % T | | 1.88 | 0.35 | 1.07 | 3.36 |
| Cooking % B | | 21.11 | 2.12 | 11.19 | 26.23 |
| Cooking % T | | 26.57 | 2.53 | 16.50 | 35.39 |
| SFB (g/mm) | | 148.96 | 41.09 | 73.73 | 280.42 |
| SFT(g/mm) | | 108.59 | 28.03 | 57.53 | 214.59 |

Percentage carcass yield: Dressing % - dressing-out percentage; AbF % - abdominal fat; BM % - breast meat, TM % - thigh meat, ToM % - total meat.
Percentage drip loss: 24 h drip % B - 24 h breast meat; 48 h drip % B - 48 h breast meat; 24 h drip % T - 24 h thigh meat; 48 h drip % T - 48 h thigh meat.
Percentage cooking loss: cooking % B - breast meat; cooking % T - thigh meat.
Shear force: SFB - breast meat; SFT - thigh meat.

and 317 for body weight, carcass yield, and meat qualities, respectively. The reasons behind the variations in sample number were unidentified ID and outlier records for certain samples, which were eliminated from the analysis. Moreover, the number of body weights used for relationship analysis (504) was slightly different from the number of body weights shown in Table 1 (510) because the samples could not be identified with their ID.

In this study, we assumed normality of data; therefore, some data, for example, percentage of abdominal fat, breast meat, thigh meat, total meat, and drip loss of breast and thigh meat at 24 h and 48 h were transformed using the common logarithm (log10). The exception was percentage of abdominal fat, which had some data points equal to zero. Therefore, 1 was added to each value and they were then transformed by log10. After completion of statistical analysis, all transformed data were back-transformed with $10^{X'}$ and $10^{X'}-1$ for percentage of abdominal fat, where $X'$ is the transformed data.

All experimental procedures were approved by the Institution Animal Care and Use Committee of the Suranaree University of Technology (The certificate ID: 24/2555).

***Genotyping***

Blood samples were collected from 510 LHKs. Genomic DNA was extracted from whole blood using a DNA mini kit for blood per manufacturer's instructions (Geneaid Biotech Ltd, New Taipei City, Taiwan). DNA was quantified spectrophotometrically and diluted to 10 $\mu g/\mu l$.

The genotypes of *IGF-I* and *IGF-II* were analyzed using PCR-restriction fragment length polymorphism (PCR-RFLP), as reported by Zhou *et al*. (2005) and Amills *et al*. (2003), respectively.

The genotypes of *MC4R* and *CAPN1* were analyzed using PCR-single strand conformation polymorphism (PCR-SSCP), as described by Wang *et al*. (2009) and Zhang *et al*. (2008), respectively.

***Statistical Analysis***

Linkage disequilibrium (LD) between *IGF-II* and *MC4R*, *IGF-II* and *CAPN1*, and *MC4R* and *CAPN1* were analyzed using GENEPOP version 3.4 (Raymond and Rousset, 2003).

Groups of loci with significant associations were rearranged as composite genotypes.

The significant effects of genotype or composite genotype on body weight, carcass yield, and meat quality were analyzed using a general linear model, with sex, genotype, and interaction between sex and genotype as fixed effects. Analysis of variance was used to test the significance of differences between measured phenotypic traits in individual genotypes. The level of significance was defined at $P<0.05$. SPSS for Windows (Release 10.0; SPSS Inc., Chicago, IL, USA) was used for the analysis.

## Results and Discussion

### *Allelic and Genotypic Frequency*

The allelic and genotypic frequencies of all genes are shown in Table 2. *IGF-II*, *MC4R*, and *CAPN1* showed a potential for use as genetic markers in selection programs because there was more than one genotype at each locus and each genotype showed a suitable frequency. However, *IGF-I* was eliminated from the analysis because the frequency of genotype *CC* exceeded 0.96, whereas the *AC* and *AA* genotypes were rare (0.04) and absent, respectively.

The low frequencies of the *AA* and *AC* genotypes of *IGF-I* observed in the present study are in agreement with the results of Promwatee *et al*. (2011), and Moe *et al*. (2009), who studied other indigenous Thai chicken lines (Pradu Hang Dam and Chee), and indigenous chickens from Asian countries (Cambodia, Laos, and Myanmar). The results are in contrast with those obtained with commercial broilers (Moe *et al*., 2009; Kadlec *et al*., 2011). Based on these results, we speculated that the main role of *IGF-I* in chickens involves growth, development, and adaptability.

### *Hardy-Weinberg Equilibrium and Linkage Disequilibrium (LD)*

Significant deviations from the Hardy-Weinberg equilibrium (HWE) were detected for *MC4R* and *CAPN1*, while *IGF-I* and *II* remained in equilibrium (Table 2). The LHK population used was randomly mated and there was no migration. However, historically, the breed was selected by farmers for cock-fighting purposes, who tended to select the stronger chickens. The allele set involved in determining these traits would thus have been indirectly selected for and passed on to the next generation. This may provide an explanation for the deviation of the genes involved in growth from the HWE. Significant associations of *MC4R* with growth performance were reported by Li and Li (2006) and Qiu *et al*. (2006). *CAPN1* also deviated significantly from the HWE. These could be explained if these genes are involved in growth or another mechanism, which could have been affected by selection; however, this speculation should be investigated in future experiments.

In the case of *IGF-I*, low frequency of the *A* allele may be common in native chickens, because natural or long periods of indirect selection almost annihilate the *A* allele and fix the *C* allele fixed. As a consequence, this locus is still in HWE. Meanwhile, for *IGF-II*, it is possible that the allelic frequencies were not affected by any kind of selection.

LD is non-random association of alleles at different loci. In the present study, significant LD was not observed. Therefore, single loci were used as genetic markers.

### *Correlation between Final Body Weight and Meat Quality*

The final body weight showed significant negative and positive correlations with cooking loss and the shear force of breast meat, respectively. For thigh meat, we observed sig-

Table 2. **Allelic and genotypic frequencies and the Hardy–Weinberg equilibrium (HWE) of *IGF-I*, *IGF-II*, *MC4R* and *CAPN1***

| Gene | No. of animals | Frequency | | | | |
|---|---|---|---|---|---|---|
| | | Genotype | | | Allele | |
| | | AA | AC | CC | A | C |
| *IGF-I* | 510 | 0 ($n=0$) | 0.04 ($n=20$) | 0.96 ($n=490$) | 0.02 | 0.98 |
| HWE | | *P*-value 1.00 | | | | |
| *IGF-II* | 510 | AA | AB | BB | A | B |
| | | 0.25 ($n=127$) | 0.50 ($n=257$) | 0.25 ($n=126$) | 0.501 | 0.499 |
| HWE | | *P*-value 0.90 | | | | |
| *MC4R* | 510 | GG | GT | TT | G | T |
| | | 0.24 ($n=124$) | 0.18 ($n=91$) | 0.58 ($n=295$) | 0.332 | 0.668 |
| HWE | | *P*-value 0.00 | | | | |
| *CAPN1* | 510 | A1A1 | A1A2 | A2A2 | A1 | A2 |
| | | 0.59 ($n=300$) | 0.15 ($n=78$) | 0.26 ($n=132$) | 0.665 | 0.335 |
| HWE | | *P*-value 0.00 | | | | |

nificant positive correlations between body weights and drip loss after 24 and 48 h of storage. However, despite the significance of these associations, the correlation coefficients were all relatively low. No significant correlation between body weights, drip loss of breast meat at 24 and 48 h, cooking loss, and shear force of thigh meat were observed. The results are presented in Table 3.

Weak and no correlation between body weight and meat qualities might be explained by the high association of growth rate with the toughness of the meat, fiber size (Dransfield and Sosnicki, 1999; and Zhao *et al*., 2011), and drip loss (Dransfield and Sosnicki, 1999). However, there was a high degree of genetic variation in the chickens used in the present study, as they were drawn from an unselected population of LHK chickens, while the same genetic background of chickens were used by Zhou *et al*. (2011). This might explain the discrepancy in the results of our study and those of previous studies. Moreover, the correlations between breast and thigh meat also varied as different fiber types and size cause differences in metabolism (aerobic and anaerobic) and texture of meat (Dransfield and Sosnicki, 1999; and Listat *et al*., 2016), which might explain our results.

Table 3. **Correlation coefficient between body weight and meat quality of Leung Hang Khao chickens**

| | Body weight at 16 weeks | Correlation coefficient | *P*-value |
|---|---|---|---|
| Breast meat | Drip loss at 24 h | 0.012 | 0.84 |
| | Drip loss at 48 h | −0.003 | 0.96 |
| | Cooking Loss | −0.139 | 0.03 |
| | Shear force | 0.143 | 0.03 |
| Thigh meat | Drip loss at 24 h | 0.123 | 0.028 |
| | Drip loss at 48 h | 0.126 | 0.025 |
| | Cooking Loss | −0.077 | 0.17 |
| | Shear force | −0.003 | 0.96 |

***Relationship between IGF-II and Body Weight, Carcass Yield, and Meat Quality Traits in LHK Chickens***

Genotype had a significant effect on body weight at 16 weeks of age (*P* value < 0.05) (Table 4). However, the genotypes did not differ significantly for carcass yield or meat quality (Tables 5 and 6).

The results for body weight are in agreement with those of previous studies (Darling and Brickell, 1996; Dransfield and Sosnicki, 1999), which confirm that *IGF-II* plays a major role in chicken growth and development by stimulating proliferation, differentiation, and metabolism of myogenic cell lines (Florini *et al*., 1996). The results are in contrast with those of Amills *et al*. (2003), who studied the same region of the gene (exon 3). The significant effect may be attributed to a *C* to *T* substitution; the current results imply that this substitution may lead to differences in peptide sequence, which may alter the activity of the hormone. Thus, differences in the genetic structure of the populations

Table 4. **Least square means and standard errors of body weight (grams) in Leung Hang Khao chickens**

| Gene | | Number of chickens | Least square mean (SE) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 wk | 2 wks | 4 wks | 6 wks | 8 wks | 10 wks | 12 wks | 14 wks | 16 wks |
| *IGF-II* | AA | 126 | 33.33 | 82.61 | 199.57 | 382.35 | 586.24 | 821.84 | 1062 | 1152 | $1413^{B}$ |
| | | | (0.27) | (1.33) | (3.60) | (6.42) | (10.24) | (13.03) | (16.04) | (19.14) | (20.91) |
| | AB | 252 | 32.96 | 84.19 | 209.30 | 388.04 | 589.14 | 827.84 | 1076 | 1190 | $1452^{AB}$ |
| | | | (0.20) | (0.96) | (2.59) | (4.55) | (7.20) | (9.29) | (11.40) | (14.05) | (14.43) |
| | BB | 126 | 33.30 | 84.17 | 204.84 | 386.59 | 590.56 | 832.53 | 1081 | 1202 | $1484^{A}$ |
| | | | (0.27) | (1.36) | (3.61) | (6.46) | (10.12) | (13.06) | (16.05) | (17.99) | (20.31) |
| *P*-value | | | 0.43 | 0.59 | 0.08 | 0.77 | 0.95 | 0.81 | 0.69 | 0.11 | 0.05 |
| *MC4R* | GG | 122 | 33.54 | 84.56 | 207.69 | 390.67 | 591.91 | 825.87 | 1074 | 1207 | 1478 |
| | | | (0.28) | (1.39) | (3.68) | (6.46) | (10.42) | (13.32) | (16.30) | (19.27) | (19.99) |
| | GT | 91 | 32.97 | 84.03 | 201.12 | 375.77 | 588.33 | 815.72 | 1048 | 1167 | 1408 |
| | | | (0.32) | (1.55) | (4.20) | (7.37) | (11.82) | (15.15) | (18.65) | (21.70) | (28.14) |
| | TT | 291 | 33.02 | 83.32 | 206.35 | 388.23 | 587.59 | 831.81 | 1083 | 1183 | 1449 |
| | | | (0.18) | (0.90) | (2.44) | (4.31) | (6.80) | (8.76) | (10.76) | (13.45) | (13.19) |
| *P*-value | | | 0.23 | 0.73 | 0.47 | 0.26 | 0.94 | 0.64 | 0.28 | 0.34 | 0.13 |
| *CAPN1* | A1A1 | 296 | $32.49^{B}$ | $87.62^{A}$ | 208.60 | $378.62^{B}$ | 580.16 | 820.59 | 1048 | 1214 | 1459 |
| | | | (0.19) | (0.97) | (2.40) | (4.14) | (6.47) | (8.35) | (10.66) | (10.97) | (12.19) |
| | A1A2 | 77 | $32.77^{AB}$ | $83.25^{B}$ | 206.01 | $379.74^{AB}$ | 575.95 | 811.44 | 1052 | 1219 | 1479 |
| | | | (0.37) | (1.91) | (4.63) | (8.08) | (12.76) | (16.38) | (20.95) | (22.55) | (28.62) |
| | A2A2 | 131 | $33.43^{A}$ | $84.31^{AB}$ | 209.72 | $397.57^{A}$ | 604.94 | 85.60 | 1087 | 1173 | 1406 |
| | | | (0.28) | (1.48) | (3.62) | (6.30) | (9.97) | (12.67) | (16.08) | (18.64) | (22.90) |
| *P*-value | | | 0.02 | 0.04 | 0.82 | 0.04 | 0.09 | 0.46 | 0.18 | 0.14 | 0.07 |

$^{A, B, C}$ different letters indicate significant differences at $P < 0.05$.
wk – week.

Table 5. **Least square means and standard errors of carcass yield traits in Leung Hang Khao chickens**

| Gene | | Number of chickens | Least square mean (SE) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Dressing % | BT_AbF % | BT_BM % | BT_TM % | BT_ToM % |
| *IGF-II* | AA | 124 | 66.25 | 0.52 | 12.25 | 15.24 | 27.54 |
| | | | (0.64) | (0.03) | (1.01) | (1.01) | (1.01) |
| | AB | 251 | 66.63 | 0.53 | 12.33 | 15.31 | 27.67 |
| | | | (0.45) | (0.02) | (1.01) | (1.01) | (1.00) |
| | BB | 125 | 66.74 | 0.66 | 12.33 | 15.28 | 27.67 |
| | | | (0.63) | (0.03) | (1.01) | (1.01) | (1.01) |
| *P*-value | | | 0.82 | 0.08 | 0.90 | 0.89 | 0.81 |
| *MC4R* | GG | 122 | 65.93 | 0.52 | 12.30 | 15.14 | 27.54 |
| | | | (0.58) | (0.02) | (1.01) | (1.01) | (1.01) |
| | GT | 90 | 65.93 | 0.52 | 12.59 | 15.14 | 27.73 |
| | | | (0.67) | (0.05) | (1.01) | (1.01) | (1.01) |
| | TT | 288 | 66.00 | 0.60 | 12.30 | 15.49 | 27.67 |
| | | | (0.38) | (0.02) | (1.01) | (1.01) | (1.01) |
| *P*-value | | | 0.99 | 0.31 | 0.26 | 0.14 | 0.77 |
| *CAPN1* | A1A1 | 295 | 65.63 | 0.55 | 12.22 | 15.21 | 27.54 |
| | | | (0.38) | (0.02) | (1.01) | (1.00) | (1.00) |
| | A1A2 | 78 | 66.35 | 0.61 | 12.36 | 15.52 | 28.18 |
| | | | (0.72) | (0.04) | (1.01) | (1.01) | (1.01) |
| | A2A2 | 127 | 66.51 | 0.57 | 12.47 | 15.28 | 28.18 |
| | | | (0.57) | (0.03) | (1.01) | (1.01) | (1.01) |
| *P*-value | | | 0.36 | 0.88 | 0.20 | 0.28 | 0.13 |

Percentage carcass yields: Dressing % - dressing-out percentage; BT_AbF % - back-transformed abdominal fat; BT_BM % - back-transformed breast meat; BT_TM % - back-transformed thigh meat; BT_ToM % - back-transformed total meat.
$^{A, B, C}$ different letters indicate significant differences at $P<0.05$.

used in this study with those used by Amills *et al*. (2003) and Zhang *et al*. (2008) might explain the inconsistent results.

Since *IGF II* is mainly associated with growth and muscle development, we expected significant differences in the quantity of meat produced in terms of carcass yield; however, our results were to the contrary. This could be explained by the differences in the magnitude of the effect of various genotypes on muscle development, which was negligible in our study. Hence, the C to T substitution may have had limited effect on muscle development. Alternatively, the genotype might affect the number of muscle fibers or fiber size. Duclos *et al*. (1999) showed that the IGFs regulate body and muscle growth. In the current study, however, these traits were not measured.

Toughness and tenderness are indicators of meat texture and quality. This can be measured using parameters such as drip loss and shear force. Dransfield and Sosnicki (1999) and Zhao *et al*. (2011) reported that growth rate is associated with muscle fiber size and number, and that fiber size is associated with toughness and tenderness. Additionally, Tesseraud *et al*. (2003) found high concentration of IGF-II in a high-quality chicken meat line. Therefore, growth rate is indirectly related to drip loss and shear force. In the current study, *IGF-II* showed significant effects on body weight, but non-significant effects on all meat quality traits. This may be because the differences in the growth rates of the chickens were not large enough to affect either fiber size or the number of muscle fibers. Alternatively, the absence of any significant effect might be explained if the traits measured in the current study, such as drip loss and shear force, depended not only on fiber size, but also on the presence of other biochemical compounds in meat, particularly collagen (Nakamura *et al*., 1975), which was not measured.

***Relationship between MC4R and Body Weight, Carcass Yield, and Meat Quality Traits in LHK Chickens***

In contrast to the hypothesized role of *MC4R*, the genotypes did not differ significantly for any of the traits measured (Tables 4, 5, and 6). This is also in contrast to the results of Li and Li (2006), Qiu *et al*. (2006), and Wang *et al*. (2009), who reported that different genotypes were associated with significantly different carcass traits and body weights. For example, Wang *et al*. (2009) showed that the *GT* genotype had a superior effect than the *GG* and *TT* genotypes. On the contrary, Li and Li (2006) studied different regions of this gene and observed that the effects of homozygous genotypes were superior to those of heterozygous genotypes. Schwartz *et al*. (1996) investigated the role of the melanocortin 4 receptor and demonstrated that it was associated with the control of food intake, energy balance, and body weight. Moreover, it might be involved in certain aspects of lipid metabolism in chickens (McMurtry *et al*., 1997). Li and Li (2006) reported that different genotypes of *MC4R*, namely the *AA* and *BB* genotypes, showed differences in protein secondary structure, possibly resulting in

Table 6. **Least square means and standard errors of meat quality traits in Leung Hang Khao chickens**

| Gene | | Number of chickens | Least square mean (SE) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | BT_24h drip % B | BT_48h drip % B | 24h drip % T | 48h drip % T | cooking % B | cooking % T | SFB (g/mm) | SFT (g/mm) |
| *IGF-II* | AA | 72 | 2.61 | 2.12 | 2.13 | 1.83 | 21.28 | 26.91 | 147.35 | 105.75 |
| | | | (1.03) | (1.03) | (0.05) | (0.04) | (0.35) | (0.30) | (6.76) | (3.29) |
| | AB | 163 | 2.58 | 2.08 | 2.17 | 1.87 | 21.26 | 26.41 | 148.63 | 108.31 |
| | | | (1.02) | (1.02) | (0.03) | (0.03) | (0.20) | (0.20) | (3.81) | (2.19) |
| | BB | 82 | 2.48 | 2.05 | 2.22 | 1.94 | 21.16 | 26.60 | 149.56 | 111.38 |
| | | | (1.02) | (1.03) | (0.04) | (0.04) | (0.29) | (0.28) | (5.71) | (3.07) |
| *P*-value | | | 0.30 | 0.59 | 0.31 | 0.18 | 0.95 | 0.38 | 0.98 | 0.46 |
| *MC4R* | GG | 84 | 2.51 | 2.04 | 2.17 | 1.86 | 21.61 | 26.59 | 144.89 | 108.48 |
| | | | (1.02) | (1.02) | (0.04) | (0.04) | (0.28) | (0.27) | (5.56) | (3.09) |
| | GT | 41 | 2.63 | 2.14 | 2.11 | 1.82 | 21.20 | 27.36 | 147.15 | 103.67 |
| | | | (1.05) | (1.05) | (0.06) | (0.05) | (0.44) | (0.39) | (8.58) | (4.38) |
| | TT | 192 | 2.57 | 2.09 | 2.19 | 1.90 | 21.01 | 26.42 | 150.37 | 109.86 |
| | | | (1.02) | (1.02) | (0.03) | (0.03) | (0.19) | (0.18) | (3.70) | (2.03) |
| *P*-value | | | 0.65 | 0.71 | 0.49 | 0.33 | 0.32 | 0.10 | 0.74 | 0.44 |
| *CAPN1* | A1A1 | 214 | 2.57 | $2.10^{B}$ | 2.18 | 1.88 | 21.29 | 26.63 | 150.35 | 109.35 |
| | | | (1.01) | (1.02) | (0.03) | (0.02) | (0.18) | (0.17) | (3.49) | (1.92) |
| | A1A2 | 41 | 2.69 | $2.23^{A}$ | 2.21 | 1.92 | 20.82 | 26.20 | 144.30 | 105.94 |
| | | | (1.04) | (1.04) | (0.06) | (0.05) | (0.36) | (0.40) | (7.11) | (4.37) |
| | A2A2 | 62 | 2.45 | $1.98^{C}$ | 2.14 | 1.85 | 21.25 | 26.70 | 142.88 | 108.28 |
| | | | (1.03) | (1.03) | (0.05) | (0.04) | (0.40) | (0.33) | (7.59) | (3.64) |
| *P*-value | | | 0.07 | 0.03 | 0.64 | 0.61 | 0.51 | 0.57 | 0.59 | 0.77 |

Percentage drip loss: BT_24 h drip % B – Back-transformed 24 h breast meat; BT_48 h drip % B – Back-transformed 48 h breast meat; 24 h drip % T - 24 h thigh meat; 48 h drip % T - 48 h thigh meat.
Percentage cooking loss: cooking % B - breast meat; cooking % T - thigh meat.
Shear force: SFB - breast meat; SFT - thigh meat.
$^{A, B, C}$ different letters indicate significant differences at $P<0.05$.

differences in its biological function. Thus, variations in animal genotype might manifest as different phenotypes. However, the chickens used in this study were taken from a relatively unselected population, which might explain the contrast between our results and those of previous studies. Each trait showed large variations, which exceeded the effect of the genotype. Thus, the observed differences were non-significant.

***Relationship between CAPN1 and Body Weight, Carcass Yield, and Meat Quality Traits in LHK Chickens***

Significant association between *CAPN1* and body weight was detected at 0, 2, and 6 weeks of age (*P* value=0.02, 0.04, and 0.02, respectively) (Table 4), and the locus was also found to be significantly associated with percentage of drip loss at 48 h (*P* value=0.03) (Table 6). The *A2A2* genotype had a positive effect on body weight at 0, 2, and 6 weeks of age, and it was also associated with improved drip loss.

Our results are consistent with those of Zhang *et al*. (2008), Felício *et al*. (2013), and Shu *et al*. (2015), who studied different breeds of chickens. The results can be explained by the results of Goll *et al*. (2003), who reported that calpains are involved in muscle growth and development. Moreover, Koohmaraie (1996) also reported that CAPN1 degrades myofibrillar proteins under postmortem conditions and appears to be the primary enzyme in the postmortem tenderization process. This may explain the effect of this gene on the measured traits; however, the reasons for the variations in the effect of different *CAPN1* genotypes remain unclear. Page *et al*. (2002) observed that mutations altered the amino acid sequence of the enzyme, which correlated with beef tenderness. It is possible that a similar phenomenon exists in chickens, the mechanism of which should be investigated in a future study.

The observations of this study suggest that meat quality may be negatively affected when growth performance is improved. Therefore, it is important that meat quality traits are monitored when selection for growth performance improvement is performed. Single loci could be used as genetic markers as no significant LD was detected. *IGF-II* and *CAPN1* could be used as genetic markers when both growth improvement and meat quality are the main goals of breeding.

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