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Association between Single Nucleotide Polymorphisms in *TYW5* Locus and Beef Amino Acids Content in Shuxuan Cattle

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ABSTRACT

Background: Shuxuan cattle is a crossed cattle breed in southwest of China, by crossing the indigenous Bashan cattle with two exotic breeds. The objective of this study was to estimate allele frequencies of the g.88925365A>C in *TYW5* gene in Shuxuan cattle and to evaluate association between this polymorphism and meat quality traits.

Methods: The SNP g.88925365A>C was genotyped in 151 Shuxuan cattle and subjected to association analysis with the 17 amino acid content and total amino acid.

Result: The AC was the main genotype with 41.06% and C was the predominant allele with 50.99% at this locus. The allele diversity based on the He (0.4998), Ne (1.9992) and PIC (0.3749) revealed moderate genetic variation within Shuxuan cattle population. Significant association ($P<0.05$) was observed between the polymorphism and six amino acid content (Glu, Asp, Val, Ile, Ser and Cys). These results provided a potential genetic marker for the genetic improvement of meat quality in Shuxuan cattle.

Key words: Amino acid content, Association analysis, Shuxuan cattle, *TYW5* gene.

INTRODUCTION

Protein accounts for 45% in the total dry matter of the human body and 70% in the total muscle. It is a necessary nutrient in human diet. The protein requirement was taken as 0.93 g/kg/d for adults and most of it from a variety of meat (Elango *et al.* 2012). The average global consumption of meat has been estimated to be 122 g/d per person, of which a fifth is beef only next to pork and chicken (Godfray *et al.* 2018). Beef is rich in protein with high digestibility and absorption rate and well-balanced amino acid composition relative to human requirement. There is 63%~68% crude protein on a dry matter basis in beef and it contains all nutritionally essential and functional amino acid for humans (Wu *et al.* 2016). Thus, beef is a quality source of dietary amino acid to human growth, development, and health. For example, sufficient amino acid is required for muscle protein synthesis and leucine enhance the rate of muscle protein synthesis and glutamate improve appetite and gastrointestinal function (Hoffer 2016). A recent study found that beef protein diet significantly upregulated the expression of immune-related proteins, which could improve the body's resistance to disease (Shi *et al.* 2018). Moreover, amino acids can impart sweet, umami, sour and other tastes of beef, contributing to the increasing consumer like flavor scores (Joo *et al.* 2017).

The Shuxuan cattle is a crossed dual-purpose breed by crossing the Chinese indigenous cattle (Bashan cattle) with Simmental cattle and Holstein cattle during the past 30 years in Sichuan province. It consists of 75% Simmental, 15% Bashan and 10% Holstein blood and mainly distributes in Southwest China (Chen *et al.* 2017b).

Wybutosine is a hyper modified guanosine found in position 37 of eukaryotic tRNA Phe and is essential for correct phenylalanine codon translation. The tRNA wybutosine-synthesizing enzyme 5 (*TYW5*) is a Jumonji C-domain-containing protein, which acts as a component of the wybutosine biosynthesis pathway (Rodriguez *et al.* 2012). Previous genome-wide association study reported that the SNP rs41594486 in *TYW5* gene is associated with carcass quality traits in crossbreed cattle (Lu *et al.* 2013). Since to our knowledge, there is no research about the genotype distribution of SNP rs41594486 in *TYW5* gene in Shuxuan cattle. The identification of genetic markers associated with

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amino acid composition of beef was potential to improve meat quality traits via marker-assisted selection (MAS). Therefore, it is preliminary and interesting work to investigated the genetic variation in the *TYW5* gene for Shuxuan cattle herd improvement.

MATERIALS AND METHODS

Animals and amino acids analysis

A total of 151 healthy male Shuxuan cattle with 18 months of age were selected in the present study. According to pedigree records, the genetic relationships within three generations were avoided for all animals. After slaughtering and chilling 48 h, the loin eye muscle between ribs 12 and 13 was sampled to extract genomic DNA and measure amino acids. Genomic DNA was extracted using TIANamp Genomic DNA kit (TianGen™, Beijing, China) and then stored at -20°C for later analysis. The content of 17 amino acids were measured by gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis was performed on a HP6840/HP5973 apparatus (Agilent Technologies, Les Ulis, France) equipped with an HP-101 capillary column (L25 m×0.2 mm×0.2 um, Agilent). The injector temperature was 280°C, with an injection volume of 1.0 µl. The oven temperature was maintained at 70°C for 3 min, increased at 5°C/min to 220°C then increased at 10°C/min to 260°C, hold for 15 min. The injection volume was 1.0 µl, the carrier gas was high purity helium at a flow rate of 20 ml/min and the split ratio of 60:1. The electron impact (EI) ion source was used for MS, ion source temperature of 200°C, with ionization voltage of 70 eV.

SNP selection and genotyping

The candidate SNP (2:g.88925365A>C with GenBank accession number of rs41594486) was selected based on previous genome-wide association study (Lu *et al.* 2013), and was genotyped using a custom-by-design SNPscan™ Kit (Genesky Biotechnologies Inc., Shanghai, China) in the present study. Briefly, 100-200 ng genomic DNA was used for producing 20 µL ligation mixture containing 1×Ligase buffer, 1×probe and 0.5 µL ligase. The reaction was performed on a ABI 2720 Thermal Cycler (Applied Biosystems, USA) under the following program: 2 min at 98°C, 4 cycles of (94°C 1 min, 58°C 4 hr), 94°C 2 min, hold at 4°C and immediately stopped by adding 20 µL of 2×Stop Buffer. Multiplex fluorescence PCR reactions were performed for each ligation product. Each PCR reaction was prepared in a 20 µL mixture containing 1×PCR Master Mix, 1 µL Primer Mix SetA or SetB and 1 µL ligation product. PCR products were separated and detected by capillary electrophoresis on ABI3730XL sequencer (Applied Biosystems, USA).

Statistical analysis

Genotype frequencies and allelic frequencies were determined by direct count and calculation. The Hardy-Weinberg equilibrium was measured through χ^2 test. Population genetic characteristics were calculated according to Nei's methods (Nei and Roychoudhury 1974), including heterozygosity (He), effective allele numbers (Ne) and polymorphism information content (PIC).

The association between genotypes and meat quality traits were investigated under the linear mixed model (LMM) as:

$$Y_{ij} = \mu + G_i + \varepsilon_{ij}$$

Where

Y_{ij} is measurement values of meat quality traits, μ is overall of mean for each trait, G_i is the effect of genotypes (AA, AC and CC); ε_{ij} is random residual effects. The model fitting and statistical tests were conducted using the R package of lmer Test (Kuznetsova *et al.* 2017). The Bonferroni method was used for adjustment for multiple comparisons (Chen *et al.* 2017a).

Ethics approval

Collection of biological samples and experimental procedures involved in this study were approved by the Institutional Animal Care and Use Committee in College of Animal Science and Technology, Sichuan Agricultural University, China (DKY2019124).

RESULTS AND DISCUSSION

In this study, the content of 17 amino acids were measured by GC-MS and the total amino acids were calculated in the Shuxuan cattle (Table 1). The decreasing order of the content of these 17 amino acids in beef were Glu, Asp, Lys, Leu, Arg, Ala, Val, Gly, Thr, Ile, Ser, His, Phe, Pro, Tyr, Met, Cys, from 3.37% to 0.14%. Among them, the sum of 7 essential amino acids was about 38.27% of the total amino acid. Except for cystine, the content of other 16 amino acids is relatively stable in beef with the coefficient of variation within 16%. The overall distribution and pairwise correlations among the 18 amino acids were shown in Fig 1. Beside the Cys, all other 17 amino acids showed the normal distribution more or less. There are 143 pairwise correlations at significant level ($P<0.05$) among all 18 amino acids. The highest correlation was observed between Ser and Asp as well as between total amino acid and Thr with spearman's coefficient of 0.97 ($P<0.001$). On the whole, there are strong pairwise correlations among the contents of the amino acids except for Cys in beef of Shuxuan cattle.

The SNP g.88925365A>C of *TYW5* gene had been successfully genotyped in all cattle. The genetic diversity and population genetic characteristics of this SNP are shown in Table 2. For this SNP, AC was the main genotype with a high frequency of 41.06% and C was the predominant allele with a medium frequency of 50.99%. The χ^2 test indicated that the genotype distribution of SNP g.88925365A>C was not in Hardy-Weinberg equilibrium ($\chi^2_{0.05}<\chi^2<\chi^2_{0.01}$). According to the allele frequencies, we calculated that He, Ne and PIC were 0.4998, 1.9992 and 0.3749, respectively. The allele diversity based on the He, Ne and

Table 1: Summary of the 18 amino acid composition of *longissimus dorsi* in Shuxuan cattle.

Traits (%)	Summaries of measurements				
	1st Quartile	Median	Mean	3rd Quartile	Variation coefficient (95% confidence interval)
Glutamic acid (Glu,%)	3.16	3.42	3.37	3.57	8.12 (8.01-8.22)
Aspartic acid (Asp,%)	1.82	1.94	1.92	2.02	7.69 (7.59-7.78)
Lysine (Lys,%)	1.74	1.86	1.83	1.93	8.64 (8.52-8.76)
Leucine (Leu,%)	1.57	1.66	1.64	1.72	7.36 (7.27-7.45)
Arginine (Arg,%)	1.21	1.28	1.28	1.35	8.77 (8.64-8.89)
Alanine (Ala,%)	1.18	1.25	1.24	1.30	7.16 (7.08-7.24)
Valine (Val,%)	0.88	0.95	0.94	0.99	8.36 (8.25-8.47)
Glycine (Gly,%)	0.85	0.89	0.91	0.95	10.61 (10.44-10.80)
Threonine (Thr,%)	0.85	0.90	0.89	0.94	7.86 (7.76-7.96)
Isoleucine (Ile,%)	0.81	0.88	0.87	0.93	9.53 (9.38-9.68)
Serine (Ser,%)	0.76	0.81	0.80	0.84	7.86 (7.76-7.96)
Histine (His,%)	0.73	0.80	0.80	0.87	11.64 (11.43-11.87)
Phenylalanine (Phe,%)	0.71	0.74	0.74	0.77	7.43 (7.34-7.52)
Proline (Pro,%)	0.67	0.72	0.72	0.76	15.41 (15.04-15.80)
Tyrosine (Tyr,%)	0.65	0.68	0.68	0.71	15.02 (14.66-15.39)
Methionine (Met,%)	0.50	0.55	0.53	0.58	12.34 (12.10-12.59)
Cystine (Cys,%)	0.04	0.05	0.14	0.28	106.83 (91.17-128.99)
Total amino acid (T.AA,%)	18.50	19.5	19.28	20.15	7.21 (7.13-7.30)

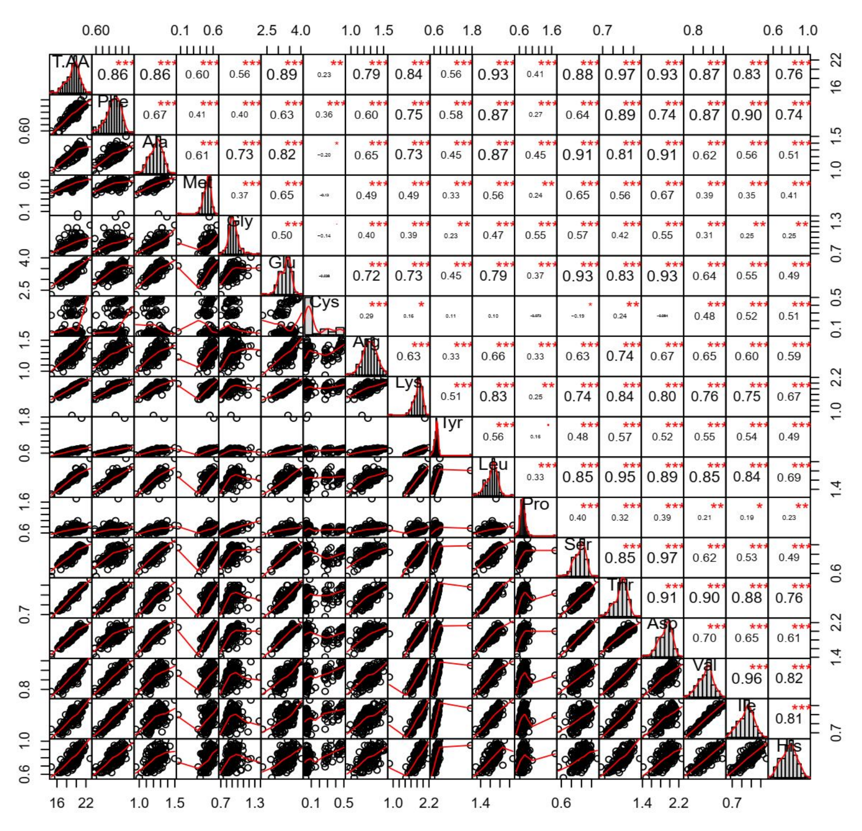

Fig 1: Correlation matrix for the amino acid composition of longissimus dorsi. The overall distribution of traits are individually demonstrated as histogram plots on the diagonal. The spearman correlation coefficient and statistical tests ("***" < 0.001, "**" < 0.01, "*" < 0.05 and "." < 0.1) are on the upper triangular. The bivariate scatter plots are placed on the lower triangular.

PIC revealed moderate genetic variation within Shuxuan cattle populations.

The association between genotypes of SNP g.88925365A>C and 18 amino acids content of longissimus dorsi were analyzed and the results are shown in Table 3. Accordingly to the stringent criterion of Bonferroni multiple adjustment, there was a significant difference in Glu, Ser, Asp, Cys, Val and Ile among the three genotypes. Individuals with genotype AC had significantly higher Glu, Ser and Asp than those of CC genotype (P<0.01). Individuals with genotype CC had significantly higher Cys, Val and Ile than those of AA genotype (P<0.01). Individuals with genotype CC also had significantly higher Cys than that of AC genotype (P<0.01). No difference was observed between any genotype of SNP g.88925365A>C and other 12 traits (P>0.05). These results suggested that g.88925365A>C could be used as a valuable marker to select excellent Shuxuan cattle with desired meat quality.

The nutritional value of meat has become an increasingly important factor influencing consumer choice of domestic animal products in China. Beef is a good source of protein for human, which has high biological value protein and all the amino acid in about the right proportions required by humans (Basulira *et al.* 2019). The amino acid content varies slightly among different cattle breeds (Prajwal *et al.* 2019; Suliman et al. 2017). Generally speaking, the most abundant amino acids in beef are Glu, Asp, Ala, Lys and Leuÿwhile the contents of Met, His and Tyr are much lower. And the essential amino acids account for more than 40% of the total amino acids in beef (Patel *et al.* 2017). In this study, the content of 17 amino acids were measured by GC-MS with the decreasing order of Glu, Asp, Lys, Leu, Arg, Ala, Val, Gly, Thr, Ile, Ser, His, Phe, Pro, Tyr, Met, Cys in the beef of Shuxuan cattle, and they showed strong correlations with each other. The sum of essential amino acids is about 38.27% of total amino acid. These results are consistent with other studies, which beef is a good source of dietary protein for human.

Table 2: Population genetic characteristics of *TYW5* gene single nucleotide polymorphism (g.88925365A>C) in Shuxuan cattle.

SNP	Observed genotype (number)	Total	Frequency			χ^2(HWE§)
			Genotype	Allele	Genetic characteristics	
g.88925365A>C	AA (N=43)	151	0.2848	A=0.4901	He* =0.4998	4.8103
	AC (N=62)		0.4106	C=0.5099	Ne†=1.9992	
	CC (N=46)		0.3046		PIC‡=0.3749	

Note:*, He= Gene heterozygosity; †, Ne= Effective allele number; ‡, PIC= Polymorphism information content; §, HWE= Hardy weinberg equilibrium; $\chi^2_{0.05(1)}$=3.84,$\chi^2_{0.01(1)}$=6.63.

Table 3: Association between g.88925365A>C genotypes of the *TYW5* gene and amino acid composition of *longissimus dorsi* in Shuxuan cattle.

Trait	Genotype (mean±SD*)		
	AA(43)	AC (62)	CC (46)
Glutamic acid (Glu,%)	3.39±0.27^{ab}	3.43±0.28^{a}	3.26±0.24^{b}
Aspartic acid (Asp,%)	1.92±0.16^{ab}	1.95±0.15^{a}	1.87±0.12^{b}
Lysine (Lys,%)	1.82±0.15	1.85±0.19	1.82±0.12
Leucine (Leu,%)	1.62±0.13	1.66±0.12	1.64±0.11
Arginine (Arg,%)	1.27±0.12	1.28±0.12	1.28±0.09
Alanine (Ala,%)	1.25±0.10	1.25±0.09	1.21±0.07
Valine (Val,%)	0.91±0.08^{b}	0.94±0.08^{ab}	0.96±0.07^{a}
Glycine (Gly,%)	0.91±0.12	0.92±0.09	0.88±0.07
Threonine (Thr,%)	0.87±0.08	0.90±0.07	0.88±0.06
Isoleucine (Ile,%)	0.83±0.09^{b}	0.87±0.08^{ab}	0.89±0.08^{a}
Serine (Ser,%)	0.80±0.06^{ab}	0.81±0.06^{a}	0.77±0.06^{b}
Histine (His,%)	0.78±0.10	0.80±0.09	0.81±0.10
Phenylalanine (Phe,%)	0.72±0.06	0.74±0.05	0.74±0.06
Proline (Pro,%)	0.72±0.10	0.74±0.14	0.71±0.06
Tyrosine (Tyr,%)	0.66±0.06	0.70±0.15	0.67±0.05
Methionine (Met,%)	0.54±0.05	0.54±0.08	0.51±0.05
Cystine (Cys,%)	0.09±0.13^{b}	0.11±0.14^{b}	0.23±0.16^{a}
Total amino acid (T.AA,%)	19.13±1.54	19.50±1.42	19.13±1.19

Note:*, SD = Standard deviation; ^{a,b,c}Means with different superscripts were signifcantly different (P<0.01).

In modern beef cattle breeding, meat and carcass traits have been widely included into the genetic selection programs. Genetic association analysis revealed that hundreds of genetic markers were significantly associated with meat and carcass traits in beef cattle (Wang *et al.* 2017; Liao *et al.* 2018; Magalhaes *et al.* 2019; He *et al.* 2020; Leal-Gutierrez *et al.* 2020). In terms of beef muscle composition, many markers are associated with fatty acid content (Gamarra *et al.* 2018; Beak *et al.* 2019). However, there are few markers associated with amino acid content. In this study, we first focused on the relationship between *TYW5* gene polymorphism and amino acid content in cattle. The SNP g.88925365A>C of *TYW5* gene were successfully genotyped in Shuxuan cattle. It was found significant associations between this SNP and content of Glu, Asp, Val, Ile, Ser and Cys. Among them, Glu and Asp are the delicious amino acids and Ile is the necessary amino acid. There are relatively more of them in individuals with AC genotypes, which indicated AC is the favoured genotype for those amino acids. The TYW5 protein, a Jumonji C (JmjC)-domain-containing protein, hydroxylates wybutosine to hydroxywybutosine at position 37 in phenylalanine tRNA using Fe(II) ion and 2-oxoglutarate (2-OG) as cofactors (Bhat *et al.* 2019). The SNP rs41594486 in the *TYW5* gene was significantly associated with lean yield grade and lean in a genome wide association study, explaining 3.68% and 2.57% of the variation in lean yield grade and lean (Lu *et al.* 2013).

CONCLUSION

In summary, we first measured the content of 17 amino acids in the Shuxuan cattle beef and then investigated their association with the genetic polymorphism of *TYW5* gene. Beef of Shuxuan cattle is rich in amino acids and the amino acid content of individuals with AC genotype is the the favoured in Shuxuan cattle. The results would help us to better understanding the meat quality and genetic resources of this breed.

Data availability

The original data used in this study are available from the corresponding author upon request.

Author contributions

Wei Wang and Songjia Lai conceived and designed the research. Maozhong Fu, Jun Yi and Donghui Fang performed the material preparation and experiments. Xianbo Jia, Shiyi Chen and Jie Wang performed the data collection and analysis. Xianbo Jia and Shiyi Chen drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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