National Institute for Agrarian and Veterinary Research

EZN CIISA FMV - ULisboa LEAF

GenPrOV

Effects of nonsynonymous SNPs at *GH2-N* and *GHR* genes on coagulation properties of Assaf ewes' milk

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Introduction

Milk production potential is a function of the number of mammary epithelial cells

Lactation performance depends on:

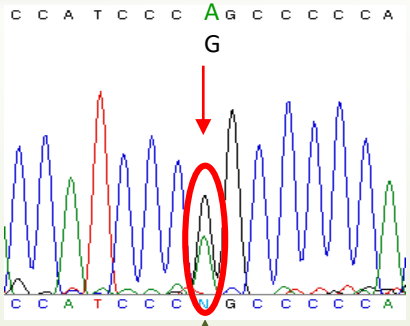
- Mammary cell proliferation (or decrease apoptosis)
- Structural and biochemical differentiation of mammary epithelium
- Synthesis and secretion of milk components

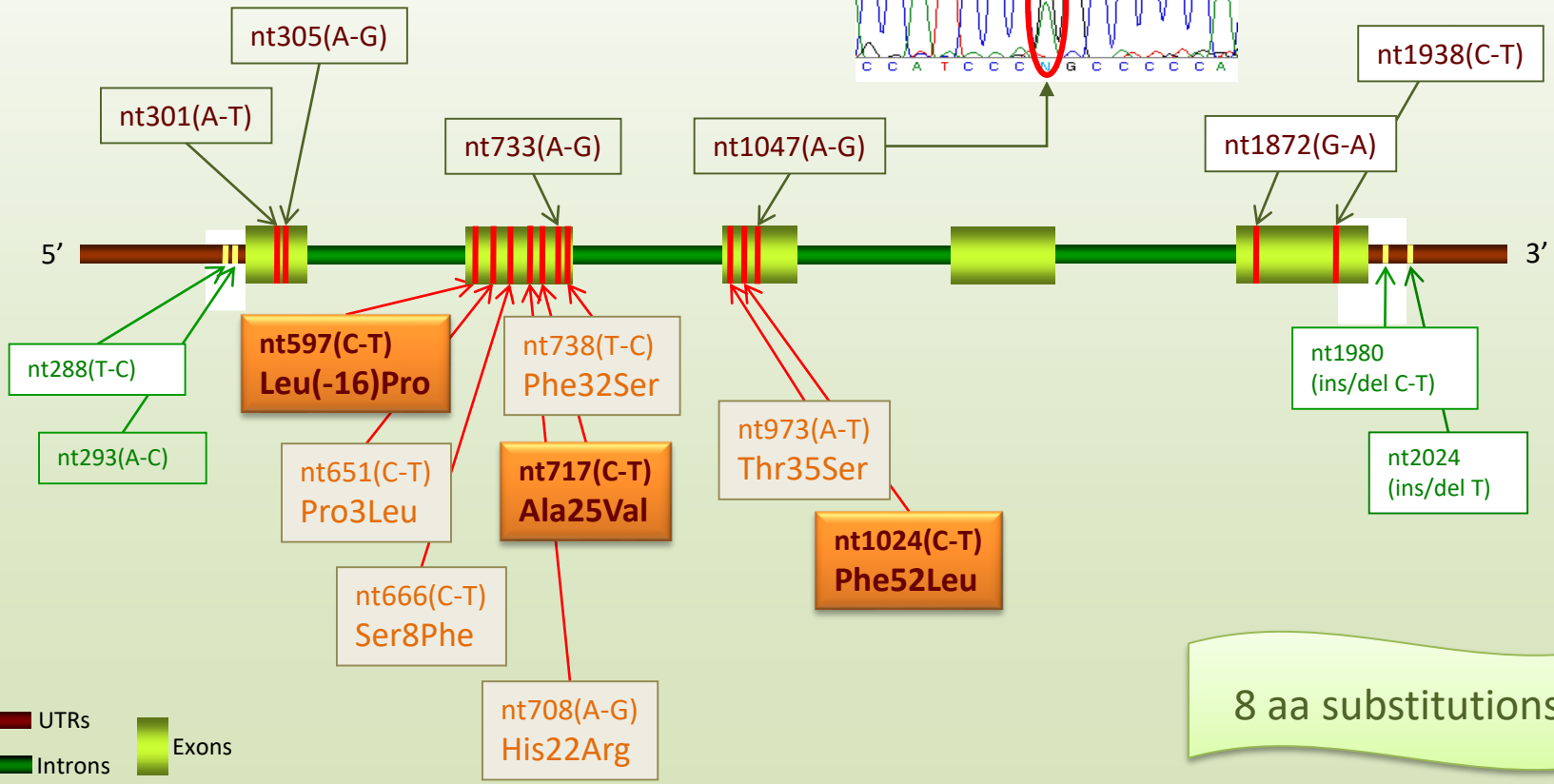
Various hormones are involved in these processes

Introduction

Growth hormone gene (*GH2-M*)

- Polymorphisms at the ***GH2-N* copy**





8 aa substitutions!

Objectives

To uncover SNPs in ovine somatotrophic axis associated with high yielding dairy ewes.

- Genotype SNPs in growth hormone copy *GH2-N* and *GHR* genes in Assaf ewes

- to identify candidate causative mutations for milk production and composition, to be used in Marker-Assisted Selection programs by dairy ewes breeders

Aiming at an increase in the sheep's breeding value for milk traits, and the flocks' profitability.

Methodology

GenPrOV

- Genotyping and phenotyping

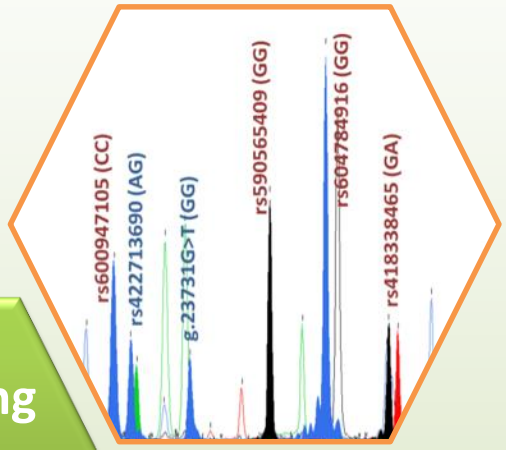
430 ewes

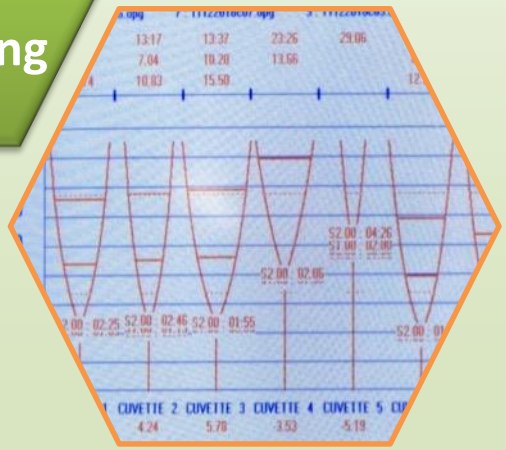
Blood collection and DNA extraction

Milk samples collection

184 ewes

Genotyping and phenotyping





Methodology

1. **Six SNPs genotyped by SnapShot analysis**
2. **Milk production** (monthly)
3. **Milk composition analysis by Milk-o-Scan:**
   - **Fat, protein, lactose, total solids and fat free total solids content** (monthly)
4. **Coagulation properties evaluated at 1st and 3th month of lactation by Optigraph:**
   - **Clotting time (R), gel firmness after 20 min. (A20)** and **after a 2R (AR) period,** and **rate of firming (OK20)**

Methodology

4. Statistical analysis

MIXED model procedure from SAS®

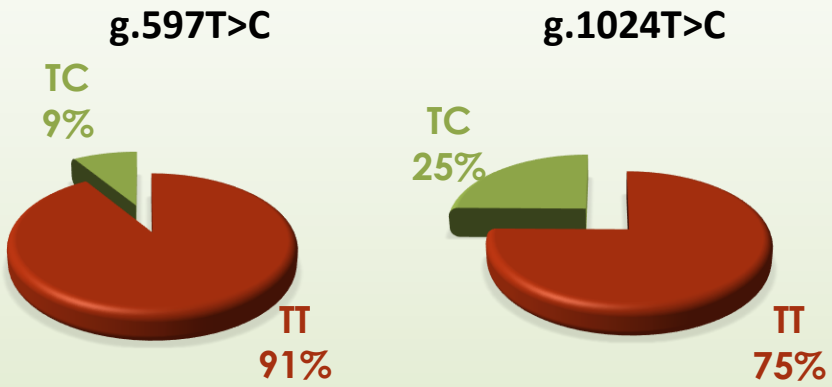
$$Y_{ijklmn} = \mu + NLact_i + SNP_j + Contr_k + \beta_1(x_{ijkl} - \bar{x}) + \beta_1(x_{ijkl} - \bar{x})^2 + Ewe_{ijklm} + \varepsilon_{ijklmn}$$

$NLact_i$ – effect of the lactation number i (1 to 6)
SNP_j – effect of the genotype for each SNP j
$Contr_k$ – effect of lactation month k (1 to 5)
$\beta_1(x_{ijkl} - \bar{x})$ – linear and quadratic effect of the age of the ewe at lambing
Ewe_{ijklm} - random effect of the ewe $ijklm$
ε_{ijklmn} - random error

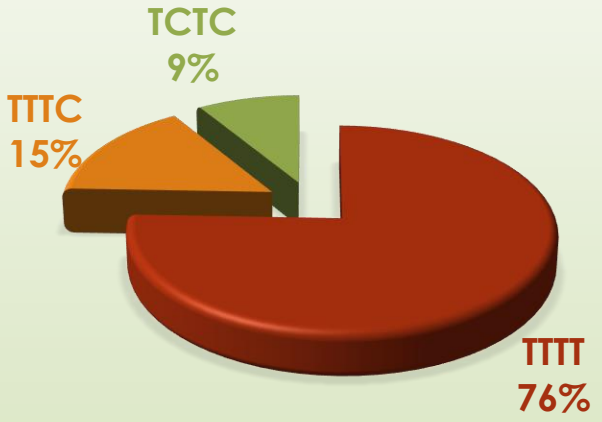
Results

GH2-N frequencies

Genotypes frequencies



Haplotypes frequencies

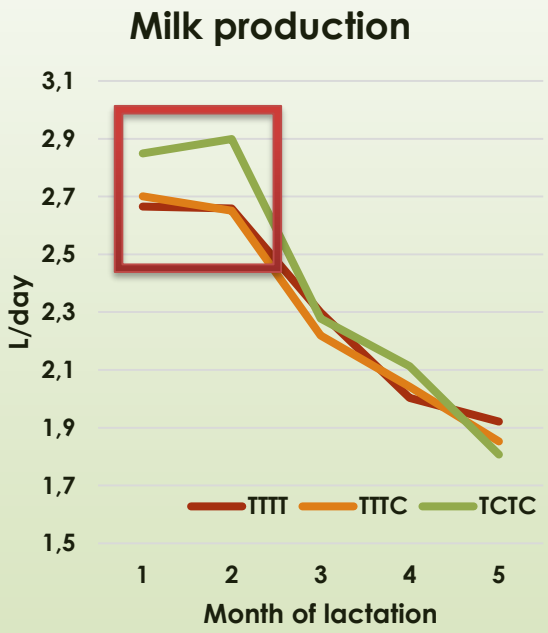


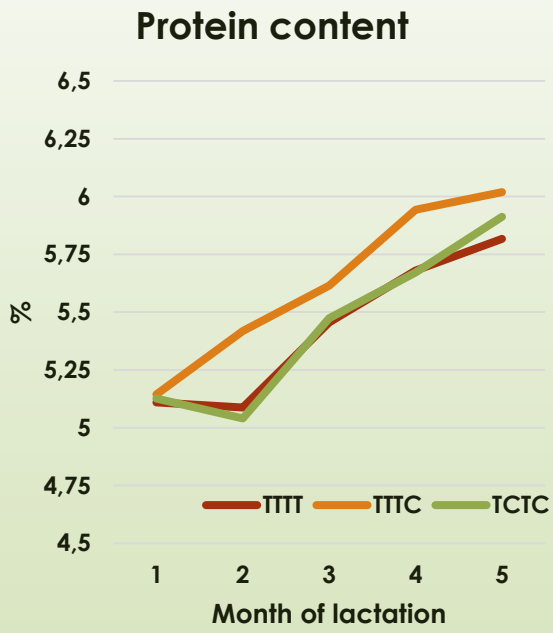
SNP*		aa**	Alelles[b]		P_{HWE}
GH2-N	**g.597T>C**	**Leu11Pro**	T (0.956)	C (0.044)	***
	g.717C>T	**Ala51Val**	C (1.000)		-
	g.1024T>C	**Phe78Leu**	T (0.876)	C (0.124)	***

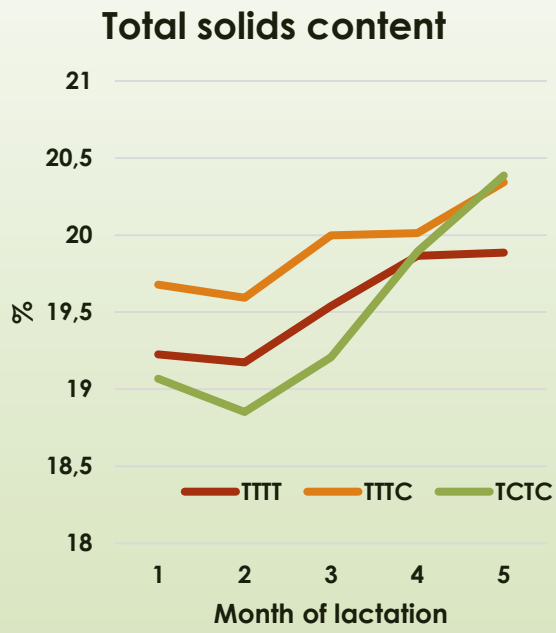
* GenBank accession number X12546;
** GenPept accession number P67930

Results
GH2-N vs milk traits

- ***GH2-N* SNPs had no effects on milk production and composition**



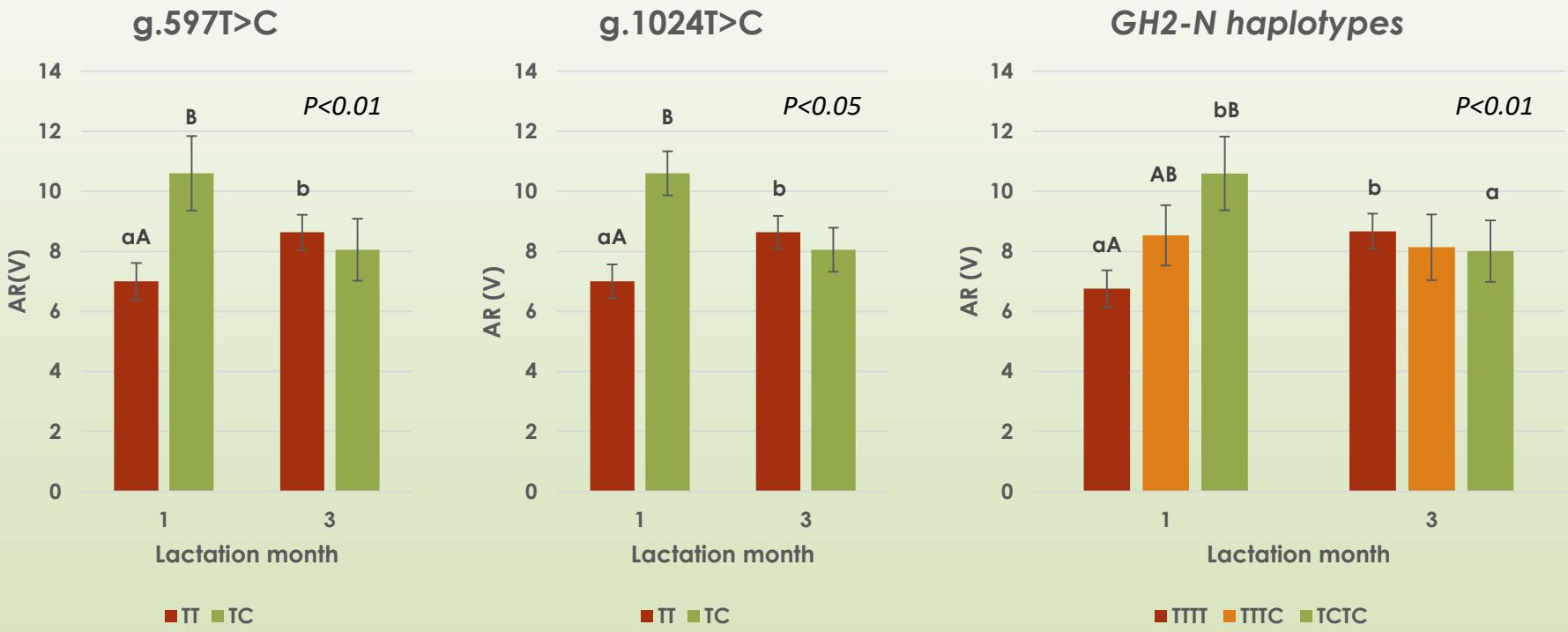




Note: haplotypes results

Results
GH2-N vs milk traits

➢ **SNPs affected gel firmness after a 2x clotting time (AR) period**



a, b – different lowercase letters correspond to significant differences between lactation month within genotypes (*P<0.05*)
A, B – different capital letters correspond to significant differences between genotypes within lactation month (*P<0.05*)

Results
GHR frequencies

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➢ **Genotypes and alleles frequencies**

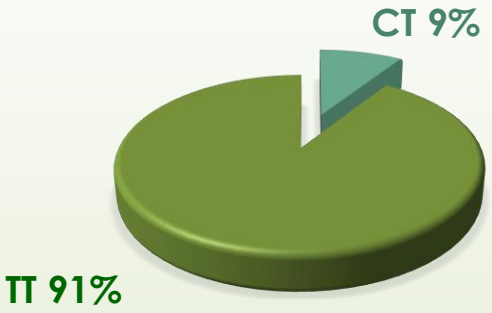
	SNP*	aa	Genotypes[a]			Alelles[b]		P_{HWE}
GHR	rs1086611503	Ser380Pro	TT (0.909)	TC (0.091)		T (0.955)	C (0.045)	***
	rs595567866	Glu392Lys	GG (0.905)	GA (0.093)	AA (0.002)	G (0.951)	A (0.049)	***
	rs597181420	Ala529Thr	CC (0.910)	CT (0.083)	TT (0.007)	C (0.952)	T (0.048)	***

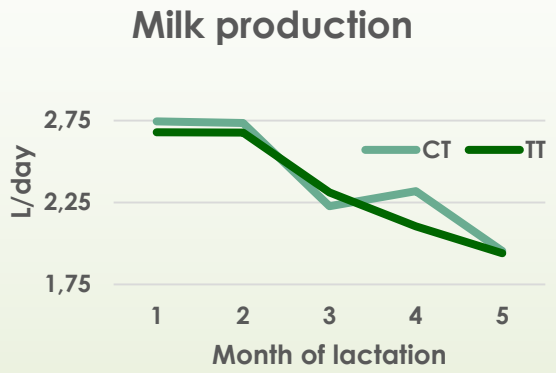
* dbSNPs;
** GenPept accession number Q28575

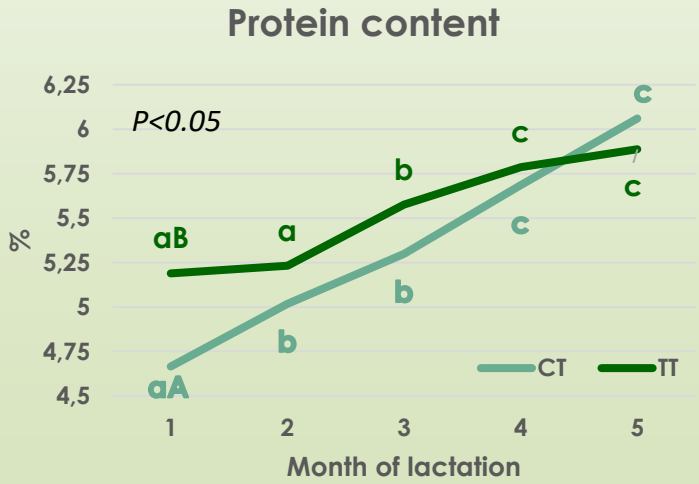
Nine haplotypes identyfied!

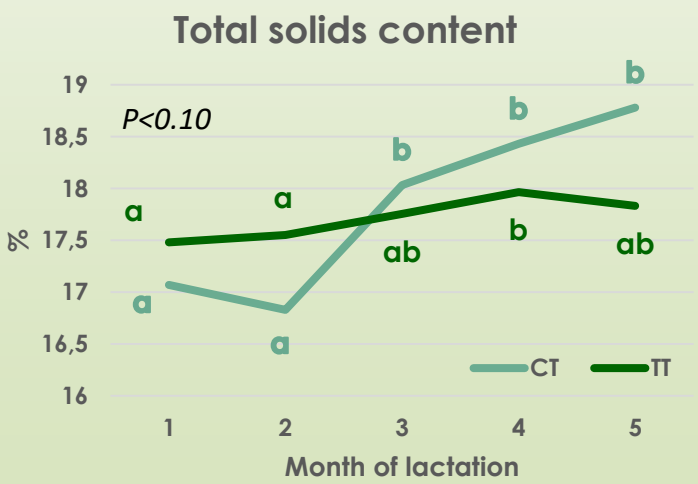
Results

GHR - rs1086611503









a, b – different lowercase letters correspond to significant differences between lactation month within genotypes (*P<0.05*)
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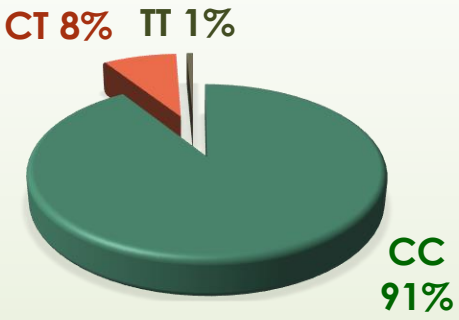
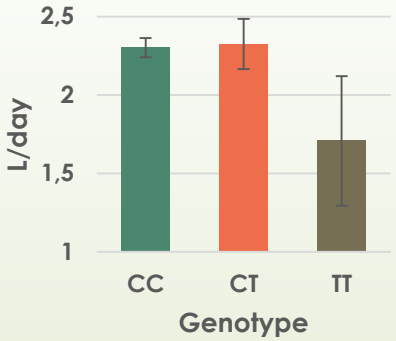
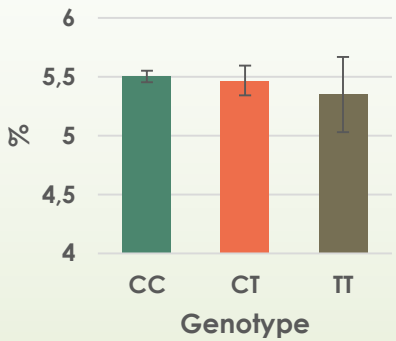
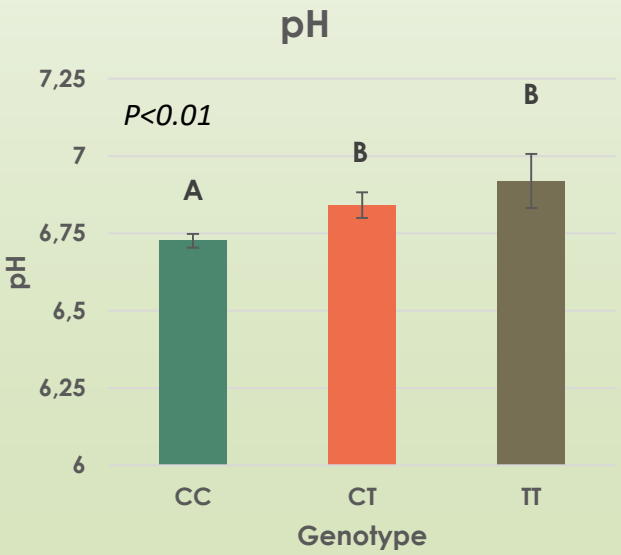
Results

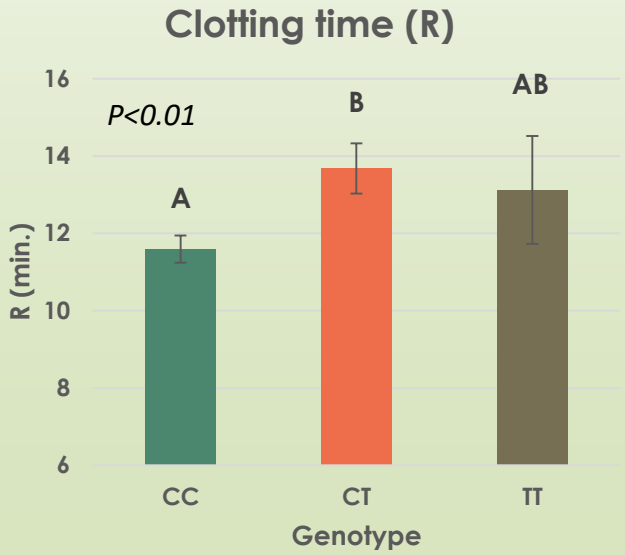
GHR - rs597181420









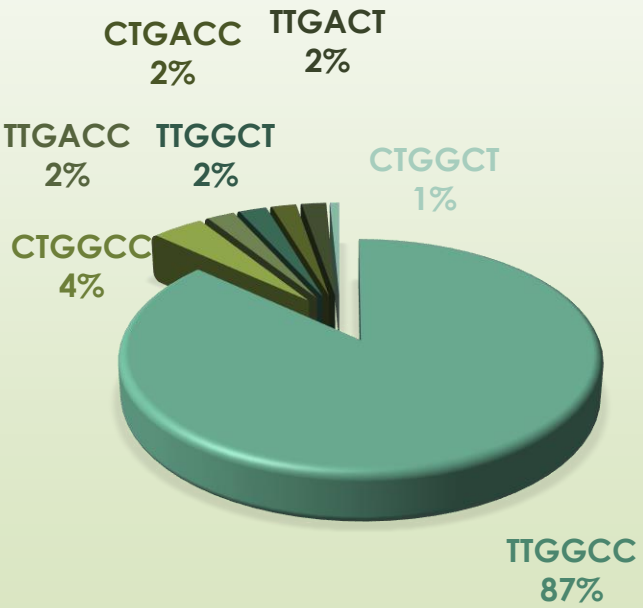


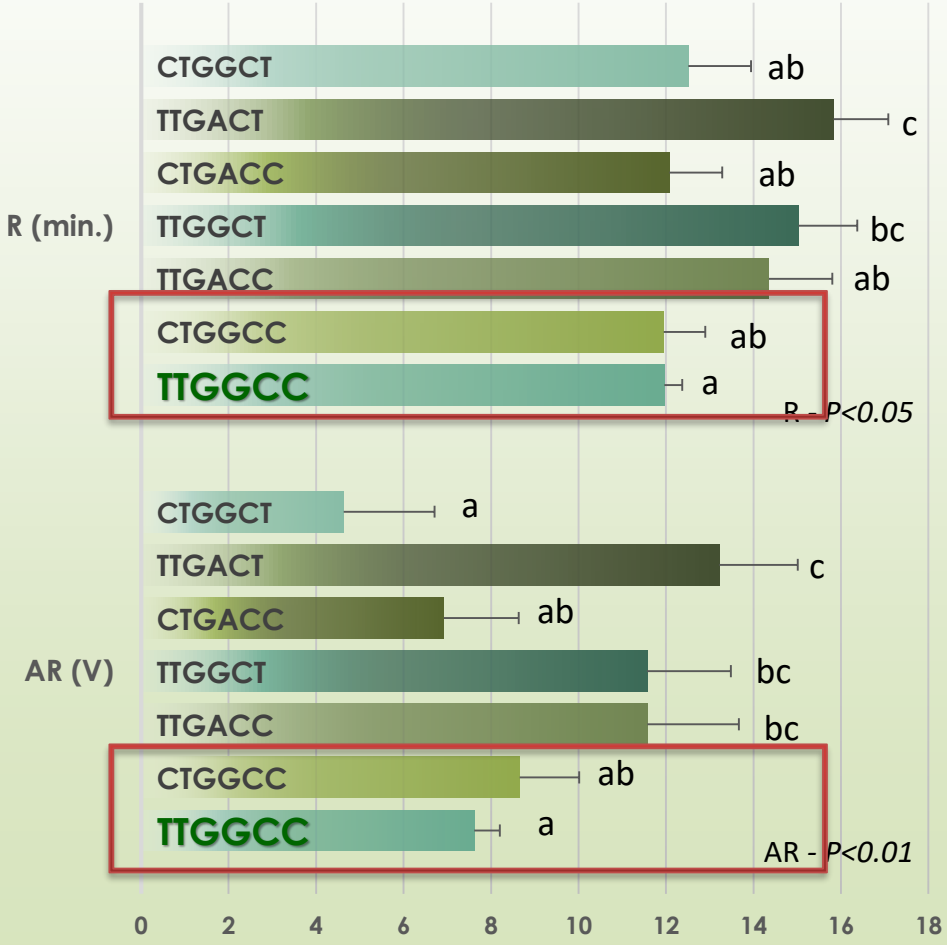
A, B – different capital letters correspond to significant differences between genotypes (*P<0.05*)

Results

GHR haplotypes *vs* milk traits

Haplotypes frequencies





a, b – different capital letters correspond to significant differences between haplotypes (*P<0.05*)

Conclusions

1. **Studied SNPs at *GH2-N* and *GHR* genes were**
   - ✓ **Polymorphic – all** but *GH2-N* g.717C>T
   - ✓ not associated with milk production traits
   - ✓ **associated with milk quality and coagulation parameters**

2. ***GH2-N* SNPs and haplotypes** ➡ gel firmness (AR)

3. ***GHR* SNPs:**
   - ✓ **rs1086611503** ➡ **milk protein** and total solids content throughout lactation
   - ✓ **rs597181420** ➡ pH and **clotting time (R)**
   - ✓ **Haplotypes** ➡ **clotting time (R)** and **gel firmness (AR)**

Perspectives

Ongoing SNP genotyping:

- *GH2-N* and *GH2-Z* genes copies and GHR·
- Othe
  - S

Selection of the panel of SNPs that best allows to estimate breeding value of the ewes and rams to be bred for the production of quality milk

Ongoi

- Colle

  prope

Associate these SNP with milk prodution traits:

- Twenty years of records for milk production traits.

Acknowledgment

Gene markers for Production and milk quality in Assaf OVine breed.

Project
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https://projects.iniav.pt/genprov/

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Thanks for your attention!