SIMULTANEOUS GENOTYPING OF SIX SNPS AT THE GH2-Z GENE IN "SERRA DA ESTRELA" OVINE BREED.

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Introduction

Growth hormone (GH):

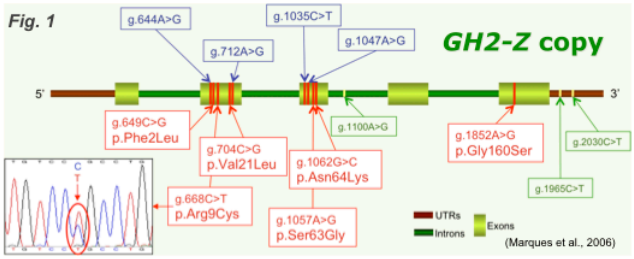
✓ Plays an essential role in milk production:

1. Involved in puberty and gestation - **MAMMOGENESIS**,
2. Modelling the mammary gland - **LACTOGENESIS**:
   a) higher milk synthesis rate, and
   b) increase of persistency of the lactation.



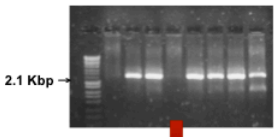
✓ ***GH2-Z*** *(Fig.1)* copy of the *GH* gene is **polymorphic**.

Fig. 1

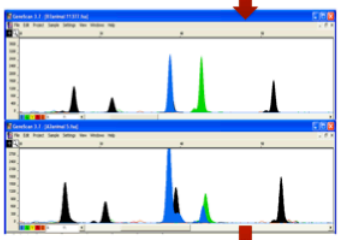


✓ ***GH2-Z*** genotypes are **significantly associated** with **milk production and quality in "Serra da Estrela" ewes**.

Methodology







➢ **GENOTYPING OPTIMIZATION:**

1. PCR amplification of a **2.1 Kbp** fragment of the ***GH2-Z* copy**.
2. SNP analysis by a single-base extension (SNaPshot) method with extension primers designed to identify the **six non-synonymous substitutions previously detected at the GH2-Z copy** (in red - Fig.1).
3. Fragment analysis performed by capillary electrophoresis with an ABI310 DNA genetic analyzer (AB) using GeneScan 120 LIZ as internal size standard.
4. Results analysed with Genescan® software and interpreted by Genotyper® to convert SNaPshot results *to GH2-Z* gene copy genotypes.

➢ ***GH2-Z* COPY SNPs ANALYSIS**

- Genomic DNA extraction from blood of **433 "Serra da Estrela" ewe lambs with less than one month of age**:
  + 15 flocks belonging to ANCOSE members.
  + INIAV flock.

Objective

To establish a genotyping procedure that allows detecting six *GH2-Z* copy SNPs simultaneously.

Results

Tab. 1 – Genotype and allele frequencies

	g.649C>G (p.F2L)	g.668C>T (p.R9C)	g.704C>G (p.L21V)	g.1057A>G (p.S63G)	g.1062G>C (p.K64N)	g.1852G>A (p.G160S)
Genotype freq. (%)	CC = 88.8	CC = 85.8	CC = 85.6	AA = 78.1	GG = 98.3	AA =100.0
	CG = 11.2	CT = 11.0	CG = 14.4	AG = 15.4	GC = 1.5	
		TT = 3.2		GG = 6.5	CC = 0.2	
Allele freq. (%)	C = 94.4	C = 91.3	C = 92.8	A = 85.8	G = 99.0	A = 100.0
	G = 5.6	T = 8.7	G = 7.2	G = 14.2	C = 1.0	

Tab. 2- GH2-Z phenopypes frequencies

	aa2	aa9	aa21	aa63	aa64	aa160	Freq. (%)
AA	FF	RR	LL	SS	KK	GG	50.81
AB		RC					8.55
AD		RC		SG			1.62
AE				SG			9.47
AI	FL		LV				8.78
AJ				SG	KN		0.92
AK			LV				2.54
BB		CC					1.85
BE		CC		SG			1.15
DK			LV	SG			0.46
EE				GG			4.62
EJ				GG	KN		0.23
EI	FL		LV	GG			0.92
FF	FL		LV	SG			0.46
IJ	FL		LV	SG	KN		0.23
JJ				GG	NN		0.23

✓ G2-Z phenotypes **AA, AE, AI and AB** were the most frequent.

✓ The obtained frequencies were **similar** to those seen in other flocks of "Serra da Estrela" dairy ewes using PCR-SSCP analysis.

Conclusions

The genotype procedure implemented:

✓ **is fast and reliable for the determination of the six *GH2-Z* copy SNPs.**

✓ **Genotypes can be determined at birth.**

✓ **It can be used for future characterization of ovine *GH2-Z* polymorphisms in other breeds,**

✓ **Is available to farmers who might consider using it as a tool to select their dairy ewe lambs at an early age.**

Future studies

80 ewes lambs of genotypes AA, AB and AE (28 lambs of each) are reared in two feeding regimes to study the effect of GH2-Z copy genotypes and growth rate during pre-puberty on mammary gland development, ewes metabolism, and milk yield and quality.



Acknowledgments:
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