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Study on the relationship between single nucleotide polymorphisms of the coccidiosis-resistance candidate zyxin gene exon 1 and carcass traits in the Jinghai yellow chicken

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Abstract: Previous research revealed that several single nucleotide polymorphisms (SNPs) of zyxin were significantly associated with coccidiosis-resistance parameters. In this research, the Jinghai yellow chicken was chosen to detect the SNPs of zyxin gene exon 1 by PCR-SSCP and DNA sequencing techniques, and was also used to analyze the effect of zyxin SNPs on carcass traits. A single mutation was found with 3 genotypes, designated as AA, AB, and BB. The results showed that BB was significantly different from AA and AB in terms of abdominal fat weight and slaughter rate for cockerel ($P < 0.05$), while there was no significant difference between AA and AB ($P > 0.05$). The heart weight, breast muscle rate, and drumstick muscle rate of AB were significantly higher than those of AA and BB for pullet ($P < 0.05$), while semieviscerated rate and eviscerated rate of AB were lower than those of AA and BB. Except for slaughter rate, all traits related to carcass traits showed significant differences between the 3 genotypes for both cockerel and pullet ($P < 0.05$). These results indicated that the selection of zyxin gene for coccidiosis resistance might affect some unimportant traits, such as abdominal organs, tissue performance, and slaughter rate.

Key words: Carcass traits, chicken, relationship, SNPs, zyxin gene

1. Introduction

Zyxin, also known as zyxin 2 protein, is a kind of intracellular protein with the function of cytoskeleton actin regulation, and its molecular weight is about 80–85 kDa. Zyxin plays the role of remodeling actin cytoskeleton during trauma repair, cell migration, cell adhesion, and epithelial-mesenchymal transformation (1–3). The N-terminal of the protein is a proline-rich region, while the C-terminal contains 3 series LIM zinc finger regions or configuration of a DNA-binding protein that resembles a finger with a base, usually cysteine and histidine binding a zinc ion (4). Zyxin affects the movement of cells, and growth and migration of tumor cells as well as the regulation of mitosis. In addition, it works as a transcription activator by synergy with other proteins in the nucleus. It also works on microfilament cytoskeleton assembly (2,5). Zyxin is expressed extensively in many animal tissues (6). However, the complete roles zyxin plays throughout the cell life are still unknown. Hong et al. (2009) studied the relativity between zyxin single nucleotide polymorphisms (SNPs) and coccidiosis-resistance parameters, and found that several zyxin SNPs were significantly associated with carotenoid and nitrate ion ($NO_2^- + NO_3^-$) concentration. The results suggest that zyxin is a candidate gene related to coccidiosis resistance (7). The objectives of the present research were to study the effect of different genotypes on carcass traits in the Jinghai yellow chicken and to explore whether it has a negative impact on slaughter performance of this chicken when using zyxin SNPs genotype as the molecular genetic marker to carry out coccidiosis resistance marker-assisted selection, and to provide a basis for Jinghai yellow chicken coccidiosis resistance selection.

2. Materials and methods

2.1. Experimental animals and performance measurements

All animal handling protocols observed were approved by the Yangzhou University Animal Care and Use Committee, Yangzhou, P. R. China. One hundred and fifty-eight 16-week-old Jinghai yellow chickens obtained from Jiangsu Jinghai Poultry Industry Group Company Limited

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were hatched on the same day, and raised in the same way following the standard protocol. Blood samples were taken from the wing vein of the chickens with heparin used as anticoagulant. Slaughter performances were measured according to NY/T 823-2004 "Performance terms and measurements for poultry" (Agricultural Industry Standard of the People's Republic of China). The traits included live weight, carcass weight, heart weight, liver weight, head weight, feet weight, gizzard weight, abdominal fat weight, semieviscerated weight, eviscerated weight, breast muscle weight, and drumstick muscle weight and slaughter rate, semieviscerated rate, eviscerated rate, breast muscle rate, and drumstick muscle rate.

2.2. Genomic DNA isolation and primer design

Genomic DNA was extracted from chicken venous blood by conventional phenol–chloroform extraction method (8). DNA was dissolved in TE buffer and stored at –20 °C. One pair of primers was designed with Primer Premier 5.0 software to amplify the exon 1 region of the zyxin gene, based on the chicken DNA sequence of the zyxin gene (GenBank accession no. NM_001004386). The primer was: Forward 5'-GCATATCCACATCTGCCACA-3' and Reverse 5'- CGGCCAGCAGTCAAAAAG-3'. The primer was synthesized by Beijing Dingguo Corporation Limited, China, and named P_1.

2.3. Polymerase chain reaction (PCR) amplification

The PCR was carried out in 20 µL of reaction mixture consisting of 1 µL of template DNA (50 ng/µL), 1 µL of each of the forward and reverse forms of the primer (10 pmol/µL), 2 µL of 10X reaction buffer, 0.2 µL of *Taq* DNA polymerase (2 U/µL), 0.4 µL of dNTPs (10 mmol/L), and 14.4 µL of double distilled water. The amplification conditions were denaturation at 95 °C for 30 s, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, and a final elongation step at 72 °C for 10 min. The PCR products generated were verified by electrophoresis on a 1% agarose gel (5 V/cm) in 0.5X TBE buffer. Gels were stained with an intercalating agent, ethidium bromide, before visualization.

2.4. SSCP detection and sequencing analysis

First, 2.5 µL of each PCR product was added to 7.5 µL of denaturation buffer (98% dimethyl formamide, 10 mmol/L EDTA (pH 8.0), 0.025% xylene cyanide FF, 0.025% bromophenol blue) exactly and 98 °C denaturized for 10 min, and then chilled in an ice bath for 5 min to make the amplification product remain in the single strand state. The PCR products were added to 10% polyacrylamide gel; firstly, 250 V electrophoresis was performed to separate them for 10 min and then 140 V electrophoresis for more than 18 h. SSCP patterns on the gel were visualized by silver staining. Selected different genotypes of PCR products were sent to Beijing Dingguo Corporation Limited, China, for sequencing.

2.5. Statistical analysis

The general linear model (GLM) was used to analyze the genotype effects of the zyxin gene on slaughter performances. Significant differences among the means were compared by least significant difference (LSD). The linear model used was of the form:

$y_{ijk} = \mu + S_i + G_j + B_{ij} + e_{ijk}$,

where y_{ijk} is the measured slaughter performances, μ is overall mean, S_i is different sex effect, G_j is genotypic effect, B_{ij} is the interaction effect between sex and genotype, and e_{ijk} is the error term. The statistical analysis was carried out with SPSS 18.0. The results obtained were presented as least squares mean and standard deviation.

3. Results

3.1. PCR amplification and SSCP analysis

The fragment length of the PCR products amplified by the primer was consistent with the expected length and could be directly analyzed by SSCP, which is depicted in Figure 1. SSCP analysis revealed that the products of primer P_1 displayed polymorphisms. Three different genotypes were detected and named AA, AB, and BB (Figure 2).

3.2. Sequencing analysis of different genotypes of individuals

Sequencing analysis results of the different genotypes of primer P_1 are depicted in Figure 3. By comparing the sequence of different genotypes, 1 nucleotide mutation (C/T) was found at the site 152 base pair (bp) of exon 1, which mutated the CTT genetic codon into TTT, leading to the change of the zyxin protein amino acid (leucine → phenylalanine), and this corresponds to 1 of the 4 SNPs of the zyxin gene associated with coccidiosis-resistance parameters (7).

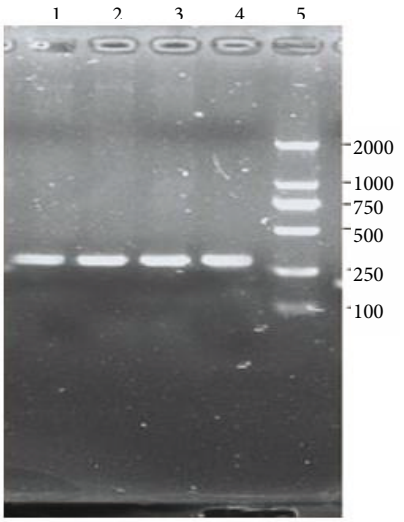

Figure 1. The agarose gel electrophoresis result of zyxin gene exon 1 PCR products amplified by the primer. Lanes 1–4: 4 individual chickens' PCR products; lane 5: DNA marker.

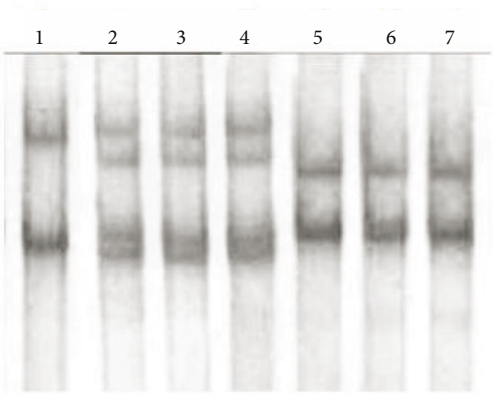

Figure 2. Polyacrylamide gel electrophoresis results of SSCP analysis of PCR products in chickens. Lane 1: BB genotype; lanes 2–4: AB genotype; lanes 5–7: AA genotype.

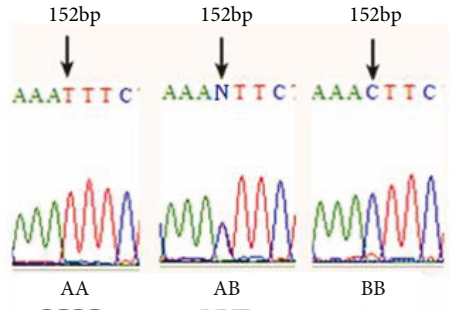

Figure 3. Sequence alignment of AA, AB, and BB genotypes of the primer in chickens.

3.3. Relative analysis of different genotypes on growth and carcass traits

According to GLM analysis, there were significant differences between cockerel and pullet in almost all measured traits, but no significant interaction effect between sex and genotype. The one-way analysis of variance used to analyze the effect of 3 genotypes on all traits considered for cockerel and pullet revealed that the abdominal fat weight and slaughter rates of genotype BB were significantly higher than those of AA and AB ($P < 0.05$) for cockerel, while AA and AB had no significant difference ($P > 0.05$). There was no distinct difference among the 3 genotypes for the other traits ($P > 0.05$) (Table 1).

Table 1. Parameter estimates of different genotypes on carcass traits for cockerel.

Traits	Genotypes			
	AA (N = 45)	AB (N = 27)	BB (N = 5)	P value
Live weight (g)	1667.29 ± 225.63^a	1668.41 ± 242.19^a	1735.40 ± 76.90^a	P = 0.812
Head weight (g)	58.19 ± 8.05^a	57.29 ± 13.00^a	60.88 ± 8.02^a	P = 0.707
Foot weight (g)	58.29 ± 8.95^a	57.46 ± 14.71^a	59.28 ± 8.29^a	P = 0.559
Gizzard weight (g)	31.62 ± 5.21^a	32.69 ± 6.69^a	28.86 ± 7.62^a	P = 0.407
Heart weight (g)	10.51 ± 2.54^a	11.26 ± 2.69^a	11.38 ± 2.42^a	P = 0.442
Liver weight (g)	30.33 ± 4.79^a	30.67 ± 4.96^a	34.16 ± 3.19^a	P = 0.242
Abdominal fat weight (g)	10.81 ± 3.04^b	11.73 ± 3.25^b	15.52 ± 2.99^a	P = 0.046
Breast muscle weight (g)	202.21 ± 37.36^a	200.75 ± 39.06^a	197.92 ± 22.60^a	P = 0.464
Drumstick muscle weight (g)	242.69 ± 44.62^a	242.22 ± 43.13^a	271.56 ± 40.60^a	P = 0.364
Carcass weight (g)	1483.50 ± 207.28^a	1488.37 ± 215.77^a	1594.42 ± 116.25^a	P = 0.521
Semi-eviscerated weight (g)	1254.65 ± 173.14^a	1253.95 ± 184.28^a	1327.38 ± 73.79^a	P = 0.662
Eviscerated weight (g)	1172.39 ± 163.09^a	1167.60 ± 177.30^a	1225.46 ± 58.03^a	P = 0.766
Slaughter rate (%)	88.94 ± 1.69^b	89.22 ± 1.93^b	91.81 ± 3.40^a	P = 0.009
Breast muscle rate (%)	17.18 ± 1.29^a	17.23 ± 2.52^a	16.12 ± 1.29^a	P = 0.443
Drumstick muscle rate (%)	20.70 ± 2.32^a	20.80 ± 2.51^a	22.08 ± 2.38^a	P = 0.434
Semi-eviscerated rate (%)	75.25 ± 1.95^a	75.36 ± 5.96^a	76.47 ± 1.91^a	P = 0.798
Eviscerated rate (%)	70.31 ± 1.87^a	70.16 ± 6.02^a	70.61 ± 3.81^a	P = 0.968

Note: Different letters in the rows indicate significantly different mean values at $P < 0.05$ and the same letters indicate no significant difference ($P > 0.05$).

The results of multiple comparison analysis of carcass traits between the genotypes for pullets are presented in Table 2. Significant differences among the 3 genotypes were found in gizzard weight, heart weight, breast muscle rate, drumstick muscle rate, and semieviscerated and eviscerated rates ($P < 0.05$). For heart weight, breast muscle rate, and drumstick muscle rate, genotype AB was significantly higher than AA and BB ($P < 0.05$), while AA and BB had no distinct difference ($P > 0.05$). For both semieviscerated rate and eviscerated rate, AB was significantly lower than AA and BB ($P < 0.05$), but AA and BB had no distinct difference ($P > 0.05$).

4. Discussion

So far, research on the zyxin gene has mainly been conducted in humans, and expression of the gene is found in a wide range of human tissues. Zyxin and other genes may form a regulating complex compound to control the mitosis process (9), and the zyxin gene plays the role of activator of transcription in the nucleus (10). Although there is about 60% sequence homology on zyxin between chickens and humans, the main function of zyxin shows limited diversities between the 2 species (11).

This study revealed that there was a C → T mutation that mutated CTT into TTT at the position of 152 bp of exon 1, which was proved to be associated with coccidiosis-resistance parameters (7) by way of SNP detection, and made leucine turn into phenylalanine. The results also showed that genotype BB was significantly higher than both AA and AB ($P < 0.05$) for abdominal fat weight and slaughter rate of cockerels, while AA and AB had no distinct difference ($P > 0.05$). Genotype AB was significantly higher than AA and BB ($P < 0.05$) for breast muscle rate and drumstick muscle rate in pullets, while the significance of semieviscerated rate and eviscerated rate was the opposite. For heart weight of pullets, there was a difference between the genotypes. It follows that the performance of slaughter traits caused by the 3 genotypes are not exactly the same for both pullet and cockerel.

Table 2. Parameter estimates of different genotypes on carcass traits for pullet.

Traits	Genotypes			
	AA (N = 50)	AB (N = 23)	BB (N = 8)	P value
Live weight (g)	1315.46 ± 192.74^{a}	1292.78 ± 199.39^{a}	1377.11 ± 187.88^{a}	P = 0.556
Head weight (g)	38.07 ± 6.58^{a}	38.77 ± 8.36^{a}	40.15 ± 8.35^{a}	P = 0.736
Foot weight (g)	39.41 ± 8.72^{a}	38.99 ± 7.87^{a}	41.20 ± 7.49^{a}	P = 0.811
Gizzard weight (g)	28.59 ± 6.63^{ab}	27.06 ± 7.64^{b}	29.06 ± 3.05^{a}	P = 0.049
Heart weight (g)	7.89 ± 2.32^{b}	10.36 ± 2.82^{a}	8.28 ± 2.07^{b}	P = 0.042
Liver weight (g)	27.49 ± 5.57^{a}	26.05 ± 6.99^{a}	29.16 ± 7.45^{a}	P = 0.431
Abdominal fat weight (g)	21.98 ± 18.07^{a}	13.84 ± 14.72^{a}	18.36 ± 29.97^{a}	P = 0.226
Breast muscle weight (g)	169.79 ± 29.98^{a}	169.18 ± 27.37^{a}	173.23 ± 17.25^{a}	P = 0.936
Drumstick muscle weight (g)	174.96 ± 28.93^{a}	177.69 ± 30.80^{a}	174.35 ± 16.63^{a}	P = 0.922
Carcass weight (g)	1488.62 ± 3.26^{a}	1487.66 ± 2.54^{a}	1488.09 ± 1.24^{a}	P = 0.494
Semi-eviscerated weight (g)	990.75 ± 144.86^{a}	913.21 ± 255.55^{a}	1006.49 ± 121.77^{a}	P = 0.206
Eviscerated weight (g)	904.79 ± 134.82^{a}	835.89 ± 245.19^{a}	921.61 ± 104.34^{a}	P = 0.239
Slaughter rate (%)	88.62 ± 3.27^{a}	87.66 ± 2.54^{a}	88.09 ± 1.24^{a}	P = 0.432
Breast muscle rate (%)	18.86 ± 2.39^{b}	24.04 ± 2.13^{a}	18.98 ± 2.62^{b}	P = 0.045
Drumstick muscle rate (%)	19.39 ± 1.91^{b}	25.12 ± 2.45^{a}	19.06 ± 2.34^{b}	P = 0.036
Semi-eviscerated rate (%)	75.47 ± 5.20^{a}	69.90 ± 4.23^{b}	73.17 ± 7.43^{a}	P = 0.046
Eviscerated rate (%)	68.92 ± 5.04^{a}	63.89 ± 6.87^{b}	67.06 ± 7.03^{a}	P = 0.037

Note: Different letters in the rows indicate significantly different mean values at $P < 0.05$ and the same letters indicate no significant difference ($P > 0.05$).

As coccidiosis can at times cause grave losses to the poultry industry, it is very important to develop new varieties of disease-resistant strains of chickens by molecular breeding methods so as to alleviate the problem of coccidiosis in poultry production enterprises, and particularly to meet food safety requirements. Based on the precise location for the quantitative trait loci (QTL) of chicken coccidiosis-resistance, Zhu et al. and Kim et al. (11,12) demonstrated that resistance to avian coccidiosis is linked with microsatellite markers LEI0071 and LEI0101 on chromosome 1, and Hong et al. found that the zyxin gene located in the region of LEI0071 and LEI0101 is related to *Eimeria* coccidiosis resistance and 4 SNP sites of the zyxin gene are significantly associated with coccidiosis-resistance parameters (7). Therefore, the SNP site of zyxin could be used as an avian *Eimeria* coccidiosis resistant breeding molecular genetic marker for marker-assisted selection.

Comprehensive analysis of the zyxin gene suggests that single nucleotide polymorphisms in exon 1 of the Jinghai yellow chicken have a certain impact on some carcass traits, such as abdominal fat weight, heart weight, and slaughter rate, but it has a nonsignificant impact on some important economic traits, such as live weight, breast weight, and drumstick muscle weight. According to the research by Hong et al. (7), the selection of this gene can increase coccidiosis resistance. Meanwhile, our study shows that the selection of this gene has little effect on major productive performance such as live weight. Whether this gene is related to other important traits such as meat quality, egg production, and many more in poultry-related activities may however be examined in further research, so as to develop better breeding programs, improve resistance to chicken coccidiosis, and develop highly resistant chicken strains or poultry lines.

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