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Effect of Microsatellite Marker on Bull Meat Traits

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Abstract: The aim of the present study was to verify associations between bull meat traits and 6 microsatellite loci. A sample of 66 weaned young bulls from Grassland Red Cattle (GLRC) and its improved hybrids by Limousine (LM) was characterized for BM2113, TGLA44, ETH225, IDVGA46, BM1824 and IDVGA55. The muscularity evaluation traits in the regulation of linear valuation and slaughtering meaty traits were used to evaluate their meat traits. The relationship between bull meat traits and 6 microsatellite loci was analyzed by SPSS. The results showed that for microsatellite BM2113, the allele 142 had a positive correlation with net meat weight and net meat rate; for microsatellite IDVGA46, the allele 211 had a negative correlation with the 5 muscularity evaluation and 4 slaughter meaty traits; for BM1824, the allele 171 had a positive correlation with Loin thickness; for microsatellite TGLA44, the allele 221 had a positive correlation with some muscularity evaluation traits such as withers, loin thickness, hip shape and some slaughtering meaty traits such as body weight, carcass weight and net meat weight.

Key words: Bull, meat trait, microsatellite loci, carcass weight, body weight, China

INTRODUCTION

As a molecular genetic marker, microsatellite DNA has large number and distributed randomly in genome of most eukarya and a few prokaryotes and it has many advantages including abundant polymorphism, easy to detect, conservation and Mendelian inheritance, etc. It was widespread applicated in animal genetics and breeding. By analysising the relationship between polymorphism of microsatellite DNA and productivity performance, some genetic markers related to productivity performance can be found which can be applied to animal molecular breeding and genetic Marker-assisted Selection (MAS).

Some researches have gotten ideal results from researchers both home and abroad such as the test in hybrid cattle of Piedmontese cattle and Chianina, the test in Nanyang cattle, Piedmontese cattle as well as their hybrid filial generation, the test in Yanbian cattle and Limousine as well as their hybrid filial generation, the test in Brahman steers, the test in live yearling bull, etc. (Napolitano *et al*., 1996; Cao *et al*., 1999; Hauguo *et al*., 2004; Smith *et al*., 2007; Bergen *et al*., 2006).

Grassland red cattle which adapt to live in Northern prairie grassland regions is a dual-purpose meat and milk variety in characteristics. They are mainly distributed in the cold alpine grasslands in North Hebei inner Mongolia and Jilin. They are deeply welcomed by the local farmers and herdsmen as they have some highlight advantages including the strong adaptability, suitable for grazing, feeding coarse-resistant, good disease-resistant ability, high milk fat percentage as well as good meat quality. However with foreign and domestic varieties of good beef cattle breeds in comparison there are some shortages such as smaller body, slower growth and lower meat production, etc. In order to improve the meat production performance of grassland red cattle, some superior abroad cattle varieties such as the Danish red cattle and Limousine cattle have been introduced to create a hybrid. The results of production performance of filial generation indicated that Limousine cattle can effectively improve the meat performance of grassland red cattle and it can be considered as a good hybrid combination partner (Yu, 1998).

In this study, microsatellite markers are implicated to study the genetic variation of grassland red cattle in the molecular level and the relations between meat performance and genetic markers in filial generation bull were analyzed so as to provide a theoretical basis for creating a new meat strain in Grassland red cattle.

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MATERIALS AND METHODS

Test samples of cattle: About 42 Purebred grassland red cattle, 24 hybrids of Limousine cattle and Grassland red cattle including 13 F_1, 6 F_2 and 5 F_3, total 66 were selected as studying group. Blood samples were collected by puncture of the jugular vein and saved in -20°C with EDTA.

Reagent: Taq DNA polymerase, 4×dNTP, Proteinase K, RNAase, Tris saturated phenol, N, N'-methylene-bis-acrylamide, EDTA, DNA Marker, TEMED, etc.

Extraction of genomic DNA: Genomic DNA was extrcted from blood samples using standard procedures referring to <molecular cloning: A laboratory manual> (Sambrook and Russell, 2002).

Primer screening, synthesis and PCR amplification: The 6 microsatellite loci related to some production performance in other cattle breed were selected from five chromosomes (Napolitano *et al*., 1996; Cao *et al*., 1999; Hauguo *et al*., 2004). Microsatellite primers were identifed basing on National Centre for Biotechnology Information (NCBI)-GenBank. Primers were synthesized by the Beijing state-ting the development of biotechnology centers and biotechnology companies. Genomic DNA was amplified at the following 6 microsatellite loci IDVGA46, TGLA44, BM1824, ETH225, BM2113 and IDVGA55 with Polymerase Chain Reaction (PCR). Six microsatellite loci primers and PCR amplification reaction conditions are shown in Table 1. Amplification profiles consisted of one cycle at 94°C during 5 min; 35 cycles with the following steps each one of 30 sec at 94°C, 55°C 45 sec (this temperature was changed for each loci according with Table 1), 45 sec at 72°C and finally one cycle of 10 min at 72°C.

PCR products detection and electrophoresis typing: PCR products were detected on 1.5% Agarose gel electrophoresis, adding a standard sample in gel. Agarose gel was observed in the UV transmittance to analyze whether there is the required strip. If so, PCR products were denaturized at 95°C for 5 min and then electrophoresed on 8% denaturing polyacrylamide-bisacrilamide gel during 2 h. The gels were stained with silver staining method then gel maps, photographs were saved to determine the genotype.

Genotype determination: All the alleles of microsatellite were analysed by UVIBAND Version 99 software. Fragments of different sizes was different alleles which named A-E and so on, respectively according to the order from small to large allele sequence.

Production performance of cattle: About 8 months of age and well-developed weaning young bull were selected and feeded for this experiment. The feeding standard was Chinese Beef Feeding Standard in the middle levels of nutrition. In the end of fattening period the slaughtering meat traits (slaughtering procedures were referred to Chinese beef slaughter test provisional standard) and beef muscle-degree linear meat traits (referring to Italy muscle-degree assessment criteria of linear shape score about Piedmontese beef) were measured (Cao, 1999).

Statistical analysis: Only varieties and genetic markers were considered in this experiment and two-factor cross-group design was used with the model of:

$$Y_{ij} = u+\alpha_i+\beta_j+e_{ij}$$

Where:

Y_{ij} = Phenotype records of individual
α_i = Variety effect
β_j = Genetic markers effect
e_{ij} = Random error

The result of genotype and production trait carried on non-equilibrium data analysis by SPSS for Windows of General Liner Model (GLM). Significance test of difference and multiple comparisons in production traits between the different marker genotypes were carried out by LSD. Finally, the effects of allele were analyzed.

Table 1: The primers characteristics and PCR reaction conditions in 6 pairs of microsatellite

Micro-satellite (D No.)	Repeats	Products	Primers	Lod-scorep	Annealing temperature/(°C)	Mg^{2}+concentration/(%)
IDVGA46	$(CA)_{11}$	205	AAATCCTTTCAAGTATGTTTTCA			
(D19S18)			ACTCACTCCAGTATTCTTGTCTG	19q16	50	1.5
TGLA44			AACTGTATATTGAGAGCCTACCATG			
(D2S3)			CACACCTTAGCGACTAAACCACCA	2q	65	1.5
BM1824	$(CA)_{15}$	187	GTTCAGGACTGGCCCTGCTAAACA			
(DIS34)			CCTCCAGCCACTTTCTCTTCTC	1q	59	1.5
ETH225	$(CA)_{18}$	149	GATCACCTTGCCACTATTTCCT			
(D9S1)			ACATGACAGCCAGCTGCTACT	9q	68	2.0
BM2113	$(CA)_{20}$	150	GCTGCCTTCTACCAAATACCC			
(D2S26)			CTTCCTGAGAGAAGCAACACC	2q	55	1.5
IDVGA55	$(AC)_{12}$	199	GTGACTGTATTTGTGAACACCTA			
(D18S16)			TCTAAAACGGAGGCAGAGATG	18q24	55	1.0

RESULTS AND DISCUSSION

Analysis of Variance (ANOVA) by GLM: The fixed model was composed with the marked effect of various microsatellite loci. The ANOVA of mean-square of production traits in 6 microsatellite loci were carried in Table 2. Most of muscle rating traits were significance of difference in variety such as withers, shoulder, waist thick, big thigh, hip shape. Some meat carcass traits were significance of difference in variety such as dressing percentage, net meat weight, net meat rate. Some loci marker had a significant effect upon the muscle-degree linear score traits and slaughtering meat traits, for microsatellite TGLA44, alleles E (221 bp) had a positive impact on the meat performance in the group which were consistent with the relevant reports. It has been confirmed that microsatellite locus TGLA44 and double muscle gene were combined in the chromosomes 2 of cattle and Limousine contained double muscle genes (Zhang *et al*., 1998; He and Lai, 2005).

Significant test of difference about genotypes: The significant tests of traits difference among genotypes of 6 microsatellite loci were carried out. About 4 microsatellite loci were found having significant effect to some beef traits which were shown in Table 3-6. The frequency in some genotypes were too low and lack the analytical value so in this test each genotype observed at least three times was considered.

Table 3 showed significant difference test results of different traits corresponding to different genotypes at TGLA44 microsatellite locus. In muscularity evaluation traits such as withers and loin thickness, etc., EE genotype was the largest which was significantly higher than the genotype AA and AD ($p<0.05$), AE genotype which contain allele E compared with AA and AD were also higher ($p>0.05$). In the hip shape, body weight, carcass weight and net meat weight, etc., EE genotype was higher than AD ($p<0.05$) and AE is also higher than AA and AD ($p>0.05$) which showed that allele E (221 bp) had a positive impact on muscle score traits such as withers, loin thickness, hip shape, etc. and slaughtering meat traits such as body weight, carcass weight and net meat weight.

Table 4 showed significant difference test results of different traits corresponding to different genotypes at BM2113 microsatellite locus. From Table 4, researchers could see in trait of net meat weight, BC genotype was significantly higher than AB ($p<0.05$) and it was higher than BB and AB ($p>0.05$) which showed that allele C (142 bp) have positive impact on net meat weight traits. In trait of net meat rate, genotype BC and AC was higher than the genotypes which did not contain allele C, AC genotype was significantly higher than AB ($p<0.01$), BC genotype was significantly higher than AB ($p<0.05$) which showed that allele C (142 bp) had a positive impact on net meat rate traits too. Table 5 showed significant difference test results of different traits corresponding to different genotypes at BM1824 microsatellite locus. In trait of loin thickness, genotype AD was significantly higher than genotype BB and DD ($p<0.05$) and was also higher than the BD and CD ($p>0.05$). It showed that allele A (171 bp) had positive effects on trait of loin thickness.

Table 6 showed significant difference test results of different traits corresponding to different genotypes at IDVGA46 microsatellite locus. From Table 4 in traits such

Table 2: The result of 6 kinds of microsatellite GLM ANOVA

	Muscularity evaluation traits							Slaughtering traits			
Microsatellite	Withers	Shoulder	Loin thickness	Big thigh	Hip shape	Body mass	ADG	Carcass weight	Dressing percentage	Net meat weight	Net meat rate
IDVGA55											
Variety	4.00*	2.10*	1.40	2.10*	1.40	539.8	16978.0	876.5	8.85**	1050.3*	8.83
Mark	1.30	0.30	0.20	0.90	0.90	94.1	3357.0	192.8	3.84	273.8	6.24
IDVGA46											
Variety	3.86**	3.69**	5.17*	7.07**	9.12**	176.7	1176.1	266.6	4.25	244.6	5.33
Mark	1.09	0.97	1.46	1.46	2.04*	382.0	2834.0	199.6	1.31	122.0	1.53
ETH225											
Variety	4.50**	3.30*	A5.50*	6.30**	7.70**	825.0	14394.0	627.2	7.58*	585.4	10.48
Mark	0.20	0.20	0.50	0.40	0.10	403.7	1864.0	430.4	3.87	394.6	6.87
BM2113											
Variety	1.43	1.50	2.14	3.23	4.30	478.4	7472.6	620.0	4.24	432.6	4.06
Mark	0.39	0.36	1.15	0.39	0.16	473.4	5256.1	91.5	3.38	111.4	1.07
BM1824											
Variety	3.45**	2.21*	3.28	3.80*	5.28**	714.7	2499.0	269.9	6.74	214.0	11.30
Mark	1.31	0.83	0.76	0.76	0.17	598.1	1451.0	95.7	5.45	91.2	4.22
TGLA44											
Variety	2.15*	1.39	2.45	3.55*	4.68**	276.5	4554.6	408.2	5.08	334.8	5.13
Mark	0.67	0.44	1.68	0.14	3.14*	687.9	4640.3	245.0	0.21	485.8*	0.27

*Means $p<0.05$; **Means $p<0.01$

as withers, shoulder, thigh muscle, hip shape, carcass weight, dressing percentage, net meat weight, net meat rate, AC genotype was significantly lower than BB, AB and BD ($p<0.05$ or $p<0.01$) also less than the AA genotype. In trait of loin thickness, genotype AC was significantly lower than genotype BD ($p<0.05$) and was also less than the genotypes AA, AB and BB. These results suggest that allele C (211 bp) had a negative impact on above bovine meat traits.

In this study, alleles E was not detected in Grassland red cattle but was detected in hybrids of Limousine and Grassland red cattle which showed that allele E came from Limousine. For microsatellite IDVGA46, study of found that the allele 211 had negative impacted on shoulder development in Nanyang cattle, Piedmontese cattle and their hybrids (Cao, 1999). The results of this study showed that allele C (211 bp) had negative impacted on shoulder development. IDVGA55 had no impact on meat traits in all studies. For microsatellite IDVGA46, study of Jin showed that allele 249 had positive and alleles 203 and 245 had negative effect on waist width traits of muscularity evaluation traits (Hauguo *et al*., 2004). In this study, alleles 203 had no effect on traits and alleles 205, 245 and 249 were not detected in the groups.

The reasons of the difference in these studies had two reasons one is the studied groups of cattle were different, the other is the size of research groups were different. In theory, the big group is better than the small. As conditions in this study, the group were small there may have a certain margin of error in study results. Therefore, this study is only a preliminary study. In the future, further investment is needed to conduct more in-depth research and ensure the accuracy of the findings. At present, muscularity evaluation traits such as withers, shoulder, loin thickness, big thigh muscles and hip shape which could reflected the development of cattle muscle were widely used to the study of beef performance. Cao (1999) considered these ratings traits as the visual assessment to muscle and they can more objective descript the development of beef cattle. Cattle's muscles growth situation can be tested by this method in the condition of having no feasible to slaughter the cattle. Jin studied the relationship between microsatellite markers and meat traits of Yanbian yellow dual-purpose cattle with service and meat by using Beef Linear Shape Score Method (Hauguo *et al*., 2004). In this study, the muscle traits of Grassland red cattle and its hybrid were scored by the muscularity evaluation traits in the regulation of linear valuation and meat production were measured. So, the study to the meat traits of Grassland red cattle was more objective and accurate.

At the same time, the application of the muscularity evaluation traits in the regulation of linear valuation on dual-purpose with milk and meat Grassland red cattle were studied. Form the results, the conclusion from two methods were consistent. For microsatellite locus IDVGA46, allele C (211 bp) had negative impact on 5 muscle severity score and 4 slaughtering meat traits. For

Table 3: Association of TGLA44 locus and traits

Genotypes	AA185/185	EE221/221	AD185/205	AE185/221
Number	7	5	10	6
Withers	5.93±1.4^{b}	7.06±0.9^{a}	6.1±0.66^{b}	6.67±0.52^{ab}
Loin thickness	5.81±1.5^{b}	7.26±0.99^{a}	6.15±1.06^{b}	6.75±0.69^{ab}
Hip shape	6.73±1.31^{ab}	7.36±0.99^{a}	6.2±1.27^{b}	6.88±0.67^{ab}
Body weight (kg^{-1})	491.1±32.6^{ab}	513.8±20.3^{a}	477.80±30.5^{b}	496.2±16.1^{ab}
Carcass weight (kg^{-1})	273.6±17.9^{ab}	291.2±11^{a}	265.30±23.9^{b}	278.9±13.6^{ab}
Net meat weight (kg^{-1})	229.8±15.6^{ab}	246.4±11^{a}	224.2±22.1^{b}	233.4±11.8^{ab}

Table 4: Association of BM2113 locus and traits

Genotypes	BB136/136	AB128/136	AC128/142	BC136/142
Number	7	15	8	6
Net meat weight (kg^{-1})	230.05±20.83^{ab}	225.04±17.75^{b}	235.63±9.21^{ab}	244.17±11.26^{a}
Net meat rate/(%)	49.21±1.75^{ab}	47.97±1.53^{bB}	49.88±1.18^{aA}	49.48±1.36^{a}

In the same row, values with different lowercase superscripts mean significant difference ($p<0.05$), values with different capital letter superscripts mean significant difference ($p<0.01$)

Table 5: Association of BM1824 locus and traits

Genotypes	BB175/175	DD187/187	AD171/187	BD175/187	CD179/187
Number	6	4	3	19	6
Loin thickness	5.42±1.16^{b}	5.88±0.75^{b}	6.83±0.76^{a}	6.27±1.41^{ab}	6.75±1.33^{ab}

Table 6: Association of IDVGA46 locus and traits

Genotype	AA201/201	BB203/203	AB201/203	AC201/211	BD203/217
Number	4	3	15	6	9
Withers	5.88±1.93^{ab}	6.67±0.58^{a}	6.43±0.98^{aA}	5.25±0.69^{bB}	6.70±0.61^{aA}
Shoulder	6.5±1.47^{ab}	7.00±0.5^{a}	6.67±0.94^{a}	5.67±0.75^{b}	6.73±0.55^{a}
Loin thickness	5.75±1.94^{ab}	6.83±0.76^{ab}	6.57±1.27^{ab}	5.5±1.1^{b}	6.87±0.89^{a}
Thigh muscle	5.88±1.93^{ab}	6.83±0.76^{aA}	6.77±1.1^{a}	5.5±0.89^{bB}	6.9±0.68^{aA}
Hip shape	6.63±1.8^{ab}	7±0.5^{a}	6.63±1.29^{a}	5.75±0.82^{bB}	7.06±0.76^{aA}
Carcass weight (kg^{-1})	267.6±21.8^{ab}	285.5±10.4^{a}	277.8±17.5^{a}	255.4±22.8^{b}	281.7±13.5^{a}
Dressing/(%)	57.7±1.65^{ab}	58.5±0.76^{a}	58.3±1.46^{aA}	56.2±1.48^{bB}	58.9±1.93^{aA}
Net meat weight (kg^{-1})	226.5±20^{ab}	240.2±10.9^{a}	234.1±15.8^{a}	214.6±20.9^{bB}	241.3±17.9^{aA}
Net meat rate/(%)	48.8±1.72^{ab}	49.2±1.33^{ab}	49.1±1.58^{ab}	47.3±1.67^{b}	50.5±3.62^{a}

locus TGLA44, allele E (221 bp) had positive effects on some muscularity evaluation traits such as withers, loin thickness, hip shape and some slaughtering meaty traits such as body weight, carcass weight and net meat weight. For some microsatellite, the conclusions of two methods did not consistent. For example, allele C (142 bp) in microsatellite locus BM2113 have positive effects on two slaughtering meat traits net meat weight and net meat rate but it had none effect on muscularity evaluation traits. For microsatellite BM1824, allele A (164 bp) had positive effects on loin thickness and it had none effect on slaughtering meaty traits. So, the two methods could not be equated completely.

CONCLUSION

The muscularity evaluation traits in the regulation of linear valuation could roughly evaluate meat performance of dual-purpose milk and meat varieties of Grassland red cattle only. Slaughtering meaty traits were more accuracy than the former.

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