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ASSOCIATION ANALYSIS OF *IGF-1* AND *GH* GENE SNPs WITH GROWTH AND DEVELOPMENT PERFORMANCE IN SHEEP

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ABSTRACT

IGF-1 and *GH* genes have been proven to be important genes that affect animal growth and development. The purpose of this experiment was to analyse the effect of single nucleotide polymorphism (SNP) of sheep *IGF-1* and *GH* genes on their growth and development performance. The results showed that a SNP site T5299C was detected in the *IGF-1* gene, while two SNP sites C408G and T364C were detected in the *GH* gene. At the T5299C site, the CC genotype in the Charolais ewes, rams, and ram lambs, as well as the TT genotype in the Charolais ewe lambs and the CT genotype in the Australian White rams were dominant genotypes. At the C408G site, the CG genotype in the Charolais ewes and ram lambs, CC genotype in the Charolais rams and Australian White rams, and GG genotype in the Charolais ewe lambs were dominant genotypes. At the T364C site, the CT genotype in the Charolais ewes, ram lambs, and Australian White rams, CC genotype in the Charolais ewe lambs, and TT genotype in the Charolais rams were dominant genotypes. And we found the dominant haplotype combinations of each breed of sheep through the haplotype combinations of different loci. This provides a certain reference for future research on the influence of *IGF-1* and *GH* genes on the growth and development performance of sheep.

Keywords: SNP, Growth and Development, Sheep, *IGF-1*, *GH*

INTRODUCTION

China is a large country with a history of sheep farming, has a rich variety of locally bred high-quality sheep breeds, which are an important component of China's livestock biodiversity **(Yang, 2021)**. Sheep is one of the earliest domesticated animals by humans, it is plump and has dense wool, with numerous breeds. Sheep have great economic value and mutton is popular worldwide. Mutton is one of the indispensable foods in people's diet, possessing high nutritional value. It contains high-quality protein, various minerals, vitamins and other nutrients, which are highly beneficial to human health. Eating mutton in moderation can help us maintain good health. However, sheep have a slower growth rate and lower average weight. To address the challenges faced today, people have employed various methods and discovered certain genes that can improve the efficiency of sheep growth and development.

Scientists have discovered through continuous research that *IGF-1* and *GH* genes can serve as candidate genes influencing the growth and development of sheep **(Ding *et al.*, 2021)**. Insulin-like growth factor-1 (*IGF-1*) is a member of the insulin-like growth factor family, which mediates the growth-promoting effects of Growth Hormone (*GH*). *GH* stimulates the liver to synthesize and secrete *IGF-1*, which in turn stimulates differentiation of various target tissues and cells through specific *IGF* receptors **(Bergan-Roller *et al.*, 2017)**. In 1978, Rinderknecht and Humbel first isolated the *IGF-1* protein from human serum and elucidated its amino acid sequence and protein structure **(Rinderknecht *et al.*, 1978)**. In 1989, the cDNA sequence of *IGF-1* was isolated and cloned from the liver of lambs **(Wong *et al.*, 1989)**. The signaling pathway of *IGF-1* not only has an important regulatory effect on protein synthesis and degradation, but also plays an important role in the growth and development of skeletal muscles **(Schiaffino *et al.*, 2013)**. The *GH* gene is a pituitary anterior lobe hormone secreted by the anterior pituitary gland **(Ding *et al.*, 2020)**. In early studies, it was believed that the *GH* gene only had significant effects during early animal development. However, it was later discovered that the *GH* gene regulates skeletal calcium balance throughout the entire lifespan and promotes animal growth and development **(Dhandare *et al.*, 2020)**.

SNP refers to the DNA sequence polymorphism at the genomic level caused by a mutation in a single nucleotide. It is a next-generation molecular marker technology. SNPs have the characteristics of easy detection and good genetic stability. In theory, SNPs can be biallelic or have 3 or 4 alleles, but in practice, the occurrence of the latter two are very rare and can be ignored, so it is commonly said that all SNPs are biallelic polymorphisms. SNPs located in the coding region directly impact protein function and synthesis, leading to genetic mutations and changes in traits. SNPS in the promoter region may affect transcriptor binding and thus the transcription level of this gene, SNPS in the non-coding region may affect the function of regulatory elements. This holds significant importance for animal genetics and breeding. **An *et al.* (2014)** found through their analysis of the role of the sheep *IGF-1* gene in the proliferation of sheep muscle cells that it effectively promotes the growth of sheep skeletal muscle cells. **Cao (2013)** discovered through experiments that there are two single nucleotide polymorphisms (SNPs) in the second exon and second intron of Small Tailed Han sheep *IGF-1*, which are dominantly associated with traits related to growth and development such as shoulder height, body height, and body length. Research has found that in animals such as chickens, cows and sheep, the *GH* gene is one of the candidate genes that are identified for polymorphisms and are associated with their growth and ketone traits **(Ip *et al.*, 2001; Ishida *et al.*, 2010; Liu *et al.*, 2011)**. **Wu *et al.* (2012)** conducted PCR-RFLP genotyping of the *GH* gene introns 2, 3 and 4 in three different breeds of ducks and performed association analysis with growth-related traits. They found a C>T mutation at position 172 bp in the second intron and identified three genotypes:CC, CT, and TT. Individuals with the TT and CT genotypes exhibited significantly higher growth-related trait indices compared to those with the CC genotype. **Zhou *et al.* (2013)** conducted a study on the *IGF-1* gene in Liangshan semi-fine wool sheep and identified two SNP sites in the promoter region and exon 3. Although these SNPs did not cause changes in amino acids, they had a certain promoting effect on the early development of sheep.

The purpose of this experiment is to investigate the impact of SNP sites in the *IGF-1* and *GH* genes on the growth and development performance of sheep. By analyzing the genetic diversity of SNPs and conducting correlation analysis with growth and development performance, the genotypes and haplotypes that affect the growth and development performance are determined. This study aims to facilitate the breeding and improvement of sheep breeds and advance the progress of sheep genetic breeding, providing theoretical support for the cultivation of sheep with higher growth and development performance.

MATERIALS AND METHODS

Experimental animals

A total of 632 healthy sheep were collected from Tianfeng Sheep Farm in Zhangwu County, Fuxin City, Liaoning Province, including 312 Charolais ewes, 120 Charolais ewe lambs, 40 Charolais rams, 120 Charolais ram lambs, and 40 Australian White rams. All animals selected for this study were under the same

feeding conditions. All animal handling procedures and protocols used in this study were approved by the Institutional Animal Care and Use Committee of Shenyang Agricultural University. Blood samples for DNA extraction were collected from the jugular vein of each sheep under the guidance of a qualified veterinarian, with 1ml of blood collected per sheep. After collection, the blood was placed in blood collection tubes containing EDTA and stored at-20°C.

Body size performance data

The body size performance data used in the experiment were provided by Tianfeng Sheep Farm in Zhangwu County, Fuxin City, Liaoning Province. These body size performance data include weight, body height, sacral height, back height, waist height, hip height, frontal width, tube circumference, chest circumference, limb length, leg length, body length, chest depth, chest width, waist angle width and hip width.

DNA extraction

Take 200μL of blood from the anticoagulant tube and transfer it to a centrifuge tube. Add 20μL of Proteinase K and mix well. Then add Buffer DL, shake vigorously, and incubate at 56°C in a water bath for 10 minutes. Next, add 200μL of anhydrous ethanol to the centrifuge tube and mix well. Transfer the liquid to a DNA adsorption column and let it stand for two minutes. Centrifuge at 10,000rpm at room temperature for 1 minute and discard the waste liquid in the collection tube. Add 500μL of GW Solution to the adsorption column, centrifuge at 10, 000rpm for 30 seconds, and discard the waste liquid. Add 700μL of Wash Solution to the adsorption column, centrifuge at 10, 000rpm for 30 seconds, and discard the waste liquid. Repeat this step twice. Then centrifuge at 12,000rpm at room temperature for 2 minutes to remove any remaining liquid. Remove the adsorption column and place it in a new centrifuge tube. Add 50μL of CE Buffer, let it stand for 3 minutes, and centrifuge at 12,000rpm at room temperature for 2 minutes. Collect the DNA solution and measure the sample's OD value using UV spectrophotometry. Store the qualified samples at-20°C.

Primer design

The reference sequences for the *IGF-1* gene and *GH* gene were obtained from the NCBI database (accession numbers NC_056056.1 and NC_056064.1, respectively) and specific primers were designed using Primer Premier 5 software. (Table 1)

Table 1 Primer design of *IGF-1* and *GH* genes

Gene	Sense primer (Forward)	Anti-sense primer (Reverse)	TM(℃)F/R	Fragment size	Regions
IGF-1	5'AGGAATGCAGAGATGGGGTAA3'	5'CACAGGCGGTCATTCAGCT3'	59/59	329bp	5116-5445
GH	5'GGAGATCAGGCGTCTAGCTC3'	5'CAGTTCCCTCCCATTGTGTG3'	59/59	303bp	259-562

PCR amplification

The PCR reaction system has a volume of 50μL, including 25μL of 2x SanTaq PCR Mix solution, 1μL of DNA template, 2μL of upstream and downstream primers, and 20μL of ddH_2O. The above reagents are added to a PCR tube, thoroughly mixed and centrifuged, and PCR amplification is performed according to the PCR reaction conditions in a PCR machine. The reaction conditions are as follows: initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 59°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes. Then, electrophoresis is performed for 20 minutes at 130V and 180W. After electrophoresis, observe whether the band of the electrophoresis result contains the target fragment (Figure. 1). If it is present, send the sample to Shanghai Sangon Biotech Co. , Ltd. for sequencing.

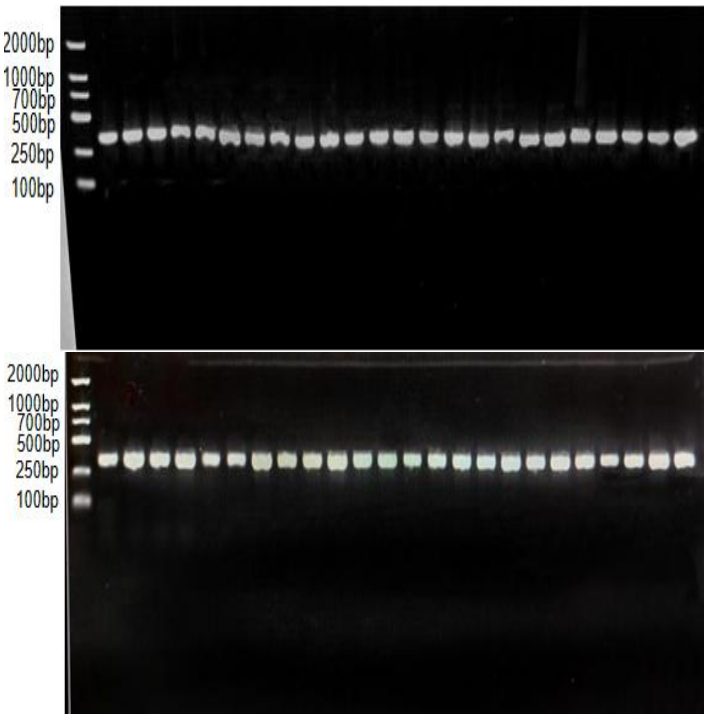

Figure 1 PCR amplification products of *IGF-1* (left) and *GH* (right)
Note:From left to right, the first to fourth bands are Charolais ewe lambs, the fifth to ninth bands are Charolais ewes, the tenth to fourteenth bands are Charolais ram lambs, the fifteenth to nineteenth bands are Charolais rams, and the twentieth to twenty-fourth bands are Australian White rams.Gel electrophoresis results for both genes were obtained using the same samples, which were selected at random.

Statistical analyses

Calculate genotype and allele frequencies, polymorphic information content (PIC), effective allele number (Ne), and heterozygosity (He). Perform single-factor analysis on the growth and development traits of Charolais sheep and Australian White rams with *IGF* and *GH* genes using SPSS software (23.0). Use the animal model integrity equation $Y_{ijkl} = \mu + h_i + p_j + s_k + m_l + e_{ijkl}$ to analyze, where Y_{ijkl} is the observed value, μ is the overall mean value, h_i is the effect of genotype or haplotype combination, P_j is the effect of season and farm, s_k is the effect of year, m_l is the effect of paternal lineage, and e_{ijkl} is the random error. If the p-value is less than 0.05, the difference is significant, and Duncan's method is used for multiple comparisons.

RESULTS

SNP identification

We compared the results and gene sequences of the *IGF-1* and *GH* genes. Using Chromas 2 and DNAMAN software, we conducted comparative analysis and found that the *IGF-1* gene has one SNP site at T5299C, while the *GH* gene has two SNP sites at T364C and C408G (Figure. 2,3).

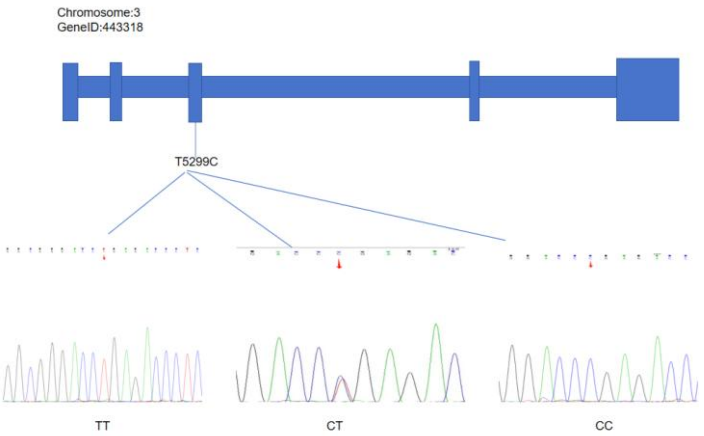

Figure 2 T5299C site of the *IGF-1* gene

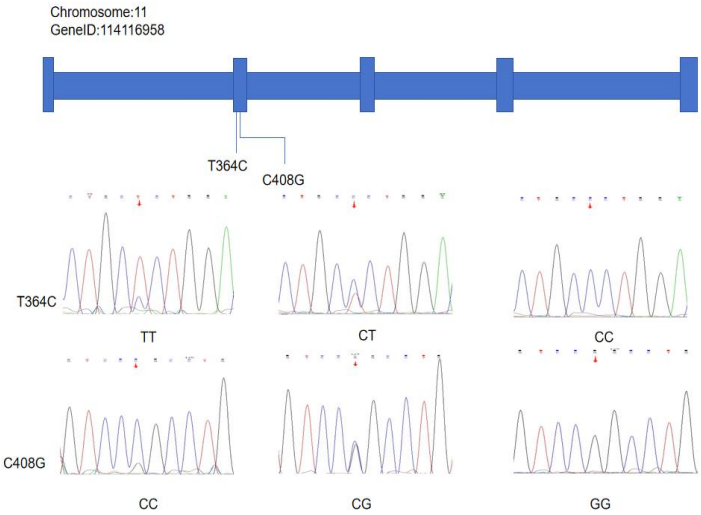

Figure 3 T364C and C408G sites of the *GH* gene

Genetic diversity of *IGF-1* and *GH* genes

The genotype and allele frequencies of the SNP sites of the *IGF-1* and *GH* genes in Charolais and Australian White rams are shown in Table 2. Genes with a frequency greater than 0.5 are considered dominant genes. The T5299C site of the *IGF-1* gene in Charolais ewes, Charolais ram lambs, and Charolais rams has a PIC value of less than 0.25, indicating a low level of polymorphism. This suggests that there is low genetic variation at this site in Charolais ewes, Charolais ram lambs, and Charolais rams. However, in Charolais ewes and Australian White rams, the PIC values range from 0.25 to 0.5, indicating a moderate level of polymorphism. This suggests that there is a higher degree of genetic variation at this site in Charolais ewes and Australian White rams, which may lead to greater genetic progress. The T364C and C408G sites of the *GH* gene in both Charolais and Australian White rams have PIC values ranging from 0.25 to 0.5, indicating a moderate level of polymorphism. This suggests that there is a higher level of genetic variation at these sites in both groups, which may lead to greater genetic progress.

Table 2 Genetic diversity analysis of the *IGF-1* and *GH* genes in sheep

Name	site	specimens	Genotype Frequency			Allelic Frequencies		PIC	He	Ne	χ^2	P
			MM	Mm	mm	M	m					
Charolais ewe lambs	T5299C	120	0.33	0.47	0.2	0.57	0.43	0.37	0.49	1.97	0.07	0.79
Charolais ewes	T5299C	312	0.1	0.13	0.77	0.17	0.83	0.24	0.28	1.38	22.62	1.97894E-06
Charolais ram lambs	T5299C	120	0	0	1	0	1	0	0	1	-	-
Charolais rams	T5299C	40	0	0	1	0	1	0	0	1	-	-
Australian White rams	T5299C	40	0.5	0.3	0.2	0.65	0.35	0.35	0.46	1.83	1.16	0.281364385
Charolais ewe lambs	C408G	120	0	0.93	0. 07	0. 47	0.53	0.37	0.50	1.99	22.97	1.64656E-06
Charolais ewes	C408G	312	0.01	0.95	0. 04	0. 49	0.51	0.37	0.50	2.00	63.00	2.0719E-15
Charolais ram lambs	C408G	120	0.03	0.93	0. 03	0. 5	0.5	0.38	0.50	2.00	22.53	2.06529E-06
Charolais rams	C408G	40	0.25	0.75	0	0.62 5	0.375	0.36	0.47	1.88	7.20	0.01
Australian White rams	C408G	40	0.2	0.7	0.1	0.55	0.45	0.37	0.50	1.98	1.72	0.19
Charolais ewe lambs	T364C	120	0	0.93	0.07	0.47	0.53	0.37	0.50	2.00	22.97	1.64656E-06
Charolais ewes	T364C	312	0.01	0.95	0.04	0.49	0.51	0.37	0.50	2.00	63.00	2.0719E-15
Charolais ram lambs	T364C	120	0	0. 97	0. 03	0.48	0.52	0.37	0.50	2.00	26. 25	2.99349E-07
Charolais rams	T364C	40	0.05	0.95	0	0.53	0.47	0.37	0.50	2.00	16.37	5. 20517E-05
Australian White rams	T364C	40	0	0.9	0.1	0.45	0.55	0.37	0.50	1.98	6. 69	0. 01

Gene substitution effect analysis

Except for Australian White rams, all other sheep show a negative effect at the T5299C site of the *IGF-1* gene. The C408G and T364C sites of the *GH* gene also exhibit a negative effect in all tested sheep, leading to a decrease in growth and development performance. Specifically, in Charolais ewes, replacing T with C at the T5299C site of the *IGF-1* gene results in a 17.80% decline in growth and development performance. At the C408G site of the *GH* gene, replacing C with G causes a 25.61% reduction in growth and development performance. Similarly, at the T386C site of the *GH* gene, replacing T with C leads to a 25.61% decrease in growth and development performance (Table 3).

Table 3 Gene substitution effect analysis

Name	site	Dominant effect d	Additive effect a	Average effect of u gene a1	Average effect of U gene a2	The average effect of u instead of U a
Charolais ewe lambs	T5299C	6	-2	0.03	-0.02	0.05
Charolais ewes	T5299C	-24	26	11.94	-5.86	17.80
Charolais ram lambs	T5299C	-15	15	6.62	-3.25	9.87
Charolais rams	T5299C	-10	10	4.42	-2.17	6.58
Australian White rams	T5299C	-0. 5	-1. 5	-1.12	0.55	-1.67
Charolais ewe lambs	C408G	27	1	6.86	-3.37	10.23
Charolais ewes	C408G	72	1	17.18	-8.43	25.61
Charolais ram lambs	C408G	27	0	6.19	-3.04	9.23
Charolais rams	C408G	12. 5	-2.5	1.19	-0.58	1.7725
Australian White rams	C408G	5. 5	-0.5	0.93	-0.45	1.38
Charolais ewe lambs	T364C	27	1	6.86	-3.37	10.23
Charolais ewes	T364C	72	1	17.18	-8.43	25.61
Charolais ram lambs	T364C	28.5	0.5	6.87	-3.37	10.24
Charolais rams	T364C	18.5	-0.5	3.91	-1.92	5.82
Australian White rams	T364C	8.5	0.5	2.28	-1.12	3.41

Analysis of the relationship between SNPs and body size

At the T5299C site in Charolais ewe lambs, the TT genotype is superior to other genotypes in terms of weight, hip height, and barrel circumference, while it performs significantly better than the CC genotype in terms of hip width. The CT genotype is superior to other genotypes in terms of limb length and waist angle width. The CC genotype is superior to other genotypes in terms of body height, back height, and waist height. At the C408G site, the CG genotype is superior to the GG genotype in terms of body height, back height, and waist height, while the GG genotype is superior to the CG genotype in terms of weight, frontal width, and chest circumference, and significantly better than the CG genotype in terms of barrel circumference. At the T364C site, the CT genotype is superior to the CC genotype in terms of body height, back height, and waist height, while the CC genotype is superior to the CT genotype in terms of weight, frontal width, and chest circumference, and significantly higher than the CT genotype in terms of barrel circumference (Table 4).

Table 4 Body size performance of T5299C, C408G and T364C sites in Charolais ewe lambs

Name	Charolais ewe lambs						
Site	T5299C	T5299C	T5299C	C408G	C408G	T364C	T364C
Genotype	TT(40/120)	CT(56/120)	CC(24/120)	CG(112/120)	GG(8/120)	CT(112/120)	CC(8/120)
Weight(kg)	8.10±0.32	7.86±0.32	7.83±0.81	7.88±0.25	8.75±0.75	7.88±0.25	8.75±0.75
body height(cm)	42.55±0.42	42.61±0.41	44.00±0.89	42.96±0.32	41.50±0.25	42.96±0.32	41.50±0.50
back height(cm)	42.50±0.58	42.11±0.57	43.08±1.08	42.52±0.41	41.25±0.25	42.52±0.41	41.25±0.25
waist height(cm)	42.40±0.49	42.70±0.59	44.33±1.37	42.98±0.45	42.50±0.00	42.98±0.45	42.50±0.00
sacral height(cm)	42.10±0.51	42.36±0.51	43.50±1.14	42.45±0.38	43.25±1.75	42.45±0.38	43.25±1.75
hip height(cm)	37.10±0.95	36.89±1.00	37.00±1.48	37.00±0.63	36.75±3.75	37.00±0.63	36.75±3.75
frontal width(cm)	10.30±0.32	9.93±0.20	10.33±0.28	10.09±0.16	10.75±0.25	10.29±0.16	10.75±0.25
tube circumference(cm)	7.90±0.16	7.86±0.19	7.60±0.19	7.75±0.11^{b}	8.75±0.25^{a}	7.75±0.11^{b}	8.75±0.25^{a}
chest circumference(cm)	48.25±1.03	47.61±0.63	47.83±1.89	47.73±0.59	49.75±1.25	47.73±0.59	49.75±1.25
limb length(cm)	15.10±0.43	15.43±0.50	14.08±0.51	14.96±0.27	16.25±3.25	14.96±0.27	16.25±3.25
leg length(cm)	32.20±0.79	28.82±2.12	31.92±1.52	30.61±1.16	30.00±1.00	30.61±1.16	30.00±1.00
body length(cm)	30.00±0.94	29.61±1.62	29.75±0.75	29.80±0.87	29.25±1.75	29.80±0.87	29.25±1.75
chest depth(cm)	16.42±0.26	16.17±0.38	15.38±0.55	16.01±0.23	17.30±0.80	16.01±0.23	17.30±0.80
chest width(cm)	11.98±0.38	11.84±0.36	11.50±0.50	11.79±0.24	12.25±0.25	11.79±0.24	12.25±0.25
waist angle width(cm)	10.05±0.44	10.34±0.43	9.15±0.33	9.99±0.26	10.20±2.30	9.99±0.26	10.20±2.30
hip width(cm)	14.49±0.67^{a}	13.58±0.59^{ab}	11.77±0.64^{b}	13.48±0.41	14.10±2.90	13.48±0.41	14.10±2.90

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

At the T5299C site in Charolais ewes, the TT genotype is superior to other genotypes in terms of weight, barrel circumference, chest circumference, and chest width, while it performs extremely significantly better than the CT genotype in terms of chest depth. The CT genotype is superior to other genotypes in terms of leg length and waist angle width. The CC genotype is superior to other genotypes in terms of recommended height, hip height, and frontal width, and significantly better than the CT genotype in terms of body height, back height, and waist height. At the C408G site, the CC genotype is superior to other genotypes in terms of limb length, leg length, and hip width. The GG genotype is superior to other genotypes in terms of weight, body height, back height, and waist height, while the CG genotype significantly outperforms the CC genotype in terms of chest circumference and performs better than other genotypes in terms of body length and waist angle width. Both CG and GG genotypes show significant superiority over the CC genotype in terms of chest width. At the T364C site, the TT genotype is superior to other genotypes in terms of leg length and hip width. The CC genotype is superior to other genotypes in terms of weight and body height, while the CT genotype significantly outperforms the TT genotype in terms of chest circumference and performs better than other genotypes in terms of body length and waist angle width. Both CT and CC genotypes demonstrate significant superiority over the TT genotype in terms of chest width (Table 5).

Table 5 Body size performance of T5299C, C408G and T364C sites in Charolais ewes

Name	Charolais ewes								
Site	T5299C	T5299C	T5299C	C408G	C408G	C408G	T364C	T364C	T364C
Genotype	TT(32/312)	CT(40/312)	CC(240/312)	CC(4/312)	CG(296/312)	GG(12/312)	TT(/4312)	CT(296/312)	CC(12/312)
Weight(kg)	81.81±5.25	73.50±3.95	79.48±1.48	69.50±0.00	78.97±1.39	81.67±9.53	69.50±0.00	78.97±1.39	81.67±9.53
body height(cm)	71.56±1.14^{a}	69.02±0.70^{b}	72.16±0.38^{a}	73.00±0.00	71.59±0.36	73.83±1.88	73.00±0.00	71.59±0.36	81.67±9.53
back height(cm)	70.44±0.97^{ab}	69.15±0.61^{b}	71.64±0.40^{a}	71.00±0.00	71.10±0.36	73.67±0.88	71.00±0.00	71.10.±0.36	73.83±1.88
waist height(cm)	70.88±1.36^{ab}	69.55±1.02b	72.21±0.39^{a}	73.00±0.00	71.60±0.38	74.67±0.88	73.00±0.00	71.60±0.38	73.67±0.88
sacral height(cm)	70.75±1.06	70.05±0.80	71.59±0.48	69.00±0.00^{b}	71.19±0.41^{ab}	75.00±0.58^{a}	69.00±0.00^{b}	71.19±0.41^{ab}	74.67±0.88
hip height(cm)	63.56±2.13	64.30±1.00	64.44±0.72	65.00±0.00	64.28±0.62	65.33±4.41	65.00±0.00	64.28±0.62	75.00±0.58^{a}
frontal width(cm)	15.31±0.27	15.30±0.15	15.56±0.12	16.00±0.00	15.46±0.10	16.50±0.29	16.00±0.00	15.46±0.10	65.33±4.41
tube circumference(cm)	9.44±0.24	9.37±0.20	9.78±0.10	10.00±0.00	9.67±0.09	10.00±0.76	10.00±0.00	9.67±0.09	16.50±0.29
chest circumference(cm)	128.5±2.65	126.00±3.42	125.16±1.23	110.00±0.00^{b}	126.00±1.09^{a}	121.17±3.32^{ab}	110.00±0.00^{b}	126.00±1.09^{a}	10.00±0.76
limb length(cm)	20.81±0.61	20.95±1.23	21.70±0.26	23.00±0.00	21.50±0.27	21.33±1.20	23.00±0.00	21.50±0.27	121.17±3.32^{ab}
leg length(cm)	46.31±2.89	48.20±2.02	46.93±0.84	55.00±0.00	46.80±0.77	50.00±3.21	55.00±0.00	46.80±0.77	21.33±1.20
body length(cm)	63.50±2.00	61.80±1.85	64.38±0.61	62.00±0.00	64.06±0.58	62.00±2.65	62.00±0.00	64.06±0.58	50.00±3.21
chest depth(cm)	37.14±0.69^{aA}	34.42±0.71^{bB}	36.63±0.30^{aAB}	34.50±0.00	36.42±0.28	36.50±1.61	34.50±0.00	36.42±0.28	62.00±2.65
chest width(cm)	35.75±1.23	34.34±1.07	34.72±0.38	29.50±0.00^{b}	34.87±0.35^{a}	34.17±1.36^{a}	29.50±0.00^{b}	34.87±0.35^{a}	36.50±1.61
waist angle width(cm)	31.34±2.43	33.02±1.07	31.98±0.71	25.50±0.00	32.19±0.63	30.60±3.32	25.50±0.00	32.19±0.63	34.17±1.36^{a}
hip width(cm)	34.39±1.21	32.86±0.97	34.44±0.43	37.00±0.00	34.20±0.39	34.00±1.89	37.00±0.00	34.20±0.39	30.60±3.32

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

At the C408G site in Charolais ram lambs, the CC genotype is superior to other genotypes in terms of barrel circumference. The CG genotype is superior to other genotypes in terms of weight, body height, and waist height, significantly outperforming the CC genotype in terms of back height and hip height, and extremely significantly better than the GG genotype in terms of chest depth. The GG genotype is significantly superior to the CC genotype in terms of limb length. At the T364C site, the TT genotype is superior to other genotypes in terms of barrel circumference. The CT genotype is superior to other genotypes in terms of weight, body height, and waist height, significantly outperforming the TT genotype in terms of back height and hip height, and extremely significantly better than the CC genotype in terms of chest depth. The CC genotype is superior to the TT genotype in terms of limb length (Table 6).

Table 6 Body size performance of T5299C, C408G and T364C sites in Charolais ram lambs

Name	Charolais ram lambs						
Site	T5299C	C408G	C408G	C408G	T364C	T364C	T364C
Genotype	CC(120/120)	CC(4/120)	CG(112/120)	GG(4/120)	TT(4/120)	CT(112/120)	CC(4/120)
Weight(kg)	8.77±0.29	7.50±0.00	8.84±0.31	8.00±0.00	7.50±0.00	8.84±0.31	8.00±0.00
body height(cm)	42.76±0.41	40.00±0.00	42.92±0.42	41.00±0.00	40.00±0.00	42.92±0.42	41.00±0.00
back height(cm)	42.27±0.43	38.00±0.00^{b}	42.50±0.42^{a}	40.00±0.00^{ab}	38.00±0.00^{b}	42.50±0.42^{a}	40.00±0.00^{ab}
waist height(cm)	43.04±0.42	41.00±0.00	43.11±0.45	43.00±0.00	41.00±0.00	43.11±0.45	43.00±0.00
sacral height(cm)	38.06±0.41	36.00±0.00	38.13±0.44	38.00±0.00	36.00±0.00	38.13±0.44	38.00±0.00
hip height(cm)	42.66±0.42	39.00±0.00^{b}	42.85±0.42^{a}	41.00±0.00^{ab}	39.00±0.00^{b}	42.85±0.42^{a}	41.00±0.00^{ab}
frontal width(cm)	10.22±0.11	10.00±0.00	10.23±0.12	10.00±0.00	10.00±0.00	10.23±0.12	10.00±0.00
tube circumference(cm)	7.75±0.14	8.00±0.00	7.77±0.15	7.00±0.00	8.00±0.00	7.77±0.15	7.00±0.00
chest circumference(cm)	48.68±0.64	43.50±0.00	48.99±0.64	45.00±0.00	43.50±0.00	48.99±0.64	45.00±0.00
limb length(cm)	15.87±0.29	13.00±0.00^{b}	15.96±0.29^{a}	16.00±0.00^{a}	13.00±0.00^{b}	15.96±0.29^{a}	16.00±0.00^{a}
leg length(cm)	31.90±0.43	30.50±0.00	31.98±0.45	31.00±0.00	30.50±0.00	31.98±0.45	31.00±0.00
body length(cm)	29.00±0.59	27.50±0.00	29.02±0.63	30.00±0.00	27.50±0.00	29.02±0.63	30.00±0.00
chest depth(cm)	16.01±0.33	13.50±0.00^{bAB}	16.25±0.30^{aA}	12.00±0.00^{bB}	13.50±0.00^{bAB}	16.25±0.30^{aA}	12.00±0.00^{bB}
chest width(cm)	12.33±0.32	11.00±0.00	12.43±0.34	11.00±0.00	11.00±0.00	12.43±0.34	11.00±0.00
waist angle width(cm)	10.54±0.42	9.50±0.00	10.56±0.44	11.00±0.00	9.50±0.00	10.56±0.44	11.00±0.00
hip width(cm)	14.06±0.46	11.50±0.00	14.17±0.48	13.50±0.00	11.50±0.00	14.17±0.48	13.50±0.00

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

At the C408G site in Charolais rams, the CC genotype is superior to the CG genotype in terms of weight, body height, and back height, while the CG genotype is superior to the CC genotype in terms of barrel circumference, limb length, and body length. At the T364C site, the TT genotype is superior to the CT genotype in terms of weight, back height, and waist height, while the CT genotype is superior to the TT genotype in terms of body height, limb length, and body length (Table 7).

Table 7 Body size performance of T5299C, C408G and T364C sites in Charolais rams

Name	Charolais rams				
Site	T5299C	C408G	C408G	T364C	T364C
Genotype	CC(40/40)	CC(10/40)	CG(30/40)	TT(2/40)	CT(38/40)
Weight(kg)	87.03±2.43	90.70±4.73	85.80±2.85	88.00±0.00	86.97±2.56
body height(cm)	70.56±0.71	71.50±1.64	70.24±0.80	67.00±0.00	70.74±0.73
back height(cm)	70.23±0.83	72.20±1.23	69.57±0.99	72.00±0.00	70.13±0.87
waist height(cm)	72.49±0.87	73.66±0.78	72.10±1.13	74.00±0.00	72.41±0.91
sacral height(cm)	72.44±0.86	73.80±0.75	71.98±1.11	76.00±0.00	72.25±0.89
hip height(cm)	65.24±1.12	67.10±2.41	64.62±1.27	70.00±0.00	64.99±1.15
frontal width(cm)	16.30±0.23	16.30±0.37	16.30±1.10	17.00±0.00	16.26±0.24
tube circumference(cm)	11.28±0.21	11.00±0.42	11.37±0.25	12.00±0.00	11.24±0.22
chest circumference(cm)	107.33±1.14	109.30±2.15	106.67±1.34	108.00±0.00	107.29±1.20
limb length(cm)	23.65±0.55	22.40±0.40	24.07±0.70	22.00±0.00	23.74±0.57
leg length(cm)	52.45±0.87	54.60±0.93	51.73±1.07	56.00±0.00	52.26±0.90
body length(cm)	65.60±0.93	65.20±1.36	65.73±1.18	65.00±0.00	65.63±0.98
chest depth(cm)	34.79±0.45	35.90±0.87	34.41±0.51	36.00±0.00	34.72±0.47
chest width(cm)	28.89±0.49	30.90±1.21	28.22±0.42	33.00±0.00^{a}	28.67±0.47^{b}
waist angle width(cm)	23.47±0.86	23.40±2.54	23.49±0.85	18.00±0.00	23.76±0.86
hip width(cm)	30.02±0.76	31.70±1.81	29.45±0.80	32.50±0.00	29.88±0.79

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

At the C408G site in Australian White rams, the CC genotype is significantly superior to the GG genotype in terms of back height, body length, and waist angle width, while the CG genotype is significantly better than the GG genotype in terms of chest width (Table 8).

Table 8 Body size performance of T5299C, C408G and T364C sites in Australian White rams

Name	Australian White rams							
Site	T5299C	T5299C	T5299C	C408G	C408G	C408G	T364C	T364C
Genotype	TT(20/40)	CT(12/40)	CC(8/40)	CC(8/40)	CG(28/40)	GG(4/40)	CT(36/40)	CC(4/40)
Weight(kg)	67.60±5.23	77.83±8.35	84.25±15.25	69.50±1.00	76.00±6.54	69.00±0.00	74.56±5.09	69.00±0.00
body height(cm)	60.50±10.08	75.17±0.73	72.00±1.00	75.75±0.75	64.21±7.36	71.00±0.00	66.78±5.87	71.00±0.00
back height(cm)	70.00±1.23	74.50±0.50	72.00±5.00	75.00±0.00^{a}	71.50±1.35^{ab}	67.00±0.00^{b}	72.28±1.15	67.00±0.00
waist height(cm)	71.46±1.07	74.83±0.93	74.25±3.25	75.75±0.25	72.54±1.13	71.00±0.00	73.26±0.98	71.00±0.00
sacral height(cm)	71.20±1.19	73.67±1.67	73.50±2.50	74.50±2.50	72.00±1.06	71.00±0.00	72.56±0.98	71.00±0.00
hip height(cm)	65.80±0.92	61.67±4.18	67.25±3.25	64.00±6.00	65.21±1.65	64.00±0.00	64.94±1.62	64.00±0.00
frontal width(cm)	16.54±0.54	16.17±0.33	17.00±0.00	16.50±0.00	16.46±0.41	17.00±0.00	16.47±0.31	17.00±0.00
tube circumference(cm)	10.80±0.68	11.83±0.44	11.00±1.00	11.50±0.50	11.21±0.54	10.00±0.00	11.28±0.43	10.00±0.00
chest circumference(cm)	97.30±2.58	101.33±4.60	103.50±2.50	96.75±0.75	100.43±2.74	101.00±0.00	99.61±2.16	101.00±0.00
limb length(cm)	23.50±1.00	21.83±1.01	22.50±0.50	21.75±1.75	23.21±0.72	22.00±0.00	22.89±0.66	22.00±0.00
leg length(cm)	50.80±1.65	52.50±3.01	52.50±0.50	51.25±4.75	51.57±1.28	53.00±0.00	51.50±1.26	53.00±0.00
body length(cm)	66.50±1.10	66.67±2.73	65.00±4.00	67.50±4.50^{a}	66.64±0.88^{ab}	61.00±0.00^{b}	66.83±1.01	61.00±0.00
chest depth(cm)	34.00±1.06	35.50±0.87	36.00±1.00	34.75±0.75	34.86±0.92	35.00±0.00	34.83±0.71	35.00±0.00
chest width(cm)	25.14±0.89	24.67±1.48	22.50±1.50	23.25±0.75^{ab}	25.31±0.73^{a}	21.00±0.00^{b}	24.86±0.65	21.00±0.00
waist angle width(cm)	21.98±0.53	24.70±2.22	20.75±4.75	22.55±0.95^{a}	23.49±1.11^{a}	16.00±0.00^{b}	23.28±0.87	16.00±0.00
hip width(cm)	25.22±1.52	26.30±2.51	29.00±2.00	23.95±1.55	26.87±1.50	27.00±0.00	26.22±1.25	27.00±0.00

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Haplotype combinations of the two SNP sites in the *GH* gene

Through the analysis using SHEsis software（http://analysis. bio-x. cn/myAnalysis. php）, it was found that there are 9 possible haplotype combinations between the *GH* gene C408G and T364C sites. However, out of the 632 experimental sheep, only 4 haplotype combinations were observed (Table 9）.

Table 9 Haplotype combinations of the two SNP sites in the *GH* gene

Haplotype	H1:CC	H2:CT	H3:TT
H1:CC	CCCC	CCCT（76/632）	CCTT（10/632）
H2:CG	CCCG	CCGT（518/632）	CGTT
H2:GG	CCGG（28/632）	CTGG	TTGG

The correlation analysis between the haplotype combination of *GH* gene C408G and T364C and body size

Among the Charolais ewe lambs, 2 haplotype combinations were identified. CCGG haplotype combination was superior to CCGT haplotype combination in sacral height, chest circumference and other aspects, so CCGG is the dominant haplotype combination (Table 10).

Table 10 Body size performance of haplotype combinations of *GH* gene C408G and T364C sites in Charolais ewe lambs

Name	Charolais ewe lambs	
Haplotype	CCGT(112/120)	CCGG(8/120)
Weight(kg)	7.88±0.25	8.75±0.75
body height(cm)	42.96±0.32	41.50±0.50
back height(cm)	42.52±0.41	41.25±0.25
waist height(cm)	42.98±0.45	42.50±0.00
sacral height(cm)	42.45±0.38	43.25±1.75
hip height(cm)	37.00±0.63	36.75±3.75
frontal width(cm)	10.09±0.16	10.75±0.25
tube circumference(cm)	7.75±0.11	8.75±0.25
chest circumference(cm)	47.73±0.59	49.75±1.25
limb length(cm)	14.96±0.27	16.25±3.25
leg length(cm)	30.61±1.16	30.00±1.00
body length(cm)	29.80±0.87	29.25±1.75
chest depth(cm)	16.01±0.23	17.30±0.80
chest width(cm)	11.79±0.24	12.25±0.25
waist angle width(cm)	9.99±0.26	10.20±2.30
hip width(cm)	13.48±0.41	14.10±2.90

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Table 11 Body size performance of haplotype combinations of *GH* gene C408G and T364C sites in Charolais ewes

Name	Charolais ewes			
Haplotype	CCCT(60/312)	CCTT(4/312)	CCGT(236/312)	CCGG(12/312)
Weight(kg)	79.67±2.74	69.50±0.00	78.80±1.61	81.67±9.53
body height(cm)	71.53±0.81	73.00±0.00	71.60±0.40	73.83±1.88
back height(cm)	71.50±0.95	71.00±0.00	71.00±0.38	73.67±0.88
waist height(cm)	71.60±0.99	73.00±0.00	71.60±0.41	74.67±0.88
sacral height(cm)	$71.81±0.77^{ab}$	$69.00±0.00^{b}$	$71.03±0.48^{ab}$	$75.00±0.58^{a}$
hip height(cm)	65.05±1.38	65.00±0.00	64.09±0.70	65.33±4.41
frontal width(cm)	15.49±0.19	16.00±0.00	15.45±0.12	16.50±0.29
tube circumference(cm)	9.48±0.26	10.00±0..00	9.72±0.90	10.00±0.76
chest circumference(cm)	$124.57±1.95^{a}$	$110.00±0.00^{b}$	$126.36±1.28^{a}$	$121.17±3.32^{ab}$
limb length(cm)	22.03±0.45	23.00±0.00	21.36±0.32	21.33±1.20
leg length(cm)	47.37±1.71	55.00±0.00	46.65±0.86	50.00±3.21
body length(cm)	64.40±1.42	62.00±0.00	63.98±0.64	62.00±2.65
chest depth(cm)	35.95±0.67	34.50±0.00	36.53±0.31	36.50±1.61
chest width(cm)	$34.50±0.90^{a}$	$29.50±0.00^{b}$	$34.97±0.38^{a}$	$34.17±1.36^{a}$
waist angle width(cm)	31.65±1.70	25.50±0.00	32.33±0.67	30.60±3.32
hip width(cm)	33.33±0.90	37.00±0.00	34.42±0.43	34.00±1.89

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Four haplotype combinations were discovered in Charollais ewes. The CCGG haplotype combination exhibited a significant advantage in terms of shoulder height compared to the CCTT haplotype combination. Additionally, the CCGT haplotype combination showed a significant advantage in terms of chest circumference and chest width compared to the CCTT haplotype combination. Therefore, the CCGG haplotype combination is considered the dominant haplotype combination (Table 11).

Three haplotype combinations were found in Charollais lambs. The CCGT haplotype combination exhibited a significant advantage in terms of back height, hip height, and other aspects compared to the CCTT haplotype combination. Additionally, the CCGT haplotype combination showed an extremely significant advantage in terms of chest depth compared to the CCTT haplotype combination. Therefore, the CCGT haplotype combination emerged as the dominant haplotype combination in Charollais lamb (Table 12).

Table 12 Body size performance of haplotype combinations of *GH* gene C408G and T364C sites in Charolais ram lambs

Name	Charolais ram lambs		
Haplotype	CCTT(4/120)	CCGT(112/120)	CCGG(4/120)
Weight(kg)	7.50±0.00	8.84±0.31	8.00±0.00
body height(cm)	40.00±0.00	42.92±0.42	41.00±0.00
back height(cm)	$38.00±0.00^{b}$	$42.50±0.42^{a}$	$40.00±0.00^{ab}$
waist height(cm)	41.00±0.00	43.11±0.45	43.00±0.00
sacral height(cm)	36.00±0.00	38.13±0.44	38.00±0.00
hip height(cm)	$39.00±0.00^{b}$	$42.85±0.42^{a}$	$41.00±0.00^{ab}$
frontal width(cm)	10.00±0.00	10.23±0.12	10.00±0.00
tube circumference(cm)	8.00±0.00	7.77±0.15	7.00±0.00
chest circumference(cm)	43.50±0.00	48.99±0.64	45.00±0.00
limb length(cm)	$13.00±0.00^{b}$	$15.96±0.29^{a}$	$16.00±0.00^{a}$
leg length(cm)	30.20±0.00	31.98±0.45	31.00±0.00
body length(cm)	27.50±0.00	29.02±0.63	30.00±0.00
chest depth(cm)	$13.50±0.00^{bAB}$	$16.25±0.30^{aA}$	$12.00±0.00^{bB}$
chest width(cm)	11.00±0.00	12.43±0.34	11.00±0.00
waist angle width(cm)	9.50±0.00	10.56±0.44	11.00±0.00
hip width(cm)	11.50±0.00	14.17±0.48	13.50±0.00

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Three haplotype combinations were found in Charollais rams. The CCCT haplotype combination showed a significant advantage in terms of body height compared to the CCTT haplotype combination. On the other hand, the CCTT haplotype combination exhibited an extremely significant advantage in terms of chest width compared to the CCGT haplotype combination. Consequently, the CCCT haplotype combination is considered the dominant haplotype combination (Table 13).

Table 13 Body size performance of haplotype combinations of *GH* gene C408G and T364C sites in Charolais rams

Name	Charolais rams		
Haplotype	CCCT(8/40)	CCTT(2/40)	CCGT(30/40)
Weight(kg)	91.38±6.04	88.00±0.00	85.80±2.85
body height(cm)	72.63±1.55^{a}	67.00±0.00^{b}	70.24±0.80^{ab}
back height(cm)	72.25±1.59	72.00±0.00	69.57±0.99
waist height(cm)	73.58±1.00	74.00±0.00	72.10±1.13
sacral height(cm)	73.25±0.66	76.00±0.00	71.98±1.11
hip height(cm)	66.38±2.97	70.00±0.00	64.62±1.27
frontal width(cm)	16.13±0.43	17.00±0.00	16.30±0.28
tube circumference(cm)	10.75±0.43	12.00±0.00	11.37±0.25
chest circumference(cm)	109.63±2.75	108.00±0.00	106.67±1.34
limb length(cm)	22.50±0.50	22..00±0.00	24.07±0.7.0
leg length(cm)	54.25±1.11	56.00±0.00	51.73±1.07
body length(cm)	65.25±1.75	65.00±0.00	65.73±1.18
chest depth(cm)	35.88±1.13	36.00±0.00	34.41±0.51
chest width(cm)	30.38±1.41^{abAB}	33.00±0.00^{aA}	28.22±0.42^{bB}
waist angle width(cm)	24.75±2.78^{a}	18.00±0.00^{a}	23.49±0.85^{ab}
hip width(cm)	31.50±2.33	32.50±0.00	29.45±0.80

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Table 14 Body size performance of haplotype combinations of *GH* gene C408G and T364C sites in Australian White rams

Name	Australian White rams		
Haplotype	CCCT(8/40)	CCGT(28/40)	CCGG(4/40)
Weight(kg)	69.50±1.00	76.00±6.53	69.00±0.00
body height(cm)	75.75±0.75	64.21±7.36	71.00±0.00
back height(cm)	75.00±0.00^{a}	71.50±1.35^{ab}	67.00±0.00^{b}
waist height(cm)	75.75±0.25	72.54±1.13	71.00±0.00
sacral height(cm)	74.50±2.50	72.00±1.06	71.00±0.00
hip height(cm)	64.00±6.00	65.21±1.65	64.00±0.00
frontal width(cm)	16.50±0.00	16.46±0.41	17.00±0.00
tube circumference(cm)	11.50±0.50	11.21±0.54	10.00±0.00
chest circumference(cm)	96.75±0.75	100.43±2.74	101.00±0.00
limb length(cm)	21.75±1.75	23.21±0.72	22.00±0.00
leg length(cm)	51.25±4.75	51.57±1.28	53.00±0.00
body length(cm)	67.50±4.50^{a}	66.64±0.88^{ab}	61.00±0.00^{b}
chest depth(cm)	34.75±0.75	34.86±0.92	35.00±0.00
chest width(cm)	23.25±0.75^{ab}	25.31±0.73^{a}	21.00±0.00^{b}
waist angle width(cm)	22.55±0.95^{a}	23.49±1.11^{a}	16.00±0.00^{b}
hip width(cm)	23.95±1.55	26.87±1.50	27.00±0.00

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level. Three haplotype combinations were found in Australian White rams. The CCGT haplotype combination exhibited a significant advantage in terms of chest width and waist angle width compared to the CCGG haplotype combination. Moreover, the CCCT haplotype combination demonstrated a significant advantage in terms of back height and body length compared to the CCGG haplotype combination. Hence, the CCCT haplotype combination was identified as the dominant haplotype combination (Table 14).

The haplotype combinations of the *IGF-1* gene T5299C site and the *GH* gene C408G site

Through the analysis using SHEsis software（http://analysis. bio-x. cn/myAnalysis. php）, it was found that there are 9 possible haplotype combinations between the *IGF-1* and *GH* (C408G) genes. However, out of the 632 experimental sheep, only 7 haplotype combinations were observed (Table 15).

Table 15 Haplotype combinations of the *IGF-1* gene T5299C site and the *GH* gene C408G site

Haplotype	H1:CC	H2:CG	H3:GG
H1:TT	H1H1:TTCC	H1H2:TTCG（88/632）	H1H3:TTGG（4/632）
H2:CT	H2H1:CTCC（8/632）	H2H2:CTCG（96/632）	H2H3:CTGG（4/632）
H3:CC	H3H1:CCCC（10/632）	H3H2:CCCG（406/632）	H3H3:CCGG（16/632）

The correlation analysis between the haplotype combination of *IGF-1* gene T5299C and *GH* gene C408G and body size

Among the Charolais ewe lambs, 5 haplotype combinations were identified. The CCCG haplotype combination showed a significant advantage in terms of body height compared to the CTGG haplotype combination. The CTGG haplotype combination demonstrated a significant advantage in sacral height compared to the TTGG haplotype combination. Furthermore, the CTGG haplotype combination exhibited an extremely significant advantage in hip height compared to the TTGG haplotype combination. In terms of tube circumference, the CTGG haplotype combination displayed a highly significant advantage over the CCCG and CTCG haplotype combinations. Additionally, the CTGG haplotype combination showed an extremely significant advantage in limb length compared to other haplotype combinations. In terms of chest depth, the CTGG haplotype combination exhibited a highly significant advantage over the CCCG haplotype combination. The TTGG haplotype combination showed a highly significant advantage in loin angle width compared to TTCG, CTGG, and CCCG haplotype combinations. Moreover, the TTGG haplotype combination demonstrated an extremely significant advantage in hip width compared to CTGG and CCCG haplotype combinations, indicating that CCCG is the dominant haplotype combination (Table 16).

Table 16 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene C408G sites in Charolais ewe lambs

Name	Charolais ewe lambs				
Haplotype	TTCG(36/120)	TTGG(4/120)	CTCG(52/120)	CTGG(4/120)	CCCG(24/120)
Weight(kg)	8.11±0.36	8.00±0.00	7.73±0.32	9.50±0.00	7.83±0.81
body height(cm)	42.61±0.47^{ab}	42.00±0.00^{ab}	42.73±0.42^{ab}	41.00±0.00^{b}	44.00±0.89^{a}
back height(cm)	42.67±0.62	41.00±0.00	42.15±0.62	41.50±0.00	43.08±1.08
waist height(cm)	42.39±0.55	42.50±0.00	42.77±0.63	42.50±0.00	44.33±1.37
sacral height(cm)	42.17±0.57^{ab}	41.50±0.00^{b}	42.15±0.51^{ab}	45.00±0.00^{a}	43.50±1.14^{ab}
hip height(cm)	37.56±0.93^{abAB}	33.00±0.00^{bB}	36.62±1.03^{abAB}	40.50±0.00^{aA}	37.00±1.48^{abAB}
frontal width(cm)	10.28±0.35	10.50±0.00	9.85±0.19	11.00±0.00	10.33±0.28
tube circumference(cm)	7.83±0.17^{bAB}	8.50±0..00^{abAB}	7.77±0.18^{bB}	9.00±0.00^{aA}	7.60±0.19^{bB}
chest circumference(cm)	48.22±1.16	48.50±0.00	47.35±0.62	51.00±0.00	47.83±1.89
limb length(cm)	15.33±0.41^{bB}	13.00±0.00^{cB}	15.12±0.42^{bB}	19.50±0.00^{aA}	14.08±0.51^{bcB}
leg length(cm)	32.33±0.87	31.00±0.00	28.81±2.29	29.00±0.00	31.92±1.52
body length(cm)	30.28±1.00	27.50±0.00	29.50±1.75	31.00±0.00	29.75±0.75
chest depth(cm)	16.40±0.29^{abAB}	16.50±0.00^{abAB}	16.02±0.37^{bAB}	18.10±0.00^{aA}	15.38±0.55^{bB}
chest width(cm)	11.98±0.42	12.00±0.00	11.79±0.38	12.50±0.00	11.50±0.50
waist angle width(cm)	9.78±0.39^{bcB}	12.50±0.00^{aA}	10.52±0.42^{bAB}	7.90±0.00^{cB}	9.15±0.33^{bcB}
hip width(cm)	14.21±0.68^{abAB}	17.00±0.00^{aA}	13.76±0.61^{bAB}	11..20±0.00^{bB}	11.77±0.64^{bB}

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Among the Charolais ewes, 4 haplotype combinations were identified. The CCGG haplotype combination showed a significant advantage in terms of body height, waist height, and chest depth compared to the CTCG haplotype combination. Additionally, the CCGG haplotype combination demonstrated a significant advantage in frontal width compared to both the CTCG and TTCG haplotype combinations. Moreover, the CCGG haplotype combination exhibited an extremely significant advantage in tube circumference compared to both the CTCG and TTCG haplotype combinations, indicating that the CCGG haplotype is the dominant one (Table 17).

Table 17 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene C408G sites in Charolais ewes

Name	Charolais ewes			
Haplotype	TTCG(32/312)	CTCG(40/312)	CCCG(232/312)	CCGG(8/312)
Weight(kg)	81.81±5.25	73.50±3.95	79.33±1.48	84.00±16.00
body height(cm)	71.56±1.14^{ab}	69.02±0.70^{b}	72.10±0.39^{ab}	73.75±3.25^{a}
back height(cm)	70.44±0.97	69.15±0.61	71.60±0.41	73.00±1.00
waist height(cm)	70.88±1.36^{ab}	69.55±1.02^{b}	72.15±0.40^{ab}	74.00±1.00^{a}
sacral height(cm)	70.75±1.06	70.05±0.80	71.49±0.49	74.50±0.50
hip height(cm)	63.56±2.13	64.30±1.00	64.52±0.73	62.00±5.00
frontal width(cm)	15.31±0.27^{b}	15.30±0.15^{b}	15.53±0.12^{ab}	16.50±0.50^{a}
tube circumference(cm)	9.44±0.24^{bB}	9.37±0.20^{bB}	9.74±0.10^{bAB}	10.75±0.25^{aA}
chest circumference(cm)	128.50±2.65	126.00±3.42	125.20±1.27	124.00±3.00
limb length(cm)	20.81±0.61	20.95±1.23	21.72±0.26	21.00±2.00
leg length(cm)	46.31±2.89	48.20±2.02	46.73±0.85	52.50±3.50
body length(cm)	63.50±2.00	61.80±1.85	64.39±0.62	64.00±3.00
chest depth(cm)	37.14±0.69^{ab}	34.42±0.71^{b}	36.59±0.31^{ab}	37.75±1.75^{a}
chest width(cm)	35.75±1.23	34.34±1.07	34.76±0.39	33.75±2.25
waist angle width(cm)	31.34±2.43	33.02±1.07	32.09±0.72	28.65±4.65
hip width(cm)	34.39±1.21	32.86±0.97	34.45±0.44	34.25±3.25

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Among the Charolais ram lambs, 2 haplotype combinations were identified. CCCG haplotype combination was superior to CCGG haplotype combination in weight, body height, back height and other aspects, so CCCG is the dominant haplotype combination (Table18).

Table 18 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene C408G sites in Charolais ram lambs

Name	Charolais ram lambs	
Haplotype	CCCG(116/120)	CCGG(4/120)
Weight(kg)	8.79±0.30	8.00±0.00
body height(cm)	42.82±0.42	41.00±0.00
back height(cm)	42.35±0.43	40.00±0.00
waist height(cm)	43.04±0.44	43.00±0.00
sacral height(cm)	38.06±0.43	38.00±0.00
hip height(cm)	42.71±0.43	41.00±0.00
frontal width(cm)	10.22±0.12	10.00±0.00
tube circumference(cm)	7.78±0.14	7.00±0.00
chest circumference(cm)	48.80±0.65	45.00±0.00
limb length(cm)	15.86±0.30	16.00±0.00
leg length(cm)	31.93±0.44	31.00±0.00
body length(cm)	28.97±0.61	30.00±0.00
chest depth(cm)	16.15±0.31	12.00±0.00
chest width(cm)	12.38±0.33	11.00±0.00
waist angle width(cm)	10.52±0.43	11.00±0.00
hip width(cm)	14.08±0.47	13.50±0.00

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Among the Charolais rams, 2 haplotype combinations were identified. CCCC haplotype combination was superior to CCCG haplotype combination in weight, body height, back height and other aspects, so CCCC is the dominant haplotype combination (Table19).

Table 19 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene C408G sites in Charolais rams

Name	Charolais rams	
Haplotype	CCCC(10/40)	CCCG(30/40)
Weight(kg)	90.70±10.57	85.80±11.04
body height(cm)	71.50±1.64	70.24±0.80
back height(cm)	72.20±1.23	69.57±0.99
waist height(cm)	73.66±0.78	72.10±1.13
sacral height(cm)	73.80±0.75	71.98±1.11
hip height(cm)	67.10±2.41	64.62±1.27
frontal width(cm)	16.30±0.37	16.30±0.28
tube circumference(cm)	11.00±0.42	11.37±0.25
chest circumference(cm)	109.30±2.15	106.67±1.34
limb length(cm)	22.40±0.40	24.07±0.70
leg length(cm)	54.60±0.93	51.73±1.07
body length(cm)	65.20±1.36	65.73±1.18
chest depth(cm)	35.90±0.87	34.41±0.51
chest width(cm)	30.90±1.21	28.22±0.42
waist angle width(cm)	23.40±2.54	23.49±0.85
hip width(cm)	31.70±1.81	29.45±0.80

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Among the Australian White rams, 5 haplotype combinations were identified. The CCCG haplotype combination exhibited an extremely significant advantage in terms of weight, back height, and waist height compared to the CCGG haplotype combination. Furthermore, in terms of hip height, the CCCG haplotype combination showed a highly significant advantage over the CTCG haplotype combination. Additionally, the CCCG haplotype combination demonstrated a significant advantage in body length compared to the CCGG haplotype combination, indicating that the CCCG haplotype combination is the dominant one (Table 20).

Table 20 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene C408G sites in Australian White rams

Name	Australian White rams				
Haplotype	TTCG(20/40)	CTCC(8/40)	CTCG(4/40)	CCCG(4/40)	CCGG(4/40)
Weight(kg)	67.60±5.23^{bB}	69.50±1.00^{bB}	94.50±0.00^{aAB}	99.50±0.00^{aA}	69.00±0.00^{bB}
body height(cm)	60.50±10.08	75.75±0.75	74,00±0.00	73.00±0.00	71.00±0.00
back height(cm)	70.00±1.23^{bcBC}	75.00±0.00^{aAB}	73.50±0.00^{abAB}	77.00±0.00^{aA}	67.00±0.00^{cC}
waist height(cm)	71.46±1.07^{cB}	75.75±0.25^{abAB}	73.00±0.00^{bcAB}	77.50±0.00^{aA}	71.00±0.00^{cB}
sacral height(cm)	71.20±1.19	74.50±2.50	72.00±0.00	76.00±0.00	71.00±0.00
hip height(cm)	65.80±0.92^{aAB}	64.00±6.00^{abAB}	57.00±0.00^{bB}	70.50±0.00^{aA}	64.00±0.00^{abAB}
frontal width(cm)	16.54±0.54	16.50±0.00	15.50±0.00	17.00±0.00	17.00±0.00
tube circumference(cm)	10.80±0.68	11.50±0.50	12.50±0.00	12.00±0.00	10.00±0.00
chest circumference(cm)	97.30±2.58^{b}	96.75±0.75^{b}	110.50±0.00^{a}	106.00±0.00^{ab}	101.00±0.00^{b}
limb length(cm)	23.50±1.00	21.75±1.75	22.00±0.00	23.00±0.00	22.00±0.00
leg length(cm)	50.80±1.65	51.25±4.75	55.00±0.00	52.00±0.00	53.00±0.00
body length(cm)	66.50±1.10^{ab}	67.50±4.50^{ab}	65.00±0.00^{ab}	69.00±0.00^{a}	61.00±0.00^{b}
chest depth(cm)	34.00±1.06	34.75±0.75	37.00±0.00	37.00±0.00	35.00±0.00
chest width(cm)	25.14±0.89^{abAB}	23.25±0.75^{bcAB}	27.50±0.00^{aA}	24.00±0.00^{bcAB}	21.00±0.00^{cB}
waist angle width(cm)	21.98±0.53^{cC}	22.55±0.95^{cBC}	29.00±0.00^{aA}	25.50±0.00^{bB}	16.00±0.00^{dD}
hip width(cm)	25.22±1.52^{ab}	23.95±1.55^{b}	31.00±0.00^{a}	31.00±0.00^{a}	27.00±0.00^{ab}

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

The haplotype combination of *IGF-1* gene T5299C site and *GH* gene T364C site

Through analysis using SHEsis software（http://analysis. bio-x. cn/myAnalysis. php）, it was found that the *IGF-1* and *GH* T364C SNP sites can form 9 haplotype combinations. However, only 7 haplotype combinations were identified among the 632 experimental sheep.

Table 21 The haplotype combination of *IGF-1* gene T5299C site and *GH* gene T364C site

Haplotype	H1:TT	H2:CT	H3:CC
H1:TT	TTTT	TTCT（88/632）	TTCC（4/632）
H2:CT	CTTT	CTCT（104/632）	CTCC（4/632）
H3:CC	CCTT（6/632）	CCCT（406/632）	CCCC（20/632）

The correlation analysis between the haplotype combination of *IGF-1* T5299C and *GH* T364C and body size

Among the Charolais ewe lambs, 5 haplotype combinations were identified. The CCCT haplotype combination exhibited a significant advantage in terms of body height compared to the CTCC haplotype combination. The CTCC haplotype combination showed a significant advantage in shoulder height compared to the TTCC haplotype combination, and a highly significant advantage in hip height compared to the TTCC haplotype combination. Additionally, the CTCC haplotype combination demonstrated a highly significant advantage in limb length compared to other haplotype combinations. In terms of chest depth, the CTCC haplotype combination showed a highly significant advantage over the CCCT haplotype combination. The TTCC haplotype combination exhibited a highly significant advantage in waist width and hip width compared to the CCCT and CTCC haplotype combinations. Therefore, the CCCT haplotype combination is the dominant one (Table 22).

Table 22 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene T364C sites in Charolais ewe lambs

Name	Charolais ewe lambs				
Haplotype	TTCT(36/120)	TTCC(4/120)	CTCT(52/120)	CTCC(4/120)	CCCT(24/120)
Weight(kg)	8.11±0.36	8.00±0.00	7.73±0.32	9.50±0.00	7.83±0.81
body height(cm)	$42.61±0.47^{ab}$	$42.00±0.00^{ab}$	$42.73±0.42^{ab}$	$41.00±0.00^{b}$	$44.00±0.89^{a}$
back height(cm)	42.67±0.62	41.00±0.00	42.15±0.62	41.50±0.00	43.08±1.08
waist height(cm)	42.39±0.55	42.50±0.00	42.77±0.63	42.50±0.00	44.33±1.37
sacral height(cm)	$42.17±0.57^{ab}$	$41.50±0.00^{b}$	$42.15±0.51^{ab}$	$45.00±0.00^{a}$	$43.50±1.14^{ab}$
hip height(cm)	$37.56±0.93^{abAB}$	$33.00±0.00^{bB}$	$36.62±1.03^{abAB}$	$40.50±0.00^{aA}$	$37.00±1.48^{abAB}$
frontal width(cm)	10.28±0.35	10.50±0.00	9.85±0.19	11.00±0.00	10.33±0.28
tube circumference(cm)	$7.83±0.17^{bAB}$	$8.50±0..00^{abAB}$	$7.77±0.18^{bB}$	$9.00±0.00^{aA}$	$7.60±0.19^{bB}$
chest circumference(cm)	48.22±1.16	48.50±0.00	47.35±0.62	51.00±0.00	47.83±1.89
limb length(cm)	$15.33±0.41^{bB}$	$13.00±0.00^{cB}$	$15.12±0.42^{bB}$	$19.50±0.00^{aA}$	$14.08±0.51^{bcB}$
leg length(cm)	32.33±0.87	31.00±0.00	28.81±2.29	29.00±0.00	31.92±1.52
body length(cm)	30.28±1.00	27.50±0.00	29.50±1.75	31.00±0.00	29.75±0.75
chest depth(cm)	$16.40±0.29^{abAB}$	$16.50±0.00^{abAB}$	$16.02±0.37^{bAB}$	$18.10±0.00^{aA}$	$15.38±0.55^{bB}$
chest width(cm)	11.98±0.42	12.00±0.00	11.79±0.38	12.50±0.00	11.50±0.50
waist angle width(cm)	$9.78±0.39^{bcB}$	$12.50±0.00^{aA}$	$10.52±0.42^{bAB}$	$7.90±0.00^{cB}$	$9.15±0.33^{bcB}$
hip width(cm)	$14.21±0.68^{abAB}$	$17.00±0.00^{aA}$	$13.76±0.61^{bAB}$	$11..20±0.00^{bB}$	$11.77±0.64^{bB}$

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Among the Charolais ewes, 5 haplotype combinations were identified. The CCCC haplotype combination exhibited a significant advantage in terms of body height, back height, and waist height compared to the CTCT haplotype combination. It also showed a significant advantage in shoulder height compared to the CCTT haplotype combination. The TTCT haplotype combination exhibited a highly significant advantage in chest circumference and chest width compared to the CCTT haplotype combination. Thus, the CCCC haplotype combination is the dominant one (Table 23).

Table 23 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene T364C sites in Charolais ewes

Name	Charolais ewes				
Haplotype	TTCT(32/312)	CTCT(40/312)	CCTT(4/312)	CCCT(224/312)	CCCC(12/312)
Weight(kg)	81.81±5.25	73.50±3.95	69.50±0.00	79.55±1.52	81.67±9.53
body height(cm)	$71.56±1.14^{ab}$	$69.02±0.70^{b}$	$73.00±0.00^{ab}$	$72.05±0.40^{ab}$	$73.83±1.88^{a}$
back height(cm)	$70.44±0.97^{ab}$	$69.15±0.61^{b}$	$71.00±0.00^{ab}$	$71.55±0.42^{ab}$	$73.67±0.88^{a}$
waist height(cm)	$70.88±1.36^{ab}$	$69.55±1.02^{b}$	$73.00±0.00^{ab}$	$72.07±0.41^{ab}$	$74.67±0.88^{a}$
sacral height(cm)	$70.75±1.06^{ab}$	$70.05±0.80^{ab}$	$69.00±0.00^{b}$	$71.45±0.50^{ab}$	$75.00±0.58^{a}$
hip height(cm)	63.56±2.13	64.30±1.00	65.00±0.00	64.38±0.75	65.33±4.41
frontal width(cm)	15.31±0.27	15.30±0.15	16.00±0.00	15.51±0.13	16.50±0.29
tube circumference(cm)	9.44±0.24	9.37±0.20	10.00±0.00	9.76±0.10	10.00±0.76
chest circumference(cm)	$128.50±2.65^{aA}$	$126.00±3.42^{aAB}$	$110.00±0.00^{bB}$	$125.65±1.27^{aAB}$	$121.17±3.32^{abAB}$
limb length(cm)	20.81±0.61	20.95±1.23	23.00±0.00	21.70±0.27	21.33±1.20
leg length(cm)	46.31±2.89	48.20±2.02	55.00±0.00	46.62±0.87	50.00±3.21
body length(cm)	63.50±2.00	61.80±1.85	62.00±0.00	64.55±0.63	62.00±2.65
chest depth(cm)	37.14±0.69	34.42±0.71	34.50±0.00	36.67±0.32	36.50±1.61
chest width(cm)	$35.75±1.23^{aA}$	$34.34±1.07^{aAB}$	$29.50±0.00^{bB}$	$34.85±0.39^{aAB}$	$34.17±1.36^{aAB}$
waist angle width(cm)	31.34±2.43	33.02±1.07	25.50±0.00	32.17±0.74	30.60±3.32
hip width(cm)	34.39±1.21	32.86±0.97	37.00±0.00	34.42±0.45	34.00±1.89

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Among the Charolais ram lambs, 2 haplotype combinations were identified. CCCT haplotype combination was superior to CCCC haplotype combination in weight, body height, back height and other aspects, so CCCT is the dominant haplotype combination (Table24).

Table 24 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene T364C sites in Charolais ram lambs

Name	Charolais ram lambs	
Haplotype	CCCT(116/120)	CCCC(4/120)
Weight(kg)	8.79±0.30	8.00±0.00
body height(cm)	42.82±0.42	41.00±0.00
back height(cm)	42.35±0.43	40.00±0.00
waist height(cm)	43.04±0.44	43.00±0.00
sacral height(cm)	38.06±0.43	38.00±0.00
hip height(cm)	42.71±0.43	41.00±0.00
frontal width(cm)	10.22±0.12	10.00±0.00
tube circumference(cm)	7.78±0.14	7.00±0.00
chest circumference(cm)	48.80±0.65	45.00±0.00
limb length(cm)	15.86±0.30	16.00±0.00
leg length(cm)	31.93±0.44	31.00±0.00
body length(cm)	28.97±0.61	30.00±0.00
chest depth(cm)	16.15±0.31	12.00±0.00
chest width(cm)	12.38±0.33	11.00±0.00
waist angle width(cm)	10.52±0.43	11.00±0.00
hip width(cm)	14.08±0.47	13.50±0.00

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Among the Charolais rams, 2 haplotype combinations were identified. CCTT haplotype combination was superior to CCCC haplotype combination in weight, back height, waist height and other aspects, so CCTT is the dominant haplotype combination (Table 25).

Table 25 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene T364C sites in Charolais rams

Name	Charolais rams	
Haplotype	CCTT(2/40)	CCCT(38/40)
Weight(kg)	88.00±0.00	86.97±2.56
body height(cm)	67.00±0.00	70.74±0.73
back height(cm)	72.00±0.00	70.13±0.87
waist height(cm)	74.00±0.00	72.41±0.91
sacral height(cm)	76.00±0.00	72.25±0.89
hip height(cm)	70.00±0.00	64.99±1.15
frontal width(cm)	17.00±0.00	16.26±0.24
tube circumference(cm)	12.00±0.00	11.24±0.22
chest circumference(cm)	108.00±0.00	107.29±1.20
limb length(cm)	22.00±0.00	23.74±0.57
leg length(cm)	56.00±0.00	52.26±0.90
body length(cm)	65.00±0.00	65.63±0.98
chest depth(cm)	36.00±0.00	34.72±0.47
chest width(cm)	33.00±0.00	28.67±0.47
waist angle width(cm)	18.00±0.00	23.76±0.86
hip width(cm)	32.50±0.00	29.88±0.79

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

Among the Australian White rams, 4 haplotype combinations were identified. The CCCT haplotype combination exhibited an advantage in terms of weight and hip height compared to the CTCT haplotype combination. It also showed a highly significant advantage in back height and waist height compared to the TTCT and CCCC haplotype combinations. In terms of body length, it exhibited a significant advantage over the CCCC haplotype combination, and in terms of waist width, it demonstrated a highly significant advantage over the CCCC haplotype combination. Therefore, the CCCT haplotype combination is the dominant one (Table 26).

Table 26 Body size performance of haplotype combinations of *IGF-1* gene T5299C and *GH* gene T364C sites in Australian White rams

Name	Australian White rams			
Haplotype	TTCT(20/40)	CTCT(12/40)	CCCT(4/40)	CCCC(4/40)
Weight(kg)	$67.60±5.23^{b}$	$77.83±8.35^{ab}$	$99.50±0.00^{a}$	$69.00±0.00^{b}$
body height(cm)	60.50±10.08	75.17±0.73	73.00±0.00	71.00±0.00
back height(cm)	$70.00±1.23^{bBC}$	$74.50±0.50^{aAB}$	$77.00±0.00^{aA}$	$67.00±0.00^{bC}$
waist height(cm)	$71.46±1.07^{bB}$	$74.83±0.93^{abAB}$	$77.50±0.00^{aA}$	$71.00±0.00^{bB}$
sacral height(cm)	71.20±1.19	73.67±1.67	76.00±0.00	71.00±0.00
hip height(cm)	$65.80±0.92^{ab}$	$61.67±4.18^{b}$	$70.50±0.00^{a}$	$64.00±0.00^{ab}$
frontal width(cm)	16.54±0.54	16.17±0.33	17.00±0.00	17.00±0.00
tube circumference(cm)	10.80±0.68	11.83±0.44	12.00±0.00	10.00±0.00
chest circumference(cm)	97.30±2.58	101.33±4.60	106.00±0.00	101.00±0.00
limb length(cm)	23.50±1.00	21..83±1.01	23.00±0.00	22.00±0.00
leg length(cm)	50.80±1.65	52.50±3.01	52.00±0.00	53.00±0.00
body length(cm)	$66.50±1.10^{ab}$	$66.67±2.73^{ab}$	$69.00±0.00^{a}$	$61.00±0.00^{b}$
chest depth(cm)	34.00±1.06	35.50±0.87	37.00±0.00	35.00±0.00
chest width(cm)	$25.14±0.89^{a}$	$23.25±0.75^{ab}$	$24.00±0.00^{ab}$	$21.00±0.00^{b}$
waist angle width(cm)	$21.98±0.53^{aAB}$	$24.70±2.22^{aA}$	$25.50±0.00^{aA}$	$16.00±0.00^{bB}$
hip width(cm)	25.22±1.52	26.30±2.51	31.00±0.00	27.00±0.00

Note: Different lower case letter superscripts indicate significant differences at the 0.05 level, while upper case letter superscripts indicate differences at the 0.01 level.

DISCUSSION

The growth and development traits of sheep are highly important economic characteristics. By selecting and breeding sheep with excellent growth and development traits, it can promote the development of the sheep industry in the country and bring significant economic benefits. In previous studies, it has been found that genes such as *MSTN*, *CLPG* **(Hu *et al.*, 2016)** and *Myf5* **(Niu *et al.*, 2014)** are associated with the growth and development of sheep. Research has shown that three SNP sites, rs6214, rs10860860 and rs2946834 in *IGF-1* are associated with the inheritance of high myopia **(Rydzanicz *et al.*, 2011)**. **Yan *et al.* (2014)** conducted their study using Suffolk rams as experimental subjects and found that the content of the *GH* gene in tissues such as the testes, spleen and mesenteric lymph nodes was significantly higher than in other tissues. Similarly, the content of the *IGF-1* gene in the liver and mesenteric lymph nodes was significantly higher than in other tissues. This indicates that the *IGF-1* gene and *GH* gene have a certain promoting effect on growth and development. **Kumar *et al.* (2023)** conducted experiments to determine the correlation between the *IGF-1* gene and performance traits in Munjal sheep. The results indicated that certain SNP site mutations in the *IGF-1* gene would lead to positive development of growth and development-related traits in Munjal sheep. **Li *et al.* (2012)** through their study, found a correlation between the mutation site in the fourth exon of the *GH* gene and the weight and body size of sheep. **Han (2016)** found three SNP sites on the *GH* gene in Tibetan sheep, among which two sites, G498C and G616A, were significantly correlated with some growth traits of Tibetan sheep. This provides assistance for breeding Tibetan sheep.

This study primarily analyzed the effects of genotypes and selected haplotype combinations at different sites of the *IGF-1* and *GH* genes on the growth and development performance of sheep.We used SNP genetic analysis, which can help us better understand the impact of genetic variation on epigenetics **(Zhu and Zhou, 2020)**. This study discovered one SNP site, T5299C, in the *IGF-1* gene, and two SNP sites, C408G and T364C, in the *GH* gene.The T5299C site of the *IGF-1* gene in Charolais ewe lambs exhibits a moderate degree of polymorphism, with a PIC value ranging from 0.25 to 0.5. The T364C site of the *GH* gene in Charolais sheep and Australian White rams also shows a moderate degree of polymorphism, with a PIC value ranging from 0.25 to 0.5. Additionally, the C408G site of the *GH* gene in Charolais sheep displays a moderate degree of polymorphism, with a PIC value ranging from 0.25 to 0.5. These findings indicate a relatively high level of genetic variation, suggesting the potential for significant genetic progress. The T5299C site of the *IGF-1* gene exhibits a relatively high He value in Charolais ewe lambs and Australian White rams, while the C408G and T364C sites of the *GH* gene display a relatively high He value in Charolais sheep and Australian White rams. These results suggest that these sites have undergone a relatively high degree of relative mutation in the corresponding populations, thus indicating a greater abundance of genetic resources and diversity.

The research conducted by **Muniasamy *et al.* (2023)** demonstrated that a mutation occurs at the A781G locus of the *GH* gene in Kilakarsal sheep, resulting in the AA genotype and AG genotype. The AG genotype was found to have significantly higher birth weight than the AA genotype (P=0.038). There was also a significant difference in body weight at 9 months of age, with a difference of 1.40 kg,Similar findings have been reported in other studies.Through the analysis of the

relationship between genotypes and growth and developmental traits, it can be determined. At the T5299C site of the *IGF-1* gene, the advantageous genotypes for body height and body length of Charolais ewes, rams, and ram lambs were CC, while the advantageous genotype for weight, chest circumference, and body length of Charolais ewe lambs was TT and the advantageous genotype for body height and body length of Australian White rams was CT. At the C408G site of the *GH* gene, the advantageous genotypes for chest circumference and body length of Charolais ewes and ram lambs were CG, while the advantageous genotype for body height of Charolais and Australian White rams were CC and the advantageous genotype for body height, sacral height of Charolais ewe lambs was GG. At the T364C site, the advantageous genotypes for body length of Charolais ewes, ram lambs, and Australian White rams were CT, while the advantageous genotype for weight, chest circumference, and tube circumference of Charolais ewe lambs was CC, and the advantageous genotype for weight and chest circumference of Charolais rams was TT. Compared to the genotype results, haplotype combinations are more convincing. Research has shown that SNP analysis of the *IGF1R* gene in Hulun Buir sheep revealed that the favorable haplotype combinations for growth and development are TGTG and TGCA **(Ding *et al.*, 2022)**. In this study, we found that in the haplotype combinations of different sites of the *GH* gene, the advantageous haplotype combination for weight of Charolais ewes and ewe lambs was CCGG, while the advantageous haplotype combination for body height of Charolais and Australian White rams was CCCT, and the advantageous haplotype combination for weight and body height of Charolais ram lambs was CCGT. In the combination of T5299C and C408G, the advantageous haplotype combination for body height, body length, and chest circumference of Charolais rams, ram lambs, and ewe lambs as well as Australian White rams was CCCG, while the advantageous haplotype combination for weight and body height of Charolais ewes was CCGG, and the advantageous haplotype combination for weight, body height, and chest circumference of Charolais rams was CCCC. In the combination of T5299C and T364C, the advantageous haplotype combination for body height and sacral height of Charolais rams, ram lambs, and ewe lambs as well as Australian White rams was CCCT, while the advantageous haplotype combination for body height and sacral height of Charolais ewes was CCCC, and the advantageous haplotype combination for weight and chest circumference of Charolais rams was CCTT. This experiment shows a negative impact of mutations in the *IGF-1* and *GH* genes on the growth and development of sheep, which contradicts the previous description. This discrepancy may be due to the fact that the sheep have already been subjected to artificial selection on the breeding farm.

CONCLUSION

This study detected one SNP in the *IGF-1* gene and two SNPs in the *GH* gene in Charolais ewe lambs, Charolais ewes, Charolais rams, Charolais ram lambs, and Australian White rams.The results indicate that at the T5299C site of the *IGF-1* gene, the advantageous genotypes for body height and body length of Charolais ewes, rams, and ram lambs were CC, while the advantageous genotype for weight, chest circumference, and body length of Charolais ewe lambs was TT and the advantageous genotype for body height and body length of Australian White rams was CT. At the C408G site of the *GH* gene, the advantageous genotypes for chest circumference and body length of Charolais ewes and ram lambs were CG, while the advantageous genotype for body height of Charolais and Australian White rams was CC and the advantageous genotype for body height, sacral height of Charolais ewe lambs was GG. At the T364C site, the advantageous genotypes for body length of Charolais ewes, ram lambs, and Australian White rams were CT, while the advantageous genotype for weight, chest circumference, and tube circumference of Charolais ewe lambs was CC, and the advantageous genotype for weight and chest circumference of Charolais rams was TT. In the haplotype combinations of different sites of the *GH* gene, the advantageous haplotype combination for weight of Charolais ewes and ewe lambs was CCGG, while the advantageous haplotype combination for body height of Charolais and Australian White rams was CCCT, and the advantageous haplotype combination for weight and body height of Charolais ram lambs was CCGT. In the combination of T5299C and C408G, the advantageous haplotype combination for body height, body length, and chest circumference of Charolais rams, ram lambs, and ewe lambs as well as Australian White rams was CCCG, while the advantageous haplotype combination for weight and body height of Charolais ewes was CCGG, and the advantageous haplotype combination for weight, body height, and chest circumference of Charolais rams was CCCC. In the combination of T5299C and T364C, the advantageous haplotype combination for body height and sacral height of Charolais rams, ram lambs, and ewe lambs as well as Australian White rams was CCCT, while the advantageous haplotype combination for body height and sacral height of Charolais ewes was CCCC, and the advantageous haplotype combination for weight and chest circumference of Charolais rams was CCTT. However, it was observed through experiments that the gene substitution effect was positive, indicating a decrease in growth and development efficiency, which is contrary to expectations. The reason for this may be that the breeding farm has already undergone selection, retaining sheep with advantageous genes and eliminating those with disadvantageous genes. It is hoped that this study can provide reference for future research.

Ethical statement: To ensure the ethical treatment of animals, all procedures and handling involving sheep are carried out in accordance with the guidelines established by the Experimental Animal Management Committee of Shenyang Agricultural University.

Data availability: The data sets used in this article can be requested from the corresponding author.

Author contributions: Shuaitong Li carried out data analysis and writing of the original draft. Lingchao Kong was in charge of experiments. Jiaqi Li, Yuan Pan, Siyi Li, Yining Liu, Sibing Hou, Qingkun Liu, Yanjun Qiao, Yinggang Sun were responsible for the sample collecting. Zeying Wang carried out the writing in the form of review and editing.

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REFERENCES

An, J., Zhang, X., Liu, C., & Liu, M. (2014). Construction of a lentiviral expression vector for sheep IGF-1 gene and its study on promoting myocyte growth. Journal of Northwest A&F University, 42(4), 15-21. https://doi.org/10.13207/j.cnki.jnwafu.2014.04.011

Bergan-Roller, H. E., Ickstadt, A. T., Kittilson, J. D., & Sheridan, M. A. (2017). Insulin and insulin-like growth factor-1 modulate the lipolytic action of growth hormone by altering signal pathway linkages. Gen Comp Endocrinol, 248, 40-48. https://doi.org/10.1016/j.ygcen.2017.04.005

Cao, H. (2013). Analysis of polymorphisms and genetic effects of ADPN and IGF1 genes in Xiaoweihan sheep. Shandong Agricultural University. http://106.52.218.247:8085/kcms2/article/abstract?v=3IEynGI71r8xPHFuBeVui-tv577dEHO5WnRwPvLeYxJWe_jf5tpdMYNGOpvvHeFpJV25DylIXEP3NX_kfH1awul-QNymW-MLO-TfQVMEu8nqJoThOhGfgFsaZ3my1LPAJcgPaRkEy_jvrjswrKhg7Q==uniplatform=NZKPTlanguage=CHS

Ding, N., Liu, S., Tian, F., Tian, D., Li, X., & Zhao, K. (2021). Polymorphism analysis of GH and IGF-1 genes in indigenous sheep breeds. Genomics and Applied Biology, 40(2), 504-515. https://doi.org/10.13417/j.gab.040.000504

Dhandare, B. C., Rather, M. A., Bhosale, B. P., Pawar, R., Guttula, P. K., & Pagarkar, A. U. (2020). Molecular modeling,docking and dynamic simulations of growth hormone receptor(GHR) of Labeo rohita. Journal of biomolecular structure & dynamics, 40(7), 3024-3037. https://doi.org/10.1080/07391102.2020.1844063

Ding, N., Tian, D., Li, X., Zhang, Z., Tian, F., Liu, S., Han, B., Liu, D., & Zhao, K. (2022). Genetic Polymorphisms of IGF-1 and IGF1R genes and their effects on growth traits in Hulun Buir sheep. Gene, 13(4),666. https://doi.org/10.3390/genes13040666

Ding, X., Wang, H., Zhang, L., Yu, J., Wu, S., Liu, Y., & Cao, X. (2020). Research progress on GH gene. Modern Animal Husbandry and Veterinary Medicine, 384(11), 56-59. https://kns.cnki.net/kcms2/article/abstract?v=rbgYNE3Bhz8SMhZ5j_2_ha03zjl6CAVg1DJg4MXRPk_ZixAVshx19l3h3ALvkOahcBbsF6p8Wdmap1to4BeJGsu2sImQnIrN59-9Vj1l-ptjy7WaJ6TESxLwllRZFZnD3qyUSE5hIVisePLM4zFf6Z_TkDmRKy62ISjc3rd3Ai1pzpZYpDbuupwj3U50-66ucxVgRyveKUo=&uniplatform=NZKPT&language=CHS

Han, Y. (2016). Association analysis of MyoG, GH, and CKM gene polymorphisms with growth and development traits in Tibetan sheep. Qinghai Normal University. http://106.52.218.247:8085/kcms2/article/abstract?v=3IEynGI71r_7a7nLdezb5YwWwiEbR6hEzfMHAK_ZKHAlXeD6V1Fr1iKnyFTEcnIkyHWeK1GlCgptP8bVPNT_vLacxcAJbc9LWx-5KzvzdaS4k-BM_nouuLHhLQPn70PpyCaUAvLEHLEb-20hRYNZCw==uniplatform=NZKPTlanguage=CHS

Hu, S., Zhang, S., Yuan, Z., Xuan, J., Ma, X., Zhang, L., Zhao, F., Wei, C., & Du, L. (2016). Polymorphisms of sheep CLPG and MSTN genes and their association analysis with growth traits. Chinese Journal of Animal Husbandry and Veterinary Medicine, 43(5), 1285-1293. https://doi.rog/10.16431/j.cnki.1671-7236.2016.05.025

Ip, S. C. Y., Zhang, X., & Leung, F. (2001). Genomic growth hormone gene polymorphisms in native Chinese chickens. Experimental Biology and Medicine, 226(5), 458-462. http://doi.org/10.1177/153537020122600511

Ishida, T., Umebayashi, A., Tsuruta, S., Akashi, R., & Harada, H. (2010). Polymorphisms in growth hormone gene and their associations with calf weight in Japanese Black cattle. Anim Sci Journal, 81(6), 623-629. https://doi.org/10.1111/j.1740-0929.2010.00783.x

Kumar, S., Dahiya, S. P., Magotra, A., Ratwan, P., & Banger, Y. (2023). Influence of single nucleotide polymorphism in the IGF-1 gene on performance and conformation traits in Munjal sheep. Zygote, 31(1), 70-77. https://doi.org/10.1017/S0967199422000545

Li, W. (2012). Polymorphism of the sheep GH gene and its correlation with body weight, body measurements, and meat quality. Gansu Agricultural University. http://106.52.218.247:8085/kcms2/article/abstract?v=3IEynGI71r9edVXNVcPNx5DljiL88m1040bWV4uAliIxV6CgyKOBwnFBBU8o58vfZ_9Sk306Qkgdc0OOAoTKZkRV88HGS7QtC8xiPXveqNRq9CZs6s89AULsdT3E9QnzAVtgEOI_Z1jHnxh7_w8fnA==uniplatform=NZKPTlanguage=CHS

Liu, Y., Lan, X., Qu, Y., Li, Z., Chen, Z., Lei, C., Fang, X., & Chen, H. (2011). Effects of genetic variability of the dairy goat growth hormone releasing hormone receptor(GHRHR) gene on growth traits. Molecular Biology Reports, 38(1), 539-544. https://doi.org/10.1007/s11033-010-0138-7

Muniasamy, S., Veerasamy, J., Ramanujam, R., & Tirumurugaan, K. G. (2023). Polymorphism of the growth hormone (GH) gene and its association with growth traits in the Kilakarsal breed of sheep. Vet. Arhiv, 93(4),427-434. https://doi.org/10.24099/vet.arhiv.1452

Niu, Z., Chen, T., Yu, J., Hao, G., Ji, Y., & Shi, H. (2014). Association analysis of Myf5 gene polymorphism with early growth and development traits in Altay sheep. Chinese Journal of Herbivorous Science, 34(5), 9-12. https://doi.org/10.3969/j.issn.2095-3887.2014.05.002

Rinderknecht, E., & Humbel, R. E. (1978). The amino acid sequence of human insulin-like growth factor 1 and its structural homology with proinsulin. The journal of biological chemistry, 253(8), 2769-2776. https://doi.org/10.1016/S0021-9258（17）：40889-1

Rydzanicz, M., Nowak, D. M., Karolak, J. A., Frajdenberg, A., Podfigurna-Musielak, M., Mrugacz, M., & Gajecka, M. (2011). IGF-1 gene polymorphisms in Polish families with high-grade myopia. Molecular Vision, 17,2428-2439. https://www.molvis.org/molvis/v17/a264

Schiaffino, S., Dyar, K. A., Ciciliot, S., Blaauw, B., & Sandri, M. (2013). Mechanisms regulating skeletal muscle growth and atrophy. The FEBS Journal, 280(17), 4294-4314. https://doi.org/10.1111/febs.12253

Wong, E. A., Ohlsen, S. M., Godfredson, J. A., Dean, D. M., & Wheaton, J. E. (1989). Cloning of ovine insulin-like growth factor-1 cDNAs: heterogeneity in the mRNA population. DNA, 8(9), 649-657. http://doi.org/10.1089/dna.1.1989.8.649

Wu, Y., Pan, A., Pi, J., Pu, Y., Du, J., Liang, Z., & Shen, Z. (2012). One novel SNP of growth hormone gene and its associations with growth and carcass traits in ducks. Molecular Biology Reports, 39(8), 8027-8033. https://doi.org/10.1007/s11033-012-1649-1

Yang, W. (2021). The potential of sheep farming industry development in China. Chinese Animal Health, 23(01), 65+69. https://doi.org/10.3969/j.issn.1008-4754.2021.01.047

Yan, Y., Yang, H., Yang, Y., Pan, X., & Ma, Q. (2014). Analysis of tissue expression levels of GH and IGF-1 genes in Suffolk sheep. Journal of Domestic Animal Ecology, 35(4), 18-22,28. https://dx.doi.org/10.3969/j.issn.0258-7033.2014.03.008

Zhu, H., & Zhou, X. (2020). Statistical methods for SNP heritability estimation and partition: a review. Computational and Structural Biotechnology Journal, 18,1557-1568. https://doi.org/10.1016/j.csbj.2020.06.011

Zhou, M., Zhang, X., Qi, S., Wang, Q., Wu, D., & Yang, P. (2013). Expression of sheep IGF-I gene and correlation analysis of its SNPs with growth traits. Chinese Journal of Animal Science and Veterinary Medicine, 44(9), 1363-1370. https://doi.org/10.11843/j.issn.0366-6964.2013.09.005