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Correlation of *PGAM2* Gene Polymorphism with Production Performance in F3 Generation of Boer × White Goat Hybrid

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ABSTRACT

Guizhou white goat is an excellent local goat breed in China, however, it has the disadvantages of smaller individuals, slower growth rate and low feed conversion rate, so, we introduced Boer goat as the male parent and crossed with Guizhou white goat to obtain F3 generation of Boer × White goat hybrid to improve its production performance. To study the effect of *PGAM2* gene polymorphisms on the performance of F3 generation hybrid goats, the relationship between *PGAM2* gene polymorphisms and slaughter traits, meat quality traits and organ coefficients was determined in 39 F3 generation hybrid goats. The results showed that the slaughtering performance of male goats was better than that of female goats, and there were highly significant ($P<0.01$) and significant ($P<0.05$) differences in live weight and carcass weight, respectively. In meat quality, the marbling score of female goats was significantly higher than that of male goats ($P<0.01$), and the shear force of female goats was significantly lower than that of male goats ($P<0.01$). Regarding organ coefficients, the large intestine and stomach of male goats was significantly higher than that of female goats ($P<0.01$), whereas the liver of female goats was significantly higher than that of male goats ($P<0.01$). Polymorphism analysis of the *PGAM2* gene showed that there were polymorphic loci in the 3'UTR (2435A→G), and there were significant ($P<0.01$) or highly significant ($P<0.05$) differences in some traits between the GG and AG or AA genotypes. Conclusion: The production performance of male goats is better than that of female goats in F3 generation hybrid goats, and the slaughtering performance of F3 generation hybrid goats was significantly improved compared with that before breeding. The correlation study showed that the slaughter performance of the GG population with the *PGAM2* gene was significantly better than that of GA and AA, which could be used as a potential molecular breeding marker for research.

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LD initiated the study and wrote the manuscript. JH, JX, MX and JC performed the experiments and analyzed the groups of data. YR and XC critically reviewed and revised the manuscript. All authors approved the final version.

Key words
Guizhou white goat, Slaughter traits, Meat traits, Organ coefficient, *PGAM2* gene

INTRODUCTION

Guizhou white goats are one of the most abundant local goat breeds in Guizhou Province. They are mainly distributed in Zunyi, Tongren, Southeast Guizhou and other places in Guizhou province, current population is more than 1 million (Yang, 2015). Guizhou white goats have the characteristics of extreme resistance to rough feeding, strong stress resistance, outstanding reproductive performance, stable genetic performance and delicious meat (Xu, 2020); they are deeply loved by consumers and have a good sales mark*et al*l over the country, but there are also disadvantages, such as small individuals, slow growth rates and low feed conversion rates (An *et al*., 2020). To improve the production performance of Guizhou white goats, the F1 generation was obtained by crossing Guizhou white goats as the female parent and Boer goats as the male parent. On this basis, Guizhou white goats, as a male parent, were continuously backcrossed to the F3 generation. Therefore, the blood relationship of the F3 hybrid goat was stable at 87.5% or more, and based on this, we further carried out F3 generation cross-crossing fixed breeding and rapid breeding to ultimately obtain a

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new Guizhou white goat population that is not only able to basically maintain the original meat flavour of Guizhou white goats but also to increase the body shape by at least 10% (Yang and Ch, 2013).

To further explore the germplasm characteristics and growth and development rules of F3 hybrid goats, 39 F3 hybrid goats were tested for slaughter traits, meat quality traits and organ coefficients, and a molecular breeding method was applied. The polymorphism of the phosphoglycerate mutase (*PGAM*) gene and its correlation with production traits in this group were assessed. The *PGAM* gene is a glycolysis enzyme. It catalyses the conversion of 3-phosphoglycerate to 2-phosphoglycerate (Shanske *et al*., 1987; Sakoda *et al*., 1988). In mammalian tissues, *PGAM* is a dimer of two independent 30 kDa subunits, including the brain form (B form, also known as *PGAM1*), which is commonly expressed, and the muscle form (M form, also known as *PGAM2*), which is expressed only in skeletal and cardiac muscles of adults (Ruiz-Lozano *et al*., 1999). The two forms are composed of three types of *PGAM* dimers (MM, BB and MB) (Zhang *et al*., 2001). In the physiological activities of animal organisms, the phosphoglycerate mutase *PGAM2* gene participates in the regulation of glycolysis and gluconeogenesis and can be specifically and highly expressed in muscle tissues (Kawashima *et al*., 1996; Wagner *et al*., 1990). The isozyme pattern of human *PGAM2* is developmentally regulated during myogenesis. Studies have shown that certain mutations lead to *PGAM2* deficiency in humans, resulting in severe muscle dysfunction, including exercise intolerance, cramps, myogloboproteinuria, muscle atrophy and hypertrophic fibres (Sidhu *et al*., 2018; Dimauro *et al*., 1981). Yang *et al*. (2016) showed that the c.360T > C mutation was found in the *PGAM2* gene, which is a favourable mutation site for improving pork water holding capacity (WHC) and an important reference parameter for genetic marker-assisted breeding. By reviewing relevant literature, it is found that *PGAM2* gene has been mainly studied in humans and other mammals, which is equivalent to that in goats. However, few studies have been performed on goat. Molecular breeding based on *PGAM2* gene polymorphisms may be of great significance in the development of a new strain and relevant results may help to accelerate the development of this new strain. It is helpful to improve the disadvantage of local variety in terms of production performance.

MATERIALS AND METHODS

Experimental animals

The F3 hybrid goats for this experiment were provided by Wuchuan Hongmu Goat Industry Co., Ltd. This group was based on the Boer crossbred goat F1 generation (Boer goat ♂ × Guizhou white goat ♀) as the female parent, and then Guizhou white goats were used as the male parent to continuously backcross to the F3 generation. After weaning, goats with good physiological condition and similar physical size were raised at the same feeding level until 12 months of age, including 21 female goats and 18 male goats. The animals used in this study strictly comply with the guidelines of the Animal Welfare Committee of Guizhou University (EAE-GZU-2022-P002, 5th May 2022). Feed composition and nutrient level are shown in Table I.

Table I. Feed composition and nutrient level.

Composition	Content (%)
Silage corn	40
Corn	33.72
Soybean meal	13.32
Rice bran	8.82
CaHPO3	0.96
Limestone	0.6
Salt	0.18
Pre-mixed feed (1)	2.4
Nutritional ingredient (2)	
Digestible energy (MJ/kg)	10.82
Crude protein	11.82
Neutral detergent fiber (NDF)	30.89
Acid detergent fiber (ADF)	13.46

Note: (1) Digestible energy is the calculated value, and the rest is the measured value; (2) Each kilogram of premix contains 38000 IU of vitamin A, 34000 IU of vitamin D, 800IU of vitamin E, 350 mg of iron, 1000 mg of zinc, 700 mg of manganese and 180 mg of copper.

Determination of slaughter traits

The animals were fasted for 24 h before slaughter, water was forbidden for 2 h before slaughter and bleeding of the neck vein to death was carried out and then the skin was peeled (Danforth, 2014), the internal organs (including the kidneys and renal fat) were removed, after removing the head, hoofs and tail, and the left half carcass was used to measure and calculate the relevant indicators. The test indicators included live weight, carcass weight, slaughter rate, net meat weight, bone weight, eye muscle area, back fat thickness, net meat rate, bone rate and meat-to-bone ratio (Yang, 2015). The determination method was carried out with reference to NY/T1236-2006 (Specification for Determination of Production Performance of Cotton and Goat). The animal study procedures were approved by the Laboratory Animal Ethics Committee of Guizhou University.

Table II. *PGAM2* gene primer information.

Primer	Primer sequences (5`→3`)	Product size/bp	Annealing temperature/°C
PGAM2-exon 1	F: CAGTGGGGAATGGTGAGGCTATT R: CCATGTTGTTCACTCCCCAAGTG	729	60
PGAM2-exon 2	F: CACTTGGGGAGTGAACAACATGGA R: CCCAGAATCTTCAGAAGGAGATTCA	486	59
PGAM2-exon 3	F: GGTCACCAAACCTGCTAAATGCAC R: GACCTCGGGCACTCTTAATTTAGAG	569	60

Determination of meat quality

The measurement indicators included pH value, meat colour, marbling, drip loss rate, cooked meat rate and shear force (texture analyser measurement) (Meng *et al*., 2011). The determination method was carried out with reference to NY/T 1236-2006 (Specification for Determination of Production Performance of Goat).

Determination of degree of organ development

The organs were weighed, including the liver, heart, lung, spleen, kidney, small intestine, large intestine, stomach, head and hoof, and coefficients calculated according to the formula organ coefficient = organ weight/ live weight before slaughter (Wang *et al*., 2021).

Primer design

According to the white goat *PGAM2* gene published in GenBank (accession number: NC_030829.1), Primer Premier 5.0 software was used to design upstream and downstream primers for exon 1 and exon 2 of the *PGAM2* gene. The sequences are shown in Table II and the primers were synthesized by Beijing Qingke Biotechnology Co., Ltd.

PCR amplification and sequencing analysis

PCR system 30 μL: 15 μL of 2×Taq PCR StarMix, 1 μL of upstream and downstream primers, 1 μL of DNA template, 12 μL of ddH2O. The PCR program was as follows: pre-denaturation at 94 °C for 3 min; denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 2 min, for a total of 35 cycles; extension at 72 °C for 7 min; and storage at 4 °C. PCR products were detected by 1.0% agarose gel electrophoresis. The PCR products were sent to Beijing Qingke Biotechnology Co., Ltd. for sequencing and DNAStar software was used to compare the sequencing results with the NCBI original sequences to screen possible SNP sites.

Data analysis and statistics

The experimental data were analysed and processed by SPSS 16.0 software, and the correlation analysis of the corresponding indicators was carried out. The results are expressed as the mean ± standard deviation.

RESULTS

Slaughter traits

It is obtained from Table III, the slaughter performance of one-year-old male goats was better than that of one-year-old female goats, and there were significant differences in live weight ($P<0.01$), loin muscle area ($P<0.01$) and carcass weight ($P<0.05$) between males and female goats.

Table III. Slaughter characteristics of F3 generation hybrid goats.

Indexes	One year old female goat	One year old male goat
Live weight (kg)	27.79±0.52	30.09±1.11**
Carcass weight (kg)	13.36±0.32	14.87±0.57*
Dressing percentage (%)	48.07±0.30	49.42±0.72
Net meat weight (kg)	9.70±0.32	11.46±0.44
Net meat percentage (%)	34.91±0.57	38.09±0.49
Bone weight (kg)	2.47±0.04	2.79±0.07
Bone rate (%)	18.50±0.51	18.81±0.64
Meat-bone ratio	3.93±0.13	4.10±0.13
Loin muscle area (cm^2)	8.10±0.73	11.06±1.37**
Back fat thickness (mm)	3.71±0.19	4.77±0.25

Note: ** indicates that the difference between the one-year-old sheep reaches a very significant level($P<0.01$), and * indicates that the difference between the one-year-old sheep reaches a significant level($P<0.05$).

Meat quality

It can be seen from Table IV that the overall difference in meat quality traits of one year old female goats is not significant, but the marbling score of one year old female goats is extremely significantly higher than that of one year old male goats ($P<0.01$), and the shearing force of one-year-old female goats is extremely significantly lower than that of one-year-old male goats ($P<0.01$).

Organ coefficient

As shown in Table V, it can be seen that the coefficient of liver organs of F3 generation one-year-old female goats

is extremely significantly higher than that of one-year-old male goats ($P<0.01$), while the coefficient of large intestine and stomach organs of one-year-old male goats is extremely significantly higher than that of one-year-old female goats ($P<0.01$); the other organ coefficients showed no significant differences.

Table IV. Meat quality traits of F3 generation hybrid goats.

Indexes	One year old female goat	One year old male goat
Meat color (min)	3.23±0.13	3.35±0.15
Marbling (min)	1.50±0.04**	1.35±0.09
pH (45 min)	6.33±0.06	6.55±0.13
pH (24 h)	5.79±0.09	5.92±0.08
Drip loss rate (%)	11.74±0.86	12.38±0.75
Cooked meat percentage (%)	58.91±1.11	62.51±1.65
Shear force (kg)	3.69±0.20	5.02±0.47**

For explanation of see Table III.

Polymorphism of PGAM2 *gene in F3 generation hybrid goats*

The *PGAM2* gene sequences of F3 generation hybrid goats were compared and analyzed by SeqMan analysis software, and it was found that an A-to-G mutation occurred at site 2435 in the 3'UTR region of the *PGAM2* gene (Fig. 1). The analysis showed that among the 39 hybrid goats, there were 10 GG genotypes, 20 GA genotypes, and 9 AA genotypes.

The heterozygosity and polymorphism calculation of this gene polymorphism locus has revealed that the frequency of the AG genotype at this locus was 51.28% that of the GG genotype was 25.64% and that of the AA genotype was 23.08% (Table VI). The gene frequency of G was 51.28% and that of A was 48.72%. The gene heterozygosity was high and showed moderate polymorphism (0.25~0.5), which conformed to Hardy-Weinberg equilibrium law.

Correlation of PGAM2 *gene polymorphisms with production traits*

Table VII shows that the preslaughter liveweight and meat to bone ratio of the GG genotype are significantly higher than those of the other genotypes ($P<0.01$), and the carcass weight is significantly higher than that of the other genotypes ($P<0.05$). The bone rate of the AG genotype and AA genotype is significantly higher than that of the GG genotype ($P<0.01$).

Table V. Organ coefficient of F3 generation hybrid goats.

Visceral organs	Indexes	One year old female goat	One year old male goat
Heart	weight (kg)	0.09±0.01	0.11±0.02
	coefficient (%)	0.32±0.02	0.35±0.04
Liver	weight (kg)	0.42±0.02	0.44±0.02
	coefficient (%)	1.51±0.06**	1.47±0.03
Spleen	weight (kg)	0.04±0.00	0.04±0.00
	coefficient (%)	0.14±0.01	0.12±0.01
Lung	weight (kg)	0.21±0.01	0.23±0.01
	coefficient (%)	0.77±0.02	0.77±0.02
Kidney	weight (kg)	0.09±0.01	0.11±0.01*
	coefficient (%)	0.33±0.01	0.37±0.01
Small intestine	weight (kg)	0.32±0.01	0.39±0.00
	coefficient (%)	1.17±0.04	1.31±0.04
Large intestine	weight (kg)	0.36±0.00	0.43±0.00
	coefficient (%)	1.28±0.01	1.41±0.04**
Stomach	weight (kg)	0.93±0.01	0.98±0.00
	coefficient (%)	3.36±0.05	3.62±0.11**
Head	weight (kg)	1.18±0.04	1.35±0.02*
	coefficient (%)	4.24±0.08	4.48±0.10
Hoof	weight (kg)	0.42±0.01	0.45±0.01
	coefficient (%)	1.51±0.01	1.51±0.04

For explanation of see Table I.

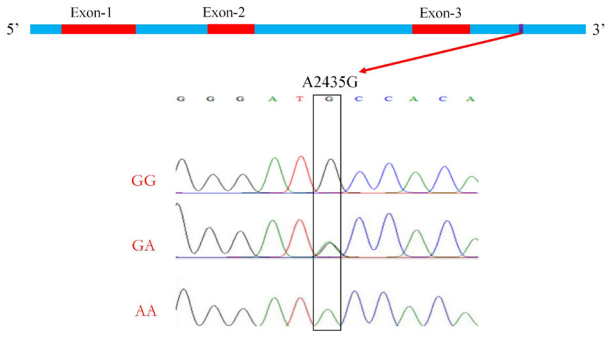

Fig. 1. PGAM2 genotype map of F3 generation hybrid goat.

Table V. Distribution of indicators at site 2435 of exon1 of *PGAM2* gene.

SNP site	Samples	Genotype frequency			Gene frequency		Heterozygosity	Polymorphism
		GG(10)	GA(20)	AA(9)	G	A		
g.2435 A→G	39	0.2564	0.5128	0.2308	0.5128	0.4872	0.4997	0.3748

Table VII. Comparison of slaughter characteristics, meat quality and organ coefficients in different *PGAM2* genotypes in F3 hybrid goats.

Indexes	GG	GA	AA
Slaughter characteristics			
Live weight (kg)	29.92±1.68**	28.67±1.22	28.07±0.91
Carcass weight (kg)	14.59±0.97*	13.95±0.83	13.70±0.70
Dressing percentage (%)	48.75±0.77	48.61±0.90	48.79±0.96
Net meat weight (kg)	10.99±1.04	10.39±0.92	10.24±0.89
Net meat percentage (%)	36.68±1.51	36.20±1.70	36.43±2.00
Bone weight (kg)	2.63±0.19	2.62±0.17	2.61±0.19
Bone rate (%)	18.05±0.57	18.78±0.35**	19.01±0.56**
Meat-bone ratio	4.17±0.15**	3.97±0.12	3.93±0.09
Eye muscle area (cm^2)	9.32±2.08	9.15±1.41	10.33±2.29
Back fat thickness (mm)	4.19±0.62	4.12±0.53	4.38±0.68
Meat quality			
Meat color (min)	3.16±0.15	3.33±0.13**	3.31±0.15*
Marbling (min)	1.41±0.11	1.44±0.10	1.42±0.11
pH (45 min)	6.42±0.115	6.43±0.17	6.45±0.14
pH (24 h)	5.86±0.09	5.85±0.12	5.85±0.07
Drip loss rate (%)	11.70±0.87	12.12±0.98	12.22±0.47
Cooked meat percentage (%)	61.34±3.32	60.15±1.82	60.65±1.79
Shear force (kg)	4.55±0.67	4.33±0.58	4.61±0.71
Organ coefficients			
Herat coefficient (%)	0.36±0.03**	0.33±0.03	0.31±0.02
Liver coefficient (%)	1.52±0.06	1.49±0.26	1.46±0.03
Spleen coefficient (%)	0.13±0.01	0.13±0.01	0.13±0.01
Lung coefficient (%)	0.78±0.02	0.76±0.02	0.76±0.02
Kidney coefficient (%)	0.36±0.02	0.34±0.03	0.34±0.02
Small intestine coefficient (%)	1.19±0.09	1.23±0.07	1.28±0.09**
Large intestine coefficient (%)	1.32±0.08	1.33±0.07	1.39±0.09**
Stomach coefficient (%)	3.21±0.10	3.32±0.06**	3.41±0.03**
Head coefficient (%)	4.33±0.05	4.32±0.15	4.45±0.20**
Hoof coefficient (%)	1.48±0.03	1.51±0.01**	1.54±0.02**

Note: Compared with the GG genotype, ** indicates that the difference between F3 hybrid goats of different genotypes reaches a very significant level($P<0.01$), and * indicates that the difference between F3 hybrid goats of different genotypes reaches a significant level($P<0.05$).

Besides that the AG and AA genotypes were extremely significantly ($P<0.01$) or significantly ($P<0.05$) higher than the GG genotype in terms of the meat colour score. There were no significant differences among the other indicators. Moreover, the organ coefficient of the heart of the GG genotype was extremely significantly higher than that of the AG and AA genotypes ($P<0.01$). The organ coefficients of the head, small intestine and large intestine of the AA genotype were extremely significantly higher than those of the GG and AG genotypes ($P<0.01$). The coefficient of stomach and hoof organs of the GG genotype was significantly lower than that of the AG and AA genotypes ($P<0.01$).

DISCUSSION

Slaughter traits of F3 hybrid goats

Livestock slaughter traits and meat quality traits are important bases for reflecting the resource advantages of breeds and are also important reference indicators for breed selection and identification. Indicators, such as slaughter rate and net meat weight in slaughter traits, can reflect the developmental status of livestock (Karamichou,2011). The performance of F3 generation Guizhou White goats is better than that of female goats. This test used the Boer goat as the male parent and the Guizhou white goat as the female parent in the hybrid and then used the parents with the Guizhou white goat for backcrossing and finally fixed crossing breeding. From slaughtering the determination results, the propagation as income F3 generation age of the Guizhou white goat a production performance was lower than that of the male parent Boer goat production performance, such as live before slaughter slaughtering performance (Sapkota *et al*., 2016; AMD *et al*., 2000). However, it was better than the live weight before slaughter of Guizhou white goat (Xu, 2020; Ran *et al*., 2016). The slaughter performance of F3 generation crossbred goat was improved compared with that before improvement.

Meat quality of F3 hybrid goats

To detect the meat traits of this group, we tested the relevant indicators of meat traits, including pH, meat colour, marbling, drip loss rate, cooked meat rate and shear force. It was found that the intermuscular fat content of the female goats was higher than that of the male goats, and the meat quality of the female goats was more tender. Compared with the reported Guizhou white goat traits, it was found that the newly bred Guizhou white goat new strains were superior to the pre-bred Guizhou white goats in terms of meat colour, marbling score and cooked meat rate, while the shear force was slightly lower than that of the Guizhou white goat before breeding, (Qian *et al*., 2015). Compared with the reported Boer goat quality traits, the newly bred Guizhou white goat new strains were slightly better than the Boer goats in terms of meat colour; the remaining indicators were not significantly different. Compared with other goat breeds, there was little difference in meat quality traits reported by (Ahmed *et al*., 2015). Compared with Haimen white goats, they have similar scores in all aspects (Chen *et al*., 2021). Therefore,

although the new cultivated strains decreased meat quality compared with the quality of Guizhou white goats, the meat quality performance of the F3 generation of Guizhou white goats remained basically unchanged.

Organ development of F3 hybrid goats

The weight of animal organs and the organ coefficient are important bases for identifying the genetic quality of animals. The organ coefficient can reflect the functional state of the animal body to a certain extent, which is of great significance for theoretical research and guiding production practice. It can also be used to measure and reflect the functional state of animals in life science research. In addition to being directly affected by changes in body weight, the organ coefficient is also easily affected by factors such as animal strain, sex, age, and rearing environmental conditions (Kessel and Shih, 1976). In our newly bred new line of Guizhou white goat, it was found that the organ coefficient of the newly bred Guizhou white goat was overall better than that of the reported Guizhou white goat (Qian *et al*., 2015), while it was slightly lower than that of the male Boer goat in the same period (Luo *et al*., 2000). Therefore, the functional state of various organs of the newly cultivated white goat is better than that of the Guizhou white goat and may be better in terms of environmental adaptation, immune regulation and feed conversion rate.

Correlation of PGAM2 *gene polymorphisms with slaughter traits*

Through polymorphism analysis, we found that there is an SNP site in the 3`UTR of the *PGAM2* gene. The 3`UTR in eukaryotes is an important functional regulatory element of mRNA and plays an important role in gene expression regulation and mRNA localization (Zhu *et al*., 2002; Kuersten and Goodwin, 2003; Chabanon *et al*., 2004; Rastinejad and Blau, 1993). The 3`UTR not only controls mRNA stability and degradation rate *in vivo*, regulates the subcellular localization and translation level of transcripts and determines the fate of a particular mRNA but it can also determine the type of cells it expresses, control the utilization efficiency of mRNA and help identify special codons. Mutation of the 3`UTR can also affect the expression of one or more genes, leading to the occurrence of diseases (Hidalgo *et al*., 2007). In addition, the 3`UTR is also an important site for interaction with other intracellular factors and plays an important role in cell phenotype, growth and differentiation (Chabanon *et al*., 2004). It was found by correlation analysis, in terms of production performance, the GG genotype had outstanding advantages in terms of live weight and carcass weight before slaughter, while the AG genotype and AA genotype had outstanding bone ratios, indicating that their net meat weight was light. Considering the GG genotype population with the one-year-old goat of other breeds, compared with the male parent, the live weight before slaughter of the male parent was still not reached. The GG genotype goat also improved in terms of the live weight before slaughter (Pérez-Baena *et al*., 2021; Tshabalala *et al*., 2003). Compared with Balkan goats, the GG genotype Guizhou white goat was slightly lower than the Balkan goat in all aspects (Memisi *et al*., 2009), but the GG genotype also significantly improved in terms of slaughter performance. This indicates that this locus could be used as a reference for improving slaughter traits.

Correlation with PGAM2 *gene polymorphisms with meat quality traits*

The correlation analysis of meat quality traits showed that among 39 F3 generation Guizhou one-year-old white goats. Compared with the Boer goat, the pH (45 min) and pH (24 h) were lower, and the meat quality of the F3 generation Guizhou white goat was slightly lower than that of the Boer goat (Tshabalala *et al*., 2003). Compared with Guizhou white goat, the F3 generation Guizhou white goat has three genotypes compared with other breeds. When compared with the pH of Saanen dairy goats as reported by Cheli *et al*. (2016) the two are almost the same. Therefore, it is necessary to strengthen the fattening of F3 generation Guizhou white goats in future breeding to promote the deposition of intramuscular fat.

Correlation of PGAM2 *gene polymorphisms with organ coefficients*

The heart, as the engine of the body, plays an important role in maintaining the body function of animals, and the liver is the largest solid organ of the body (Prothero,1979). The difference in development is not significant, indicating that the difference in the organ coefficient between the genotypes is not large. Compared with the Boer goat (Solaiman *et al*., 2011), the differences between goat of each genotype were small, and compared with Guizhou white goats, it was found that the GG genotype group had better organ development. Therefore, the differences in organ coefficients among different genotype populations are difficult to use as an important reference for selection indicators.

CONCLUSION

The production performance of F3 generation old Guizhou white goats is significantly improved compared with that before breeding, which provides a preliminary basis for the breeding and promotion and application of

new Guizhou white goats.

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IRB approval

The IRB approval was granted by Guizhou Provincial Department of Science and Technology.

Ethical statement

This study was approved by the Guizhou University Subcommittee of Experimental Animal Ethics (Approval no: EAE-GZU-2022-P002), and which complies with all relevant China legislations.

Statement of conflicts of interest

The authors have declared no conflict of interest.

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