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# Research Article
# Association of the Locus A3971G of Insulin Gene with Some Economic Traits in Local Noi Chicken Breed

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## Abstract

**Background and Objective:** It is known that insulin plays important roles in hepatic cells, muscle cells and adipose tissue cells. However, only a few studies on genetic variation of insulin gene on economic traits in farm animals, especially in chickens were reported. Thus, the purpose of this study was to look at the genetic variation of insulin candidate gene associated with important economic traits in the Noi population, one of the recognized indigenous chicken breeds in Vietnam. **Materials and Methods:** A total of 355 indigenous Noi broilers at 5 weeks old were selected and kept in private cages until 13 weeks old. During the experimental time, traits for growth and Feed Conversion Ratio (FCR) were recorded in each stage. At the end of the experiment, traits for the carcass, meat quality and chemical compositions of meat were collected. Additionally, two Single Nucleotide Polymorphism (SNPs) at loci T3737C (INS2) and A3971G (INS3) of insulin gene were genotype by using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. **Results:** Genetic variations at both loci were detected where the CC genotype (0.0167) with the lowest frequency followed by the CT (0.2630) and TT (0.7200) was found at locus INS2 and the GG genotype (0.1240) with the lowest frequency followed by the AA (0.3560) and AG (0.5230) was identified at locus INS3. Moreover, the INS3 genetic association with the observed traits such as body weight as well as pH value, cooking loss, colour ones (L*, a*, b*) of meat was determined ($p<0.05$). **Conclusion:** INS3 locus should be considered as a potential genetic resource for selecting Vietnamese indigenous Noi broilers in the future.

Key words: Insulin gene, Noi chicken, growth performances, carcass characteristics, meat quality, feed conversion ratio, abdominal fat

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The genes of the somatotropic axis play a crucial role in chicken growth and development in which insulin, one of the essential components and a peptide hormone was secreted by the β cells of the pancreatic islets of Langerhans[1]. The chicken insulin gene located an intermediate position on the fifth chromosome (Genbank-AY438372) with a total length of 4.074 bp containing 4 exons and 3 introns. Four SNPs like A428G, C1549T, T3737C and A3971G were detected were the first three belonged to the second intron and the last located on the 3'-UTR[2].

Several previous studies revealed that the SNPs of the insulin gene were associated with the traits of growth, muscle composition and fat deposition, representing the basic points to start the economical genetic improvement in poultry[2]. The insulin gene, therefore, was considered as a candidate gene in the genetic analysis due to the wide association with many of the production parameters[2-4].

Indigenous Noi chickens, one of the famous breeds in the Mekong Delta of Vietnam, have valuable characteristics such as their high adaptation ability in hard raising conditions (lack of proper health care, poor nutrition and management conditions), good natural disease resistance (Although there is not much conclusive evidence for this), delicious meat satisfying to demands of Vietnamese customers[5-8]. Thus, this breed is included in the strategy of sustainable poultry development in Vietnam. However, such important performance traits as growth rate, carcass and meat quality need to be improved or clarified in Noi chickens.

It was implied that using a molecular marker or a genetic marker from analyzed blood or tissue samples was a good tool in selection programs to improve performance and economic traits in farm animals[9]. This study aimed to considered to apply the molecular markers or genetic markers to Noi chickens based on the genetic variation of the insulin candidate gene.

## MATERIALS AND METHODS

**Study area:** This study was conducted from July, 2018-July, 2019 on the farm. The sample was analyzed at the labs during 2019-2020.

**Animals:** A resource population of 355 Noi chickens (164 males and 191 females) as described in previous studies was used[5,6].

**Sampling:** Blood samples were collected from Noi chickens at 84 days old before extraction of genomic DNA using Proteinase K digestion, followed by phenol-chloroform extraction and precipitation with ethanol[10]. At 91 days old, all chickens were slaughtered to evaluate traits of (i) Growth performance like Body Weight (BW, g/bird), Average Daily Gain (ADG, g/day/bird), Feed Conversion Ratio (FCR) at three time points of 28, 56 and 84 days old[6], (ii) Carcass performance like Live Weight (LW, g), Killed Weight (KW, g), De-Feather Weight (DFW, g), Carcass Weight (CW, g), Head Weight (HW, g), Weight of Breast Meat (WBM, g), Weight of Thigh Meat (WTM, g), Wing Weight (WW, g), Drumstick Weight (DW, g), Shank Weight (SW, g), Weight of Internal Organs (WIO, g), Gizzard Weight (GW, g), Liver Weight (LRW, g), Heart Weight (HTW, g), Weight of Abdominal Fat (WAF, g), Length of Small Intestine (LSI, mm) and Caeca Length (CL, mm)[8], (iii) Meat quality like pH value, color, cooking loss and drip loss of both breast and thigh meat at time 3, 24 and 48 hrs post-mortem[7] and (iv) Chemical compositions of breast muscle like dry matter (%), crude protein (%) and ether extract (%)[7].

**Genotyping:** Two primer pairs used to detect SNPs at loci T3737C and A3971G were displayed in Table 1. PCR was performed in a 25 μL reaction containing 1×PCR Buffer, 1.5 mM $MgCl_2$, 1.25 mM each dNTPs, 5 pM primers, 1U Taq-polymerase (thermo fisher scientific) and 100 ng genomic DNA.

PCR amplification was begun at 94°C for 3 min, followed by 35 cycles (94°C for 45 sec, 60°C for 45 sec and 72°C for 90 sec) and at 72°C for 7 min on the Veriti™ 96-Well Thermal Cycler (Applied Bio systems). PCR products were digested with restriction enzyme (*Msp*I) at 37°C overnight and then separated on 2.0% agarose gel.

**Statistical analysis:** Genetic frequency was tested by using chi-square analysis according to Hardy-Weinberg Equilibrium. Marker-trait linkage analysis was performed by a GLM procedure of the Minitab ver. 16 according to the model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha * \beta)_{ij} + \varepsilon_{ijk}$$

In which are as $y_{ijk}$ is the dependent variable, μ overall population mean, $\alpha_i$ fixed effect of sexes (I = 1-2), $\beta_j$ fixed effect of genotypes (i = 1-3), $(\alpha*\beta)_{ij}$ fixed effect of sex and genotype interaction and $\epsilon_{ijk}$-the random error. The means were considered significant when the p-value was less than 0.05.

## RESULTS

**Genotypic frequency:** Three genotypes were detected for each SNP but with different frequencies (Fig. 1 and 2). At locus INS2, the CC genotype appeared with a very low frequency

Table 1: Used primers for SNPs detection

| SNPs | Sequence (5'-3') | Accession number | Amplicon size (bp) | Ta (°C) | RE | Source |
|---|---|---|---|---|---|---|
| T3737C | F: CTCCATGTGGCTTCCCTGTA | AY438372 | 370 | 60 | *Msp*I | Qiu *et al.*[2] |
| (INS2) | R: GGCTTCTTGGCTAGTTGCAGT | (intron 2) | | | | |
| A3971G | F: GGTATCTGAAAAGCGGGTCTC | AY438372 | 280 | 60 | *Msp*I | |
| (INS3) | R: AATGCTTTGAAGGTGCGATAG | (3'-UTR) | | | | |

RE: Restriction enzyme, Ta: Temperature for amplification

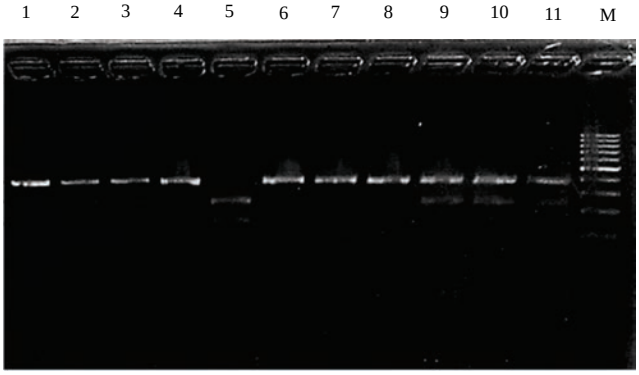


Fig. 1: Representative pattern of the PCR-RFLP/*Msp*I (INS2)
M: DNA ladder, Lane 1: Control without enzyme digestion, Lane 4,6,7,8: TT genotype, Lane 5: CC genotype, Lane 2,3,9,10, 11: CT genotype

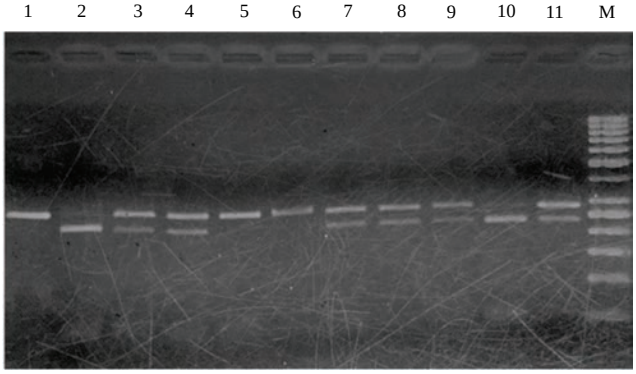


Fig. 2: Representative pattern of the PCR-RFLP/*Msp*I (INS3)
M: DNA ladder, Lane 1: Control without enzyme digestion, Lane 2, 10: GG genotype, Lane 3, 4, 7, 8, 9, 11: AG genotype, Land 5,6: AA genotype

whereas only a female in the population had this genotype. The female and the male had similar frequencies in the same genotype. Most chickens carried the TT genotype. At locus INS3, the AG heterozygous genotype frequency was highest, followed by the homologous AA and GG genotypes, where males and females had an equal frequency in the same genotype (Table 2). Therefore, the INS3 was used to analyze the genetic variation of the insulin gene with observed traits.

**Association of the INS3 with growth traits:** Significant differences ($p<0.05$) between the genotypes and BW at the stage of 28-56 days old were found (Table 3). There was also a statistically significant difference in the interaction ($p<0.05$) between genotype and sex for growth traits in the INS3 locus (Table 4).

**Association of the INS3 with carcass traits:** Significant difference ($p<0.05$) was found for the GW among genotypes whereas the AA genotype was highest (Table 5). However, this trait was not important in selection. The interaction between genotypes and sexes also showed that males differed from females for the observed traits ($p<0.05$), excepted for the WAF ($p = 0.660$) (Table 6). Generally, (i) Carcass parts of males were always higher than those of female and (ii) female had a lower carcass performance than males of the same genotype did.

**Associations of the INS3 with meat quality traits:** The data of Table 7 shows significant effects of the INS3 on physicochemical properties (pH, cooking loss, L*, a*, b*) at a certain time ($p<0.05$), for example, the highest (i) cooking loss of breast meat at 3 and 24 hrs post-mortem for the GG genotype (28.23 and 33.48%, respectively), pH value of breast meat at 24 hrs for the GG (5.62%), (ii) colour a* of thigh meat at 3 hrs (5.62) for the AA, cooking loss of thigh meat at 3 hrs for the GG (30.64%), pH value of thigh meat at 24 hrs for the GG (5.91), pH of thigh meat at 48 hrs for the GG (5.89), L* of thigh meat at 48 hrs for the CG (56.40). Furthermore, the interactions between sexes and genotypes were found significantly in some economic traits (Table 8), for example, the highest (i) colour b* of breast meat at 3 hrs for the AA female (13.06) ($p = 0.002$), cooking loss of breast meat at 3 hrs for the GG female (28.94%) ($p = 0.039$), pH value of breast meat at 24 hrs for the GG male (5.68) ($p = 0.000$), colour b* of breast meat at 24 hrs for the AG female (12.39) ($p = 0.001$), pH value of breast meat at 48 hrs for the GG female (5.62) ($p = 0.001$), colour b* of breast meat at 48 hrs for the AG female (12.85) ($p = 0.000$), (ii) cooking loss of thigh meat at 3 hrs for the GG female (31.57%) ($p = 0.022$) and pH value of thigh meat at 48 hrs for the GG male (5.91) ($p = 0.015$).

**Association of INS3 with chemical compositions of breast meat:** There was no significant difference ($p>0.05$) among genotypes for chemical compositions of breast meat as well as

Table 2: Genotypic and allelic frequency of insulin gene in the Noi population

| | Observed population | | | | | Expected population | | | |
|---|---|---|---|---|---|---|---|---|---|
| Locus | Genotype | | | Allele | | Genotype | | | |
| INS2 | CC | CT | TT | C | T | CC | CT | TT | HWE $\chi^2$ |
| Population | (n = 6) | (n = 93) | (n = 255) | | | | | | 0.565 |
| (n = 354) | 0.0167 | 0.2630 | 0.7200 | 0.1483 | 0.8517 | 0.022 | 0.253 | 0.7250 | 0.754$^{ns}$ |
| Male | (n = 5) | (n = 46) | (n = 112) | | | | | | 0.011 |
| (n = 163) | 0.0306 | 0.2822 | 0.6871 | 0.1718 | 0.8282 | 0.0295 | 0.2845 | 0.6860 | 0.9945$^{ns}$ |
| Female | (n = 1) | (n = 47) | (n = 143) | | | | | | 1.9224 |
| (n = 191) | 0.0052 | 0.2461 | 0.7487 | 0.1283 | 0.8717 | 0.0165 | 0.2236 | 0.7599 | 0.3824$^{ns}$ |
| INS3 | GG | AG | AA | G | A | GG | AG | AA | HWE $\chi^2$ |
| Population | (n = 44) | (n = 185) | (n = 126) | | | | | | 3.621 |
| (n = 355) | 0.1240 | 0.5230 | 0.3560 | 0.3845 | 0.6155 | 0.1480 | 0.475 | 0.3800 | 0.164$^{ns}$ |
| Males | (n = 20) | (n = 81) | (n = 63) | | | | | | 0.6048 |
| (n = 164) | 0.1220 | 0.4939 | 0.3841 | 0.3689 | 0.6311 | 0.1361 | 0.4656 | 0.3983 | 0.7390$^{ns}$ |
| Females | (n = 24) | (n = 104) | (n = 63) | | | | | | 3.5527 |
| (n = 191) | 0.1257 | 0.5445 | 0.3298 | 0.3997 | 0.6021 | 0.1583 | 0.4792 | 0.3625 | 0.1692$^{ns}$ |

ns: Not significant goodness-of-fit chi-square test for Hardy-Weinberg Equilibrium (HWE). INS2 (intron 2) and INS3 (3'-UTR)

Table 3: Effects of the INS3 on the growth traits

| Traits | GG (n = 44) | AG (n = 185) | AA (n = 126) | SEM | p-value |
|---|---|---|---|---|---|
| **BW (g/bird)** | | | | | |
| 28 day | 221.23$^{a}$ | 257.55$^{b}$ | 259.14$^{b}$ | 5.42 | 0.000 |
| 56 day | 740.59$^{a}$ | 793.81$^{ab}$ | 797.62$^{b}$ | 14.16 | 0.043 |
| 84 day | 1299.09 | 1379.19 | 1377.78 | 26.59 | 0.154 |
| **ADG (g/day/bird)** | | | | | |
| 28-56 day | 18.55 | 19.15 | 19.23 | 0.40 | 0.573 |
| 56-84 day | 19.95 | 20.91 | 20.72 | 0.56 | 0.567 |
| 28-84 day | 19.25 | 20.03 | 19.98 | 0.43 | 0.510 |
| **FI (g/day/bird)** | | | | | |
| 28-56 day | 55.39 | 55.46 | 55.74 | 1.02 | 0.962 |
| 56-84 day | 70.42 | 72.60 | 72.29 | 1.73 | 0.733 |
| 28-84 day | 62.90 | 64.03 | 64.02 | 1.22 | 0.836 |
| **FCR** | | | | | |
| 28-56 day | 3.01 | 2.92 | 2.92 | 0.03 | 0.075 |
| 56-84 day | 3.59 | 3.51 | 3.52 | 0.03 | 0.243 |
| 28-84 day | 3.29 | 3.22 | 3.22 | 0.02 | 0.086 |

Mean values in the same row having different superscripts are significantly different ($p<0.05$). BW: Body weight, ADG: Average daily gain, FI: Feed intake, FCR: Feed conversion ratio. SEM: Standard error of the mean

Table 4: Interaction of sex and INS3 on the growth traits

| | GG | | AG | | AA | | | |
|---|---|---|---|---|---|---|---|---|
| Traits | Male (n = 20) | Female (n = 24) | Male (n = 81) | Female (n = 104) | Male (n = 63) | Female (n = 63) | SEM | p-value |
| **BW (g/bird)** | | | | | | | | |
| 28 day | 235.85$^{ab}$ | 209.04$^{b}$ | 269.38$^{a}$ | 248.33$^{a}$ | 270.14$^{a}$ | 248.14$^{a}$ | 7.54 | 0.000 |
| 56 day | 787.50$^{ab}$ | 701.50$^{b}$ | 864.69$^{a}$ | 738.60$^{b}$ | 874.13$^{a}$ | 721.11$^{b}$ | 17.60 | 0.000 |
| 84 day | 1424.00$^{ab}$ | 1195.00$^{c}$ | 1513.46$^{a}$ | 1274.62$^{bc}$ | 1541.75$^{a}$ | 1213.81$^{c}$ | 31.96 | 0.000 |
| **ADG (g/day/bird)** | | | | | | | | |
| 28-56 day | 19.70$^{ab}$ | 17.59$^{bc}$ | 21.26$^{a}$ | 17.51$^{bc}$ | 21.57$^{a}$ | 16.89$^{c}$ | 0.48 | 0.000 |
| 56-84 day | 22.73$^{a}$ | 17.63$^{b}$ | 23.17$^{a}$ | 19.14$^{b}$ | 23.84$^{a}$ | 17.60$^{b}$ | 0.70 | 0.000 |
| 28-84 day | 21.22$^{a}$ | 17.61$^{b}$ | 22.22$^{a}$ | 18.33$^{b}$ | 22.71$^{a}$ | 17.24$^{b}$ | 0.51 | 0.000 |
| **FI (g/day/bird)** | | | | | | | | |
| 28-56 day | 58.49$^{ab}$ | 52.81$^{bc}$ | 60.74$^{a}$ | 51.35$^{c}$ | 61.31$^{a}$ | 50.18$^{c}$ | 1.26 | 0.000 |
| 56-84 day | 78.32$^{a}$ | 63.84$^{b}$ | 79.92$^{a}$ | 66.89$^{b}$ | 81.35$^{a}$ | 63.22$^{b}$ | 2.18 | 0.000 |
| 28-84 day | 68.40$^{a}$ | 58.32$^{b}$ | 70.33$^{a}$ | 59.12$^{b}$ | 71.33$^{a}$ | 56.70$^{b}$ | 1.46 | 0.000 |
| **FCR** | | | | | | | | |
| 28-56 day | 2.99$^{ab}$ | 3.03$^{b}$ | 2.87$^{a}$ | 2.96$^{ab}$ | 2.85$^{a}$ | 2.99$^{b}$ | 0.04 | 0.001 |
| 56-84 day | 3.47$^{ab}$ | 3.69$^{b}$ | 3.48$^{a}$ | 3.54$^{ab}$ | 3.43$^{a}$ | 3.61$^{b}$ | 0.04 | 0.000 |
| 28-84 day | 3.24$^{abc}$ | 3.34$^{a}$ | 3.18$^{bc}$ | 3.25$^{ab}$ | 3.15$^{c}$ | 3.30$^{a}$ | 0.03 | 0.000 |

BW: Body weight, ADG: Average daily gain, FI: Feed intake, FCR: Feed conversion ratio. Mean values in the same row having different superscripts are significantly different ($p<0.05$). SEM: Standard error of the mean

Table 5: Effects of the INS3 on the carcass traits

| Traits | GG (n = 44) | AG (n = 185) | AA (n = 126) | SEM | p-value |
|---|---|---|---|---|---|
| LW | 1375.00 | 1438.43 | 1434.21 | 29.91 | 0.406 |
| KW | 1340.00 | 1401.73 | 1398.02 | 29.12 | 0.405 |
| DFW | 1249.20 | 1304.06 | 1300.08 | 27.77 | 0.458 |
| CW | 973.16 | 1016.71 | 1011.75 | 22.75 | 0.487 |
| HW | 84.25 | 79.44 | 80.05 | 2.35 | 0.442 |
| WBM | 141.86 | 146.41 | 149.21 | 3.34 | 0.409 |
| WTM | 98.50 | 102.48 | 102.62 | 2.57 | 0.596 |
| WW | 111.36 | 116.10 | 118.10 | 3.04 | 0.417 |
| DW | 169.32 | 182.32 | 185.62 | 4.79 | 0.127 |
| SW | 56.36 | 59.59 | 60.87 | 1.87 | 0.357 |
| WIO | 142.43 | 150.16 | 149.40 | 3.08 | 0.290 |
| GW | 14.72$^{a}$ | 16.07$^{ab}$ | 16.59$^{b}$ | 0.37 | 0.013 |
| LRW | 23.76 | 25.41 | 24.76 | 0.67 | 0.275 |
| HTW | 6.53 | 7.39 | 7.20 | 0.23 | 0.065 |
| WAF | 23.41 | 26.30 | 23.37 | 1.65 | 0.219 |
| LSI | 1264.86 | 1320.98 | 1322.07 | 19.11 | 0.160 |
| CL | 298.14 | 305.45 | 308.02 | 4.28 | 0.389 |

LW (g): Live weight, KW (g): Killed weight, DFW (g): De-Feather weight, CW (g): Carcass weight, HW (g): Head weight, WBM (g): Weight of breast meat, WTM (g): Weight of thigh meat, WW (g): Wing weight, DW (g): Drumstick weight, SW (g): Shank weight, WIO (g): Weight of internal organs, GW (g): Gizzard weight, LRW (g): Liver weight, HTW (g): Heart weight, WAF (g): Weight of abdominal fat, LSI (mm): Length of small intestine, CL (mm): Caeca length. Mean values in the same row having different superscripts are significantly different (p<0.05). SEM: Standard error of the mean

Table 6: Interaction between sex and INS3 on the carcass traits

| | GG | | AG | | AA | | | |
|---|---|---|---|---|---|---|---|---|
| Traits | Male (n = 20) | Female (n = 24) | Male (n = 81) | Female (n = 104) | Male (n = 63) | Female (n = 63) | SEM | p-value |
| LW | 1527.50$^{a}$ | 1247.92$^{b}$ | 1581.85$^{a}$ | 1326.73$^{b}$ | 1613.81$^{a}$ | 1254.60$^{b}$ | 36.33 | 0.000 |
| KW | 1490.00$^{a}$ | 1215.00$^{b}$ | 1540.99$^{a}$ | 1293.27$^{b}$ | 1574.92$^{a}$ | 1221.11$^{b}$ | 35.28 | 0.000 |
| DFW | 1389.25$^{a}$ | 1132.50$^{b}$ | 1438.77$^{a}$ | 1199.15$^{b}$ | 1466.51$^{a}$ | 1133.65$^{b}$ | 33.69 | 0.000 |
| CW | 1091.85$^{a}$ | 874.25$^{b}$ | 1123.23$^{a}$ | 933.75$^{b}$ | 1147.52$^{a}$ | 875.98$^{b}$ | 27.72 | 0.000 |
| HW | 96.05$^{a}$ | 74.42$^{b}$ | 88.35$^{a}$ | 72.50$^{b}$ | 91.67$^{a}$ | 68.43$^{b}$ | 3.02 | 0.000 |
| WBM | 150.00$^{ab}$ | 135.08$^{b}$ | 155.88$^{a}$ | 139.04$^{b}$ | 161.84$^{a}$ | 136.57$^{b}$ | 4.51 | 0.000 |
| WTM | 109.60$^{a}$ | 89.25$^{b}$ | 114.67$^{a}$ | 92.98$^{b}$ | 117.11$^{a}$ | 88.13$^{b}$ | 3.19 | 0.000 |
| WW | 123.00$^{a}$ | 101.67$^{b}$ | 131.36$^{a}$ | 104.21$^{b}$ | 137.46$^{a}$ | 98.73$^{b}$ | 3.65 | 0.000 |
| DW | 189.50$^{ab}$ | 152.50$^{c}$ | 205.56$^{a}$ | 164.23$^{bc}$ | 215.21$^{a}$ | 156.03$^{c}$ | 5.82 | 0.000 |
| SW | 65.00$^{a}$ | 49.17$^{b}$ | 70.06$^{a}$ | 51.44$^{b}$ | 72.94$^{a}$ | 48.81$^{b}$ | 2.19 | 0.000 |
| WIO | 152.50$^{ab}$ | 134.04$^{b}$ | 159.05$^{a}$ | 143.24$^{b}$ | 159.78$^{a}$ | 139.02$^{b}$ | 4.18 | 0.000 |
| GW | 15.61$^{abc}$ | 13.99$^{a}$ | 16.54$^{ab}$ | 15.70$^{bc}$ | 17.35$^{a}$ | 15.82$^{abc}$ | 0.52 | 0.002 |
| LRW | 27.67$^{a}$ | 20.51$^{b}$ | 28.10$^{a}$ | 23.32$^{b}$ | 27.20$^{a}$ | 22.31$^{b}$ | 0.87 | 0.000 |
| HTW | 7.78$^{ab}$ | 5.49$^{d}$ | 8.14$^{a}$ | 6.81$^{bc}$ | 8.20$^{a}$ | 6.19$^{cd}$ | 0.30 | 0.000 |
| WAF | 23.86 | 23.03 | 25.78 | 26.71 | 23.68 | 23.05 | 2.34 | 0.660 |
| LSI | 1302.95$^{ab}$ | 1233.13$^{b}$ | 1371.17$^{a}$ | 1281.88$^{b}$ | 1368.35$^{a}$ | 1275.79$^{b}$ | 26.42 | 0.000 |
| CL | 315.65$^{ab}$ | 283.54$^{b}$ | 319.51$^{a}$ | 294.51$^{b}$ | 319.81$^{a}$ | 296.24$^{b}$ | 5.80 | 0.000 |

LW (g): Live weight, KW (g): Killed weight, DFW (g): De-Feather weight, CW (g): Carcass weight, HW (g): Head weight, WBM (g): Weight of breast meat, WTM (g): Weight of thigh meat, WW (g): Wing weight, DW (g): Drumstick weight, SW (g): Shank weight, WIO (g): Weight of internal organs, GW (g): Gizzard weight, LRW (g): Liver weight, HTW (g): Heart weight, WAF (g): Weight of abdominal fat, LSI (mm): Length of small intestine, CL (mm): Caeca length. Mean values in the same row having different superscripts are significantly different (p<0.05). SEM: Standard error of the mean

in interaction between sexes and chemical compositions of breast meat (Table 9). Additionally, there was no interaction between sexes and genotypes according to the quality of breast meat, which is shown in Table 10.

## DISCUSSION

In this study, both the INS2 (intron 2) and INS3 (3'-UTR) was not defined within the coding region of insulin but it was understood that the SNPs in non-coding regions like 5'UTR, 3'UTR and introns could also affect gene expression levels because of regulatory elements present in 5'UTR or 3'UTR regions[11].

Insulin hormone is considered as one of the peptide hormones secreted from the Beta cell of Langerhans Island of the pancreas. Insulin plays main roles in cellular, regulating carbohydrate, lipid and protein metabolism as well as promoting cell division and growth[12,13]. In early studies, the SNPs within insulin were identified in the different populations of chicken like Noi, Tau Vang, Cobb500 at loci C1549T, T3737C

Table 7: Effects of the INS3 on the meat quality traits

| Traits | GG (n = 44) | AG (n = 185) | AA (n = 126) | SEM | p-value |
|---|---|---|---|---|---|
| **Breast meat** | | | | | |
| **3 hrs post-mortem** | | | | | |
| pH value | 5.64 | 5.62 | 5.63 | 0.02 | 0.791 |
| L* | 57.44 | 57.41 | 57.41 | 0.48 | 0.999 |
| a* | 1.38 | 1.02 | 1.14 | 0.22 | 0.584 |
| b* | 12.32 | 12.31 | 12.35 | 0.33 | 0.994 |
| Cooking loss (%) | 28.23$^{a}$ | 24.87$^{ab}$ | 25.45$^{b}$ | 0.74 | 0.018 |
| Drip loss (%) | 2.69 | 2.62 | 2.81 | 0.11 | 0.333 |
| **24 hrs post-mortem** | | | | | |
| pH value | 5.62$^{a}$ | 5.54$^{ab}$ | 5.57$^{b}$ | 0.02 | 0.016 |
| L* | 57.32 | 57.35 | 57.06 | 0.47 | 0.846 |
| a* | 1.70 | 1.40 | 1.54 | 0.24 | 0.703 |
| b* | 10.91 | 11.57 | 11.31 | 0.35 | 0.477 |
| Cooking loss (%) | 33.48$^{a}$ | 29.53$^{ab}$ | 30.96$^{b}$ | 0.90 | 0.020 |
| Drip loss (%) | 2.27 | 2.08 | 1.95 | 0.09 | 0.118 |
| **48 hrs post-mortem** | | | | | |
| pH value | 5.58 | 5.53 | 5.56 | 0.02 | 0.068 |
| L* | 56.85 | 56.79 | 56.69 | 0.43 | 0.968 |
| a* | 1.65 | 1.83 | 1.77 | 0.27 | 0.917 |
| b* | 12.16 | 11.93 | 12.38 | 0.34 | 0.475 |
| Cooking loss (%) | 29.65 | 30.18 | 31.25 | 0.86 | 0.410 |
| Drip loss (%) | 1.74 | 1.58 | 1.62 | 0.10 | 0.608 |
| **Thigh meat** | | | | | |
| **3 hrs post-mortem** | | | | | |
| pH value | 5.95 | 5.93 | 5.97 | 0.02 | 0.332 |
| L* | 57.14 | 56.58 | 55.65 | 0.47 | 0.082 |
| a* | 4.44$^{a}$ | 4.83$^{a}$ | 5.62$^{b}$ | 0.28 | 0.011 |
| b* | 11.72 | 10.89 | 10.62 | 0.44 | 0.332 |
| Cooking loss (%) | 30.64$^{a}$ | 27.17$^{b}$ | 28.83$^{ab}$ | 0.74 | 0.007 |
| Drip loss (%) | 2.56 | 2.57 | 2.85 | 0.16 | 0.228 |
| **24 hrs post-mortem** | | | | | |
| pH value | 5.91$^{a}$ | 5.81$^{b}$ | 5.84$^{ab}$ | 0.03 | 0.033 |
| L* | 57.32 | 56.04 | 55.91 | 0.42 | 0.124 |
| a* | 4.59 | 5.20 | 5.71 | 0.32 | 0.091 |
| b* | 10.32 | 11.23 | 10.50 | 0.46 | 0.244 |
| Cooking loss (%) | 32.47 | 32.21 | 31.17 | 0.97 | 0.560 |
| Drip loss (%) | 2.36 | 2.29 | 2.44 | 0.11 | 0.459 |
| **48 hrs post-mortem** | | | | | |
| pH value | 5.89$^{a}$ | 5.78$^{b}$ | 5.82$^{ab}$ | 0.02 | 0.017 |
| L* | 55.93$^{ab}$ | 56.40$^{a}$ | 55.19$^{b}$ | 0.43 | 0.043 |
| a* | 6.94 | 6.22 | 6.65 | 0.44 | 0.491 |
| b* | 13.04 | 12.28 | 12.05 | 0.57 | 0.582 |
| Cooking loss (%) | 31.02 | 31.44 | 31.34 | 0.83 | 0.949 |
| Drip loss (%) | 2.12 | 1.97 | 2.10 | 0.10 | 0.455 |

Mean values in the same row having different superscripts are significantly different ($p<0.05$). SEM: Standard error of the mean, L*: Lightness, a*: Redness, b*: Yellowness

and A3971G[14] and Ross308 at loci C1549T and G3971A[15]. Moreover, Nie *et al.*[1] detected 7 SNPs two of which were located on 5'UTR (C195T, AY438372, *BsuR*I) or 3'UTR (A3971G, AY438372, *Msp*I) and five of which (T409C, AY438372, *Taq*I, A428G, AY438372, *Nde*I, C1218A, AY438372, *Nde*I, C1549T, AY438372, *Msp*I, T3737C, AY438372, *Msp*I) belonged to the intron 2 by using the PCR-RFLP method. They were found in Chinese Taihe Silkies and Xinghua chickens instead of the two commercial breeds (Leghorn and White Recessive Rock). This could be due to (i) the selective pressure on the commercial herds was formed quite early and ineffective genotypes were removed from the herd, (ii) indigenous chickens in some Asian countries often owned variable genotypes in the uncontrolled crossing of farmers intending to improve chicken performance by using hybrid advantage while genetic technology was not applied in the selection support.

In the early study conducted by Qiu *et al.*[2], the association of haplotypes combined with the SNPs at loci A428G, C1549T, T3737C and A3971G of insulin gene with growth and body composition traits were found in the White Recessive Rock line x Xinghua chickens. It was implied that insulin plays a different role in poultry than in mammals and insulin might

Table 8: Interaction between sex and INS3 on the meat quality traits

| | GG | | AG | | AA | | | |
|---|---|---|---|---|---|---|---|---|
| Traits | Male (n = 20) | Female (n = 24) | Male (n = 81) | Female (n = 104) | Male (n = 63) | Female (n = 63) | SEM | p-value |
| **Breast meat** | | | | | | | | |
| **3 hrs post-mortem** | | | | | | | | |
| pH value | 5.70 | 5.59 | 5.65 | 5.60 | 5.62 | 5.64 | 0.02 | 0.077 |
| L* | 59.31 | 55.88 | 57.57 | 57.28 | 57.61 | 57.20 | 0.68 | 0.254 |
| a* | 1.21 | 1.53 | 1.13 | 0.94 | 1.03 | 1.25 | 0.32 | 0.847 |
| b* | 11.56$^{ab}$ | 12.96$^{ab}$ | 11.46$^{a}$ | 12.96$^{b}$ | 11.63$^{ab}$ | 13.06$^{b}$ | 0.46 | 0.002 |
| Cooking loss (%) | 27.38$^{ab}$ | 28.94$^{a}$ | 25.93$^{ab}$ | 24.05$^{b}$ | 25.41$^{ab}$ | 25.49$^{ab}$ | 1.04 | 0.039 |
| Drip loss (%) | 2.42 | 2.92 | 2.72 | 2.54 | 2.74 | 2.87 | 0.16 | 0.300 |
| **24 hrs post-mortem** | | | | | | | | |
| pH value | 5.68$^{ab}$ | 5.57$^{a}$ | 5.57$^{ab}$ | 5.52$^{b}$ | 5.60$^{ab}$ | 5.53$^{ab}$ | 0.02 | 0.000 |
| L* | 58.36 | 56.46 | 57.81 | 56.99 | 57.21 | 56.90 | 0.66 | 0.552 |
| a* | 1.50 | 1.88 | 1.43 | 1.38 | 1.40 | 1.67 | 0.34 | 0.919 |
| b* | 10.15$^{ab}$ | 11.54$^{ab}$ | 10.51$^{b}$ | 12.39$^{a}$ | 10.88$^{b}$ | 11.75$^{ab}$ | 0.49 | 0.001 |
| Cooking loss (%) | 31.52 | 35.11 | 30.16 | 29.04 | 30.97 | 30.95 | 1.28 | 0.064 |
| Drip loss (%) | 2.30 | 2.24 | 2.08 | 2.09 | 1.90 | 1.99 | 0.14 | 0.466 |
| **48 hrs post-mortem** | | | | | | | | |
| pH value | 5.62$^{a}$ | 5.55$^{ab}$ | 5.56$^{ab}$ | 5.50$^{b}$ | 5.59$^{a}$ | 5.53$^{ab}$ | 0.02 | 0.001 |
| L* | 57.66 | 56.17 | 56.68 | 56.87 | 56.57 | 56.81 | 0.61 | 0.889 |
| a* | 1.53 | 1.75 | 2.04 | 1.65 | 1.52 | 2.01 | 0.38 | 0.789 |
| b* | 11.40$^{ab}$ | 12.79$^{ab}$ | 10.75$^{a}$ | 12.85$^{b}$ | 11.93$^{ab}$ | 12.84$^{b}$ | 0.46 | 0.000 |
| Cooking loss (%) | 30.64 | 28.83 | 29.98 | 30.33 | 31.11 | 31.39 | 1.23 | 0.790 |
| Drip loss (%) | 1.57 | 1.87 | 1.55 | 1.61 | 1.47 | 1.77 | 0.14 | 0.321 |
| **Thigh meat** | | | | | | | | |
| **3 hrs post-mortem** | | | | | | | | |
| pH value | 5.97 | 5.93 | 5.93 | 5.94 | 6.00 | 5.94 | 0.03 | 0.323 |
| L* | 56.94 | 57.31 | 56.72 | 56.48 | 55.98 | 55.32 | 0.66 | 0.319 |
| a* | 4.38 | 4.50 | 4.83 | 4.83 | 5.47 | 5.76 | 0.40 | 0.096 |
| b* | 11.40 | 11.99 | 10.34 | 11.33 | 10.38 | 10.85 | 0.63 | 0.388 |
| Cooking loss (%) | 29.51$^{ab}$ | 31.57$^{a}$ | 26.82$^{b}$ | 27.44$^{ab}$ | 29.71$^{ab}$ | 27.94$^{ab}$ | 1.05 | 0.022 |
| Drip loss (%) | 2.38 | 2.70 | 2.56 | 2.57 | 2.79 | 2.91 | 0.22 | 0.597 |
| **24 hrs post-mortem** | | | | | | | | |
| pH value | 5.90 | 5.92 | 5.80 | 5.81 | 5.85 | 5.82 | 0.04 | 0.201 |
| L* | 58.74 | 56.13 | 56.17 | 55.94 | 56.34 | 55.53 | 0.60 | 0.075 |
| a* | 3.93 | 5.15 | 5.01 | 5.35 | 5.53 | 5.87 | 0.46 | 0.192 |
| b* | 10.73 | 9.97 | 11.09 | 11.33 | 9.84 | 11.06 | 0.65 | 0.334 |
| Cooking loss (%) | 27.96 | 36.26 | 32.15 | 32.26 | 30.63 | 31.64 | 1.36 | 0.067 |
| Drip loss (%) | 2.32 | 2.38 | 2.33 | 2.26 | 2.40 | 2.47 | 0.15 | 0.854 |
| **48 hrs post-mortem** | | | | | | | | |
| pH value | 5.91$^{ab}$ | 5.87$^{ab}$ | 5.81$^{ab}$ | 5.76$^{a}$ | 5.86$^{b}$ | 5.79$^{ab}$ | 0.03 | 0.015 |
| L* | 55.63 | 56.17 | 56.61 | 56.25 | 55.34 | 55.04 | 0.61 | 0.222 |
| a* | 6.89 | 6.98 | 6.34 | 6.13 | 6.48 | 6.82 | 0.63 | 0.887 |
| b* | 13.31 | 12.82 | 11.97 | 12.50 | 10.92 | 13.14 | 0.81 | 0.257 |
| Cooking loss (%) | 30.03 | 31.83 | 30.71 | 31.96 | 30.70 | 31.96 | 1.18 | 0.769 |
| Drip loss (%) | 2.34 | 1.93 | 1.87 | 2.05 | 1.92 | 2.28 | 0.15 | 0.108 |

Mean values in the same row having different superscripts are significantly different ($p<0.05$). SEM: Standard error of the mean, L*: Lightness, a*: Redness, b*: Yellowness

be associated with early growth[2]. This was also observed in Noi chickens at 28 and 56 days of age where the Noi ones with the AA genotype had the highest BW, followed by the AG and GG ones. Rapid growth in the early stages of age was significant in animal production in general and poultry production in particular. Normally, animal individuals or herds that performed a strong growth in the immature stage would have high feed-intake, health status and survival rate. This had positive effects on their subsequent development as well as economic efficiency. According to Qiu *et al.*[2], the association of the insulin gene with the early growth of chickens might be helpful to understand the genetic mechanism underlying poultry growth. Particularly, at locus T3737C of the insulin gene, a significant association among genotypes with Body Weight (BW) at 10-12 weeks of age and Average Daily Gain (ADG) at 6-8 weeks of age was displayed in the Mia Vietnamese local chicken breed population[16].

Table 9: Effects of the INS3 on the chemical compositions of breast meat

| | Genotypes | | | | |
|---|---|---|---|---|---|
| Traits | GG (n = 44) | AG (n = 185) | AA (n = 126) | SEM | p-value |
| DM | 24.79 | 25.18 | 25.17 | 0.12 | 0.118 |
| CP | 24.01 | 23.86 | 23.79 | 0.12 | 0.550 |
| EE | 0.47 | 0.53 | 0.54 | 0.02 | 0.174 |

DM: Dry matter, CP: Crude protein, EE: Ether extract, SEM: Standard error of the mean

Table 10: Interaction between sex and INS3 on the chemical compositions of breast meat

| | GG | | AG | | AA | | | |
|---|---|---|---|---|---|---|---|---|
| Traits | Male (n = 20) | Female (n = 24) | Male (n = 81) | Female (n = 104) | Male (n = 63) | Female (n = 63) | SEM | p-value |
| DM | 24.70 | 24.87 | 25.10 | 25.23 | 25.08 | 25.26 | 0.17 | 0.318 |
| CP | 23.68 | 24.28 | 23.75 | 23.95 | 23.83 | 23.75 | 0.16 | 0.312 |
| EE | 0.50 | 0.44 | 0.51 | 0.55 | 0.57 | 0.51 | 0.03 | 0.173 |

DM: Dry matter, CP: Crude protein, EE: Ether extract, SEM: Standard error of the mean

Interaction between sexes and genotypes on BW, ADG, FI and FCR was significantly different ($p<0.05$). These were due to difference between sexes, not among genotypes. It was demonstrated that males always had a higher BW, ADG and FI than females did, while the FCR did the opposite. This is consistent with the characteristics of growth and feed consumption in poultry normally.

A linkage of three insulin's SNPs like G11303145A, C11304264T, T11306685C and C11306451T with chicken fatness and muscle fibre traits indicated that (i) the locus G11303145A was associated with the transversal area of the leg muscle fibre and the transversal area of the breast muscle fibre. Positive additive genetic influences were observed at a highly significant level for the transversal area of the leg muscle fibre ($p<0.01$) and highly significant negative additive genetic influences were observed for the transversal area of the breast muscle fibre ($p<0.05$), while (ii) the locus T11306685C was linked with the fat width and the crude fatty content of the leg muscle ($p<0.01$) and the locus C11306451T was associated ($p<0.05$) with the crude fatty content of the leg muscle[3]. Additionally, in another Vietnamese local breed, Tau Vang chicken, Khoa[17] found a significant association between A3971G SNP and weight of abdominal fat, rate of breast and thigh meat. Moreover, in Ross308 hybrid chickens, Shayma, R.U. and E.H. Al-Anbari[14] indicated that there were significant effects of A3971G locus on economic traits such as carcass weight, live body weight, relative cuts weights and breast weight where males had a higher performance of these traits than females did. Additionally, an estimation of breeding value and genetic variations for the C1549T of insulin gene hormones on average body weight data in Ross 308 broilers were calculated and analyzed[18]. It is known that the genes of the somatotropic axis played crucial roles in the regulation of growth, development and differentiation.

Chicken meat quality could be noticed on traits of appearance, colour, taste, fat content, texture and tenderness usually depending on species, genetic background, metabolic status of the pre-mortem animal, the protein complement of the muscle and environmental factors. In this study, the significant effects of the INS3 on meat quality traits such as pH, cooking loss, colour (L*, a*, b*) were demonstrated. However, they were not consistent at all-time points of the investigation.

Poultry meat, breast muscles, in particular, contains several important classes of high nutritive values. Noi chicken has ever been considered as a potential resource for meat quality which was suitable for consumers' tastes[6-8], similarly to other slow-growing chickens[19-21]. Furthermore, it was implied that the chemical compositions of muscles were modified by genotypes[22,23]. However, in this study, the INS3 genetic associations of insulin with chemical compositions were not found in the Noi population. Using a high intake of dietary animal protein led to a reduction of insulin sensitivity by as much as 25%[24] while swapping animal meat for soy protein (like tofu) significantly improved insulin sensitivity[25]. It was indicated that the capacity of insulin to enhance the accumulation by a muscle of several amino acids such as threonine or beta-alanine and gamma-aminobutyric acid uptake was not confirmed or did not respond to the hormone, respectively[26]. Present data provided a useful information to recommend that INS3 is a potential genetic resource for selecting Vietnamese indigenous Noi chickens.

## CONCLUSION

It was indicated that two SNPs were detected at loci T3737C (INS2) and A3971G (INS3) where as the frequency of the CC genotype at locus INS2 was low. The locus INS3 linked to BW at an early stage of age as well as pH value, colour and

cooking loss of meat which were valuable genetic resources for breeding programs of Noi chicken, one of the recognized and favourite chicken breeds in Vietnam, although the contribution of the insulin on the observed traits was not much.

## SIGNIFICANCE STATEMENT

The obtained results in this study could be applied in breeding programs of Noi chicken whereas the INS3 should be considered as one of the potential markers for assisting selection across the Noi population individually and Vietnamese local poultry chickens generally.

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