Faculty of Agrobiology, Food and Natural Resources

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Genomic insights into Milk Coagulation Properties and Curd Firmness in Pag Island Sheep: a Genome-Wide Association Study

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PAG ISLAND SHEEP

Source: https://www.hrvaska.net/hr/destinacije/otok_pag_hr.htm

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PAG ISLAND SHEEP

- From improved indigenous island Pramenka breed (rams of the Negretti, Bergamo and Gentile di Puglia).
- Before – wool production, last decades - primarily milk production
- Famous, full-fat, hard sheep cheese – Pag Cheese.
- Secondary – lamb production - small carcass weight and high-quality meat, highly sought after in Croatian market.
- Salt in the soil, affect the structure and quality of the vegetation - reflects on the quality of the milk and meat.
- Milk – lactation 160 days, 150kg per lactation, 7% milk fat, 6% milk protein

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MILK COAGULATION PROPERTIES (MCP)

- **expressed through:**
  - **time from rennet addition to the beginning of coagulation (RCT, min)**
  - **time needed for the curd to obtain firmness (CF, mm) of 20 mm (k_{20}, min)**
  - **curd firmness at the end of the analysis (a_{30}, mm).**

- **Traditional milk coagulation properties analysis is limited by:**
  - **slow-coagulating (SC) or non-coagulating (NC) samples**
  - **k_{20} can't be estimated in samples with high RCT value**
  - **dependence of a_{30} parameter on RCT value**

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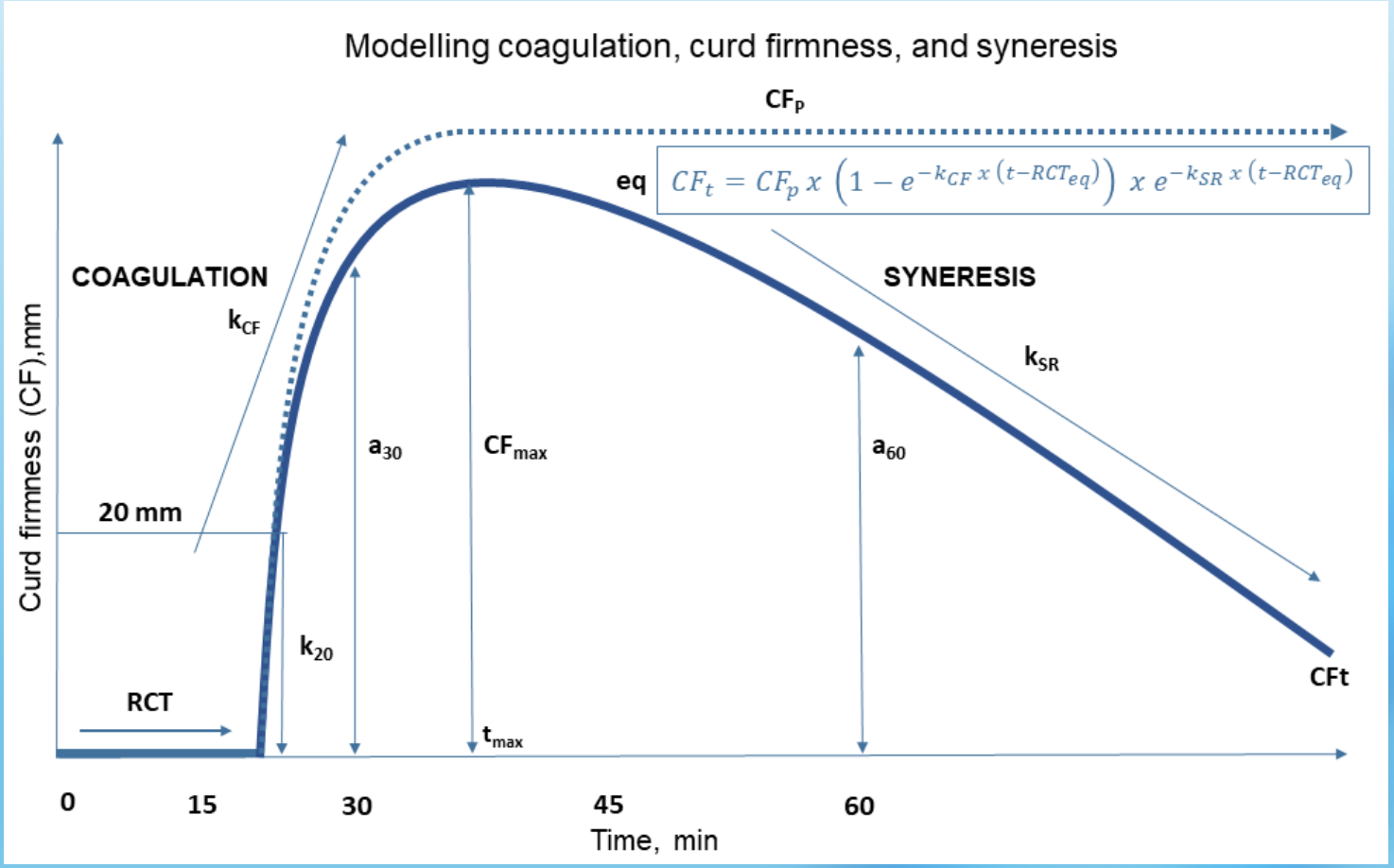
COAGULATION, CURD FIRMNESS (CF), AND SYNERESIS MODELING



Bittante *et al*. 2013.

- CF_t - curd firmness at time t (mm)
- *RCT - rennet coagulation time (min)*
- k_{20} *– time when curd =20mm (min)*
- a_{30} *– CF at 30 min after rennet addition (mm)*
- a_{60} *- CF at 60 min after rennet addition (mm)*
- t_{max} *- time at* a_{max} *(min)*
- RCT_{eq} - rennet coagulation time from model (min)
- k_{CF} - curd firming rate constant (%/min)
- k_{SR} - syneresis rate constant (%/min)
- CF_P - potential asymptotic CF in absence of syneresis (mm)
- CF_{max} – max CF_t (mm)

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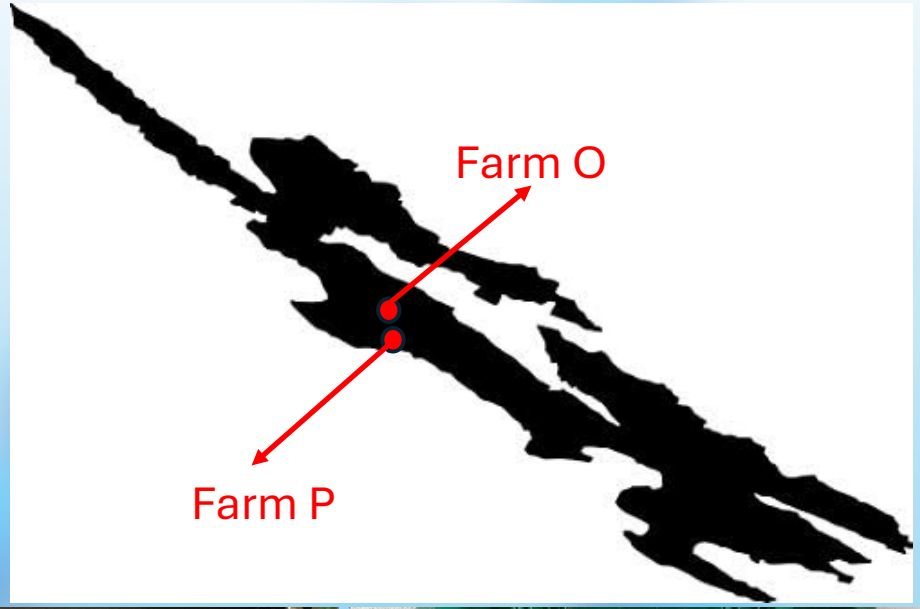
WHY DO WE CARE?

- Selection program based on milk composition and yield (MY) - not optimal!
- MCP should be among the most important selection criteria.
- A better understanding of MCP in Pag sheep milk.
- Identification of genetic polymorphisms affecting MCP.
- Improving cheese-making ability, cheese yield, selection programs, and thorough characterization of milk from Mediterranean sheep breeds.

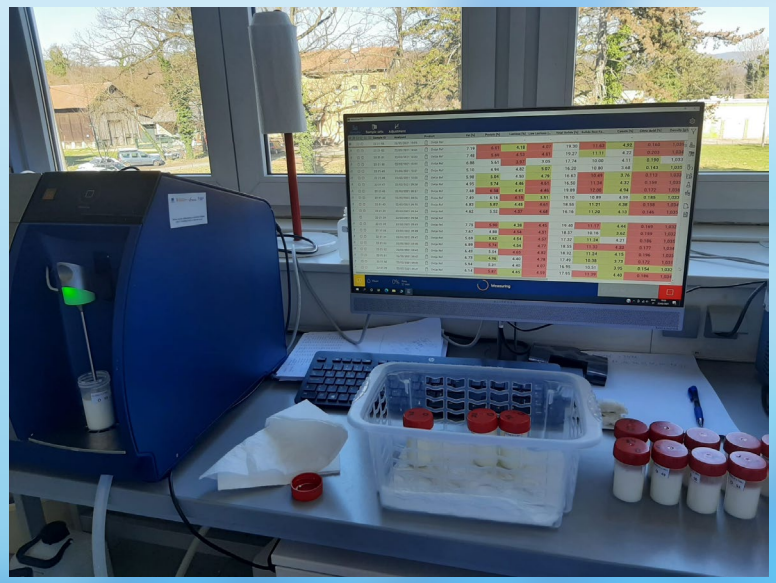
MILK SAMPLING AND HANDLING

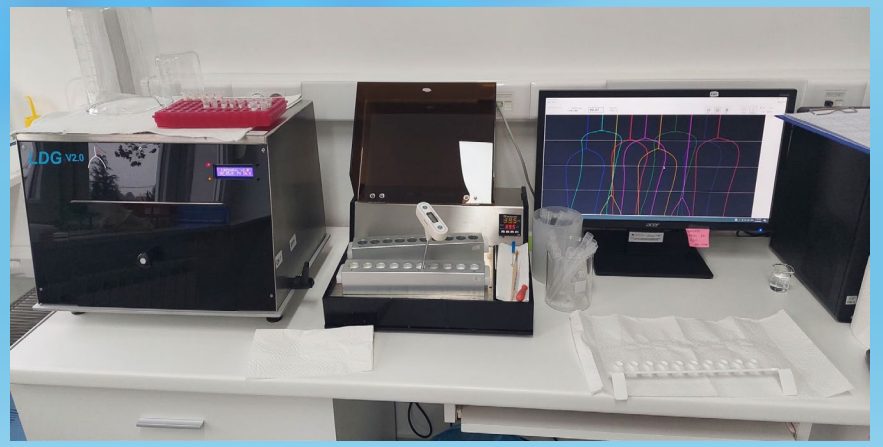
- 2 farms with total of 370 sheep in lactation
- AT sampling method (ICAR, 2018)
- 5 samples per sheep in lactation
- 20 mL immediately preserved with Na-azidiol for somatic cell count and total bacterial count
- 60 mL non-preserved for analysis of the chemical composition, physical parameters, and coagulation properties.



MILK SAMPLING AND HANDLING

- shock-frozen to -30°C in 15 minutes and stored at -20°C for MCP determination.
- Fossomatic Minor (Foss Electric, Hileroed, Denmark).
- Bactoscan FC (Foss Electric, Hileroed, Denmark).
- Milkoscan FT3 (Foss Electric, Hileroed, Denmark)
- Lactodinamograph or Formagraph (MA.PE SYSTEM SRL, Firenze, Italy)
- Rennet used - Bioren Premium PHA100 (Christian Hansen, Sweden) with declared strength 791 IMCU/gr





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DNA SAMPLING AND ANALYSIS

- Farm O
  - N=135 (7 M , 128 F)
- Farma P
  - N =232 (14 M, 218 F)
- Milk and tissue (Tissue Sample Unit Applicator, Allflex)
- Dneasy Blood & Tissue Kit (Qiagen, Germany)
- GeneSeek® Genomic Profiler™ Ovine 50k (Neogen, GB)

- Removed SNPs
  - GenTrain Score = 0,6
  - GenCall Score = 0,7
  - X and not mapped
  - Missing per SNP < 0,9
  - MAF 0,01
  - 51867 → 32885 SNPs
- Removed animals
  - Missing SNPs > 10%
  - When discrepant with pedigree
  - 325 → 291 sheep (F)
- SAS 9.4, PLINK 1.9, JMP 17 PRO

STATISTICS AND GWAS

- SAS 9.4, R and JMP 17 PRO
  - Data cleaning and visualisation
  - 1476 samples → 1173
  - Model building with BESTSUBSET option
- BLUPF90 family of programs
  - renumf90
  - blupf90+ - REML estimating genetic parameters
  - blupf90+ - predict genomic BW
  - postGSf90 – ssGWAS

PEDIGREE → 820 records, 3 gen

- Tested dependent variables (all continuous)
  - RCT, k_{20}, A_{30}, A_{60}, RCT_{eq}, k_{CF}, k_{SR}, CF_P, CF_{max}, t_{max}
- Independent variables
  - FARM – farms O and P
  - DAYTIME – AM or PM
  - DAYS IN MILK
  - AGE (in years)
- QTL Regions and Gene Annotation
  - GALLO R package
  - Genome assembly Oar_v4.0
  - AnimalQTLdb (Sheep)
  - QTL enrichment – p = 0.05 (FDR)

SOME MILK RESULTS (STATS)...

Trait	Mean	Std Dev	Min	Max
DIM (days)	100,25	43,92	12,00	202,00
AGE (years)	4,63	2,06	2,00	14,00
RCT (min)	14,84	8,75	5,00	60,00
k_{20} (min)	1,38	1,01	0,00	8,87
a_{30} (mm)	40,97	13,90	0,00	66,64
a_{60} (mm)	32,50	13,95	0,00	66,15
a_{max} (mm)	52,47	9,81	0,00	67,62
t_{max} (min)	24,72	10,57	0,00	60,00
RCT_{eq} (min)	14,08	8,84	3,94	60,00
k_{CF} (%/min)	0,34	0,18	0,00	1,30
k_{SR} (%/min)	0,02	0,01	0,00	0,08
CF_P (mm)	62,50	24,87	0,00	367,91
Cf_{max} (mm)	48,22	8,99	0,00	63,78

measured

estimated

PCA with neighbors (sheep from close islands, regions…)

PC2 (7,6%)

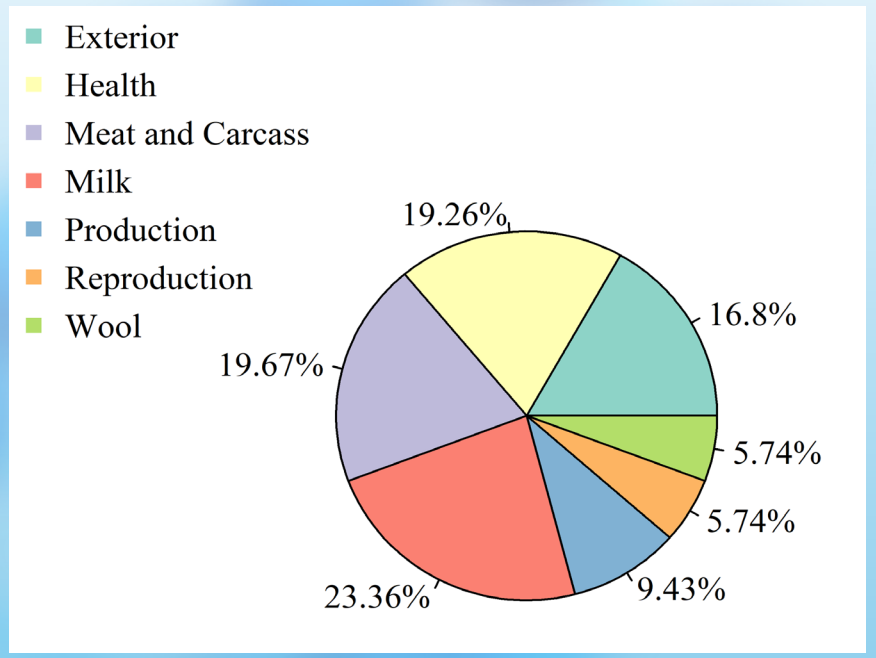
PC1 (13,6%)

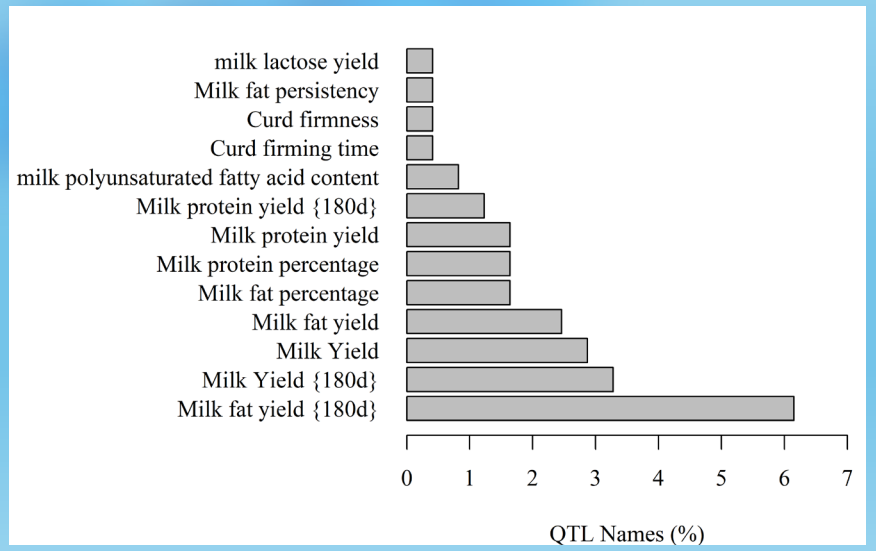
RCT – Beginning of coagulation

CURDF

TCURDF

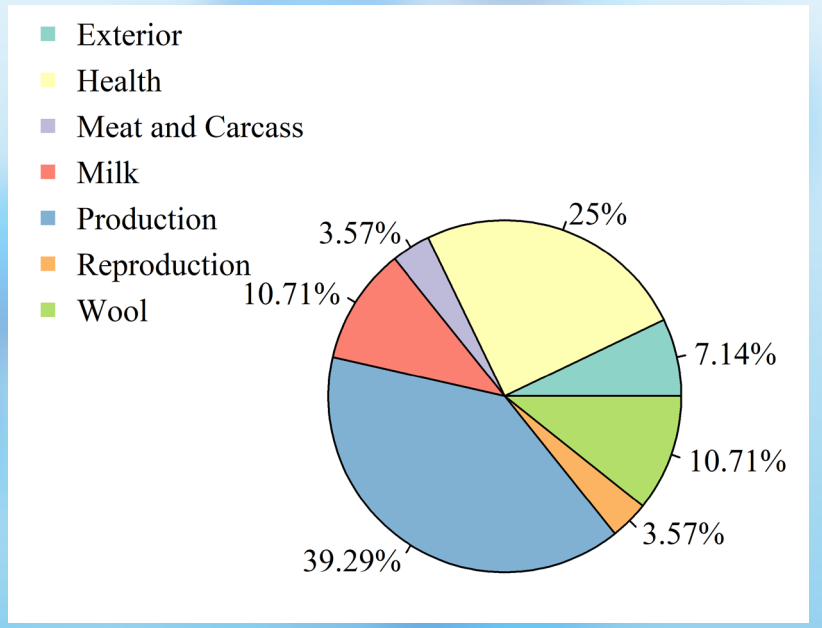
1 12

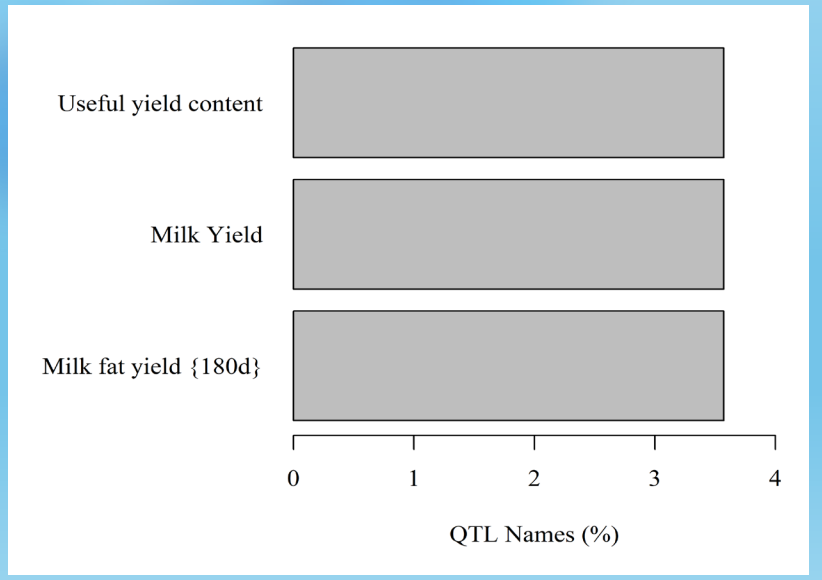




- 50 significant SNPs (17 negative effect)
- 4% V_G explained
- 45 region → 542 genes → 244 QTLs (57 for milk)

a_{max} – maximum CF during analysis



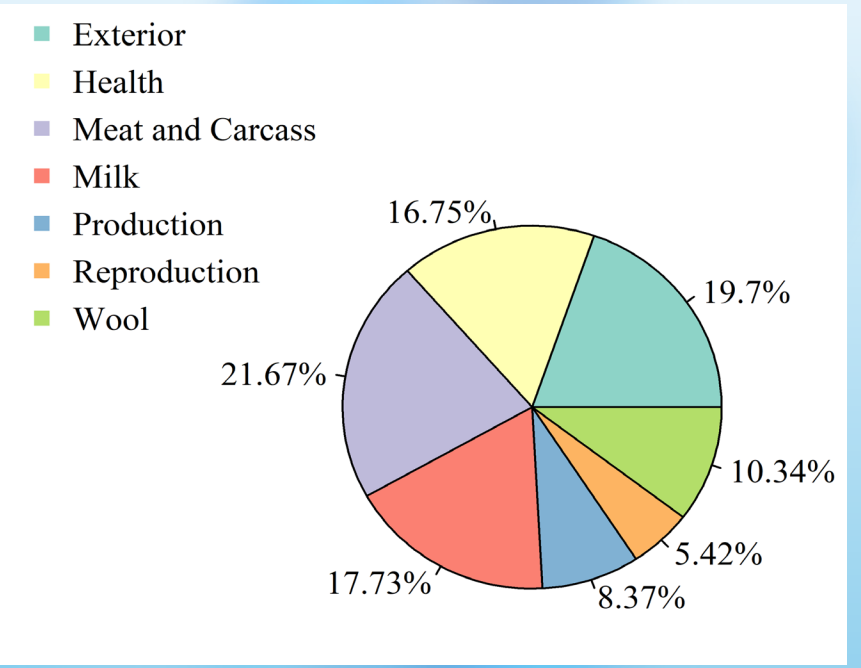


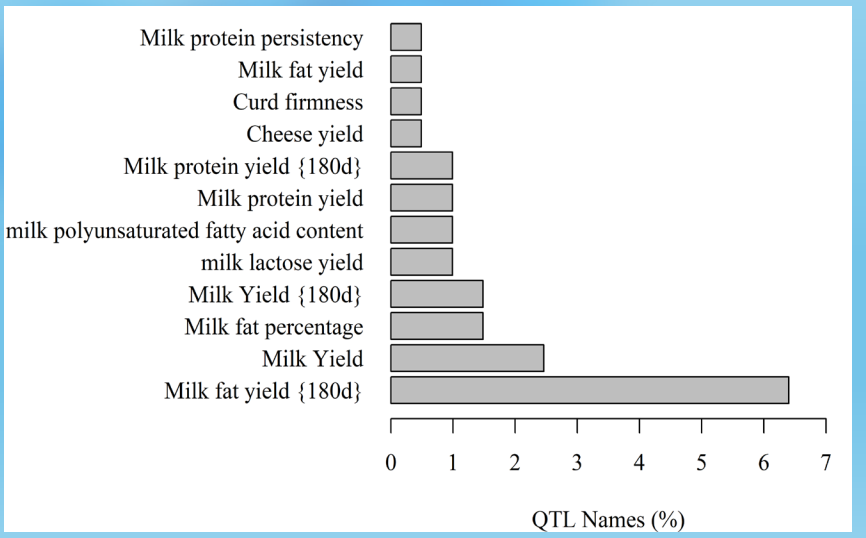
- 9 significant SNPs (7 negative effect)
- ≈ 1% V_G explained
- 7 region → 74 genes → 28 QTLs (3 for milk)

t_{max} – time at a_{max}

CURDF

CHYLD





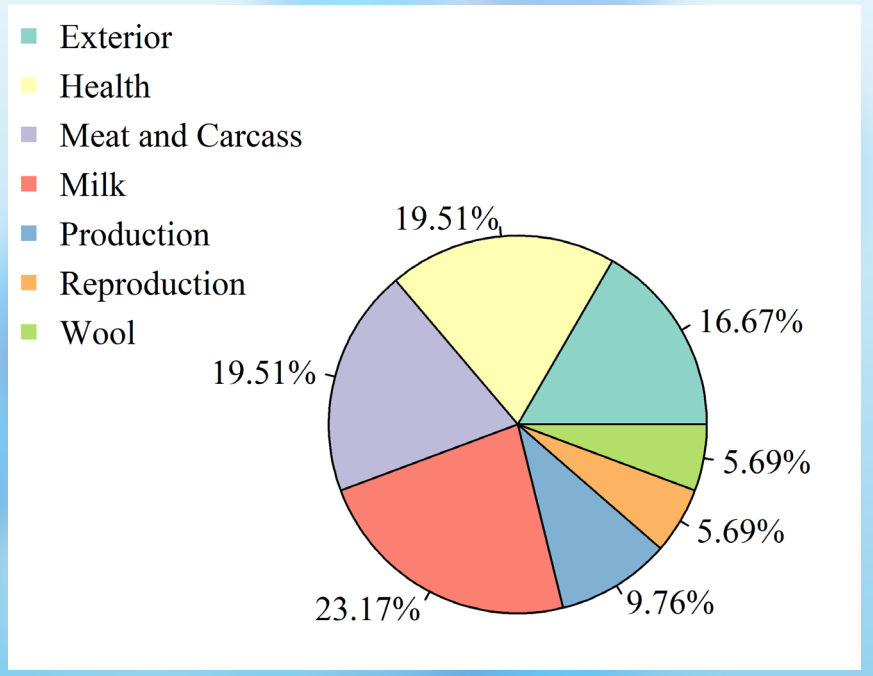
- 37 significant SNPs (12 negative effect)
- 3% V_G explained
- 31 region → 396 genes → 203 QTLs (36 for milk)

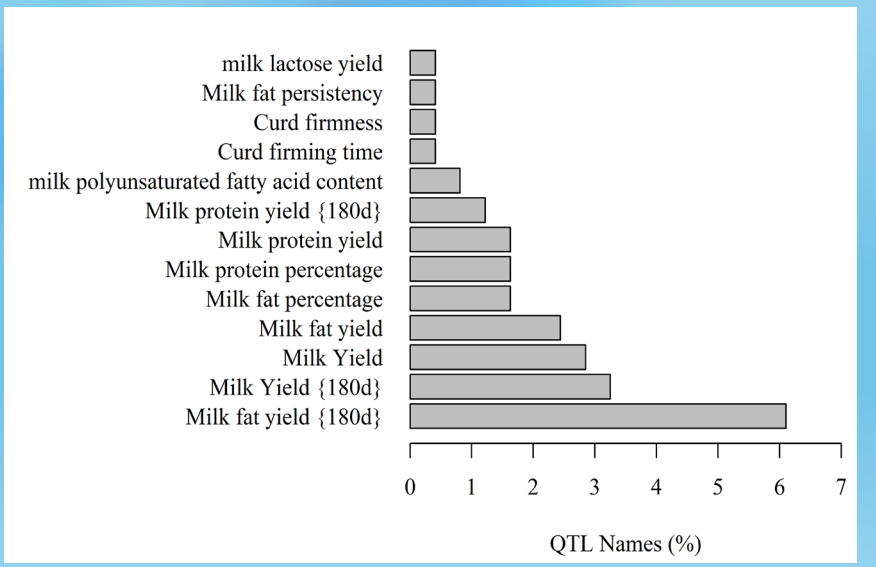
3

RCT_{eq} – Beginning of coagulation estimated

CURDF

TCURDF





- 52 significant SNPs (17 negative effect)
- 4% V_G explained
- 47 region → 555 genes → 246 QTLs (57 for milk)

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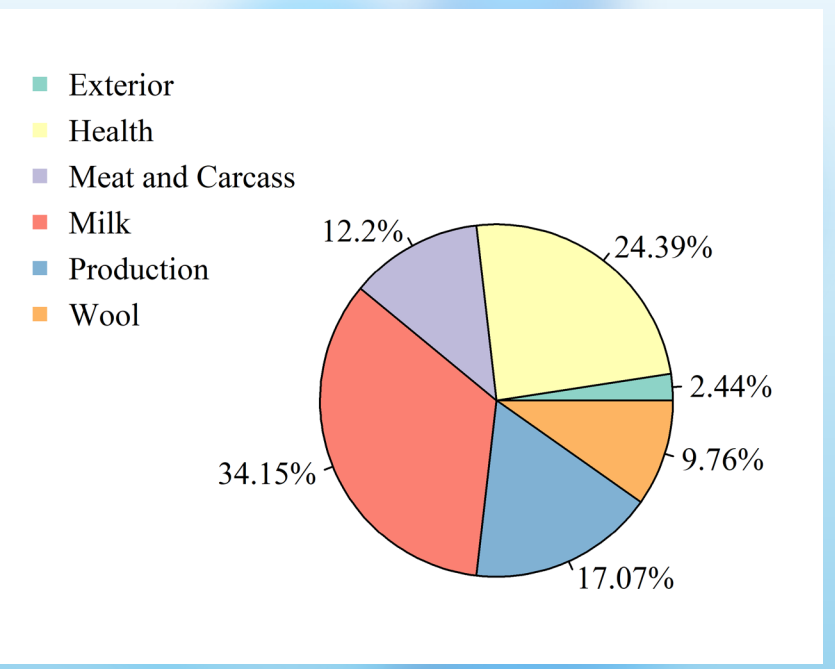
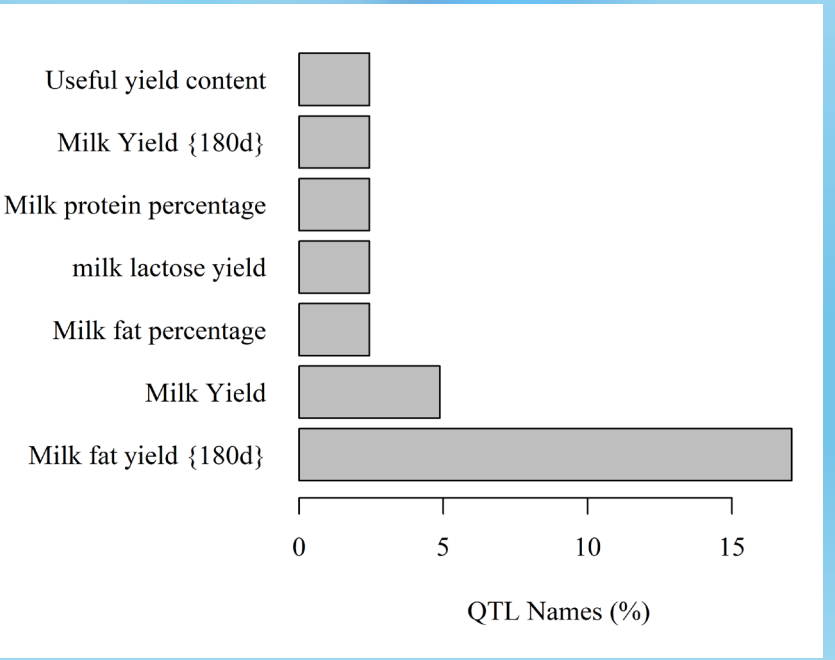
k_{CF} – constant curd firming rate estimated

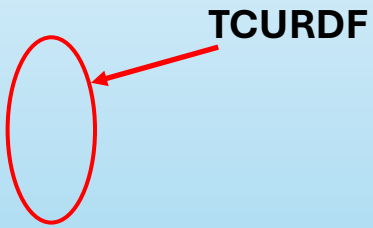


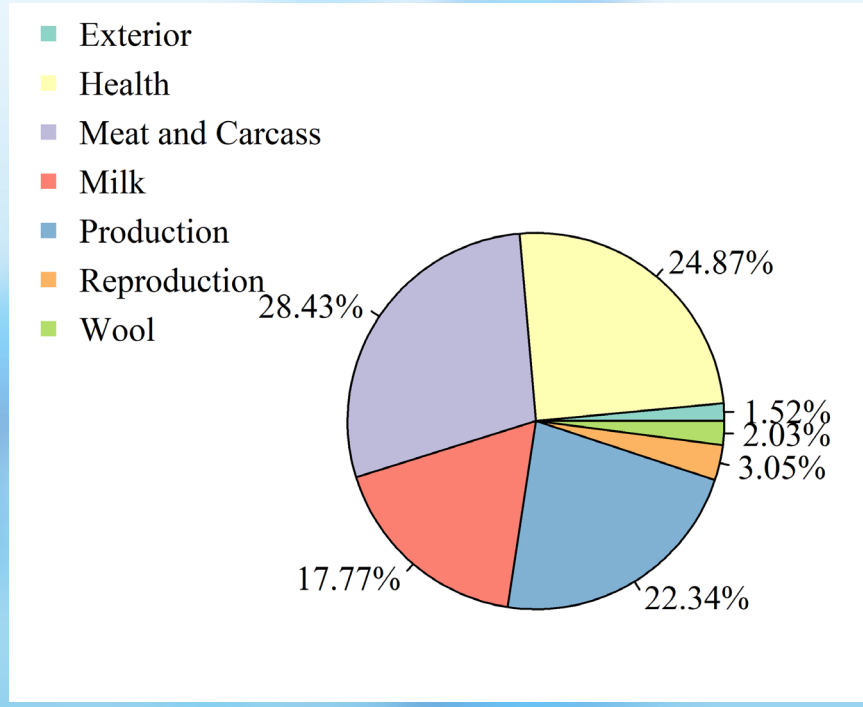


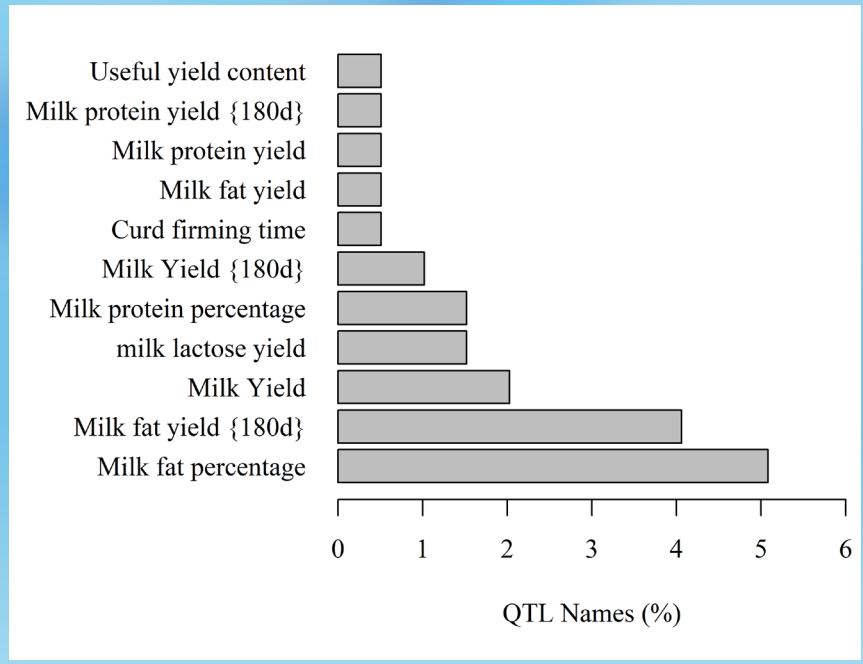
- 14 significant SNPs (9 negative effect)
- ≈ 1% V_G explained
- 11 region → 146 genes → 41 QTLs (14 for milk)

3

CF_p – potential asymptotical CF at infinite time - estimated



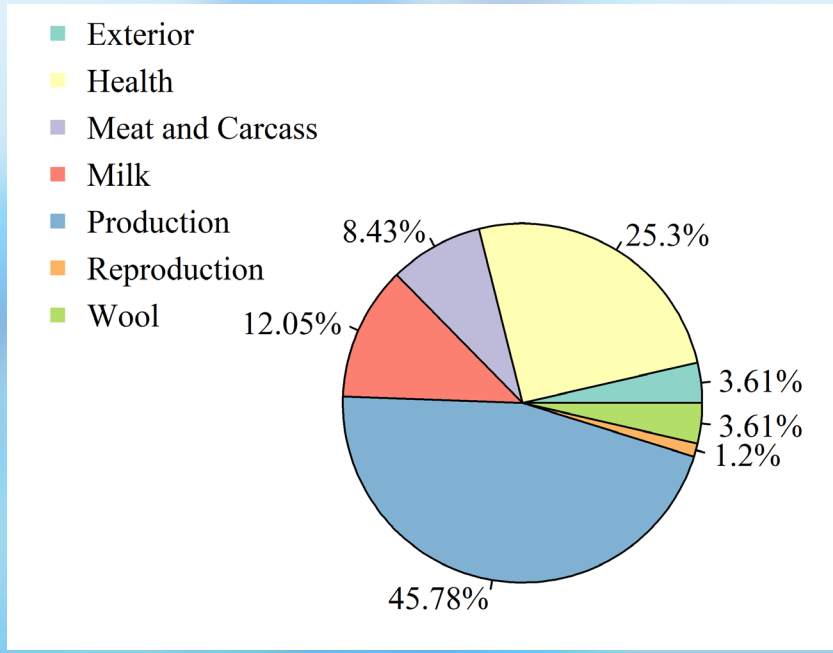


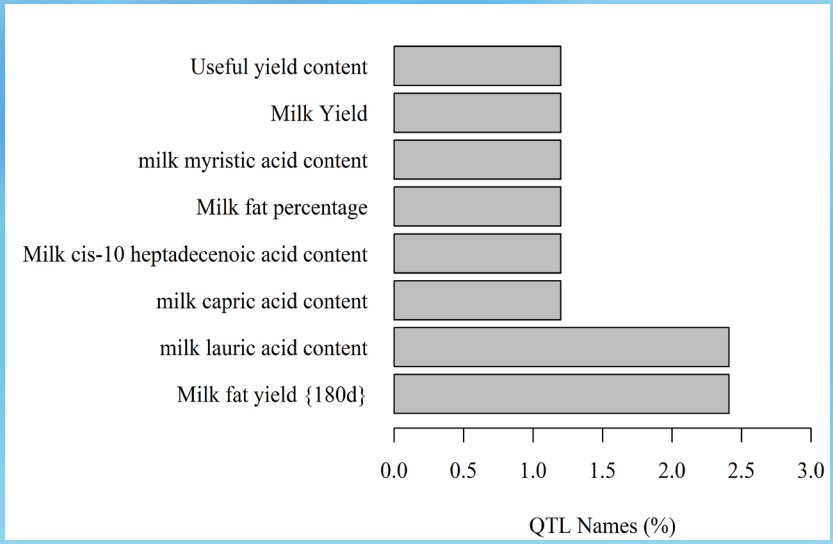


- 39 significant SNPs (17 negative effect)
- 7% V_G explained
- 28 region → 283 genes → 197 QTLs (35 for milk)

3

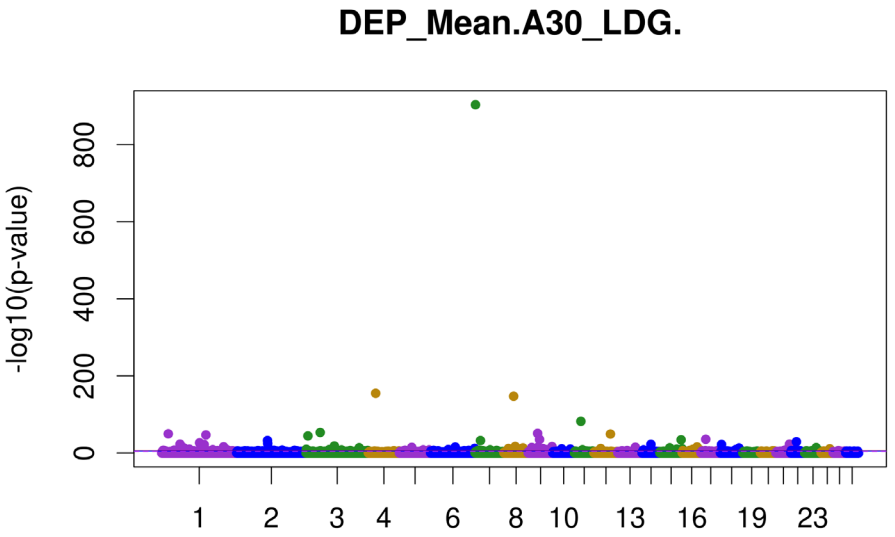
CF_{max} – Maximum curd firmness estimated

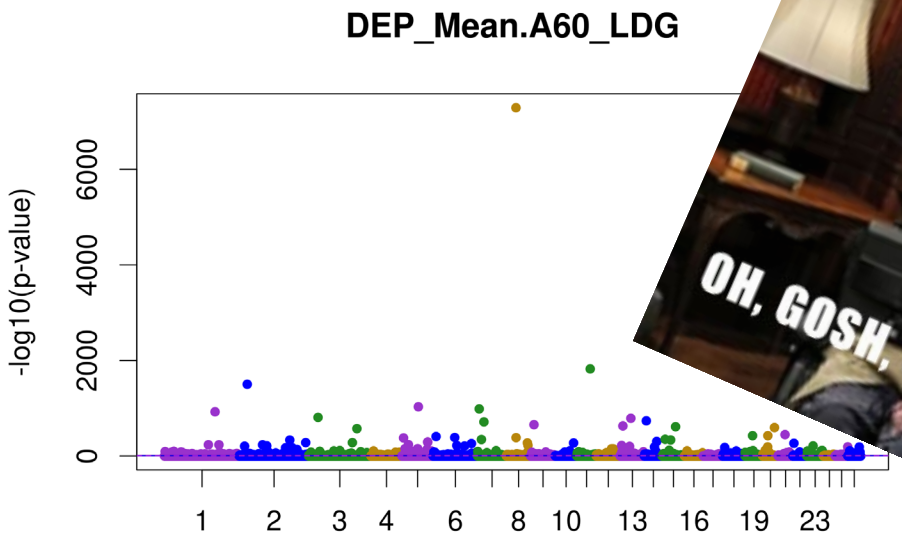


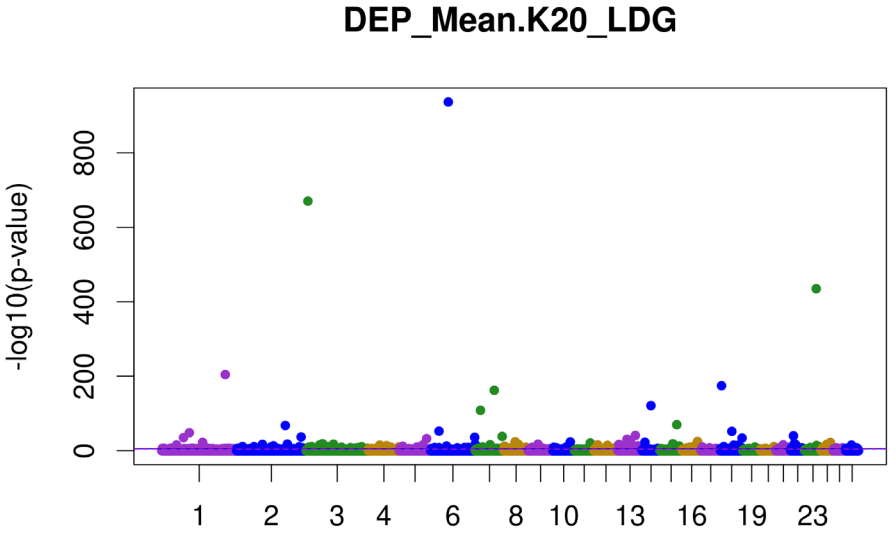


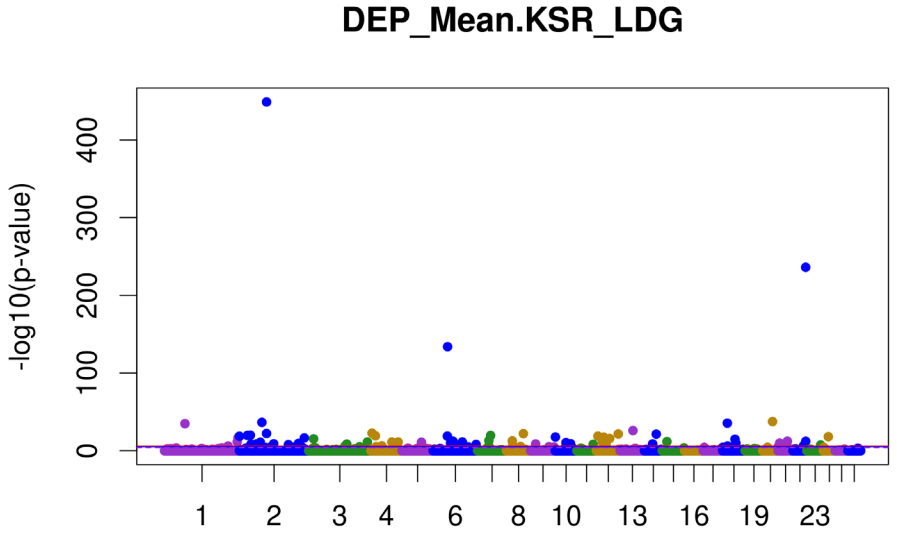
- 15 significant SNPs (10 negative effect)
- ≈ 1% V_G explained
- 11 region → 153 genes → 83 QTLs (10 for milk)

SOME TRAITS ARE MISSING...









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