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Improving Muscle Inosine Monophosphate (IMP) Contents in Wenchang Chicken by Pyramiding Favorable Genotypes of ADSL and GARS-AIRS-GART Genes

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Abstract: This study was designed to improve muscle Inosine Monophosphate (IMP) contents in Wenchang chicken by pyramiding favorable genotypes of the Adenylosuccinatelyase (ADSL) gene and Glycinamide Ribonucleotide Synthetase-Aminoimidazole Ribonucleotide Synthetase-Glycinamide Ribonucleotide Transformylase (GARS-AIRS-GART) gene. PCR-SSCP method and DNA sequencing were used to identify the Single Nucleotide Polymorphisms (SNPs) in exon 2 of ADSL gene and 5'-flanking of GARS-AIRS-GART gene. Association between the detected SNPs and different pyramiding genotypes with IMP contents were tested by least square analysis. The early growth and production performance of individuals with different pyramiding genotypes were also compared. The results showed two SNPs were detected, C/T substitution at position 3484 in exon 2 of ADSL gene and T/C substitution at position -179 in 5'-flanking of GARS-AIRS-GART gene, each had three kinds of genotypes (TT, CT, CC). The association analysis indicated the two SNPs had significant correlation with IMP contents ($p<0.05$), there were higher ($p<0.05$) IMP contents in birds that were TT genotype than those of CC and CT genotypes for both ADSL and GARS-AIRS-GART genes and the additive effects of the favorable TT genotypes on IMP contents were respectively 0.248 and 0.227 mg $^{-1}$ g. After gene pyramiding, the association analysis showed birds with pyramiding TTTT genotype had higher IMP contents (2.720 mg g^{-1}, $p<0.05$) than those with other pyramiding genotypes. This value was increased by 10.34 and 13.23% compared to the IMP contents of single genotype TT for ADSL and GARS-AIRS-GART genes. Factor test also showed the two favorable TT genotypes played significant role ($p<0.05$) on IMP contents in pyramiding genotype, however, their interaction effect was not significant ($p>0.05$). The average 90 days old weight, age at first egg and 72 weeks old laying performance in different pyramiding genotype populations had no difference ($p>0.05$). The muscle IMP contents of Wenchang chicken can be markedly improved by pyramiding favorable genotypes of ADSL and GARS-AIRS-GART genes and still maintain the original production performance.

Key words: ADSL gene, GARS-AIRS-GART gene, wenchang chicken, gene pyramiding, IMP content, China

INTRODUCTION

China was one of the countries that had the richest chicken breed resources in the world. These native breeds had formed specific germplasm characters through long term selection and breeding especially be famous for its meat quality. In the past 10 years, the researches about meat quality in chicken mainly focused on improvements of muscle intramuscular fat content. Many candidate genes such as A-FABP gene (Wang *et al*., 2006), PPAR gene (Meng *et al*., 2002), OBR gene (Wang *et al*., 2004), UCP gene (Zhao *et al*., 2002), INS gene (Qiu *et al*., 2006) had been reported to have association with fatness traits. Few reports were found about the candidate genes or genetic markers related to muscle flavor traits. However, flavor has been determined as an important aspect in evaluating chicken meat quality. Scientific evidences indicated that Inosine Monophosphate acid (IMP) was one of the key components for meat flavor, it had positive correlation with monosodium glutamate could improve the delicate flavor of monosodium glutamate and restrain bitterness and sourness (Maga, 1987). IMP contents were various in different chicken breeds (Khan *et al*., 1968; Song *et al*., 2002), its heritability was about 0.413-0.603 (Chen *et al*., 2002, 2005). The synthesis of IMP in body had two pathways, de novo synthesis and salvage synthesis (Aimi *et al*., 1990a).

Of which, the de novo synthesis was the main pathway and was invariant in all organisms thus far studied. It refered to ten kinds of catalyzed enzymes.

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Adenylosuccinate Lyase (ADSL) is an essential enzyme involved in de novo purine biosynthesis which catalyzes two steps in the synthesis of purine nucleotides. While GARS-AIRS-GART enzyme activities catalyzing the 2nd, 3rd and 5th steps in de novo purine synthesis. The chicken cDNA sequences of ADSL and GARS-AIRS-GART genes had been cloned (Aimi *et al*., 1990b). Wenchang chicken was a famous Chinese indigenous breed for its meat flavor and laying performance. However, the IMP contents varied greatly among individuals under the same diet and management. In this study, ADSL gene and GARS-AIRS-GART gene were served as candidate genes, the association between the candidate genes and muscle IMP contents were analyzed. The objective of this study was to improve muscle IMP contents and establish new high-IMP strain in Wenchang populations by pyramiding favorable genotypes of ADSL and GARS-AIRS-GART genes.

MATERIALS AND METHODS

Experimental population: The Wenchang chicken conserving populations were reared in State Gene Bank of China Poultry Breeding (Institute of Poultry Science, Chinese Academy of Agricultural Science). All birds had free access to feed and water. At age of 12 weeks, 191 male individuals and 209 female individuals were randomly selected.

DNA extraction and IMP content measurement: About 0.5 mL venous blood and approximately 2 g of breast muscles was collected from each individual. Genomic DNA was extracted from blood samples using a DNA extraction kit (sangon biotechnology company, shanghai, china). Muscle IMP content was measured using the method described by Chen *et al.* (2000).

PCR amplifications and genotyping: Two pairs of primers were designed according to the submitted sequences of ADSL gene (Accession No: AY665559) and GARS-AIRS-GART gene (Accession No: DQ078254). F1-5'CTTT CTCCTCCGCAGTCAC3'andR1-5'AGCACC TTCGTCTTCGTTTT3' was used to amplify 279 bp fragments of ADSL exon 2, F2-5'ACAGTTGCCA GTCTGATTA3' and R2- 5'CATCGCCAGAGTTAGAAGT3' was used to amplify 263 bp fragments of GARS-AIRS-GART 5'-flanking domain. The PCR was performed in a final volume of 25 uL consisted of 10× buffer, 200 μmol L^{-1} of each dNTP, 1.5 mmol L^{-1} of $MgCl_2$, 1.0 μmol L^{-1} of each primer, 1 U Taq DNA polymerase (Takara Biotechnology Co., Japanese) and 50 ng of genomic DNA on an ABI2700 thermal cycler (Applied Biosystems, Foster City, CA). The profile were: thermal denature for 5 min at 94°C followed by 30 cycles at 94°C for 30 sec, annealing at 56°C (primer pair 1) or 58°C (primer pair 2) for 30 sec, extension at 72°C for 30 sec and a final elongation at 72°C for 7 min.

PCR products were checked on 1% agarose gel stained with ethidium bromide. Only 1.5 μL PCR products of each individual was mixed with 6 uL loading buffer (98% formamide off ions, 0.01 M EDTA, 0.025% Bromophenol blue, 0.025% Xylene cyanol FF, 10% glycerol), then denatured at 98°C for 10 min and followed by a rapid chill on ice for 5 min. The denatured PCR products were subjected to 10% neutral polyacrylmide gel (acrylamide:bisacrylamide = 39:1), electrophoresed at a constant voltage (8 V cm^{-1}) for 12-14 h. The DNA bands on the gel were viewed by silver staining.

Sequencing of polymorphism fragments: PCR products of homozygous individuals were purified by Gel Extraction Mini Kit (Watson Biotechnologies, Inc., Shanghai, China) and sequenced in forward and reverse directions with an ABI 3730 sequencer (Sangon Biotechnology Company, Shanghai, China).

Pyramiding methods: In selection groups, after genotype identification and association analysis with IMP contents, individuals with different genotypes were selected for breeding propagation. Then association between IMP contents and pyramiding genotypes was analyzed.

Statistical analysis: Results are expressed as means±SEM (Standard Error of Mean). Frequencies of the polymorphisms were estimated by allele counting. Association of genotypes with IMP contents were analyzed using the GLM procedure of SAS (SAS Institute, Inc., Cary, NC). The model was assumed to be:

$$Y_{ik} = \mu + G_i + I_k + B_{ik} + E_{ik}$$

Where:

Y_{ik} = Dependent variable
μ = The overall population mean
G_i = Genotype effect of ADSL
I_k = Genotype effect of GARS-AIRS-GART
B_{ik} = Interaction effect
E_{ik} = The random error

Dominant effect d = TC-(TT+CC)/2, additive effect a = (TT-CC)/2, degree of dominance D = d/a. The level of $p<0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

The lengths of PCR products amplified were coincidence with the designed fragments. The homozygotes exhibited two distinct bands with altered mobility whereas heterozygotes showed 3-4 DNA

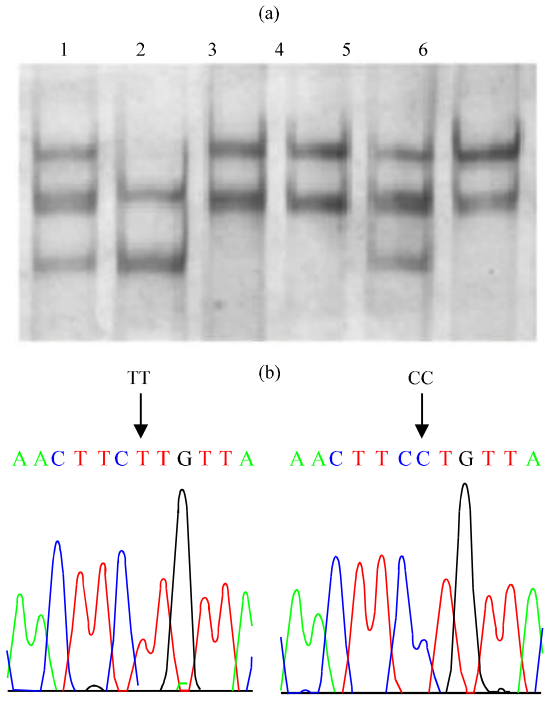

Fig. 1: (a) Polymorphism; (b): mutation sites of exon 2 in ADSL gene. Land 5, CT; 3, 4 and 6 CC; 2, TT

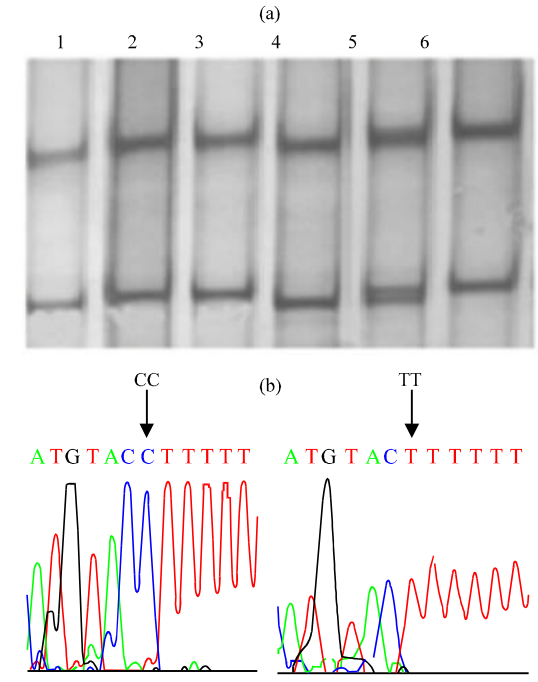

Fig. 2: (a) Polymorphism; (b) mutation sites of 5'flanking region in GARS-AIRS-GART gene. Land 1 and 4 TT; 2, 3 and 6 CC; 5, CT

bands (Fig. 1a and 2a). DNA sequencing revealed two polymorphic sites, C3484T of ADSL gene and C-179T of GARS-AIRS-GART gene (Fig. 1b and 2b).

Few reports were found about the candidate genes or genetic markers related to muscle IMP content. Zhang *et al.* (2006) also cloned the ADSL gene cDNA sequence in different chicken breeds, five nucleotide differences were detected and led to two amino acid mutation T→A (305), A→V (443), prompted ADSL gene might play impact on on chicken muscle IMP content. Zhang *et al.* (2004) analyzed the sequence diversity of AMPD1 gene and its relationship with IMP content in various chicken breeds, two polymorphism sites A120 G and A355G were deduced may have significant effects on IMP contents.

However, Chai *et al.* (2005) analyzed a 500 bp fragment of AMPD1 gene using PCR-SSCP method but analysis of variance indicated that this AMPD1 genotypes did not significantly influence the IMP content in chicken.

Different chicken materials might contribute to the results. In this study, there were significant association between the two detected polymorphic sites of ADSL and GARS-AIRS-GART genes with IMP contents ($p<0.05$) in Wenchang chicken. Effects (least square means) of the genotypes on IMP contents were showed in Table 1.

At the GARS-AIRS-GART location, there were higher ($p<0.05$) IMP contents in birds that were TT genotype (2.402 mg g^{-1}) than those of CC genotype (1.948 mg g^{-1}) and CT genotype (2.096 mg g^{-1}) . At the ADSL location, there were higher ($p<0.05$) IMP contents in birds that were TT (2.465 mg g^{-1}) genotype than those of CC (1.970 mg g^{-1}) and CT (2.034 mg g^{-1}) genotypes. Effect analysis of ADSL gene and GARS-AIRS-GART gene were showed in Table 2.

The additive effects of allele T for ADSL gene and GARS-AIRS-GART gene were respectively 0.227 and 0.248 mg g^{-1} which could be transmitted to the next generation (Edriss *et al.*, 2006). The frequencies of the two favorable TT genotypes were respectively 0.35 and 0.36 in tested populations, so through the selection means enabling favorable single gene pyramiding is feasible.

In a total of nine pyramiding genotypes were tested. Effects (least square means) of the pyramiding genotypes on IMP contents were showed in Table 3. The genotype TTTT pyramiding by two favorable TTs was still the most advantageous, there were higher ($p<0.05$) IMP contents in birds that were TTTT genotype (2.720 mg g^{-1}) than those birds with other eight genotypes. Effect pattern

Table 1: Association between different genotypes of ADSL and GARS-AIRS-GART genes with muscle IMP contents

Gene	Genotype	Genotype frequency	Sample size	IMP content (mg g^{-1})
GARS-AIRS	TT	0.35	140	2.402 ± 0.039^{A}
GART	CT	0.40	160	2.096 ± 0.042^{B}
	CC	0.25	100	1.948 ± 0.051^{B}
ADSL	TT	0.36	144	2.465 ± 0.026^{A}
	CT	0.28	112	2.034 ± 0.055^{B}
	CC	0.36	144	1.970 ± 0.061^{B}

Different capital letters means significant difference ($p<0.05$)

Table 2: Effect analysis of ADSL and GARS-AIRS-GART genes

	Gene effect		
Gene	Additive effect (mg g^{-1})	Dominant effect (mg g^{-1})	Degree of dominance
GARS-AIRS-GART	0.227	-0.079	-0.348
ADSL	0.248	-0.183	-0.738

Table 3: Association between different pyramiding genotypes of ADSL and GARS-AIRS-GART genes with IMP contents

Pyramiding genotype	Sample size	IMP content (mg g^{-1})
TTTT	41	2.720 ± 0.062^{D}
CCCC	49	1.983 ± 0.077^{A}
CCCT	36	2.106 ± 0.102^{AB}
CCTT	40	2.290 ± 0.094^{BC}
CTCC	40	2.117 ± 0.0680^{ABC}
CTCT	50	2.138 ± 0.082^{ABC}
CTTT	40	2.301 ± 0.081^{BC}
TTCC	39	2.285 ± 0.107^{BC}
TTCT	40	2.324 ± 0.055^{C}

Different capital letters means significant difference ($p<0.05$)

Table 4: Effect pattern analysis of single TT_{ADSL} and TT_{GAG} in pyramiding TTTT genotype

Source	F	Sig.	Eta squared
Corrected model	7.45	0.000	0.039
Intercept	2967.33	0.000	0.812
TT_{ADSL}	10.78	0.000	0.030
TT_{GAG}	9.52	0.003	0.021
TT_{ADSL}* TT_{GAG}	0.12	0.724	0.000

Computed using alpha = 0.05; Model: Intercept + TT_{ADSL} + TT_{GAG} + TT_{ADSL} *TT_{GAG}

analysis of single TT_{ADSL} and TT_{GAG} in the pyramiding TTTT genotype was showed in Table 4. The TT_{ADSL} and TT_{GAG} both played significant roles ($p<0.05$) on IMP content after pyramiding.

The IMP content had been increased by 10.34 and 13.23% compared to that of single genotype TT for ADSL and GARS-AIRS-GART genes. The genetic improvement had short cycle and higher efficiency compared with traditional selection based on observable phenotype (Grace *et al*., 2009).

The 90 days old body weight, age at first egg and 72 weeks old egg production of birds with different pyramiding genotypes were compared (Table 5). The conserving populations were treated as control group. The results showed pyramiding had no influence on production performance of Wenchang chicken, the average body weight, age at first egg and 72 weeks old egg production had no difference ($p>0.05$) among the birds with different pyramiding genotypes. Therefore, It was feasible to improve meat quality of Wenchang chicken by pyramiding favourable genotypes of ADSL and GARS-AIRS-GART genes.

Table 5: Production performance comparison of individuals with different pyramiding genotypes

Genotype	Sample size	Average body weight (g)	Age at first egg (day)	Egg production
TTTT	30	982.16±28.32	110.7±9.1	189.2±5.6
TTCC	30	981.38±30.19	113.3±10.4	188.3±6.3
TTCT	30	980.04±31.56	112.2±12.2	190.6±7.8
CTCC	30	982.14±28.09	109.0±10.9	188.7±6.6
CTCT	30	981.36±40.01	110.7±15.0	189.3±7.8
CTTT	30	983.12±37.46	110.2±11.5	190.1±9.2
CCCC	30	982.06±32.48	113.3±14.7	189.4±10.0
CCCT	30	984.00±35.17	111.1±13.5	187.6±10.3
CCTT	30	981.11±38.63	113.3±15.2	190.2±9.7
Control	300	982.22±39.03	110.1±12.6	189.6±9.2

CONCLUSION

It is concluded that the muscle IMP contents of Wenchang chicken can be markedly improved by pyramiding favorable genotypes of ADSL and GARS-AIRS-GART genes and still maintain the original production performance.

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