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RESEARCH ARTICLE

Manhong YE, Jie WEN, Honghe CAO, Hongbin LI, Jilan CHEN, Guiping ZHAO, Maiqing ZHENG

Association of polymorphisms in adipocyte fatty acid binding protein gene with fat-related traits in chicken

Abstract PCR-SSCP analysis was used to detect polymorphic sites in chicken adipocyte fatty acid binding protein (*A-FABP*) gene. Six Chinese local breeds, Beijing-You chicken, Dwarf chicken, Taihe silky chicken, Chongrenma chicken, Xiayan chicken, Luyuan chicken and an introduced foreign breed, Arbor Acre broiler, were used as test populations. Three PCR-SSCP loci were detected. Statistical results showed that frequencies of genotypes and alleles were significantly different in the test populations. Sequence analysis revealed that C→T, G→A, and C→T transitions were responsible for the polymorphisms. Some fat-related traits such as body weight, content of intramuscular fat (IMF) and percentage of abdominal fat (AFP) were measured in Dwarf chickens and male Beijing-You chickens. We found out that chicken quality was significantly related to different genotypes in these two populations.

Keywords *A-FABP* gene, PCR-SSCP, intramuscular fat, chicken

1 Introduction

As an important factor that has an effect on chicken quality, intramuscular fat (IMF) is attracting more and more attention from chicken breeders and consumers. Fatty acid binding protein (FABPs) belong to a family of small intracellular lipid-binding proteins. Up to now, at least 9 FABP types with characteristic tissue and cellular distribution have been named after the first tissue from which they were isolated. FABPs are postulated to be involved in the uptake of long-chain fatty acids and their consequent translocation to sites of intracellular utilization, such as mitochondria, where β-oxidation takes place, and other cellular compartments where they are esterified into triacylglycerols or phospholipids (Veerkamp and Maatman, 1995). FABPs present in tissues indicate the presence of a large reserve intracellular binding capacity for fatty acids, which can be transformed to acyl-CoA esters (Veerkamp et al., 1991). Since FABPs can modulate the concentration of cellular fatty acids, they are involved in this way in the regulation of lipid metabolism.

Adipocyte-type FABP (A-FABP) is expressed uniquely in adipocytes. Since it is involved directly in the accumulation of triacylglycerols and phospholipids in muscle adipocytes, which is an effective way to increase IMF content, the *A-FABP* gene is studied as a candidate gene that has effects on the content of IMF in chicken muscles. Up till now, abundant research conducted in swine (Gerbens et al., 1997, 1998, 2001, 2000, 1999) has not only determined the localization of the *A-FABP* gene in a specific chromosome but also revealed that different genetic variations in the *A-FABP* gene are significantly related to the difference in IMF content of the Duroc population (Zhang et al., 2002). Research on chicken *A-FABP* gene as a candidate gene associated with fat-related traits was conducted several years ago (Ye, 2003). In this paper we detect potential polymorphisms in chicken *A-FABP* gene by PCR-SSCP analysis in Beijing-You chicken, Dwarf chicken, Taihe Silky chicken, Chongrenma chicken, Luyuan chicken, Xiayan chicken and Arbor Acre broiler. Frequencies of different genotypes and alleles at each polymorphic site were analyzed in seven chicken breeds. We found out that there was a significant difference in the distribution of genotypes and alleles between different populations. Associations between different genotypes and some fat-related traits were analyzed in Dwarf chicken and male Beijing-You chicken to testify to the possibility of studying chicken *A-FABP* gene further as

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Manhong YE
College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225002, China

Jie WEN (✉), Honghe CAO, Hongbin LI, Jilan CHEN, Guiping ZHAO, Maiqing ZHENG
National Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100094, China
E-mail: wenjoffice@263.net

a useful candidate gene, which will validate the application of molecular markers for the breeding of quality chicken in the future.

2 Materials and methods

2.1 Collection of blood samples

Two hundred and sixty-one Beijing-You chickens, 273 Dwarf chickens, 36 Taihe Silky chickens, 41 Chongrenma chickens, 40 Luyuan chickens, 40 Xiayan chickens and 37 Arbor Acre broilers were obtained from the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. One mL venous blood sample from each chicken was collected in the presence of acid-citrate-dextrose (ACD) anticoagulant and stored at $-20^{\circ}C$ before use.

2.2 Preparation of genomic DNA

Extraction of chicken genomic DNA from blood was conducted as described by Meng et al.(1993).

2.3 PCR amplification

Three pairs of primers were designed according to the sequence of chicken *A-FABP* gene, the first exon (GenBank AF432506) and the first intron (GenBank AF432507), by Primer 5.0 software. Primers were synthesized by SAIBAISHENG Bioengineering Co., Ltd. Beijing, China. The sequence of each primer, expected size of PCR products, and their localization in chicken *A-FABP* gene are shown in Table 1.

PCR was performed in a reaction volume of 12 μL containing about 60 ng genomic DNA, 0.5 $\mu mol \cdot L^{-1}$ of each primer, 1 × PCR buffer [10 $mmol \cdot L^{-1}$ Tris-HCl (pH 9.0), 1.5 $mmol \cdot L^{-1}$ $MgCl_2$], 0.2 $mmol \cdot L^{-1}$ dNTPs and 2 U of *Taq* DNA polymerase. The first PCR cycle was 5 min at 94°C, followed by 32 cycles of 30 s at 94°C, 30 s at 58°C (P_1), 56°C (P_2), 53°C (P_3) respectively, and 30 s at 72°C, ending with a 10 min extension phase at 72°C. PCR products were viewed in 2% agarose gel. Specific products with the expected size were analyzed by PCR-SSCP analysis.

2.4 SSCP analysis

SSCP analysis was carried out by vertical polyacrylamide gel electrophoresis (PAGE). One to two μL of the PCR products was diluted with 5 μL of solution containing 98% deionized formamide, 10 $mmol \cdot L^{-1}$ EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol and 10% glycerol. The mixture was then denatured at 98°C for 10 min, cooled in ice for at least 5 min and loaded onto a nondenatured 12% acrylamide:bis-acrylamide (29:1) gel. Electrophoresis was performed in 1 × Tris Borate (pH 8.0) EDTA buffer at 4°C overnight. DNA bands were detected by silver staining.

2.5 Transformation of PCR products and sequence analysis

DNA fragments that displayed a modified electrophoretic pattern were selected for sequence analysis. PCR products of the homozygous genotype were purified by glass-milk and ligated to pGEM T-easy Vector (Promega). Aliquots of the ligation mixtures were used to transform *Escherichia coli* JM109. Positive clones were subjected to sequence analysis on PE377 by the Beijing HUADA Company.

2.6 Measurement of fat-related traits

Beijing-You and Dwarf chickens were raised in the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. Brooding chickens were fed a diet with metabolic energy of 11.61 $MJ \cdot kg^{-1}$ from the 1st to 42nd days. From the 43rd to 90th days the metabolic energy in the diet of growing chickens was reduced to 11.23 $MJ \cdot kg^{-1}$. Chickens were slaughtered at 90 days old. Fat-related traits were measured according to standard methods. IMF content was determined by Soxlet's extraction method.

2.7 Related analysis of polymorphic loci with fat-related traits

The distribution of different genotypes at polymorphic sites was analyzed and statistical results were obtained using SAS software by GLM (generalized linear model).

Table 1 Primer sequence, expected size of PCR products and their localizations in the *A-FABP* gene

primer sequence	expected size/bp	localization in *A-FABP* gene
P_1: F_1: 5′- ACT GCT ACC TGG CCT GAC - 3′ R_1: 5′- GGA ATG TGA CAA CGC TAA - 3′	275	containing the first exon and partial sequence of the first intron
P_2: F_2: 5′-GGA TGG GGG AAG TAT GGG - 3′ R_2: 5′-ATC GGT GGC TAG GCT GAA - 3′	218	within the first intron
P_3: F_3: 5′- AGC ACA CAA GTT AGG GCA - 3′ R_3: 5′- GGG ATG AGA CAA GGC AAT - 3′	144	within the first intron

Significance of difference was tested between chickens carrying different genotypes.

3 Results

3.1 PCR amplification using three primer pairs (viewed in 2% agarose gel)

Results showed that PCR products had satisfied the specificity of expected size, which could be used for the next SSCP analysis (Figs. 1–3).

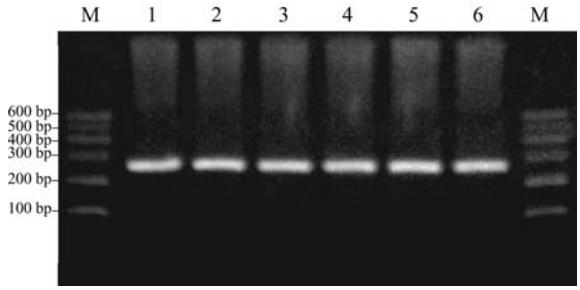

Fig. 1 275 bp PCR products
Note: M represents the marker for the 100 bp ladder; Lanes 1–6 represent the products of individual samples. The same in Figs. 2, 3.

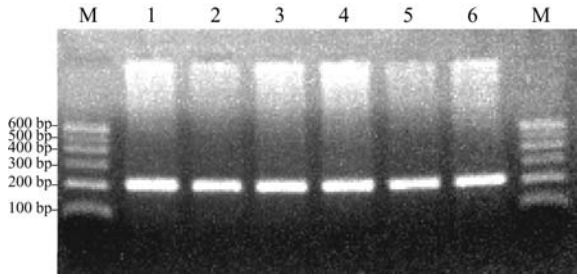

Fig. 2 218 bp PCR products

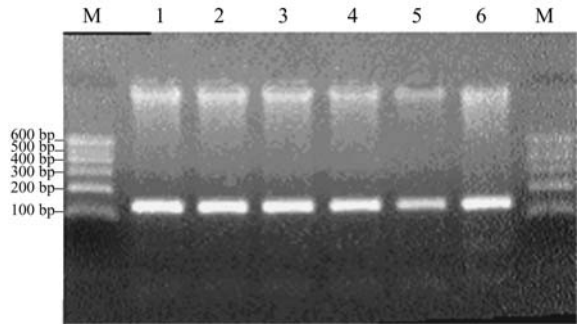

Fig. 3 144 bp PCR products

3.2 PCR-SSCP analysis (12% nondenatured PAGE)

Figures 4, 5 and 6 show all three possible genotypes, two homozygous types and one heterozygous type, detected at three PCR-SSCP loci.

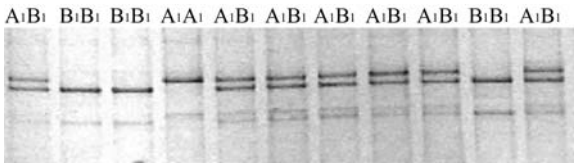

Fig. 4 PCR-SSCP analysis of 275 bp PCR products

3.3 Frequencies of genotypes and alleles and genetic polymorphism information content (PIC) of respective loci in different chicken populations.

Statistical results from 728 samples in seven test chicken populations in Table 2 revealed that: (1) all the possible genotypes existed in the test populations at three PCR-SSCP loci except for the B_1B_1 genotype at the P_1-SSCP locus, which was not detected in AA population. (2) Test populations had a medium PIC at three PCR-SSCP loci except for low PIC being detected at the P_3-SSCP locus in Dwarf chicken. (3) Frequencies of genotypes and alleles at three PCR-SSCP loci were all consistent with the Hardy-Weinberg distribution ($P > 0.05$) except that the distribution at the P_3-SSCP locus in Xiayan chickens was distorted from the Hardy–Weinberg equilibrium ($P < 0.01$). (4) The distribution of different genotypes at the P_1, P_2 and P_3-SSCP loci differed significantly, with Chi-square values of 64.84, 69.46 and 199.90 within different test populations, respectively ($P < 0.01$, $\chi^2_{0.05} = 21.03$, $\chi^2_{0.01} = 26.22$, $df = 12$) (Table 3).

In our experiment, Chinese local chicken breeds and the Arbor Acre broiler were slaughtered at the ages of 90 d and 49 d, respectively. Average IMF content measured in breast muscles showed a decreasing trend as follows: Dwarf-Chongrenma-Xiayan-BeijingYou-Arbor Acre broiler-Taihe silky-Luyuan. Chi-square values in Table 3 show the difference in the distribution of genotypes at three PCR-SSCP loci in seven chicken breeds compared with each other. Each chicken had a total of 18 Chi-square values. Luyuan chicken, which had the lowest IMF content in breast muscles, had a significantly different distribution pattern (15/18, $P < 0.01$) than any other chicken breed. The same result could be found in Dwarf chicken, which had the highest IMF content in breast muscles. Compared with other Chinese local chicken breeds, the Arbor Acre broiler had a (significantly) different distribution of genotypes at the P_1-SSCP locus. The Chongrenma chicken, with an average IMF content only lower than that of Dwarf chicken, also showed a significantly different pattern of distribution (3/6, $P < 0.01$ and 1/6, $P < 0.05$) at the P_2-SSCP locus. At the P_3-SSCP locus the distribution of genotypes in Xiayan chicken was significantly different from other chicken breeds except the Arbor Acre broiler ($P < 0.01$).

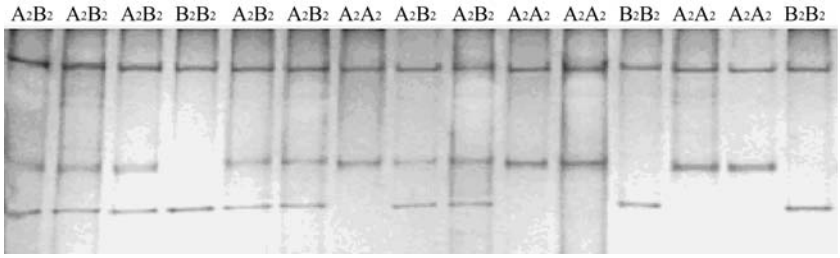

Fig. 5 PCR-SSCP analysis of 218 bp PCR products

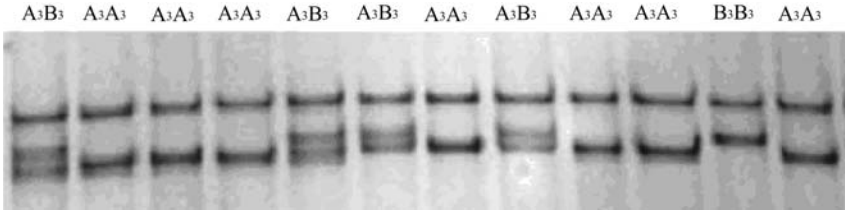

Fig. 6 PCR-SSCP analysis of 144 bp PCR products

Table 2 Frequencies of genotypes and alleles and PIC of respective loci in seven populations

genotype	B	D	T	C	L	X	A
A_1A_1	0.249(64/257)	0.251(64/255)	0.257(9/35)	0.146(6/41)	0.667(26/39)	0.075(3/40)	0.514(19/37)
A_1B_1	0.514(132/257)	0.482(123/255)	0.486(17/35)	0.610(25/41)	0.230(9/39)	0.500(20/40)	0.486(18/37)
B_1B_1	0.237(61/257)	0.267(68/255)	0.257(9/35)	0.244(10/41)	0.103 (4/39)	0.425(17/40)	0
A_1	0.506	0.492	0.500	0.451	0.782	0.325	0.757
B_1	0.494	0.508	0.500	0.549	0.218	0.675	0.243
PIC	0.3750	0.3749	0.3750	0.3726	0.2828	0.3425	0.3002
A_2A_2	0.308(80/260)	0.278(76/273)	0.111(4/36)	0.366(15/41)	0.105(4/38)	0.150(6/40)	O.108(4/37)
A_2B_2	0.480(125/260)	0.440(120/273)	0.250(9/36)	0.439(18/41)	0.263(10/38)	0.300(12/40)	0.460(17/37)
B_2B_2	0.212(55/260)	0.282(77/273)	0.639(23/36)	0.195(8/41)	0.632(24/38)	0.550(22/40)	0.432(16/37)
A_2	0.548	0.498	0.236	0.585	0.237	0.300	0.338
B_2	0.452	0.502	0.764	0.415	0.763	0.700	0.662
PIC	0.3727	0.3750	0.2956	0.3677	0.2963	0.3318	0.3474
A_3A_3	0.571(149/261)	0.781(210/269)	0.486(17/35)	0.622(23/37)	0.175(7/40)	0.263(10/38)	0.432(16/37)
A_3B_3	0.387(101/261)	0.200(54/269)	0.371(13/35)	0.324(12/37)	0.325(13/40)	0.711(27/38)	0.460(17/37)
B_3B_3	0.042(11/261)	0.019(5/269)	0.143(5/35)	0.054 (2/37)	0.500(20/40)	0.026(1/38)	0.108(4/37)
A_3	0.764	0.881	0.671	0.784	0.337	0.618	0.662
B_3	0.236	0.119	0.329	0.216	0.663	0.382	0.338
PIC	0.2956	0.1878	0.3441	0.2813	0.3470	0.3607	0.3474

Note: B, D, T, C, L, X and A represent Beijing-You, Dwarf, Taihe Silky, Chongrenma, Luyuan, Xiayan and Arbor Acre broiler, respectively.

3.4 Sequence analysis

Sequence analysis revealed that P_1 and P_3-SSCP were caused by a transition of C to T. P_2-SSCP was caused by a transition of G to A. P_1-SSCP was localized in the first exon of the chicken *A-FABP* gene. It resulted in a change of code from TTC to TTT, with amino acids unaltered.

3.5 Related analysis of different genotypes with fat-related traits

3.5.1 Related analysis in the male Beijing-You population

Table 4 shows that the male Beijing-You population chickens carrying the B_1B_1 genotype at the P_1-SSCP locus not only had the highest IMF content, which was significantly higher than that of chickens with A_1A_1 genotype ($P < 0.01$) and A_1B_1 genotype ($P < 0.05$), but also had the highest least square means in such traits as percentage of abdominal fat, subcutaneous fat thickness, fat strip width, and body weight. Therefore, chickens with a homozygous B_1B_1 genotype are likely to accumulate more fat, have a higher growth rate, and need less time to reach sexual maturity.

Statistical results shown in Table 5 reveal that the male Beijing-You chickens carrying a homozygous A_2A_2 genotype had a significantly higher IMF content and percentage of abdominal fat than those chickens with a heterozygous A_2B_2 genotype. They also had the highest least square value in subcutaneous fat thickness and fat strip width. It can be inferred that chickens with a homozygous A_2A_2 genotype have the potential to accumulate more fat in their bodies.

Table 3 Chi-square test of the distribution of different genotypes at three PCR-SSCP loci in seven populations of chicken

breed		B	D	T	C	A	L	X
average IMF/%		2.70	3.48	2.46	3.40	2.63	2.37	2.86
P_1	B		0.69	0.11	2.21	16.84**	27.91**	9.30**
	D			0.016	2.85	17.70**	27.58**	7.75*
	T				1.71	12.55**	12.46**	5.40
	C					17.74**	22.57**	3.36
	A						8.04*	28.67**
	L							30.45**
P_2	B		3.56	29.94**	0.56	11.20**	30.35**	20.96**
	D			18.69**	1.94	6.15*	18.92**	11.73**
	T				16.37**	3.71	0.02	0.64
	C					8.88*	16.56**	11.58**
	A						3.40	2.10
	L							0.62
P_3	B		26.75**	6.21*	0.59	4.35	81.44**	14.23**
	D			22.24**	5.17	23.59**	120.42**	45.34**
	T				2.17	0.62	12.89**	9.24**
	C					2.79	23.22**	11.21**
	A						14.63**	5.45
	L							22.58**

Note: Values with ** and * differ significantly at $P < 0.01$ and $P < 0.05$, respectively. $\chi^2_{0.05}$ ($df = 2$) = 5.99, $\chi^2_{0.01}$ ($df = 2$) = 9.21. B, D, T, C, L, X and A represent Beijing-You, Dwarf, Taihe Silky, Chongrenma, Luyuan, Xiayan and Arbor Acre broiler, respectively.

Table 4 Least square analysis of different genotypes at the P_1-SSCP locus in the male Beijing-You population

genotype	A_1A_1	A_1B_1	B_1B_1
sample number, $n = 220$	61	108	51
BW/g	1025.5 ± 14.79	1015.0 ± 11.11	1047.8 ± 16.17
IMF/%	2.489 ± 0.102^{Aa}	2.722 ± 0.076^{a}	3.028 ± 0.108^{Bb}
AFP/%	0.549 ± 0.037	0.541 ± 0.029	0.616 ± 0.039
SFT/mm	3.059 ± 0.127	3.123 ± 0.096	3.315 ± 0.140
FSW/mm	5.502 ± 0.176	5.410 ± 0.133	5.805 ± 0.195

Note: BW, AFP, SFT and FSW represent body weight, percentage of abdominal fat, subcutaneous fat thickness, and fat strip width, respectively. Data with different letters (A, B, C) and (a, b, c) within the same column differ significantly at $P < 0.01$ and $P < 0.05$, respectively. The table below shows the same.

Table 5 Least square analysis of different genotypes at the P_2-SSCP locus in male Beijing-You population

genotype	A_2A_2	A_2B_2	B_2B_2
sample number, $n = 255$	136	109	10
BW/g	1035.3 ± 9.93	1016.0 ± 11.82	1062.6 ± 36.62
IMF/%	2.807 ± 0.068^{a}	2.588 ± 0.081^{b}	2.778 ± 0.262^{ab}
AFP/%	0.581 ± 0.025^{a}	0.496 ± 0.031^{b}	0.522 ± 0.081^{ab}
SFT/mm	3.276 ± 0.083	3.032 ± 0.100	2.668 ± 0.344
FSW/mm	5.560 ± 0.119	5.277 ± 0.143	5.584 ± 0.437

No significant difference among the different genotypes at P_3-SSCP was detected in the traits measured in male Beijing-You chickens.

3.5.2 Related analysis in the Dwarf chicken population

The IMF content of the male chickens carrying different genotypes at the P_1-SSCP locus in the Dwarf population differed significantly, which was similar to the results in the male Beijing-You population. In the male Dwarf population it was the A_1A_1 genotype that was related to the highest IMF content in breast muscles, while in the male Beijing-You population it was the B_1B_1 genotype that had the highest IMF content (Table 6). This effect could not be detected in female Dwarf chickens.

In the Dwarf population, chickens with different genotypes at the P_2-SSCP locus did not have significant differences in the traits measured except that the A_2A_2 genotype was related to a significantly higher fat strip width than those with A_2B_2 genotype in male Dwarf chickens ($P < 0.05$).

Table 6 Least square analysis of different genotypes at P_1-SSCP locus in Dwarf population

genotype	A_1A_1	A_1B_1	B_1B_1
sample number, $n = 218$	52	110	56
IMF/%	3.620 ± 0.125^{a}	3.492 ± 0.086^{ab}	3.240 ± 0.121^{b}
	3.645 ± 0.193^{a}*	3.440 ± 0.127^{ab}*	3.100 ± 0.182^{b}*
FSW/mm	5.066 ± 0.192	5.293 ± 0.136	5.456 ± 0.185
AFP/%	2.212 ± 0.111	2.153 ± 0.078	2.104 ± 0.106
BW/g	1181.0 ± 21.15	1186.8 ± 14.75	1190.7 ± 20.04

Note: *represents statistics in male Dwarf chickens. The sample number was 111.

A significant sex-related effect on fat-related traits was detected in Dwarf chickens at the P_3-SSCP locus. Chickens with A_3B_3 genotype in the Dwarf population had a significantly higher growth rate than the chickens with B_3B_3 genotype (Table 7). The body weight of chickens carrying the A_3B_3 genotype was significantly higher

than that of chickens with other genotypes. These chickens also had the largest least square means in IMF content. We found out that the effect of the A_3B_3 genotype on body weight was found mainly in the male Dwarf chickens. There was no difference in the body weight between female Dwarf chickens with different genotypes. When the trait of fat strip width was measured, the female Dwarf chickens with A_3A_3 genotype had significantly higher least square means than those with A_3B_3 genotype. They also had the largest least square means in the percentage of abdominal fat.

Table 7 Least square analysis of different genotypes at the P_3-SSCP locus in Dwarf population

genotype	A_3A_3	A_3B_3	B_3B_3
sample number, $n = 220$	175	41	4
IMF/%	3.448 ± 0.071	3.484 ± 0.138	3.160 ± 0.458
FSW/mm	5.416 ± 0.107^{a}	4.831 ± 0.212^{b}	4.801 ± 0.764^{ab}
	$5.430 \pm 0.159^{a\#}$	$4.661 \pm 0.306^{b\#}$	$5.400 \pm 1.400^{ab\#}$
AFP/%	2.172 ± 0.061	2.0820 ± 0.131	2.010 ± 0.394
BW/g	1187.7 ± 11.52^{a}	1200.6 ± 23.81^{Aa}	1055.3 ± 76.23^{Bb}
	$1296.9 \pm 12.65^{a*}$	$1292.4 \pm 25.92^{ab*}$	$1106.5 \pm 81.97^{c*}$

Note: * and # represent statistics in male Dwarf chickens and female Dwarf chickens, with sample numbers of 108 and 112, respectively.

4 Discussion

IMF is an important factor that has effects on the flavor of chicken. Compared with broilers, most Chinese local chicken breeds have a relatively higher content of IMF and evenly distributed fat in their bodies, which can improve the flavor of chicken. Since IMF content is difficult to measure in living animals, the breeding process depending on traditional methods to improve IMF content in muscles develops slowly. We expected to find the molecular mechanisms underlying the advantage of flavor in local Chinese chicken breeds, and used molecular markers in the selection of quality chicken breeds with excellent performances.

In our study we selected chicken *A-FABP* gene, which is expressed specifically in adipocytes and is involved directly in the physiological and biochemical process of fat accumulation, as a candidate gene that has effects on IMF content in muscles. Our results showed that the distribution of genotypes and alleles at the PCR-SSCP loci detected in the *A-FABP* gene was significantly different among local Chinese chicken breeds and the Arbor Acre broiler, which provided a theoretical foundation for the utilization of local Chinese chicken breeds in future breeding schemes favorable to the improvement of IMF content. We found out that the genetic variation within the chicken *A-FABP* gene is significantly related to the fat-related traits in chickens. Effects of certain genotypes on chicken performance varied within different chicken breeds or different sexual populations in the same breed. Our continuing research will analyze the relationship between the expression level of mRNA and FABP in muscle cells and fat-related traits, to validate the practical applicability of those polymorphic loci in chicken breeding as useful molecular markers.

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