



## Association between *PON1* Gene SNPs and Growth and Carcass Traits in Beef Cattle\*

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**ABSTRACT** : Paraoxonase-1 (PON1), like lipoprotein lipase (LPL), plays a key role in the metabolism and physiology of mammalian growth. The objectives of this study were to estimate the allele and genotype frequencies at the *PON1/EcoRV* and *PON1/AluI* loci in three genetic groups of beef cattle and to determine associations between these polymorphisms and growth and carcass traits. Genotyping was performed on 30 Angus, 32 Hereford and 26 Simmental. The association analysis was carried out using the GLM procedure of SAS 9.1 and the least squares means of the genotypes were compared by the Tukey's test. Animals with AG genotype at the *PON1/EcoRV* locus had higher weight at the time of entry into the fattening corrals (329.97±6.08 kg) and close to the time of slaughter (577.56±8.32 kg) and net meat weight (275.89±4.05 kg) and fitted tenderness (3.10±0.19 kg) ( $p < 0.05$ ). Animals with AA genotype at the *PON1/AluI* locus had higher weight at the time of entry (333.37±8.93 kg) and slaughter (576.82±13.18 kg) and net meat weight (275.49±6.43 kg) and average daily gain (0.68±0.02 kg/d) ( $p < 0.05$ ). The meat color score was also significantly higher ( $p < 0.05$ ). Between genotypes and breeds, there were significant differences observed except for TBW, REMG, MBS, REA and MCS. As a metabolism gene, genotypes of the SNPs of *PON1* gene might be reflecting BFT directly, such as  $A_eA_eG_aG_a$  genotype in this experiment. (**Key Words** : Beef Cattle, *PON1* Gene, SNPs, Growth Traits, Carcass Traits)

### INTRODUCTION

Like lipoprotein lipase (LPL), paraoxonase-1 (PON1) plays a key role in the metabolism and physiology of mammalian growth (van Himbergen et al., 2006). Recently, the crystal structure of a recombinant human PON variant was elucidated which showed a six-bladed  $\beta$ -propeller with each blade consisting of four  $\beta$ -sheets, and contained in the central tunnel of the enzyme are two calcium atoms needed for the stabilization of the structure and the catalytic activity. Three  $\alpha$  helices, located at the top of the propeller, are involved in anchoring to the HDL particle (Harel et al., 2004). The clarification of the crystal structure led to more

understanding of the catalytic mechanisms underlying PON1's wide substrate range (Harel et al., 2004). Further investigations have suggested that the hydrolytic activity towards lactones (cyclic esters) is the native activity (Khersonsky et al., 2005). The molecular functions were related to aryltriphosphatase activity, phosphotriesterase activity, A-esterase activity, paraoxon hydrolase activity and paraoxonase activity. For example, catalysis of the hydrolysis of various bonds, e.g. C-O, C-N, C-C, phosphoric anhydride bonds, etc. Hydrolase is the systematic name for any enzyme of EC class 3 (<http://www.ncbi.nlm.nih.gov/entrez/disposim.cgi?id=168820>). The PON1 protein has the ability of cooperating with insulin, steapsin, growth hormone, lipoprotein lipase and leptin (Curia et al., 2005; 2006). Essentially, it enables the directed movement of lipids into, out of, within or between cells (Jonathan et al., 2003; Paul et al., 2006). For the relationships between PONs, Sarghera et al. (2008) demonstrated that genetic variation in *PON3* affects serum PON1 activity independently of the known effect of the *PON1* genetic variation. PON1 levels are important in

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determining resistance to specific organophosphorus compounds; the time course of appearance of PON1 in newborns is of great interest. The mouse PON1 expression level plateaus between 6 to 15 months of age, and the age at which PON1 levels plateau is quite variable among individuals (Cole et al., 2003). Some studies indicated that polymorphisms of the PON1 gene may relate to birth weight in humans (Ye et al., 2002; Hakanen et al., 2006). Low PON1 activity during early development may be involved in the enhanced sensitivity of the young toward organophosphate toxicity.

Despite the physiological function of the *PONs* gene being focused on cardiovascular diseases in humans, association between the *PON2* gene and birth weight of neonates with preterm have been detected (Busch et al., 1999; Liang et al., 2002; Lucio et al., 2006). Based on its function in lipid metabolism, this work was carried out to identify the association between polymorphism and bovine economic traits. Although previous research has focused on growth hormone (GH), insulin-like growth factor I (IGF-I) and growth hormone releasing hormone (GHRH) (Curi et al., 2005), leptin gene (Shin et al., 2007), Carboxypeptidase E (CPE) (Shin et al., 2007), there was little information about this gene in the bovine. Questions then arose about its function in growth, lipoprotein and fat metabolism in beef cattle and whether it is an important candidate gene. To address the last question, association analysis was carried out to detect the effects of the gene in beef cattle.

## MATERIALS AND METHODS

### Animals

For the experiment, blood was collected from 86 bulls belonging to three different breeds differing in body size, including 30 Angus, 32 Hereford and 26 Simmental. These animals represented the progeny of 45 different bulls. This study was carried out in the experimental feedlot facility of the Sanyuan company in Beijing. Calves were weaned at 210 days of age using a creep-feeding system and at the beginning of the experiment they were approximately 730 days old. The animals were identified individually, treated against endo- and ectoparasites, divided into groups of five animals each in confinement corrals according to breed and size, fed diets formulated according to the guidelines of the National Research Council (NRC, 1996) for a mean daily weight gain higher than 1.2 kg and, according to the breed, the animals were fed appropriately. After entering the corrals, the animals were allowed to adapt for a period of about 20 days. The animals were slaughtered at a commercial slaughter facility after an average confinement period of 348 days, with an average age of 2.5 years. The animals were weighed at the time of entry into the fattening corrals (BW1) and again close to the time of slaughter

(SBW). The average daily gain (ADG) during the finishing period was calculated. Rib eye area (REA) and backfat thickness (BFT) were assessed by ultrasound using the method of Perkins, as modified by Gresham (1998), during the last weight measurement. Hot carcass weight (HCW) and tenderness (TN) were measured after slaughter. Net meat weight (NMW), total bone weight (TBW), meat color score (MCS) (grade = 1-5), marbling score (MBS) (grade = 1-6) and fat color score (FCS) (grade = 1-6) were measured at carcass dissection time.

### DNA extraction and genotyping

Five milliliters of total blood was collected into a vacuum tube containing 7.5-mg EDTA by puncturing the left jugular vein. Genomic DNA was extracted from a 300- $\mu$ l aliquot of total blood using the standard method with hydroxybenzene-chloroform. The preliminary DNA sequence was amplified from bovine genomic DNA using the primer Exon6 derived from the bovine EST-contig. Starting from the known sequence, a primer walking strategy was used to identify three overlapping genomic fragments for further polymorphism search. The primer walking involved one primer designed from the bovine EST-contig and the other generated from the intron region determined with primer pair Forward: 3'-TTCAAGCCTCCCTCAGACCAACT-5', Reverse: 3'-AGCATGTGACTTCCAAAGACCCC-5'. The PCR temperature profile was 95°C for 5 min followed by 35 cycles for 30 s at 94°C, 30 s annealing at 57.3°C, 30 s at 72°C and a final extension of 10 min at 72°C. The fragment was amplified in a PTC-200 Peltier Thermal Cycler (Bio-RAD, USA). The PCR fragments were sequenced directly with the same primers used for the amplification.

### Statistical analysis

The genotype and allele frequencies at each locus were calculated and differences in the allele frequencies within and between genetic groups and Hardy-Weinberg equilibria were determined by using the POPGENE32 Version 1.31 software (Francis, 1999).

For the association studies, the traits of interest were analyzed using the General Linear Model (GLM) procedure of the SAS 9.1 program (Statistical Analysis System, 1999) and the least squares means of the genotypes were compared by the Tukey's test. The linear model used to fit the quantitative traits included, in addition to the genotype effect, the contemporary group effect which considered the effects of breeds and groups as follows:

$$Y_{ijk} = \mu + G_i + GC_j + e_{ijk}$$

where  $Y_{ijk}$  = traits of growth and carcass,  $\mu$  = overall mean,  $G_i$  = fixed effect of the  $i^{\text{th}}$  genotype,  $GC_j$  = fixed effect of the  $j^{\text{th}}$  contemporary group, and  $e_{ijk}$  = random error.

**Table 1.** Allele and genotype frequencies at the *PONI/EcoRV* locus obtained in the three breeds

Breed	Number	Allele frequency		Genotype frequency			PIC	H	$\chi^2$ test (p)
		A	G	AA	AG	GG			
Angus	30	0.5167 <sup>a</sup>	0.4833 <sup>a</sup>	0.2627	0.5079	0.2294	0.3747	0.5000	0.9308
Hereford	30	0.5167 <sup>a</sup>	0.4833 <sup>a</sup>	0.2627	0.5079	0.2294	0.3747	0.3667	0.1213
Simmental	28	0.5400 <sup>a</sup>	0.4600 <sup>b</sup>	0.2865	0.5069	0.2065	0.3734	0.2800	0.0223
Total	88	0.5235 <sup>a</sup>	0.4765 <sup>b</sup>	0.2726	0.5018	0.2255	0.3744	0.3882	0.0357

Frequencies with different superscripts differ (p<0.05).

**Table 2.** Allele and genotype frequencies at the *PONI/AluI* locus obtained in the three breeds

Breed	Number	Allele frequency		Genotype frequency			PIC	H	$\chi^2$ test (p)
		A	G	AA	AG	GG			
Angus	30	0.4500	0.5500	0.1983	0.5043	0.2983	0.3725	0.5667	0.4836
Hereford	30	0.5667	0.4333	0.3169	0.4994	0.1836	0.3705	0.5333	0.7052
Simmental	28	0.4200	0.5800	0.1714	0.4971	0.3314	0.3685	0.4400	0.5571
Total	88	0.4824	0.5176	0.2312	0.5023	0.2665	0.3747	0.5176	0.7774

Genotypes with small sample sizes and low frequencies were excluded from the analysis for the purpose of accuracy. The maternal effect was not included in the model since the number of genotyped animals which were progeny of the same mother was very small.

## RESULTS

### Genotype and allele frequencies

Two genetic variants (A and G) at the *PONI/EcoRV* locus were observed in the genetic groups studied (Table 1). PAGE was carried out for the amplification of its product, and three RFLP patterns were observed. Three genotypes designated AA, GG, and AG, respectively were observed. Genotype GG was characterized by the presence of two restriction fragments of 226 and 150 bp, while genotype AA was determined by the presence of a single 376 bp fragment. AG individuals presented three fragments of 376, 226 and 150 bp. The frequency of allele A at the *PONI/EcoRV* locus was significantly higher (p<0.05) than that of allele G in the Simmental genetic group. A significant difference (p<0.05) in allele frequencies was observed between the cattle groups. All three genotypes were observed in all groups.

Two different alleles (A and G) were found at the

*PONI/AluI* locus (Table 2). Agarose gel electrophoresis was carried out for the amplification product of it and three RFLP patterns were observed. Three genotypes designated AA, GG, and AG, respectively were observed. Genotype AA was characterized by the presence of three restriction fragments of 153, 134 and 89 bp. Genotype GG was determined by the presence of a single 376 bp fragment. Heterozygous individuals (AG) should present four fragments of 376, 153, 134 and 89 bp, but the 89 bp fragment was not presented since it may be overlapped by the dimer. The allele frequencies of the *PONI/AluI* polymorphism were observed to be in equilibrium among and within the three genetic groups studied. All three genotypes were observed in all groups.

### Association analysis

Table 3 shows a comparison of the least squares means and their respective standard errors of quantitative growth and carcass traits, which had significant differences involving the genotypes at the *PONI/EcoRV* and *PONI/AluI* loci, respectively.

All genotypes AA, AG and GG were considered in the association analysis between *PONI/EcoRV* locus genotypes and production traits. Significant associations were

**Table 3.** LSM ( $\pm$ standard error) of growth and carcass traits obtained for the genotypes at the *EcoRV* and *AluI* loci

Locus	Genotype	Growth and carcass traits					
		BW1 (kg)	SBW (kg)	NMW (kg)	ADG (kg)	TN (kg)	MCS
<i>PONI/EcoRV</i>	AA(30)	309.97 $\pm$ 6.19 <sup>a</sup>	555.72 $\pm$ 11.79 <sup>a</sup>	265.19 $\pm$ 5.71 <sup>a</sup>	0.66 $\pm$ 0.02 <sup>a</sup>	3.73 $\pm$ 0.23 <sup>a</sup>	4.16 $\pm$ 0.16 <sup>a</sup>
	AG(32)	329.97 $\pm$ 6.08 <sup>b</sup>	577.56 $\pm$ 8.32 <sup>b</sup>	275.89 $\pm$ 4.05 <sup>b</sup>	0.67 $\pm$ 0.01 <sup>a</sup>	3.10 $\pm$ 0.19 <sup>b</sup>	4.44 $\pm$ 0.15 <sup>a</sup>
	GG(26)	311.54 $\pm$ 6.49 <sup>a</sup>	553.92 $\pm$ 9.19 <sup>a</sup>	264.06 $\pm$ 4.44 <sup>a</sup>	0.65 $\pm$ 0.01 <sup>a</sup>	3.31 $\pm$ 0.19 <sup>ab</sup>	4.04 $\pm$ 0.15 <sup>a</sup>
<i>PONI/AluI</i>	AA(19)	333.37 $\pm$ 8.93 <sup>a</sup>	576.82 $\pm$ 13.18 <sup>a</sup>	275.49 $\pm$ 6.43 <sup>a</sup>	0.68 $\pm$ 0.02 <sup>a</sup>	3.36 $\pm$ 0.34 <sup>a</sup>	4.58 $\pm$ 0.19 <sup>a</sup>
	AG(46)	320.85 $\pm$ 4.64 <sup>a</sup>	566.01 $\pm$ 7.46 <sup>ab</sup>	270.08 $\pm$ 3.61 <sup>ab</sup>	0.67 $\pm$ 0.01 <sup>a</sup>	3.25 $\pm$ 0.14 <sup>a</sup>	4.22 $\pm$ 0.13 <sup>ab</sup>
	GG(23)	289.48 $\pm$ 6.63 <sup>b</sup>	546.07 $\pm$ 11.76 <sup>b</sup>	260.52 $\pm$ 5.67 <sup>b</sup>	0.63 $\pm$ 0.01 <sup>b</sup>	3.65 $\pm$ 0.21 <sup>a</sup>	4.00 $\pm$ 0.14 <sup>b</sup>

BW1 = Body weight at the beginning of confinement, SBW = Body weight at the time of slaughter, HCW = Hot carcass weight, NMW = Net meat weight, TBW = Total bone weight, REMG = Rib eye meat weight and ADG = Average daily gain, REA = Rib eye area. BFT = Backfat thickness, TN = Tenderness, MBS = Marbling score, MCS = Meat color score, FCS = Fat color score.

Means with different superscript within the same locus differ (p<0.05).

**Table 4.** LSM ( $\pm$ standard error) of growth and carcass traits obtained for three breeds

Breed	BW1 (kg)	SBW (kg)	ADG (kg)	HCW (kg)	TBW (kg)	NMW (kg)	REMG (kg)	BFT (cm)	TN (kg)	MBS	REA (cm <sup>2</sup> )	MCS	FCS
Angus	326.87 $\pm$ 5.55 <sup>a</sup>	576.55 $\pm$ 7.88 <sup>a</sup>	0.66 $\pm$ 0.01 <sup>ab</sup>	319.10 $\pm$ 4.91 <sup>a</sup>	25.84 $\pm$ 0.47 <sup>b</sup>	275.70 $\pm$ 7.67 <sup>a</sup>	1.42 $\pm$ 0.03 <sup>ab</sup>	0.99 $\pm$ 0.06 <sup>a</sup>	2.76 $\pm$ 0.13 <sup>b</sup>	1.97 $\pm$ 0.13	66.80 $\pm$ 1.44	4.33 $\pm$ 0.16	1.32 $\pm$ 0.06
Hereford	332.03 $\pm$ 6.82 <sup>a</sup>	592.12 $\pm$ 8.77 <sup>a</sup>	0.70 $\pm$ 0.01 <sup>a</sup>	317.33 $\pm$ 5.98 <sup>a</sup>	26.68 $\pm$ 0.37 <sup>a</sup>	282.72 $\pm$ 4.26 <sup>a</sup>	1.50 $\pm$ 0.04 <sup>a</sup>	0.99 $\pm$ 0.09 <sup>a</sup>	3.29 $\pm$ 0.16 <sup>ab</sup>	2.65 $\pm$ 0.18	62.27 $\pm$ 1.29	4.08 $\pm$ 0.15	1.16 $\pm$ 0.06
Simmental	292.54 $\pm$ 3.86 <sup>b</sup>	517.70 $\pm$ 7.79 <sup>b</sup>	0.63 $\pm$ 0.01 <sup>b</sup>	276.88 $\pm$ 4.34 <sup>b</sup>	24.28 $\pm$ 0.46 <sup>b</sup>	246.71 $\pm$ 3.73 <sup>b</sup>	1.32 $\pm$ 0.03 <sup>b</sup>	0.64 $\pm$ 0.04 <sup>b</sup>	4.13 $\pm$ 0.26 <sup>a</sup>	2.45 $\pm$ 0.19	63.25 $\pm$ 1.20	4.16 $\pm$ 0.15	1.23 $\pm$ 0.06

BW1 = Body weight at the beginning of confinement, SBW = Body weight at the time of slaughter, HCW = Hot carcass weight, NMW = Net meat weight, TBW = Total bone weight, REMG = Rib eye meat weight and ADG = Average daily gain, REA = Rib eye area, BFT = Backfat thickness, TN = Tenderness, MBS = Marbling score, MCS = Meat color score, FCS = Fat color score.

The same note is used in the following tables.

<sup>a, b</sup> Means with different superscripts within the same locus differ ( $p < 0.05$ ).

**Table 5.** LSM ( $\pm$ standard error) of growth and carcass traits obtained for the genotype of the *PONI/EcoRV* locus in three breeds

Breed	Genotype	BW1 (kg)	SBW (kg)	ADG (kg)	HCW (kg)	TBW (kg)	NMW (kg)	REMG (kg)	BFT (cm)	TN (kg)	MBS	REA (cm <sup>2</sup> )	MCS	FCS
Angus	AA	315.00 $\pm$ 12.26	543.25 $\pm$ 20.28 <sup>a</sup>	0.62 $\pm$ 0.03 <sup>a</sup>	300.95 $\pm$ 10.72	24.58 $\pm$ 0.79	259.33 $\pm$ 9.77 <sup>a</sup>	1.35 $\pm$ 0.06	0.98 $\pm$ 0.07	2.94 $\pm$ 0.12 <sup>a</sup>	2.00 $\pm$ 0.27	66.00 $\pm$ 1.61	4.15 $\pm$ 0.28	1.19 $\pm$ 0.13
	AG	331.73 $\pm$ 7.80	590.77 $\pm$ 8.52 <sup>a</sup>	0.67 $\pm$ 0.02 <sup>a</sup>	325.40 $\pm$ 6.39	25.72 $\pm$ 0.62	282.52 $\pm$ 4.21 <sup>a</sup>	1.44 $\pm$ 0.03	0.92 $\pm$ 0.10	2.54 $\pm$ 0.17 <sup>a</sup>	1.97 $\pm$ 0.19	66.53 $\pm$ 2.41	4.67 $\pm$ 0.25	1.43 $\pm$ 0.08
	GG	330.00 $\pm$ 9.86	584.14 $\pm$ 9.13 <sup>a</sup>	0.66 $\pm$ 0.01 <sup>a</sup>	326.20 $\pm$ 7.57	27.57 $\pm$ 0.98	278.28 $\pm$ 4.44 <sup>a</sup>	1.44 $\pm$ 0.10	1.17 $\pm$ 0.13	3.01 $\pm$ 0.37 <sup>a</sup>	1.93 $\pm$ 0.23	68.29 $\pm$ 3.14	4.29 $\pm$ 0.31	1.21 $\pm$ 0.10
Hereford	AA	327.60 $\pm$ 12.17	623.35 $\pm$ 8.50 <sup>b</sup>	0.75 $\pm$ 0.01 <sup>b</sup>	332.49 $\pm$ 7.77	27.32 $\pm$ 0.51	298.01 $\pm$ 4.17 <sup>b</sup>	1.44 $\pm$ 0.06	1.03 $\pm$ 0.21	3.45 $\pm$ 0.24 <sup>a</sup>	2.20 $\pm$ 0.27	65.20 $\pm$ 1.60	3.80 $\pm$ 0.24	1.20 $\pm$ 0.11
	AG	345.36 $\pm$ 10.12	587.86 $\pm$ 12.57 <sup>a</sup>	0.69 $\pm$ 0.02 <sup>a</sup>	317.09 $\pm$ 7.94	26.67 $\pm$ 0.68	280.59 $\pm$ 6.02 <sup>a</sup>	1.47 $\pm$ 0.04	1.14 $\pm$ 0.13	3.34 $\pm$ 0.36 <sup>a</sup>	2.64 $\pm$ 0.29	62.55 $\pm$ 1.78	4.18 $\pm$ 0.24	1.05 $\pm$ 0.05
	GG	320.67 $\pm$ 13.21	562.61 $\pm$ 18.74 <sup>a</sup>	0.67 $\pm$ 0.02 <sup>a</sup>	300.78 $\pm$ 13.91	25.97 $\pm$ 0.70	263.32 $\pm$ 9.13 <sup>a</sup>	1.45 $\pm$ 0.12	0.77 $\pm$ 0.11	3.05 $\pm$ 0.20 <sup>a</sup>	3.17 $\pm$ 0.33	58.67 $\pm$ 3.04	4.28 $\pm$ 0.29	1.28 $\pm$ 0.12
Simmental	AA	291.92 $\pm$ 5.73	507.67 $\pm$ 11.28 <sup>a</sup>	0.61 $\pm$ 0.02 <sup>a</sup>	272.73 $\pm$ 6.37	24.18 $\pm$ 0.82	241.72 $\pm$ 5.32 <sup>a</sup>	1.35 $\pm$ 0.05	0.62 $\pm$ 0.07	4.48 $\pm$ 0.45 <sup>b</sup>	2.50 $\pm$ 0.35	63.17 $\pm$ 2.11	4.50 $\pm$ 0.28	1.33 $\pm$ 0.09
	AG	297.33 $\pm$ 10.99	525.67 $\pm$ 23.23 <sup>a</sup>	0.65 $\pm$ 0.03 <sup>a</sup>	277.43 $\pm$ 12.79	24.56 $\pm$ 0.95	250.70 $\pm$ 11.24 <sup>a</sup>	1.33 $\pm$ 0.08	0.63 $\pm$ 0.07	4.08 $\pm$ 0.51 <sup>a</sup>	2.67 $\pm$ 0.31	65.83 $\pm$ 1.30	4.33 $\pm$ 0.17	1.33 $\pm$ 0.17
	GG	290.40 $\pm$ 5.86	524.95 $\pm$ 10.94 <sup>a</sup>	0.64 $\pm$ 0.01 <sup>a</sup>	281.53 $\pm$ 6.27	24.41 $\pm$ 0.67	250.27 $\pm$ 5.25 <sup>a</sup>	1.27 $\pm$ 0.06	0.68 $\pm$ 0.07	3.75 $\pm$ 0.37 <sup>a</sup>	2.25 $\pm$ 0.30	61.80 $\pm$ 2.08	3.65 $\pm$ 0.17	1.05 $\pm$ 0.05

LSM of the same trait with different superscripts differ ( $p < 0.05$ ).

observed between genotypes and growth traits BW1, SBW and NMW ( $p < 0.05$ ) and carcass trait TN ( $p < 0.05$ ), with AG animals being considered higher than AA and GG animals for BW1, SBW, NMW and TN. There were no significant associations with HCW, TBW, REMW, ADG, REA, BFT, MBS, MCS and FCS.

The association analysis involving the *PONI/AluI* locus genotypes and traits of interest were significant between

genotypes and BW1, SBW, NMW, ADG and MCS ( $p < 0.05$ ). AA and AG animals were considered to be more favorable than the GG animals for these traits. No other significant associations between genotypes and quantitative traits were observed.

Table 4, 5, 6 and 7 show the comparison of the least square means of growth and carcass traits involving among breeds, between genotypes and breeds and dual genotype

**Table 6.** Least squares means of growth and carcass traits obtained for the genotype of the *PONI/AluI* locus in three breeds

Breed	Genotype	BW1 (kg)	SBW (kg)	ADG (kg)	HCW (kg)	TBW (kg)	NMW (kg)	REMG (kg)	BFT (cm)	TN (kg)	MBS	REA (cm <sup>2</sup> )	MCS	FCS
Angus	AA	333.40 $\pm$ 14.39 <sup>a</sup>	582.20 $\pm$ 15.20 <sup>a</sup>	0.69 $\pm$ 0.03 <sup>a</sup>	317.28 $\pm$ 9.99 <sup>a</sup>	25.30 $\pm$ 1.23	273.45 $\pm$ 7.74 <sup>a</sup>	1.46 $\pm$ 0.04	0.92 $\pm$ 0.16	2.52 $\pm$ 0.27 <sup>a</sup>	1.70 $\pm$ 0.37	65.20 $\pm$ 3.65	4.80 $\pm$ 0.20	1.40 $\pm$ 0.19
	AG	327.82 $\pm$ 7.04 <sup>a</sup>	586.65 $\pm$ 9.50 <sup>a</sup>	0.68 $\pm$ 0.01 <sup>a</sup>	324.56 $\pm$ 6.12 <sup>a</sup>	26.26 $\pm$ 0.58	280.19 $\pm$ 4.56 <sup>a</sup>	1.45 $\pm$ 0.05	0.94 $\pm$ 0.07	2.70 $\pm$ 0.19 <sup>a</sup>	1.88 $\pm$ 0.17	68.94 $\pm$ 1.52	4.35 $\pm$ 0.26	1.29 $\pm$ 0.07
	GG	320.75 $\pm$ 12.44 <sup>a</sup>	551.56 $\pm$ 17.95 <sup>a</sup>	0.59 $\pm$ 0.03 <sup>b</sup>	308.50 $\pm$ 11.48 <sup>a</sup>	25.31 $\pm$ 1.05	263.13 $\pm$ 8.75 <sup>a</sup>	1.31 $\pm$ 0.45	1.16 $\pm$ 0.16	3.04 $\pm$ 0.21 <sup>a</sup>	2.31 $\pm$ 0.19	63.25 $\pm$ 3.57	4.38 $\pm$ 0.25	1.31 $\pm$ 0.13
Hereford	AA	352.00 $\pm$ 13.43 <sup>a</sup>	610.11 $\pm$ 15.31 <sup>a</sup>	0.72 $\pm$ 0.02 <sup>a</sup>	330.16 $\pm$ 8.84 <sup>a</sup>	26.99 $\pm$ 0.71	291.56 $\pm$ 7.47 <sup>a</sup>	1.44 $\pm$ 0.05	1.19 $\pm$ 0.21	3.11 $\pm$ 0.19 <sup>a</sup>	2.50 $\pm$ 0.30	63.11 $\pm$ 2.28	4.17 $\pm$ 0.26	1.11 $\pm$ 0.07
	AG	330.25 $\pm$ 8.63 <sup>a</sup>	577.66 $\pm$ 16.26 <sup>a</sup>	0.69 $\pm$ 0.01 <sup>a</sup>	306.58 $\pm$ 8.60 <sup>a</sup>	26.44 $\pm$ 0.42	275.61 $\pm$ 5.99 <sup>a</sup>	1.43 $\pm$ 0.07	0.84 $\pm$ 0.08	3.20 $\pm$ 0.19 <sup>a</sup>	2.84 $\pm$ 0.25	61.44 $\pm$ 1.81	4.13 $\pm$ 0.22	1.22 $\pm$ 0.09
	GG	301.80 $\pm$ 8.43 <sup>a</sup>	606.00 $\pm$ 18.36 <sup>a</sup>	0.71 $\pm$ 0.02 <sup>a</sup>	328.64 $\pm$ 13.62 <sup>a</sup>	26.85 $\pm$ 1.38	289.57 $\pm$ 9.55 <sup>a</sup>	1.58 $\pm$ 0.12	1.10 $\pm$ 0.30	4.02 $\pm$ 0.65 <sup>a</sup>	2.30 $\pm$ 0.54	63.40 $\pm$ 3.61	3.80 $\pm$ 0.25	1.10 $\pm$ 0.10
Simmental	AA	299.80 $\pm$ 8.28 <sup>a</sup>	511.50 $\pm$ 17.91 <sup>b</sup>	0.62 $\pm$ 0.03 <sup>a</sup>	273.32 $\pm$ 9.60 <sup>b</sup>	24.24 $\pm$ 1.07	243.63 $\pm$ 9.57 <sup>b</sup>	1.18 $\pm$ 0.05	0.76 $\pm$ 0.11	4.50 $\pm$ 1.11 <sup>b</sup>	2.30 $\pm$ 0.51	64.60 $\pm$ 3.04	5.10 $\pm$ 0.40	1.40 $\pm$ 0.19
	AG	300.15 $\pm$ 5.83 <sup>a</sup>	524.69 $\pm$ 12.17 <sup>a</sup>	0.64 $\pm$ 0.02 <sup>a</sup>	280.72 $\pm$ 6.69 <sup>b</sup>	24.60 $\pm$ 0.74	250.05 $\pm$ 5.82 <sup>a</sup>	1.39 $\pm$ 0.05	0.67 $\pm$ 0.07	4.13 $\pm$ 0.32 <sup>b</sup>	2.23 $\pm$ 0.28	63.69 $\pm$ 2.03	4.15 $\pm$ 0.18	1.27 $\pm$ 0.09
	GG	279.00 $\pm$ 4.38 <sup>b</sup>	511.70 $\pm$ 12.99 <sup>b</sup>	0.62 $\pm$ 0.02 <sup>a</sup>	273.67 $\pm$ 7.49 <sup>b</sup>	23.88 $\pm$ 0.72	243.91 $\pm$ 6.24 <sup>b</sup>	1.30 $\pm$ 0.06	0.55 $\pm$ 0.05	3.96 $\pm$ 0.28 <sup>b</sup>	2.75 $\pm$ 0.33	62.00 $\pm$ 1.59	3.70 $\pm$ 0.17	1.10 $\pm$ 0.07

LSM of the same trait with different superscripts differ ( $p < 0.05$ ).

**Table 7.** Least squares means of growth and carcass traits obtained for the linkage genotype at the *PONI/EcoRV* and *AluI* loci

Genotype	BW1 (kg)	SBW (kg)	ADG (kg)	HCW (kg)	TBW (kg)	NMW (kg)	REMG (kg)	BFT (cm)	TN (kg)	MBS	REA (cm <sup>2</sup> )	MCS	FCS
A <sub>c</sub> A <sub>c</sub> A <sub>e</sub> A <sub>a</sub>	354.33 <sup>a</sup>	583.39	0.70	313.73	25.36	279.02	1.36	1.16 <sup>ab</sup>	3.55	1.83	66.67	4.67	1.22
A <sub>c</sub> A <sub>c</sub> A <sub>a</sub> G <sub>a</sub>	301.61 <sup>b</sup>	559.63	0.66	301.82	25.67	266.97	1.43	0.87 <sup>ab</sup>	3.58	2.08	66.01	3.84	1.26
A <sub>c</sub> A <sub>c</sub> G <sub>a</sub> G <sub>a</sub>	294.94 <sup>b</sup>	545.72	0.63	299.87	25.08	260.32	1.36	0.62 <sup>b</sup>	3.69	2.65	62.47	3.94	1.24
A <sub>c</sub> G <sub>c</sub> A <sub>a</sub> A <sub>a</sub>	321.03 <sup>ab</sup>	563.04	0.66	307.63	24.73	269.16	1.37	0.82 <sup>ab</sup>	2.86	2.08	64.83	4.63	1.33
A <sub>c</sub> G <sub>c</sub> A <sub>a</sub> G <sub>a</sub>	331.91 <sup>ab</sup>	575.79	0.69	309.20	25.62	275.09	1.46	0.79 <sup>ab</sup>	3.29	2.60	66.01	4.35	1.31
A <sub>c</sub> G <sub>c</sub> G <sub>a</sub> G <sub>a</sub>	313.11 <sup>ab</sup>	555.56	0.63	300.02	25.56	264.99	1.37	1.24 <sup>a</sup>	3.78	2.28	62.17	4.31	1.22
G <sub>c</sub> G <sub>c</sub> A <sub>a</sub> A <sub>a</sub>	313.67 <sup>ab</sup>	562.17	0.65	309.43	26.95	267.61	1.38	0.89 <sup>ab</sup>	3.30	2.17	64.67	4.17	1.25
G <sub>c</sub> G <sub>c</sub> A <sub>a</sub> G <sub>a</sub>	322.18 <sup>ab</sup>	549.97	0.65	297.79	25.77	262.10	1.38	0.86 <sup>ab</sup>	3.20	2.42	61.62	4.27	1.22
G <sub>c</sub> G <sub>c</sub> G <sub>a</sub> G <sub>a</sub>	290.33 <sup>b</sup>	562.07	0.66	307.98	26.01	268.03	1.45	1.02 <sup>ab</sup>	3.66	2.50	64.60	3.33	1.00

LSM with different superscripts differ ( $p < 0.05$ ); Subscripts for genotypes "e" and "a" are *EcoRV* and *AluI*.

analysis of the *PONI/EcoRV* and *PONI/AluI* loci.

Among breeds it was observed that the specific beef breeds (e.g. Angus and Hereford in this paper) had higher growth quality than dual purpose breeds (e.g. Simmental) and in carcass traits Simmental presented lower quality except for BFT, TN, BMS, MCS and FCS. Between genotypes and breeds, there were significant differences observed except for TBW, REMG, MBS, REA and MCS. From the linkage analysis, the difference in BW1 may be indicating which haploid has potential early growth ability while, with regards to its role as a metabolism gene, genotypes of the SNPs of *PONI* might be reflecting BFT directly, such as the  $A_eA_eG_aG_a$  genotype in this experiment.

## DISCUSSION

### Statistical model

In fitting the model, the maternal effects and the permanent environmental effects were excluded since the number of genotyped animals which were progeny of the same cow was very small, and all the animals were fed under the same environment. The small sample size also influenced the interaction analysis between loci in the same groups, and this would be the focus in a further study. Also, all the animals were fed in a feedlot, so the effects of the environment were ignored in the statistical model.

### *PONI/EcoRV* polymorphism

The *PONI/EcoRV* polymorphism is an A (allele A) to G (allele G) transition located in the exon6 147 of the *PONI* gene. In this work, we calculated the frequencies of the alleles and genotypes in the three cattle genetic groups (Table 1). There were two alleles, A and G, and three genotypes AA, AG and GG. The Chi-square test indicated that the allele frequencies were in disequilibrium between groups, and within the group of Simmental ( $p = 0.022307$ ). This might be the result of selection pressure. However, the possibility of sampling error should be considered. For the fitted model, *PONI/EcoRV* genotypes had no unique effects, while the genetic breed effects were important for all traits.

The present association results demonstrated that the AG genotype was superior in growth and carcass traits. Animals with AG genotype generally had high body weight at the beginning of confinement, indicating the possibility that the animals were already heavier at weaning. At the end of the confinement, these animals continued to be heavier, although weight gain was not significantly different statistically during the finishing period. This higher live weight of the AG animals resulted in larger net meat weight. Tenderness of the AG ( $3.10 \pm 0.19$ ) animals was significantly better than those of the AA ( $3.73 \pm 0.23$ ) and GG ( $3.31 \pm 0.19$ ) animals.

### *PONI/AluI* polymorphism

The *PONI/AluI* polymorphism is an A (allele A) to G (allele G) transition located in the exon6 162 of the *PONI* gene. In this work, we calculated the frequencies of the alleles and genotypes in the three groups (Table 2). There were two alleles, A and G, and three genotypes AA, AG and GG. The Chi-square test indicated that the allele frequencies were in equilibrium between groups and within all three groups.

We obtained frequencies of 0.4824 for allele A and 0.5176 for allele G in total for the three genetic groups, and observed significant associations of AA and AG animals with higher body weight at the beginning of confinement, at slaughter and with net meat weight ( $p < 0.05$ ), and better meat color score than GG animals in beef cattle. Comparing the genotypes at this locus, AA animals might be the superior genotype for growth and carcass traits, because AA animals had high growth ability and carcass quality besides body weight at the beginning of confinement.

At the same age, the difference of BW1 among breeds, between genotypes and breeds, and in linkage analysis may be due to two reasons, earlier nutrition and breeds. However, the difference among linkage genotypes of the carcass trait BFT could be reflecting the haploid genotype effects on this trait.

The *PONs* gene family was used to identify the relationship with birth weight and shortened gestation in the neonate (Busch et al., 1999). The results indicated that PON2 Ser311Ser in neonates was significantly associated with shortened gestation (Liang et al., 2002). Further study indicated that PON1 and PON3 may exert their physiological roles through distinct substrates but could contribute synergistically towards preventing cardiovascular disease (Sarghera et al., 2008). Its possible involvement in human longevity, focusing on the relationship between genetic polymorphisms and enzyme activity and its capability to counteract oxidative stress, was based on the free radical theory of aging (Marchegiani et al., 2008). With slow-activity PON1 or PON1192, urinary diethyl phosphates (Sigma DEPs) were associated with lower birth weight and dimethyl phosphates (Sigma DMPs) with shorter birth length (Wolff et al., 2007). These results may suggest that *PONs* should have a similar function in animals and, based on this, we may choose the *PONs* polymorphisms to predict the results of early selection in animals.

## CONCLUSION

The association analysis between genotypes at the *PONI/EcoRV* and *PONI/AluI* loci and growth and carcass traits indicated that animals with AG genotype at the *PONI/EcoRV* locus could be selected for genetic gain in

tenderness, and for net meat weight; animals with AA genotype at the *PONI/AluI* locus could be selected for genetic gain in growth traits and meat color score. The  $A_eA_eG_aG_a$  genotype may be used as the marker for the selection of the BFT trait. The *PONI* gene might play a major role in the traits of interest. Further studies should be conducted to check the effects of these two loci in the expression pattern of the *PONI* gene.

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