

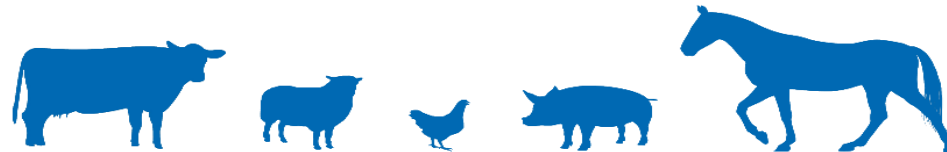
# Genome wide associations for methane emissions in dairy cows

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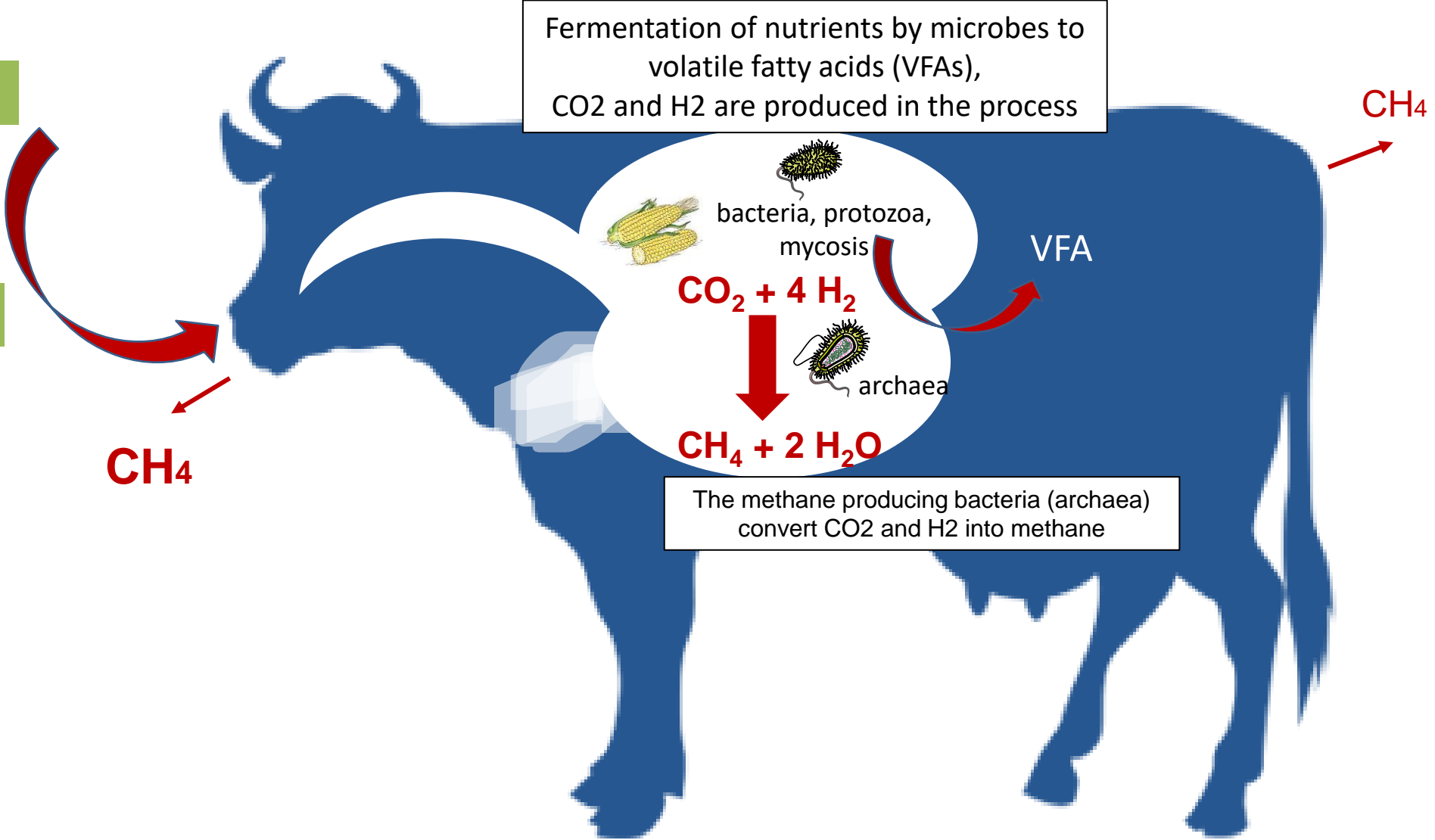
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# Methane production in a cow (Methanogenesis)

- Nutrients:
- Starch
- Glucose
- Cellulose
- Proteins



The methane producing bacteria (archaea) convert CO<sub>2</sub> and H<sub>2</sub> into methane

# Aims of this study

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- Estimation of genetic parameters for methane as a trait
- Determination of breeding value correlations between methane traits and other breeding goal traits
- Carrying out genome-wide association studies (GWAS)

[www.cccfarming.eu](http://www.cccfarming.eu)



# Farm descriptions

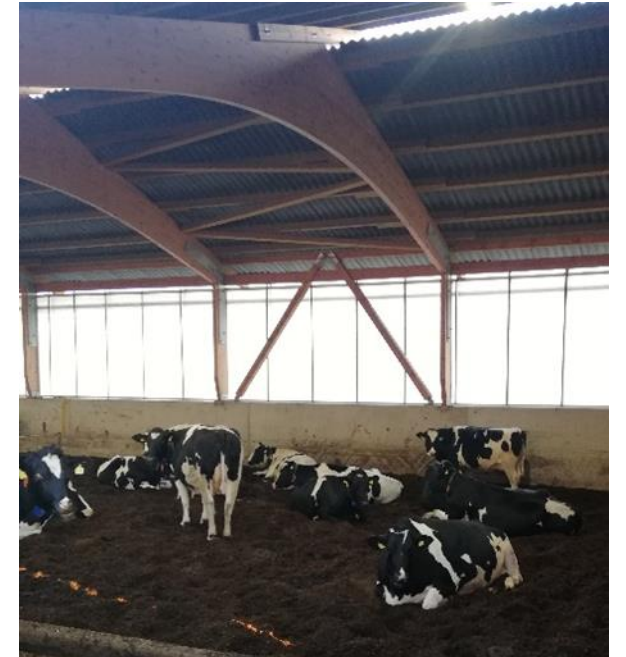
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- 7 dairy cow herds
- Compost bedded pack barn & cubicle housing
- Located in Hesse and Rhineland Palatinate
- 60 – 700 cows per herd
- Organic and conventional producing
- Holstein Friesian and German Black Pied Cattle



# Data recording

- 2,015 observations with Laser methane detector (LMD)
- 1,360 dairy cows
- 1,028 cows genotyped with 50K *Illumina BovineSNP50 v2 BeadChip* and 38614 SNPs



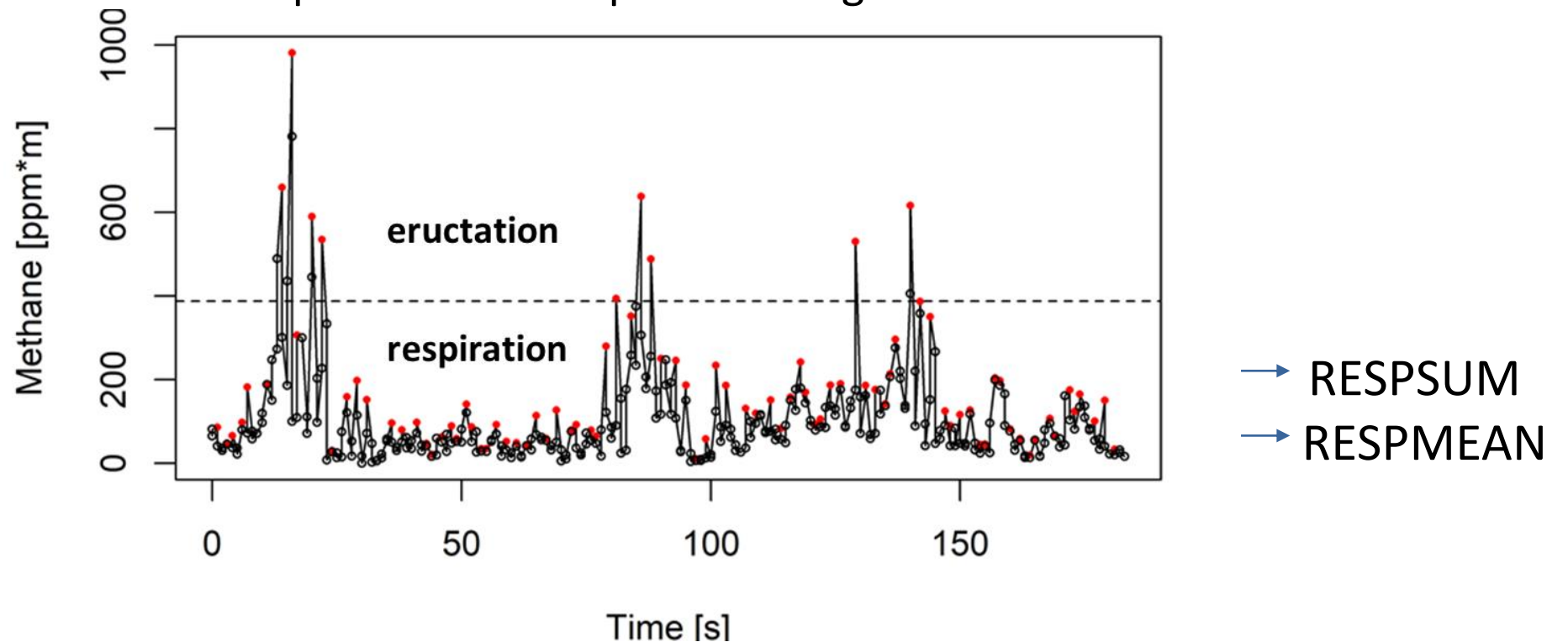
# Methane measurements with a LMD

- Laser methane detector mini (Crowcon Laser Methane Mini, Tokyo Gas Engineering Co Ltd., Tokyo, Japan)
- Original use for leak detection in pipelines or mines
- Cow fixed for the duration of the measurement (three minutes)
- Green visible laser is orientated towards the cow's nostril
- ~ 1 m distance between LMD and cow
- Measured value is expressed in ppm\*m, approx. 360 methane values per observation, 2 values/sec
- LMD connected via bluetooth to a tablet, save values as single excel sheets for every observation
- Recording of „disturbing environmental impact“
  - > Humidity, wind speed, temperature



# Trait preparation

- Measured values filtered by direct emission and diffusion effect in air (low values)
  - low values from methane emission profiles of individual animals are not considered
- Adaptation of individual profiles to physiological aspects of methane production (Chagunda et al., 2013 & Berends et al., 2014) → partitioning into respiration and eructation fractions
- Statistical method for this adaptation: use of quantiles to generate a threshold



# Pre-correction for environmental effects and technical effects

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$$\text{Model 1: } y_{ijklm} = W_i * T_j * H_k + \text{Time}_l + \text{LMD\_O}_m + e_{ijklm}$$

$y_{ijklm}$  =  $\sqrt{\text{RESPSUM}}$ ,  $\sqrt{\text{RESPMEAN}}$  in ppm\*m

$W_i$  = covariable windspeed (m/s) as polynomial of 4th order

$T_j$  = covariable temperature (°C) as polynomial of 3rd order

$H_k$  = covariable humidity (%) as polynomial of 3rd order

$\text{Time}_l$  = fixed effect observation-time (5 classes: a 2 hours)

$\text{LMD\_O}_m$  = fixed LMD\*observer effect (4 classes: 2 LMD devices combined with 3 observers)

$e_{ijklm}$  = random residual effect



# Estimation of genetic parameters and GWAS

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$$\text{Model 2: } y_{ijklmnop} = CA_i + LA_j + HS_k + CY_l + DC_m + R_n + a_o + pe_p + e_{ijklmnop}$$

$y_{ijklmnop}$	= residuals of the methane traits (RESPMEAN, RESPSUM)
$CA_i$	= covariable calving age (month)
$LA_j$	= fixed lactation effect (1., 2., $\geq 3.$ lactation)
$HS_k$	= fixed herd*season effect (season 1-4*7 different farms)
$CY_l$	= fixed effect calving year (2017 – 2021)
$DC_m$	= fixed effect lactation stage (3 classes: dry cow, heifer, dairy cow)
$R_n$	= fixed effect of feeding ration (4 classes: high energy, medium energy, low energy and experimental ration (ammonia/phosphorus reduced))
$a_o$	= random additive-genetic animal effect (pedigree & genomic)
$pe_p$	= random permanental environment effect
$e_{ijklmnop}$	= random residual effect

# Detection of candidate genes

- Results from GWAS -> significant single nucleotide polymorphism (SNP) above suggestive threshold
- Searching for potential candidate genes 100k ± significant SNPs at the chromosome
- Potential candidate genes close to SNP are related to the methane traits (RESPMEAN or RESPSUM)

**e!Ensembl** BLAST/BLAT | VEP | Tools | BioMart | Downloads | Help & Docs | Blog

**Cow (ARS-UCD1.2)**

Location: 7:8,179,440-8,205,483 Gene: SLC1A6 Transcript: SLC1A6-201

**Gene-based displays**

- Summary
  - Splice variants
  - Transcript comparison
  - Gene alleles
- Sequence
  - Secondary Structure
- Comparative Genomics
  - Genomic alignments
  - Gene tree
  - Gene gain/loss tree
  - Orthologues
  - Paralogues
  - Ensembl protein families
- Ontologies
  - GO: Molecular function
  - GO: Cellular component
  - GO: Biological process
- Phenotypes
- Genetic Variation
  - Variant table
  - Variant image
  - Structural variants
- Gene expression
- Pathway
- Regulation
- External references
- Supporting evidence
- ID History
  - Gene history

**Gene: SLC1A6** ENSBTAG00000013502

**Description** solute carrier family 1 member 6 [Source:VGNC Symbol;Acc:VGNC:52881]

**Location** [Primary assembly 7: 8,179,440-8,205,483](#) forward strand.  
ARS-UCD1.2:CM008174.2

**About this gene** This gene has 1 transcript ([splice variant](#)), [196 orthologues](#) and [6 paralogues](#).

**Transcripts** [Hide transcript table](#)

Transcript ID	Name	bp	Protein	Biotype	UniProt Match	Flags
<a href="#">ENSBTAT00000017953.6</a>	SLC1A6-201	2003	<a href="#">564aa</a>	Protein coding	<a href="#">A6H774</a>	Ensembl Canonical APPRIS P1

**Summary**

**Name** [SLC1A6](#) (VGNC Symbol)

**Ensembl version** ENSBTAG00000013502.6

**Gene type** Protein coding

**Annotation method** Annotation produced by the Ensembl [genebuild](#).

[Go to Region in Detail](#) for more tracks and navigation options (e.g. zooming)

**Genomic Track:** 8.17Mb to 8.19Mb. Tracks include Genes (Ensembl), Contigs, and Genes (Ensembl). The SLC1A6-201 gene is highlighted in green, and the ENSBTAT00000084320 gene is highlighted in red. The reverse strand is indicated at the bottom.

**Configuration:** Configure this page, Custom tracks, Export data, Share this page, Bookmark this page

# Genetic parameters for methane

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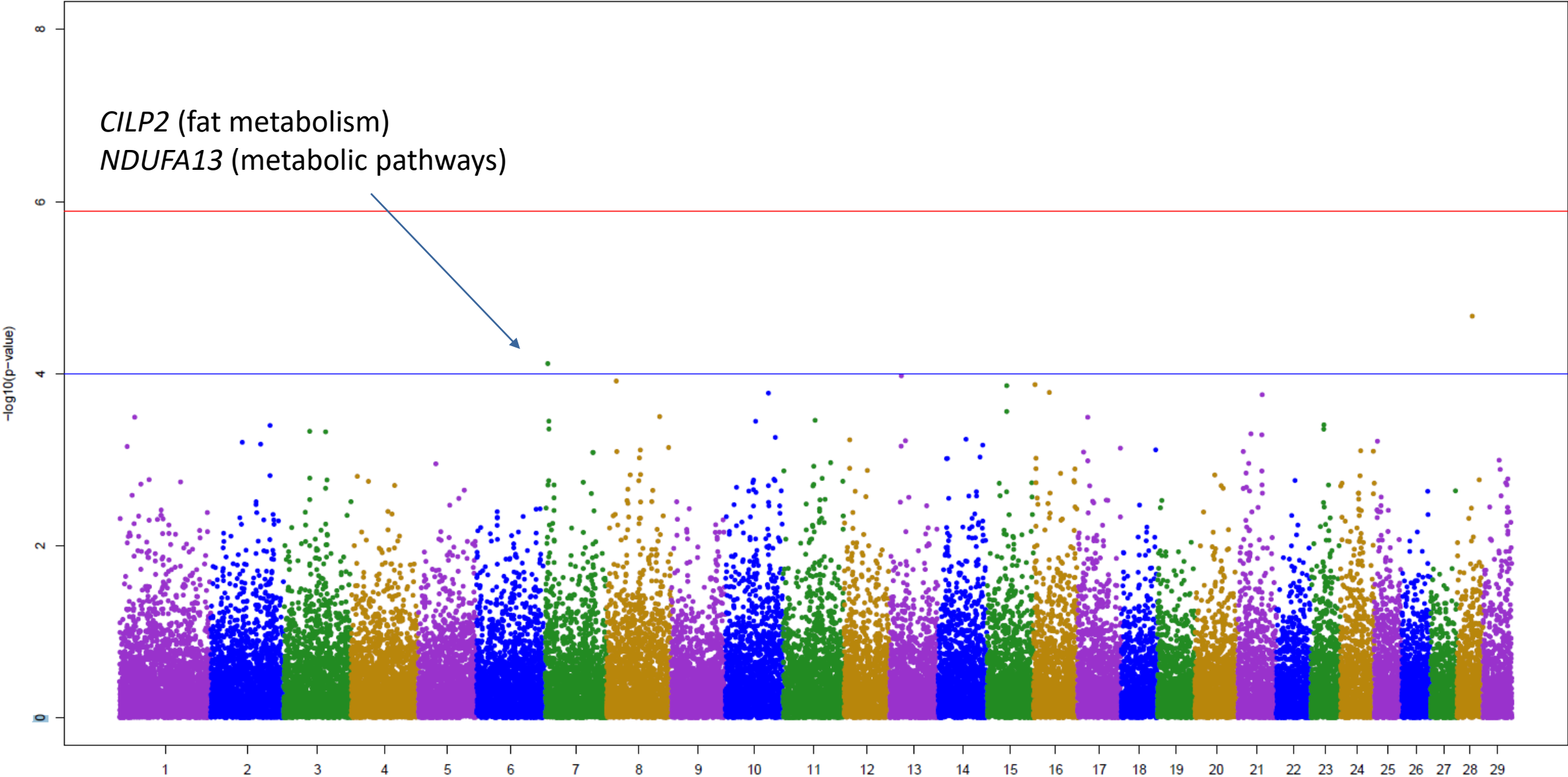
Trait	h <sup>2</sup> (SE)	$\sigma_a^2$ (additive-genetic variance)	$\sigma_e^2$ (residual variance)	r <sub>p</sub>
RESPSUM	0.024 (0.03)	1.96	179.45	0.97
RESPMEAN	0.025 (0.02)	0.04	2.47	

# Breeding value correlations between CH4 and other traits

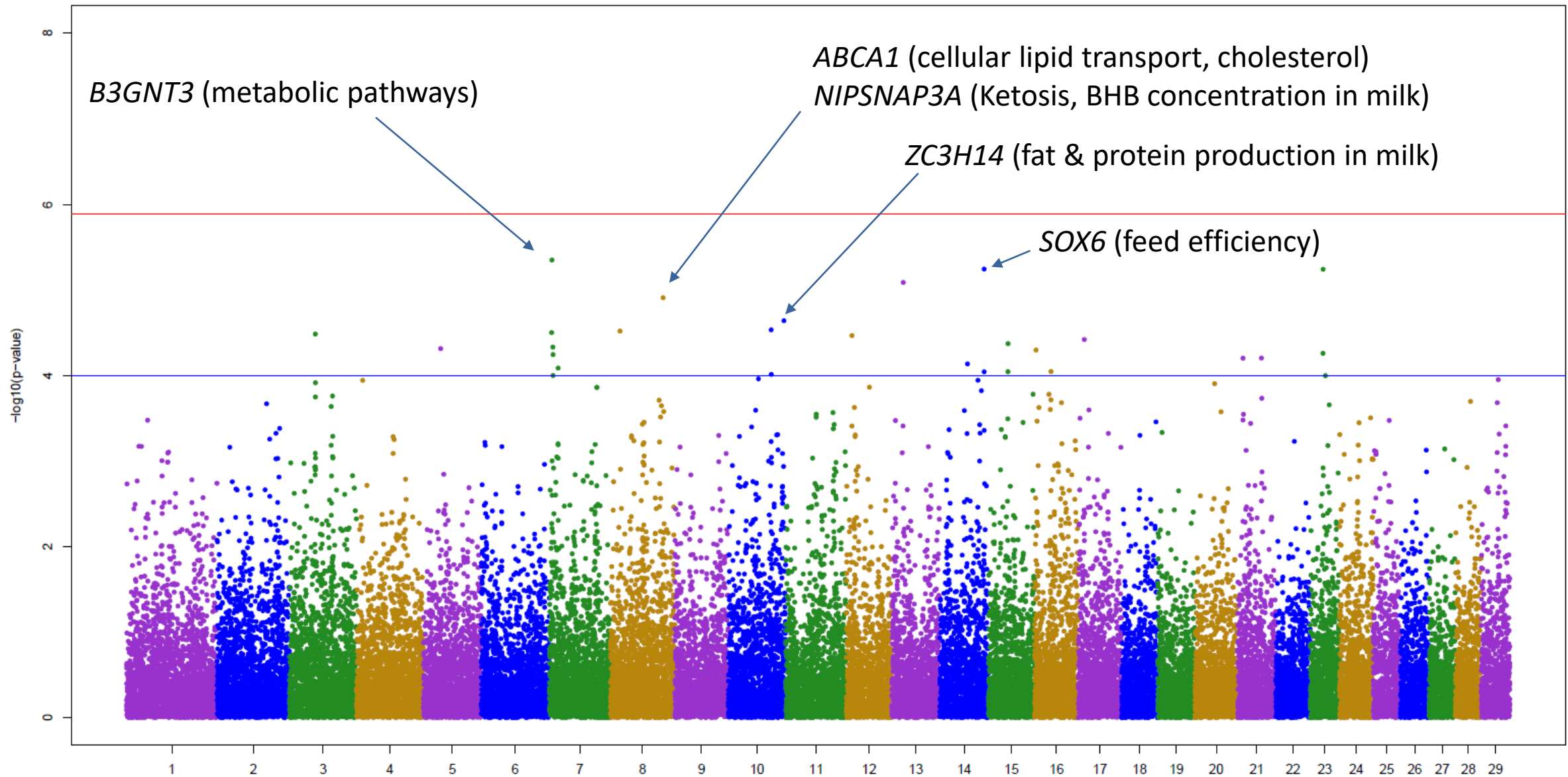
Trait	RESPSUM	RESPMEAN
Milk yield	0.02	0.02
ECM	0.03	0.07
SCS	0.03	0.06
Protein yield	0.11	0.12
Protein-%	0.16	0.17
Fat yield	0.05	0.07
Fat-%	-0.05	0.06
Age at first calving	0.36	0.28
Time from calving to first insemination	-0.15	-0.13

n = 126 sires with at least 5 daughter performances

# GWAS for RESPMEAN



# GWAS for RESPSUM



# Conclusion

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- Low heritabilities, based on measuring method with LMD, higher heritabilities possible with other measuring methods
- RESPSUM trait more interesting from a breeding perspective, because of quite high additive-genetic variance
- Genetic correlations between methane traits and other breeding goal traits close to zero: Breeding on reduced methane no detrimental impact on production and functional traits
- GWAS: No significant SNP-effects above the strong Bonferroni-threshold: Indication that methane is a polygenic trait. Nevertheless, annotated potential candidate genes have functions in metabolic pathways
- Further genomic studies should focus on the genetic architecture of the rumen microbiome

# Thank you for your attention!

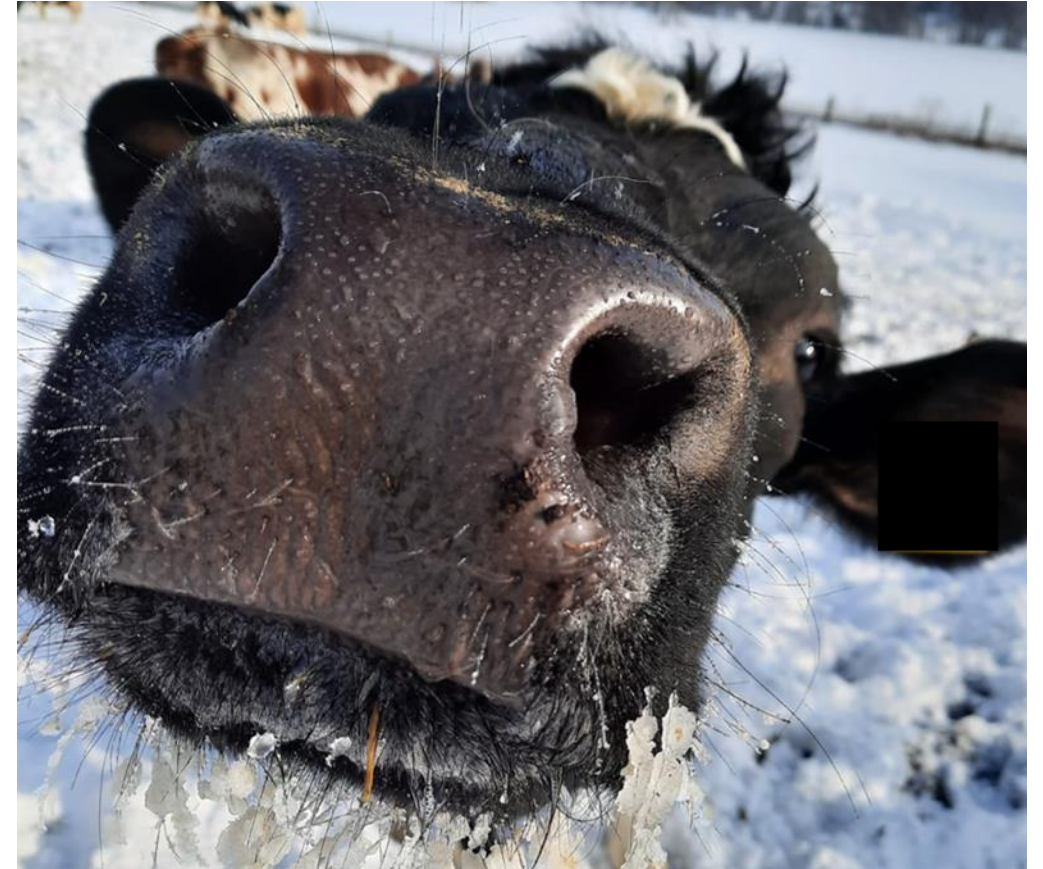
This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE), grant number 2819ERA09A



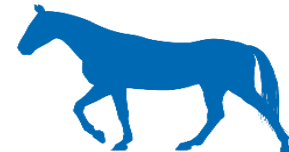
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