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Study on the Correlation Between Microsatellite Markers and Meat Quality Traits in Chicken

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Abstract: The genomes of 500 individuals in F_2 population of Recessive White Chicken and Xianju Chicken were screened using 26 microsatellite markers. The correlation between microsatellite markers and meat quality traits in chickens were analyzed. The results showed that: ADL212 on chromosome 2 was significantly associated with pH value and tenderness. MCW004 on chromosome 3 and MCW223 on chromosome 5 were significantly associated with meat color. Four microsatellite markers were significantly associated with water lost rate, they were: MCW264 and ADL212 on chromosome 2, MCW223 on chromosome 5 and ADL211 on chromosome 9.

Key words: Chicken, microsatellites, meat quality traits, chromosome, population

INTRODUCTION

Microsatellites, or Simple Sequence Repeat (SSR), Short Tandem Repeat polymorphisms (STRs), have gained widespread use in phylogenetics, conservation genetics, genome mapping, assessing genetic diversity and structure in population studies and identifying individuals and parentage due to their abundance and random distribution over the genome, high polymorphism, codominant nature, high reproducibility and relative ease of scoring by the Polymerase Chain Reaction (PCR) (Weigend *et al*., 2001; Arranze *et al*., 1996; Chung *et al*., 2006).

In the last decade, selection for broiler chickens has been focused on the improvement of production performance and carcass characteristics and has got great progress because meat and abdominal fat output has relative high level of heritability. This kind of selection can improve output and current profit, but it also resulted in the decrease of the meat quality. With the development of people's standard of living, higher meat flavor quality of chicken is demanded. Improvement and increasement of the meat quality has become one of the predominant directions in chicken breeding. However, chicken meat quality traits must be measured after slaughter, so they can't be selected directly during breeding. Marker-Assisted Selection (MAS) is a method of choice to choose meat quality traits. So searching for genetic markers associated with meat quality or Quantitative Trait Loci (QTL) controlling these traits are of great importance. Therefore, based on the polymorphisms of the 26, this study was aimed at analyzing correlation between microsatellite markers and meat quality traits, then searching appropriate microsatellite markers which affect chicken meat quality. The results may provide evidence for QTL mapping and MAS of chicken meat quality traits.

MATERIALS AND METHODS

Chicken population: With F2 design, Recessive White chicken and Xianju chicken were selected as parents to establish a resource population. A total of 500 individuals originating from this resource population were analysed in this study. Breast muscle tenderness, pH value, meat color and water lost rate were measured after slaughter at the age of 12 weeks.

DNA isolation: Per individual, 0.4 mL whole blood was collected from the ulnar vein with heparin as anticoagulant. Then, 4 mL of DNA lysate solution [2M urea, 100 mM Tris-HCl (pH 8.0), 1% SDS, 100 mM EDTA] was added and the mixture was stored at 4°C. DNA was isolated by using a phenol/chloroform based method (Sambrook *et al.,* 2001).

Microsatellite genotyping: Twenty six microsatellite markers were selected according to the published genetic maps of three resource populations. The information of these 26 markers were listed in Table 1.

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Table 1: The information of the 26 microsatellite markers

Markers	Chromosome	Total no. of alleles	Range of allele sizes (bp)
MCW 145	Chr.1	6	197~242
MCW248	Chr.1	6	208~258
ADL105	Chr.1	3	147~167
ADL185	Chr.2	6	118~163
MCW264	Chr.2	7	204~274
ADL212	Chr.2	5	100~129
MCW185	Chr.2	8	202~253
LEI147	Chr.2	7	254~327
MCW150	Chr.3	5	217~269
MCW004	Chr.3	4	181~240
ADL136	Chr.4	6	140~180
ADL166	Chr.5	6	128~173
MCW223	Chr.5	3	175~203
MCW95	Chr.8	3	227~263
ADL301	Chr.8	4	126~147
ABR322	Chr.8	4	134~160
MCW135	Chr.9	6	131~180
ADL211	Chr.9	5	103~141
ADL231	Chr.10	5	114~159
MCW67	Chr.10	4	177~209
ADL210	Chr.11	5	106~153
MCW44	Chr.12	5	166~198
ADL225	Chr.13	4	152~182
MCW104	Chr.13	5	192~248
ADL289	Chr.23	4	171~187
ADL123	E47W24	5	105~144

The 25 uL PCR volume included 50 ng of genomic DNA template, 1.0uM of each primer, 200 uM of each dNTP, 1.5 mM $MgCl_2$ and 1 U *Taq* DNA polymerase. The amplification involved initial denaturation at 95°C (10 min), followed by 30 cycles of denaturation at 95°C (1 min), primer annealing at temperature 48-66°C (1 min), extension at 72°C (1 min) and a final extension at 72°C (10 min).The obtained fragments were detected on 2.0% agarose gel.

The PCR products were subjected to 10% polyacrylamide gel in 1×TBE buffer and electrophoresed at 200 voltages for 2 hours. The DNA bands on the gel were viewed by silver staining. Allele-size scoring was performed with KDSZD 2.0 software.

Statistical analysis: Statistical analysis of associations between different genotypes and meat quality traits was performed using the GLM and CORR-procedure of SAS 8.0. The data were given in the mean±standard error format.

RESULTS

Correlation between chicken meat quality traits: The correlation between the four meat quality traits (tenderness, pH value, meat color and water lost rate) measured in the present study are shown in Table 2. From this table, we could obtain that water lost rate was significantly negative related with muscle tenderness ($p<0.01$), but significantly positive correlated with meat color ($p<0.01$), While pH value had negative correlation with meat color ($p<0.05$).

Table 2: Correlation between meat quality traits

Trait	pH value	Meat color	Tenderness	Water lost rate
pH value	1			
Meat color	-0.088*	1		
Tenderness	0.83	-0.053	1	
Water lost rate	-0.195**	0.191**	-0.227**	1

* p<0.05, ** p<0.01

Table 3: pH value and tenderness of individuals with different genotype

Genotype	Sample size	pHvalue	Tenderness
AB	47	5.94 ± 0.25^{a}	2.04 ± 0.69^{ab}
AC	229	5.72 ± 0.41^{b}	2.04 ± 0.68^{ab}
AD	76	5.73 ± 0.19^{ab}	2.35 ± 0.85^{a}
BD	64	5.75 ± 0.14^{ab}	1.98 ± 0.78^{ab}
CD	28	5.68 ± 0.13^{b}	2.11 ± 0.76^{a}
CE	17	5.69 ± 0.14^{b}	1.56 ± 0.43^{b}
DE	24	5.74 ± 0.15^{ab}	1.94 ± 0.81^{ab}

Note: Means with the different superscripts within the same column differs significantly (p<0.05)

Table 4: Meat color of individuals with different genotype

Locus	Genotypea	Sample size	Meat color
MCW004	AA	10	0.94 ± 0.50^{a}
	AB	203	0.50 ± 0.30^{b}
	AC	77	0.51 ± 0.27^{b}
	BC	112	0.61 ± 0.34^{b}
	CD	86	0.62 ± 0.33^{b}
MCW223	AA	43	0.71 ± 0.35^{ab}
	AB	178	0.54 ± 0.32^{ab}
	AC	139	0.50 ± 0.25^{a}
	BB	31	0.73 ± 0.36^{b}
	BC	87	0.55 ± 0.34^{ab}

Note: Means with the different superscripts within the same column differs significantly (p<0.05)

Microsatellite markers related with tenderness and pH value: Among 26 microsatellite loci, only locus ADL 212 on Chromosome 2 showed significant correlation with muscle pH value and tenderness. A total of 5 alleles and 7 genotypes were detected in ADL 212, Statistical analysis was applied to test different genotypic effects on pH value and tenderness. AB genotype birds had significant higher pH value than AC, CD and CE genotype birds. Individuals with CE genotype had significant lower tenderness than individuals with AD and CD genotype (Table 3).

Microsatellite markers related with meat colour: The least square analysis showed that MCW004 on chromosome 3 and MCW223 on chromosome 5 had significant correlation with meat color. The effects of different genotypes on meat color of the two microsatellite loci are presented in Table 4. On locus MCW004, four alleles and five genotypes were observed, among which individuals with AA genotype had significant higher meat color than individuals with other genotypes. There were 3 alleles and 5 genotypes on locus MCW223, where BB genotype birds had significant higher meat color than AC genotype birds.

Table 5: Water lost rate of individuals with different genotype

Locus	Genotype	Sample size	Water lost rate
MCW264	AD	52	32.24±4.42 [ab]
	AE	13	33.92±4.18 [ab]
	BD	14	30.67±6.54 [b]
	BE	162	31.04±4.50 [ab]
	BF	15	32.16±5.59 [ab]
	BG	13	35.00±3.94 [a]
	CF	160	33.08±3.43 [ab]
	CG	56	34.02±3.67 [ab]
ADL211	AB	25	32.81±2.69 [ab]
	AC	21	34.07±3.06 [a]
	AD	39	31.90±4.83 [ab]
	BB	12	34.81±4.69 [a]
	BD	105	31.20±4.85 [ab]
	BE	10	29.34±5.48 [b]
	CE	11	29.48±8.01 [b]
	DE	262	32.89±4.85 [ab]
MCW223	AA	44	33.49±5.03 [ab]
	AB	156	31.68±5.34 [b]
	AC	141	32.32±4.17 [b]
	BB	29	35.21±2.83 [a]
	BC	115	32.32±4.49 [b]
ADL212	AB	47	29.93±4.92 [b]
	AC	229	32.70±4.32 [ab]
	AD	76	31.99±6.37 [ab]
	BD	64	32.71±4.46 [ab]
	CD	28	32.03±3.74 [ab]
	CE	17	34.79±4.29 [a]
	DE	24	33.43±3.56 [a]

Note: Means with the different superscripts within the same column differs significantly ($p<0.05$)

Microsatellite markers related with water lost rate: The least square analysis showed that four markers associated with muscle water lost rate, they were MCW 264 and ADL 212 on chromosome 2, MCW223 on chromosome 5 and ADL211 on chromosome 9 (Table 5). On locus MCW264, BG genotype birds had significant higher water lost rate than BD genotype birds. On locus ADL 211, individuals with BB and AC genotype had significant higher water lost rate than individuals with CE and BE genotype. BB genotype chickens had significantly higher water lost rate than BC, AC and AB genotype chickens on locus MCW 223, while chickens with AB genotype had significant lower water lost rate than birds with CE and DE genotype.

DISCUSSION

Chicken meat quality trait is a synthetic character, including meat color, pH value, water lost rate, tenderness and so on. Meat color itself has no effect on meat flavor, but it influences people's sense to a great extent; water lost rate is a reflection of storage time (Bi *et al*., 2000); pH value is an important index which reflects the rate of in vivo glycolusis; meat tenderness can directly effect the taste of chicken. There have been some domestic and international reports about relationship between chicken meat quality traits and QTL mapping (Bi *et al*., 2000; Deng *et al*., 2001; Huang *et al*., 2004; Kaam, 1999; Knott *et al*., 1996; Wang *et al*., 2004; Zeng *et al*., 2003; Zeng, 1994).

Correlations between four meat quality traits, meat color, pH value, water lost rate and tenderness were analyzed in the present study. The results showed that pH value was significantly negative related with meat color, water lost rate had negative correlation with pH value and tenderness and was positively correlated with meat color. Hou *et al*. (2003) studied the relationship between microsatellite markers and pig meat quality traits, they found that pH value was significantly positive related with water lost rate. Polymorphisms of microsatellites are usually used to assess animal genetic diversity, classify, conserve and utilize of livestock and poultry breeds. Furthermore, it can also used in the marker assisted selection of important economic traits in animal. Sewalem *et al*. (2001) analyzed interval mapping QTLs in an F2 chicken population established from a cross of a broiler sire-line and an egg laying (White Leghorn) line at 3, 6 and 9 wk body weights, using 101 microsatellite markers, the QTL significant at the genome wide level that affected body weight at two ages were identified on chromosomes 1, 2, 4, 7 and 8 and a QTL on Chromosome 13 influenced body weight at all three ages. In the present study, the polymorphisms of 26 microsatellite markers associated with meat quality traits were analyzed, locus ADL 212 on chromosome 2 showed significant correlation with muscle pH value and tenderness, MCW004 on chromosome 3 and MCW223 on chromosome 5 had significant correlation with meat color, MCW 264 and ADL 212 on chromosome 2, MCW223 on chromosome 5 and ADL211 on chromosome 9 significantly associated with water lost rate.

CONCLUSION

The correlation between microsatellite markers and meat quality traits were analyzed, according to the polymorphisms of 26 microsatellite loci in 500 individuals originating from "Recessive White chicken" ¡Á "Xianju chicken" resource population. The results may provide evidence for QTL mapping and MAS of chicken meat quality traits.

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