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Research Article

The Association between Apolipoprotein B2 (Apob2) Gene with Body Weight and Carcass Traits of Noi Native Chickens

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Abstract | The present study was carried out to determine how ApoB2 gene polymorphisms are related to growth-related traits and carcass characteristics of Noi chickens. A total of 183 birds were examined in the study. Body weight was recorded individually at days 1, 35, 63, and 91, and carcass parameters were determined at 91 days for all chickens. For determining single nucleotide polymorphisms, the PCR-RFLP method was used with the help of *Acy*I restriction enzyme. A polymorphic site was found in the population, with the frequencies of 11, 12, and 22 genotypes being 0.18, 0.67, and 0.15, respectively. At the end of the experiment, better body weight (965-976 g/bird) and daily weight gain (10.5-10.6 g/bird/day) were found in chickens with 11 and 12 genotypes. Carcass parameters were superior in chickens carrying these genotypes (P<0.05) except for thigh and drumstick weight. In breeding selection for improving growth and carcass traits in Noi native chickens, allele 1 was more advantageous.

Keywords | ApoB2, Carcass, Chicken, Growth, Linkage

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INTRODUCTION

The advances in molecular techniques, including quantitative trait locus (QTL) analysis and candidate gene approach, have improved our ability to uncover genetic markers for economically significant animal traits (Zahra et al., 2011). Understanding functional genes aids in the selection of animals with desirable breeding characteristics and enhances growth rates, body weight, and carcass characteristics (Manjula et al., 2018). It is of particular interest when applied to indigenous breeds, as they are becoming an increasingly favored food source due to their distinctive meat quality.

Apolipoprotein B (ApoB) is one of the most hydrophobic apolipoproteins with a molecular weight of 210 kDa. It serves as the ligand and the primary structural protein for the triglyceride-rich low-density lipoprotein receptor, which is involved in the transport and metabolism of lipids and energy (Glickman et al., 1986; Schumaker et al., 1994). It is synthesized in the liver and is required to transport lipids and cholesterol, and is an ApoB receptor activator that promotes cell aggregation and low-density lipoprotein internal activity (Kirchgessner et al., 1987). Additionally, the blood ApoB concentration is tightly linked with the body weight characteristic, abdominal fat, and weight gain in animals (Sato, 2016; Zhang et al., 2006). A polymorphic site in exon 26 of the Apolipoprotein B2 (ApoB2) gene (Zhang et al., 2006), a mutation producing truncations in the ApoB gene family, has been proved to associate with body growth and fatness trait on Iranian commercial broiler lines (Seyedabadi et al., 2010) and body weight of Thai

native chicken (Supakorn, 2016). Information related to this ApoB2 gene polymorphism in the indigenous chicken breed is still limited; therefore, the objective of this study was to identify this locus and assess its linkage with growth and carcass traits of Noi chicken, a local breed popular in the Mekong Delta of Vietnam.

MATERIALS AND METHODS

Bird care and feeding

Chickens from different brooding stocks of a breeding farm were cared for and handled in accordance with the Animal Husbandry Law of Vietnam (32/2018/QH14). A total of 183 Noi chickens were raised at the experimental farm of Tra Vinh University with the rations according to the diet formula shown in Table 1. The feeding diet comprised yellow maize, soybean meal, rice bran, lysine and methionine, dicalcium phosphate, shell, salt, vitamin and mineral premix. From 1-28 days of age, the chicks were brooded on rice husks with a density of 50 chickens/m^2, and then they were raised on the floor with 12 chickens/m^2. The birds were fed *ad libitum,* and freshwater was given all time. All birds were numbered individually for phenotypic identification.

Table 1: Experimental diets of Noi chickens

Items	Experimental period		
	1-28 day	29-56 day	57-91 day
Ingredients			
Yellow maize (%)	56.3	56.1	53.6
Soybean meal (%)	29.3	23.4	17.0
Rice bran (%)	9.53	15.7	25.4
Lysine (%)	0.62	0.54	0.45
Methionin + cysteine (%)	0.19	0.18	0.17
Dicalcium phosphate (%)	1.60	1.60	1.00
Shell (kg)	1.80	1.80	1.76
Premix* (%)	0.25	0.25	0.25
Salt (%)	0.40	0.40	0.40
Nutritional values			
Dry matter (%)	86.9	86.9	86.9
Crude protein (%)	19.0	17.0	15.0
ME (kcal/kg)	2,900	2,900	2,900
Lysin (%)	1.12	1.03	0.90
Methionin + cysteine (%)	0.79	0.72	0.64
Threonine (%)	0.86	0.81	0.78
Calcium (%)	1.18	1.18	1.03
Phosphorus (%)	0.84	0.89	0.80

*: premix includes vitamin and micro-minerals

Bodyweight and carcass characteristics

At 1, 35, 63, and 91 days of age, chickens were weighed individually at 6 a.m. before feeding to determine body weight and calculate daily weight gain. At the end of the experiment, all broilers were slaughtered by exsanguination for carcass evaluation, of which the breast, thigh and drumstick muscle were collected as described by Goliomytis et al. (2003).

Genotyping of chickens

Chicken feather quills were collected for DNA isolation using the phenol/chloroform procedure of Bello et al. (2001). The ApoB2 polymorphism was genotyped by the Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) method with the *Acy*I restriction enzyme. The forward and reverse primer sequences were 5'-CATATTTCTAATGGCATCCAG-3' and 5'-TTCCCAGCGTTATTTCCG-3', respectively (Zhang et al., 2006). Briefly, each PCR reaction was performed using a volume of 10 µL with 50 ng of genomic DNA, 0.25 µM each primer, 0.25 µM each dNTP, 1X PCR buffer, and 1 U *Taq* DNA polymerase. The thermal conditions were as follows: 94°C for 10 min; 35 cycles of 94°C for 40 s, 57°C within 40 s, and 72°C within 45 s; and 72°C within 10 min for the final extension. PCR products were further digested at 37°C overnight with *Acy*I restriction enzyme, and the genotypes were determined under UV light after electrophoresis on 2.0% agarose gel at 80 V, 400 mA within 30 min.

Statistical analysis

The genotypic and allelic frequencies were analyzed using Microsoft Excel, and the method of Rodriguez et al. (2009) was applied to estimate the Hardy-Weinberg equilibrium. The association between genotypes and chicken growth and carcass traits was investigated using the General Linear Model procedure of the Minitab version 16.2 software, with the effects of sex and genotypes included in the model. Tukey's pairwise comparisons ($P<0.05$) were applied to determine the differences between genotypes.

RESULTS AND DISCUSSION

Genotype and allele frequency

The ApoB2_*Acy*I polymorphism in the ApoB2 gene was investigated. The PCR products had a length of 779 bp (Figure 1), and the PCR-RFLP results indicated two allele forms that corresponded to three genotypes: 11 (779 bp), 12 (779 bp, 658 bp, 121 bp), and 22 (658 bp, 121 bp) (Figure 2; the 121 bp band was not visible on the gel). The genotypic and allelic frequencies of the ApoB2_*Acy*I polymorphism in Noi broilers are shown in Table 2. At this locus, allele 1 was slightly more common than allele 2, while the frequency of the heterozygous genotype 12 appearing

Table 2: Genotype and allele frequency of the polymorphism in Noi chicken

Locus	Observed genotype[1]			Total	Allelic frequency		χ^2 (HW)	*p* (HW)
ApoB2_*Acy*I	11	12	22		1	2		
	33 (0.18)	122 (0.67)	28 (0.15)	183	0.52	0.48	20.5	<0.001

[1]Data presented as an individual number followed the frequency in parenthesis; HW: Hardy-Weinberg

Table 3: Association of the ApoB2_*Acy*I polymorphism with growth traits

Traits	Genotypes			P
	11	12	22	
Body weight (g/bird)				
Day 1	32.1 ± 0.52	31.5 ± 0.27	32.3 ± 0.56	0.346
Day 35	184 ± 4.21	180 ± 2.19	170 ± 4.50	0.059
Day 63	538 ± 14.9^{a}	540 ± 7.76^{a}	480 ± 16.0^{b}	0.004
Day 91	965 ± 22.2^{ab}	976 ± 11.5^{a}	910 ± 23.8^{b}	0.044
Daily weight gain (g/bird/day)				
1-35 day	4.50 ± 0.13	4.40 ± 0.07	4.10 ± 0.13	0.052
36-63 day	13.1 ± 0.50^{ab}	13.3 ± 0.26^{a}	11.5 ± 0.53^{b}	0.009
64-91 day	15.8 ± 0.56	16.2 ± 0.29	15.9 ± 0.59	0.825
Overall (1-91 day)	10.5 ± 0.25^{ab}	10.6 ± 0.13^{a}	9.9 ± 0.27^{b}	0.041

^{a,b}: means with different letters in the same row are significantly different (P<0.05)

Table 4: Association of the ApoB2_*Acy*I polymorphism with carcass characteristics

Traits	Genotypes			P
	11	12	22	
Carcass weight (CW), g	641 ± 17.2^{ab}	666 ± 8.91^{a}	614 ± 18.4^{b}	0.032
Breast weight, g	86.3 ± 1.66^{a}	85.2 ± 0.86^{a}	77.2 ± 1.77^{b}	0.000
Thigh + drumstick, g	147 ± 2.39	148 ± 1.24	143 ± 2.56	0.225
Carcass, % BW	66.3 ± 0.41^{b}	68.0 ± 0.21^{a}	67.3 ± 0.44^{ab}	0.001
Breast, % CW	13.5 ± 0.22^{a}	13.0 ± 0.11^{ab}	12.7 ± 0.23^{b}	0.029
Thigh + drumstick, % CW	23.0 ± 0.34^{ab}	22.5 ± 0.18^{b}	23.7 ± 0.36^{a}	0.018

^{a,b}: means with different letters in the same row are significantly different (P<0.05)

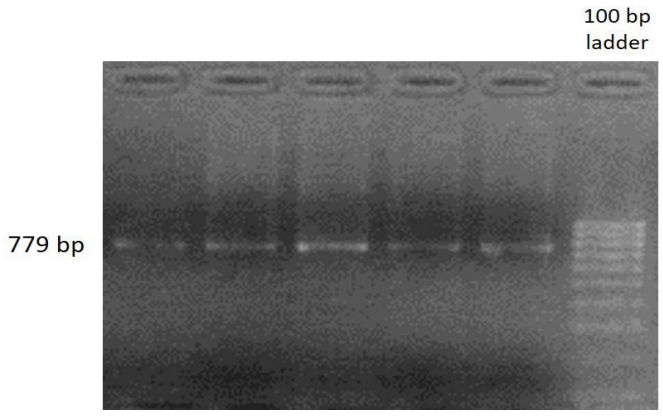

Figure 1: PCR product of the ApoB2 gene

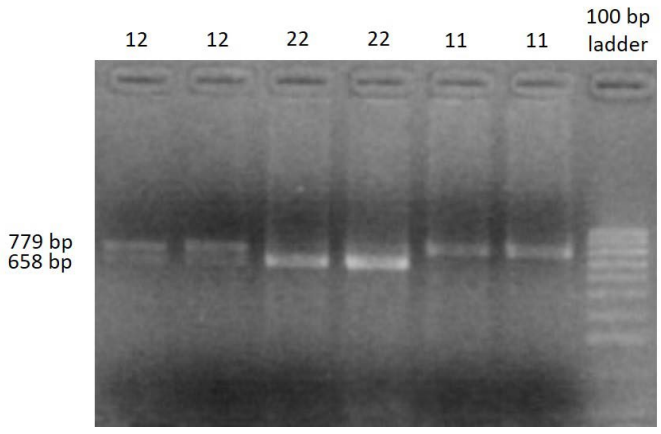

Figure 2: PCR-RFLP patterns of different genotypes

in a population was higher than that of the two homozygous genotypes, and the genotype frequency of this polymorphism varied from the Hardy-Weinberg equilibrium (P<0.001). The findings were comparable with those of Supakorn (2016), who observed that the genotype frequency of the heterozygous 12 in the ApoB2 gene was higher than those of the two homozygous types 11 and 22. Cruz et al.

(2015) also observed that the ApoB2 gene polymorphism has a greater incidence of heterozygotes than homozygotes. Similar results were additionally reported in commercial broilers, native and crossbred chickens of Thailand (Buasook et al., 2014; Seyedabadi et al., 2010; Zhang et al., 2006). However, Shahabi et al. (2012) discovered that genotype 22 appeared more significant than the other two genotypes when investigating the Khazak chicken breed. Furthermore, when Hardy-Weinberg equilibrium was postulated in the current study, a substantial difference in genotype and allele frequencies was discovered. Random selection in a population was a dominant heritable aspect of Hardy-Weinberg equilibrium. The Noi chicken population came from the same animal breeding farm, which may explain why gene polymorphism did not follow this principle.

Association of the ApoB2_*Acy*I polymorphism with growth traits

Table 3 shows the influence of ApoB2 genetic polymorphism on the body weight gain of the birds at different days of age. In general, the growth rate of Noi broilers with the 12 and 11 genotypes was higher than that of carrying the 22 genotype. Innerarity et al. (1996) and Van Hemert (2007) reported that the ApoB gene was required for energy transfer and metabolism and involved lipid transport, digestion, and meal absorption. It may directly or indirectly affect fat storage and development, leading to a change in body weight. Hendricks et al. (2009) discovered that four-week-old chickens had a particular level of plasma adiponectin, and age-related changes or rapid growth may cause a decrease in the amount of circulating adiponectin. ApoB has also been reported to associate with body weight, abdominal fat, and other growth-related characteristics (Decai et al., 2017). The present study indicated a significant influence of the ApoB2_*Acy*I locus on growth-related traits of Noi chickens, in which chickens with 11 and 12 genotypes were superior.

Similarly, Seyedabadi et al. (2010) also found that chickens with the CC and CD genotypes (equivalent to the 11 and 12 genotypes) gained more rapidly. In addition, Zhang et al. (2006) showed the influence of the ApoB2 gene on body weight in commercial broiler lines at weeks 1 and 3 with greater body weight in chickens of TT (11) and TG (12) genotypes. Our results were also in line with the conclusion of Supakorn (2016) that the ApoB2_*Acy*I polymorphic site was closely associated with body weight at 12 weeks in an indigenous Thai chicken breed, of which allele 1 was of interest for breeding selection. Research by Supakorn (2016) showed that Luenghangkhao chickens carrying the 11 genotype had a higher body weight; whereas those of the Pradhuhangdum, Chee, and the Red Jungle Fowls breeds with 11 and 22 genotypes grew faster.

Association of the ApoB2_*Acy*I polymorphism with carcass characteristics

The ApoB2 gene polymorphism influenced most carcass parameters examined, except for thigh and drumstick weight (Table 4). The advantage of allele 1 was also demonstrated. Previously, Seyedabadi et al. (2010) found a linkage between ApoB2 genotype and carcass indicators, comprising carcass weight, breast, and drumstick weight percentages, with higher values in birds carrying 11 and 12 genotypes. Our study revealed that allele 1 contributed to higher carcass parameters than allele 2, which was consistent with the findings of Supakorn (2016) and confirmed the report of Seyedabadi et al. (2010) that allele 1 was linked to the QTL of higher growth and body composition traits. It was stated by Cruz et al. (2015) that ApoB2 gene polymorphism affected feed intake and breast weight. Fat storage in chickens is related to body weight, which was utilized as a selection criterion. The ApoB2 gene may modify thigh and breast fat metabolism, feed consumption, and breast protein storage in broiler chickens; thus, it may be the QTL that dictates chicken growth and body composition.

CONCLUSIONS

A mutation at the ApoB2_*Acy*I locus was detected with two alleles, and three genotypes available in the chicken population studied. Allele 1 of this polymorphism was more favorable in breeding selection for improving growth and carcass traits in Noi native chickens.

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CONFLICT OF INTEREST

We certify that there is not any conflict of interest with any financial, personal, or other organization related to the material mentioned in the manuscript.

NOVELTY STATEMENT

Allele 1 of the ApoB2_AcyI polymorphism was more advantageous in breeding selection for enhancing growth and carcass quality in Noi native chickens.

AUTHORS CONTRIBUTION

LTTL, LTH and NTN contributed to the design of the study. NTAT, NTHN and LTP conducted the data collec-

tion and analysis. LTTL, LTH and NTN prepared the first draft and revised the manuscript. All authors have read and approved the final manuscript.

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