Institute of Agricultural and Nutritional Sciences
Martin-Luther-University Halle-Wittenberg
Group Animal Breeding

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and Industry Meeting
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German Projects for Genomic Based Breeding Programs in Holstein

H. H. Swalve
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New phenotypes

- Precision on the genetic side also demands precision on the phenotype side

- New possibilities due to new technologies
  - Markers in the milk
  - Mid-Infrared spectral data
  - Measuring methane
  - etc.

- "Old" traits - revisited
  - Early cell counts
  - Early culling
  - Fat : protein ratio at fixed points in the course of the lactation
  - New definitions for calving ease and stillbirth

➜ **Develop new traits, especially for improved health**

➜ **Increase precision in traditional traits**

➜ **Give considerations to specific environments (➜ G x E)**

In the era of genomics
phenotype is the king

(Coffey, 2010, WCGALP Leipzig)

Test herds (contract herds / cooperator herds)

❖ Traditional: test inseminations for young bulls

❖ Today: Collect more and precise data, genotype cows, establish genomic selection for traits that can not be recorded in the entire population

Why test herds for health traits?
➔ Different levels of data collection

Ordinary collection of cases
(if a cow is treated – record this)

⬇

Assess health status of entire contemporary group (herd)
(classify diseased and healthy cows at one point in time)

⬇

Record markers that are informative for a pre-disposition
(without the animals being diseased)

Züchterische Möglichkeiten zur Verbesserung der Tiergesundheit

H. H. SWALVE[1]

Test herds in Mecklenburg-Vorpommern und Brandenburg

Living animals

	RBB	RMV (RA)
Test herds	62	30
Project started	Oct. 2009	Oct. 2005
Ø # of cows/herd (Sep 2013)	587 cows (58 TH)	869 cows (29 TH)
N calvings Year 2012/13	37.418	27.321

Already collected:

	RBB	RMV (RA)
Diagnoses	1.157.336	1.105.039
First diagnoses	558.609	574.968
Claw trimming	225.856	6.130
Herds w claw trimming established	51	3

Plus:
Weights of calves
Weights of heifers

The Kuh-L project (pronounce: **cool**)
Calibration sample made up from cows: Traits

❖ All standard traits
- Dairy production
- Somatic cell count
- Conformation / Type
- Reproduction
- Calving ease and stillbirth (done the traditional way)
- Longevity

❖ Traits recorded without additional costs / already implemented
- Calving ease and stillbirth with supervised recording and weight of calf (living calf / dead calf)
- All diseases /treatments / disorders according to health key codes

❖ Based on standard milk recording but neglected so far
- Fat : protein ratio in early lactation
- Energy balance from indirect calculations
- Spectral data / fatty acids in milk

❖ Specific, new recordings
- Claw disorders recorded at time of hoof trimming

The Kuh-L project: Partners

Praxis	Funktion
FBF e.V. (Holstein organisations)	Coordinator
Rinder Allianz (test herds)	Service in all aspects of phenotyping; Samples for genotyping
RBB (test herds)	Service in all aspects of phenotyping; Samples for genotyping

Science and vit	Expertise
Group Swalve, Halle	Pilot projects, breeding program, Genomic selection, estimation of BV
Group König, Kassel	Pilot projects, breeding program, Genomic selection, estimation of BV
vit	Estimation of BV, genomic selection

Imputing for animals without sample for genotyping
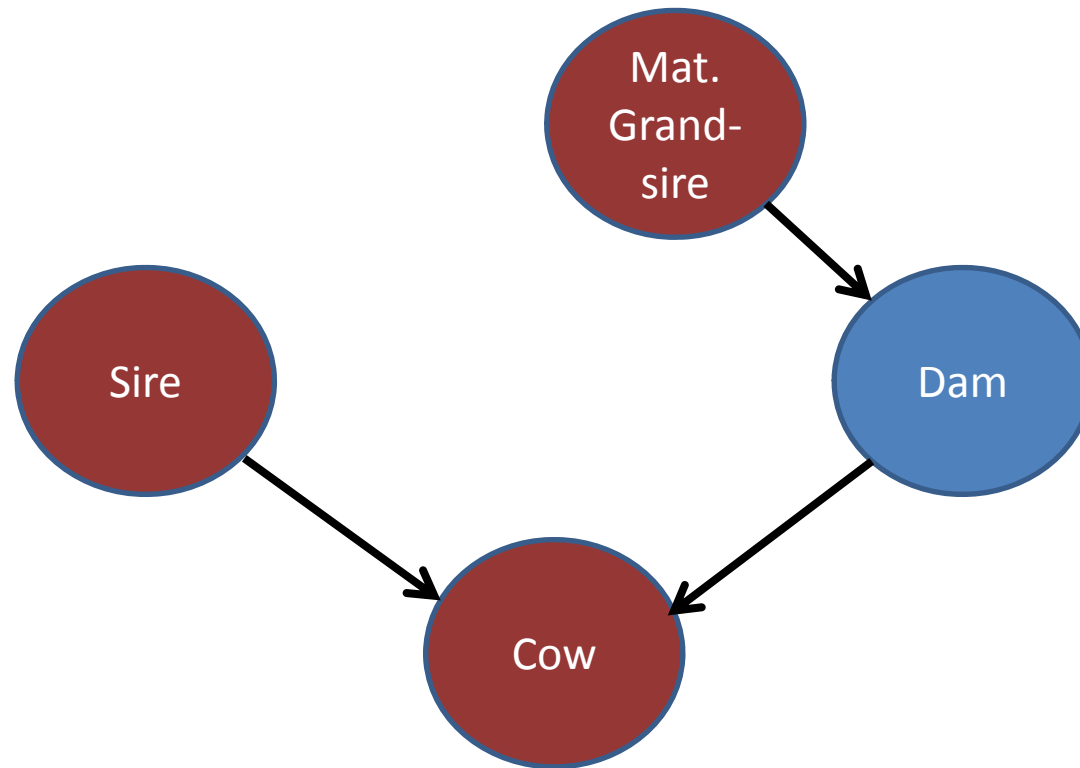
➔ Algorithms have been developed by vit and group König/Swalve

➔ it will be feasible to genotype 20.000 cows and also use 20.000 dams

Kuh-L project (pronounce: cool): As of now

❖ Selection of herds from RA and RBB test herds:
22 RA herds plus 33 RBB herds

❖ Sampling for genotyping of heifers and young cows
such they will complete a first lactation with two years
from now

❖ Preparations for claw trimming data: seminars, etc.

❖ Preparations for data flow of spectral data

❖ Samples taken: around 10,000 as of early 2015, genotypes
processed for around 7000

The SNP era: Genomic selection and GWAS

❖ Size of reference sample

❖ Accuracy of phenotypic information ➜ EBV

EBV ➜ $\hat{Y}$, weights

corrected phenotypes ⌐

h²
n records
relationships

reliability
= h² if 1 own record;
more complicated
otherwise
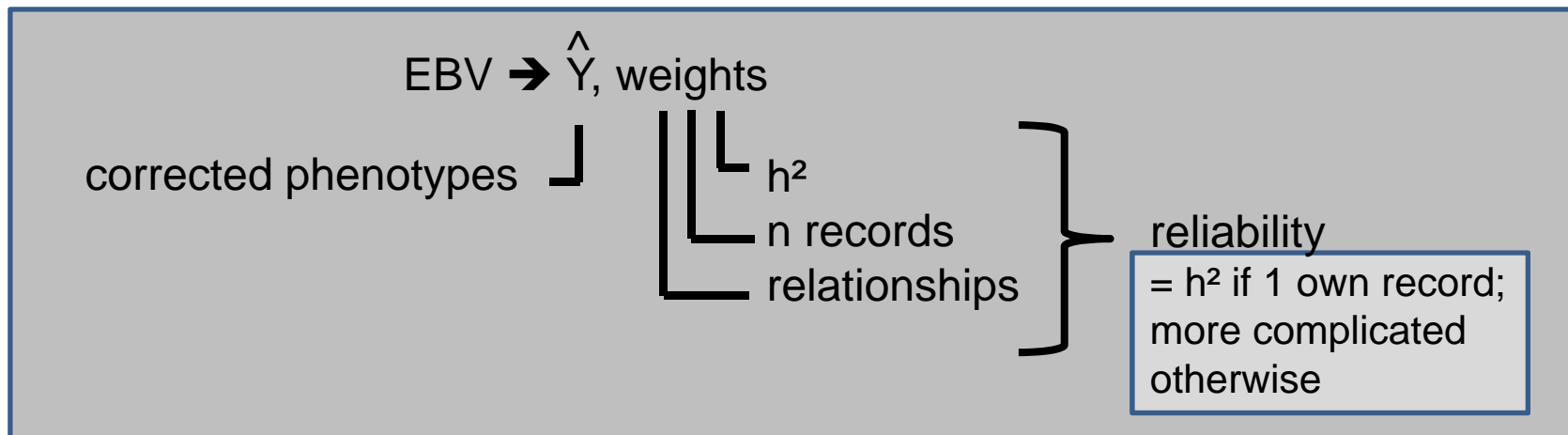
❖ Other aspects
- density of markers   today: not a problem ➜ sequence data
- extent of LD   Not a problem in most dairy breeds!?
- number of QTL   Not too many big ones, at least not in
                           standard traits

So, do we need any knowledge on functional mutations?

Individual functional mutations – why look for them?

➔ Gain more knowledge on the genetic architecture of a trait
  ➔ this will also help to understand genetic relationships between traits

❖ Problem: The identification of individual functional mutations is difficult for a number of reasons
  ➔ "missing heritability" phenomenon

"Missing" h² for diseases (other traits): reasons

❖ Sample size not sufficient

❖ Density of SNP not sufficient ➔ need sequence data

❖ CNV important?

❖ Epistasis?

❖ Other reasons?

Functional mutations: Is it a G x E problem?
Dirty field work …
➜ Diseases of the bovine hoof

Interdigital Hyperplasia (Tyloma / Limax)

Dermatitis Digitalis (BDD, "Mortellaro")

Laminitis

Dermatitis interdigitalis

White line disease

Sole ulcer

Functional mutations: Is it a G x E problem?
Sole hemorrhage / laminitis
(Swalve et al., 2014)

Laminitis

- ❖ is a non-infectious disease caused by multiple factors

- ❖ one factor is dietary carbohydrate overload leading to sub-acute ruminal acidosis

- ❖ manifests as sole hemorrhage

- ❖ we recorded any case, mild, or more severe to form one binary trait for analysis

Functional mutations:
Is it a G x E problem?
Sole hemorrhage /
Laminitis (cont.)
(Swalve et al., 2014)

- N = 1962 cows
- 7 large herds
- Slatted floor only

- Some cohorts
  with extreme
  prevalence

- 1174 cows picked
  for initial analysis
  using 384-chip
➤ Forget about
  extreme cohorts!

- 2nd analysis:
  full data

Herd-Visit	# cows	Proportion of entire data (%)	Prevalence in cohort
A_1	75	3,82	0,39
A_2	47	2,40	0,66
B_1	92	4,69	0,30
B_2	80	4,08	0,50
B_3	79	4,03	0,92
B_4	69	3,52	0,71
C_1	87	4,43	0,55
C_2	73	3,72	0,53
C_3	70	3,57	0,66
C_4	165	8,41	0,68
D_1	114	5,81	0,49
D_2	80	4,08	0,25
D_3	93	4,74	0,54
D_4	84	4,28	0,73
E_1	154	7,85	0,55
E_2	31	1,58	0,61
F_1	40	2,04	0,43
F_2	82	4,18	0,35
F_3	70	3,57	0,43
F_4	65	3,31	0,40
F_5	82	4,18	0,70
G_1	75	3,82	0,87
G_2	80	4,08	0,59
G_3	75	3,82	0,89

Functional mutations: Is it a G x E problem?
Sole hemorrhage / laminitis
(Swalve et al., 2014)

Results:

❖ (intronic) SNP within IQGAP1 = Ras GTPase-activating-like protein (BTA 21) is significant

❖ in humans and mice this gene is known to regulate neo-vascularization, preferably in limbs (knock-out mice show impaired vascularization)

Probability / Genotype	Probability for status = 1	
	Full data	Initial data
P(y = 1| AA)	.506	.369
P(y = 1| AG)	.578	.519
P(y = 1| GG)	.615	.559
Difference P(GG) – P(AA)	10.9**	19.0***

Functional mutations: Is it a G x E problem?
Sole hemorrhage / laminitis
(Swalve et al., 2014)

❖ Most likely a G x E problem, since

  ▪ on soft floors, like straw bedding, hardly any case of sole hemorrhage can be found

  ▪ on slatted floors, prevalence of laminitis / sole hemorrhage is highly dependend on "correctness" of feeding
    ➔ no overload in highly digestible carbohydrates – no disease!

  ➔ Even for a non-infectious disease, the identification of influential genes is highly dependent on the environment!

Functional mutations:
Is it a G x E problem?
➔ Interdigital hyperplasia
(Sammler et al., Ms. in prep.)

www.rene-pijl.de

IH:
➔ Highly
   heritable!

❖ Same data set as in sole hemorrhage study
❖ In total 107 cases
❖ Two herds with high prevalence
❖ Little difference in use of bulls among
   all 7 herds
❖ Drastic differences between cohorts!

Obs	Herd-visit	ncow	ih0	ih1	ihboth
1	Ahr_1	75	74	1	1
2	Ahr_2	47	40	7	4
3	Bur_1	92	90	2	1
4	Bur_2	80	74	6	5
5	Bur_3	79	69	10	8
6	Bur_4	69	66	3	2
7	Ded_1	87	85	2	0
8	Ded_2	73	73	0	0
9	Ded_3	70	68	2	2
10	Ded_4	165	160	5	5
11	GM_1	114	114	0	0
12	GM_2	80	79	1	1
13	GM_3	93	93	0	0
14	GM_4	84	83	1	1
15	Goe_1	154	153	1	1
16	Goe_2	31	31	0	0
17	Pl_1	40	33	7	4
18	Pl_2	82	66	16	11
19	Pl_3	70	63	7	2
20	Pl_4	65	59	6	3
21	Pl_5	82	69	13	9
22	Wol_1	75	64	11	7
23	Wol_2	80	79	1	1
24	Wol_3	75	70	5	3

Distribution of EBV for tyloma of sons of important sires-of-sons across quartiles of EBV (8 sires-of-sons with > 20 sons each)

		B	P	L1	A	S	R	L2	J
		nson=30	nson=27	nson=19	nson=20	nson=24	nson=27	nson=23	nson=23
		ndau=7	ndau=12	ndau=5	ndau=28	ndau=36	ndau=138	ndau=195	ndau=32
		ngdau=803	ngdau=658	ngdau=849	ngdau=500	ngdau=470	ngdau=632	ngdau=442	ngdau=657
		nob=2988	nob=2727	nob=3154	nob=1746	nob=1538	nob=2177	nob=1403	nob=1474
Best quarter	N	6	26	*15	6	16	4	2	1
	P%	2.0	0.9	1.2	1.5	1.5	1.9	1.3	2.2
Medium half	N	15	1	3	11	7	17	13	12
	P%	5.4	2.9	5.7	5.6	4.7	5.2	6.8	7.9
Worst quarter	N	9	0	1	3	1	6	8	10
	P%	23.0	0	15.3	17.2	15.9	18.4	15.8	16.8

Functional mutations: Is it a G x E problem? ➜ Interdigital hyperplasia
Results:

all cows

Herd PI

affected ↕ **controls**

SNP 8
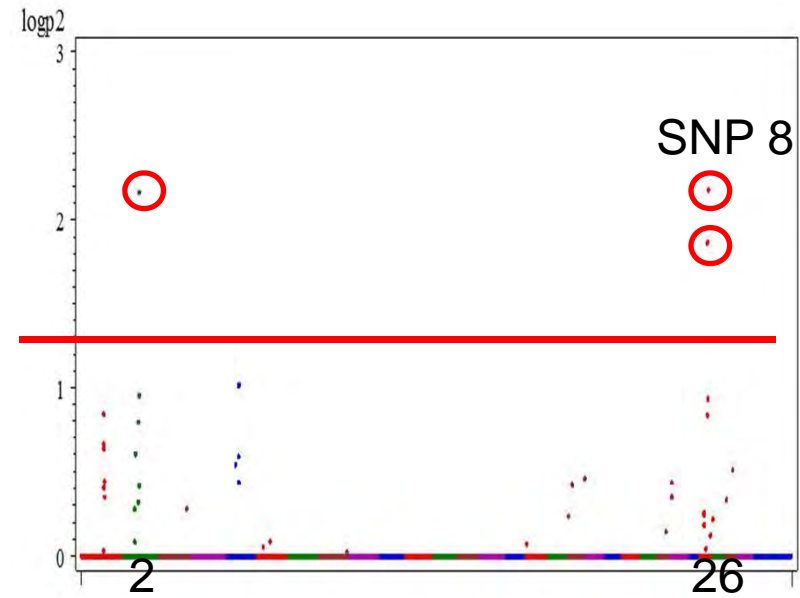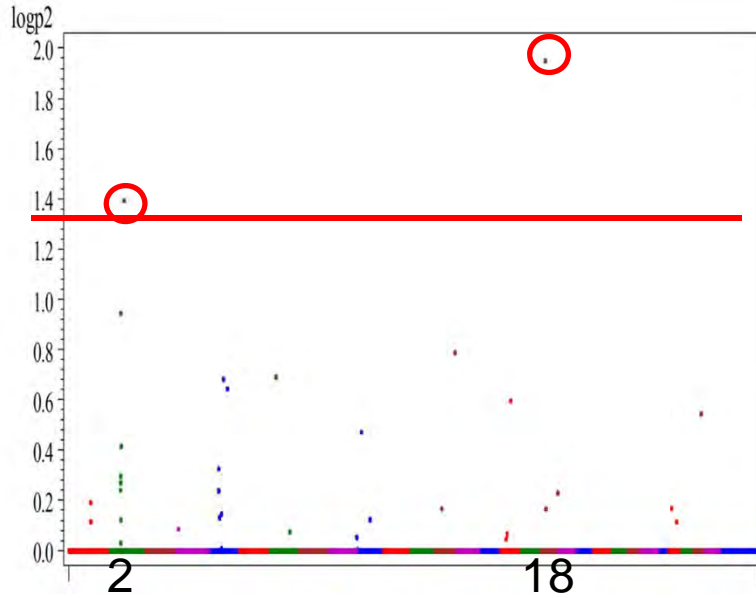
pairwise affected ↕ **controls**

SNP 4

SNP 5

SNP 8
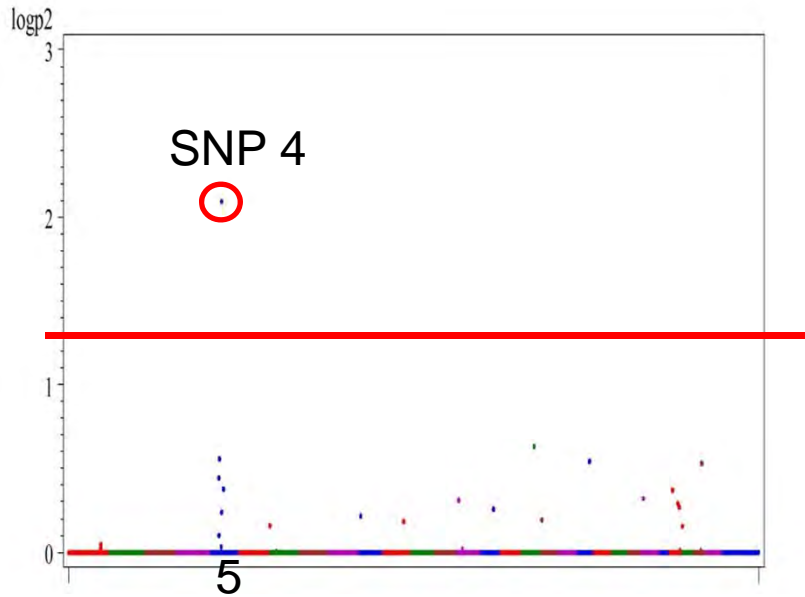
BDD / Mortellaro's Disease

= Dermatitis digitalis
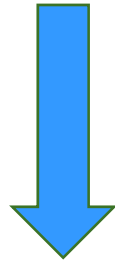
= Bovine digital dermatitis (BDD)

= Hairy heel warts

Bildquelle: Zinpro corporation 2014

- Increasing problem in Europe (S -> N)
- Proportion of animals affected varies considerably between herds
- Most often spreading at a rapid speed
- infectious

BDD – a multi-factorial disease

... bacterial infection

Treponema pallidum

Treponema (*Treponema spp.*)

- scre-shaped, actively moving

- reservoirs in deeper tissue layers

Spiral Treponemes
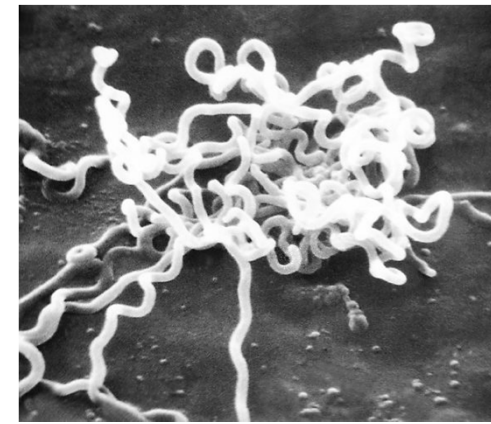
Encysted Treponemes, so-called "round-body" form
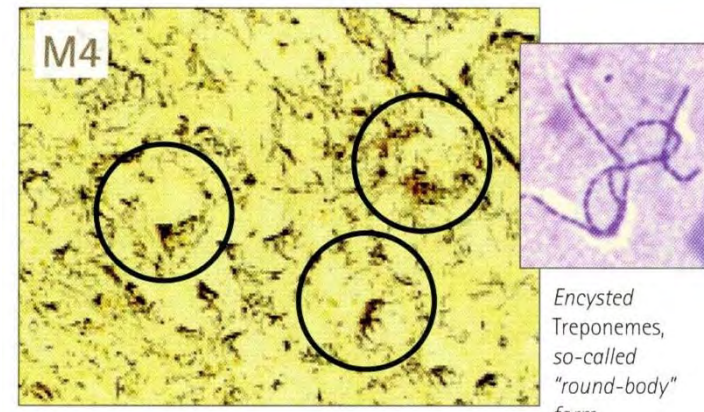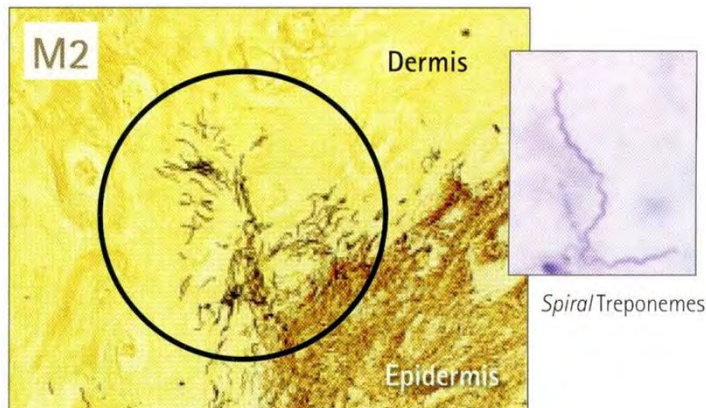
Genetic background of BDD

Knowledge so far:

- Estimates of heritability tend to be small (h^2: 0.03 – 0.14)

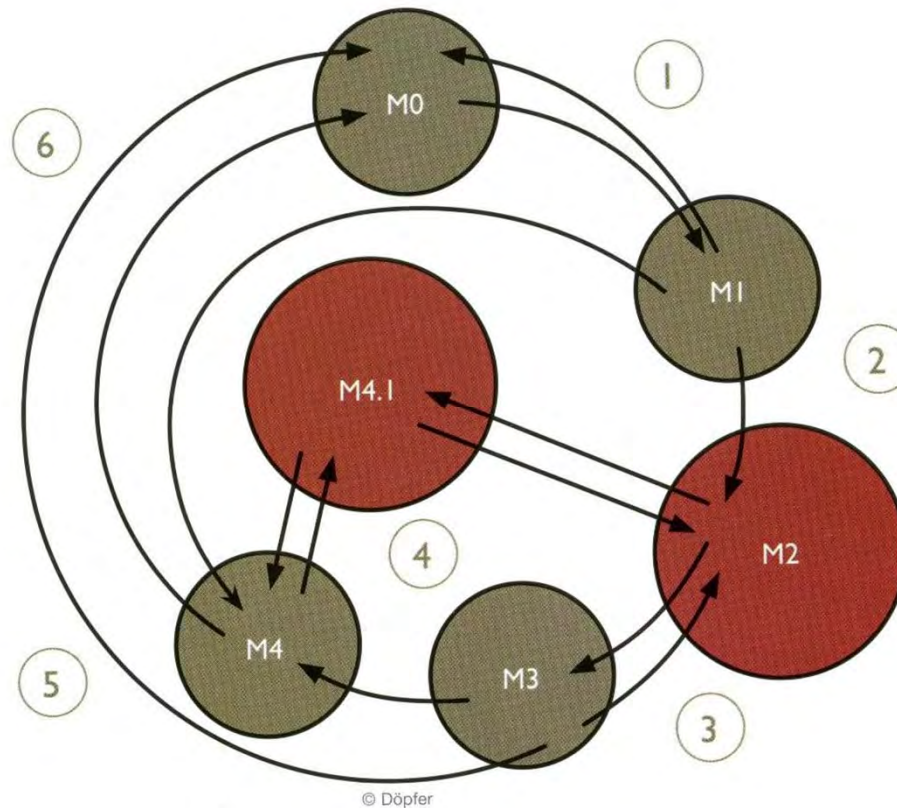-> but are mostly based on a one-time assessment or an assesment in long intervals

-> taken as binary

=>healthy / diseased (0/1)

-> Development of stages of BDD   Holzhauer et al. 2008

-> Makroscopic classification of BDD   Döpfer et al. 1997
                                        Berry et al. 2012

Classification of individual stages of BDD

© Döpfer

M0=0   healthy
M1=1   early, small
M2=2   acute, painful, active
M3=3   healing, dry
M4=4   chronic
M4.1=5 chronic, but active

→ A very dynamic disease !

DD - Stages

M1
Early stage

M2
Acute and infectious stage

M3
Healing stage

M4
Chronic stage

M4.1
Chronic stage, repeated cases, infectious

Lessons learnt from looking at individual genes

- ❖ Sample size is important
- ❖ Density of SNP is an issue ➔ may need sequence data
- ❖ CNV important?
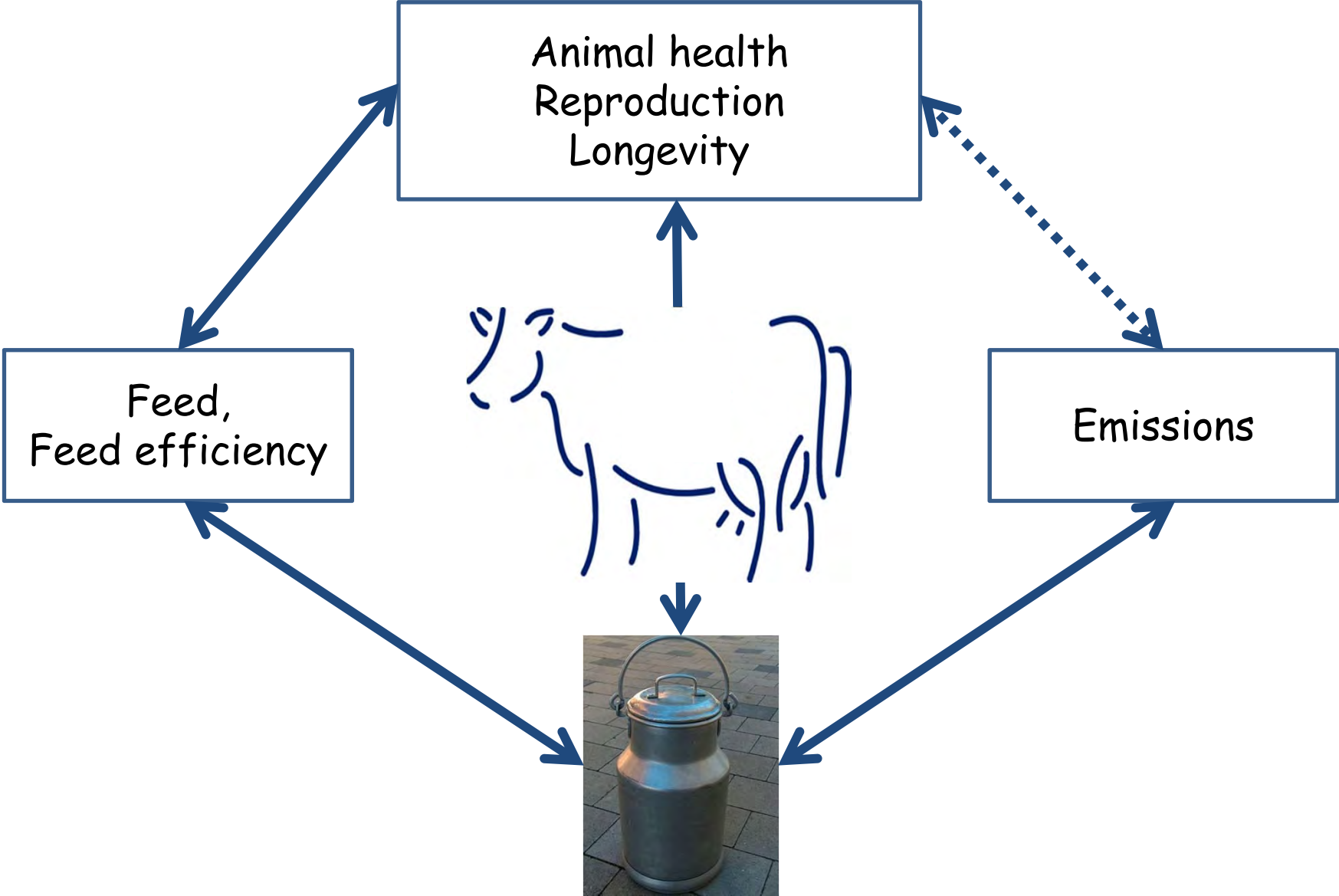- ❖ Epistasis?

- ❖ Precision of phenotypes

- ❖ G x E may mask associations in many cases

➔ In Kuh-L, we will attempt to
- ▪ Use genotypes and phenotypes for GS
  (Two-step – one-step)

- ▪ Identify functional mutations, especially for health traits

- ▪ Focus on the precision of phenotypes

Next: Ressource efficiency

Animal health
Reproduction
Longevity

Feed,
Feed efficiency

Emissions

# Residual feed intake

❖ is an animal breeder's terminology (Arendonk et al., 1991)

❖ defined as
      Actual feed consumed : Calculated requirement
                                  (Maintenance, production,
                                  change of weight)

❖ is an <u>estimated</u> figure

❖ varies by up to 15 % between cows (all corrections done, identical feed) – Connor et al. (2012)

Residual Feed Intake (RFI):
Dashed line = feed intake as calculated,
Colored points = individual cows
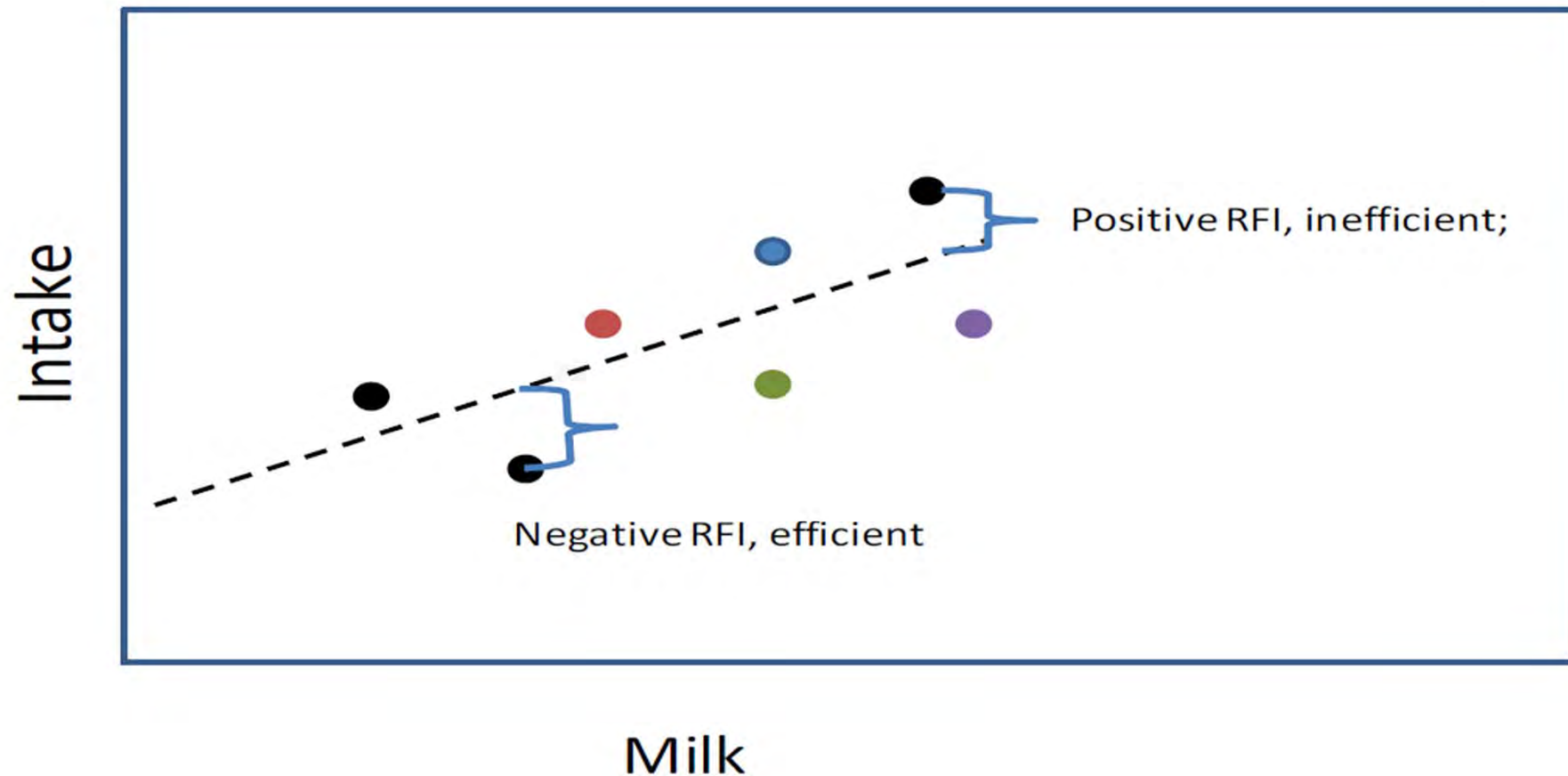
Positive RFI ➔ cow is not efficient
Negative RFI ➔ cow is efficient

Possibilities for breeding

❖ RFI exhibits heritability of 40 %

❖ RFI is correlated with feed intake

❖ Heritabilities and correlations vary in the course of lactations

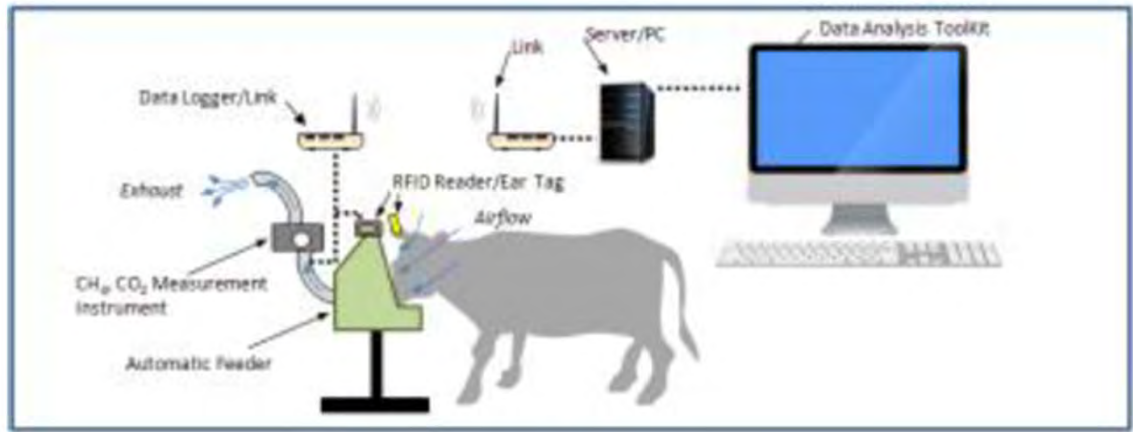❖ Optimale definition of RFI in the course of the lactation yet unclear

Predicted methane emission (PME) and RFI
(de Haas et al., 2011)

❖ both parameters are heritable (0.35 to 0.40)

❖ Selection would be possible

❖ Genetic correlations between PME and RFI vary
  in the course of the lactation:

0-42	1-5	6-10	11-15	16-20	21-25	26-30
0.32	0.84	0.50	0.18	0.21	0.34	0.43

❖ Need to collect data on methane emissions to verify this

Measuring of methane emissions in a mobile way on farms
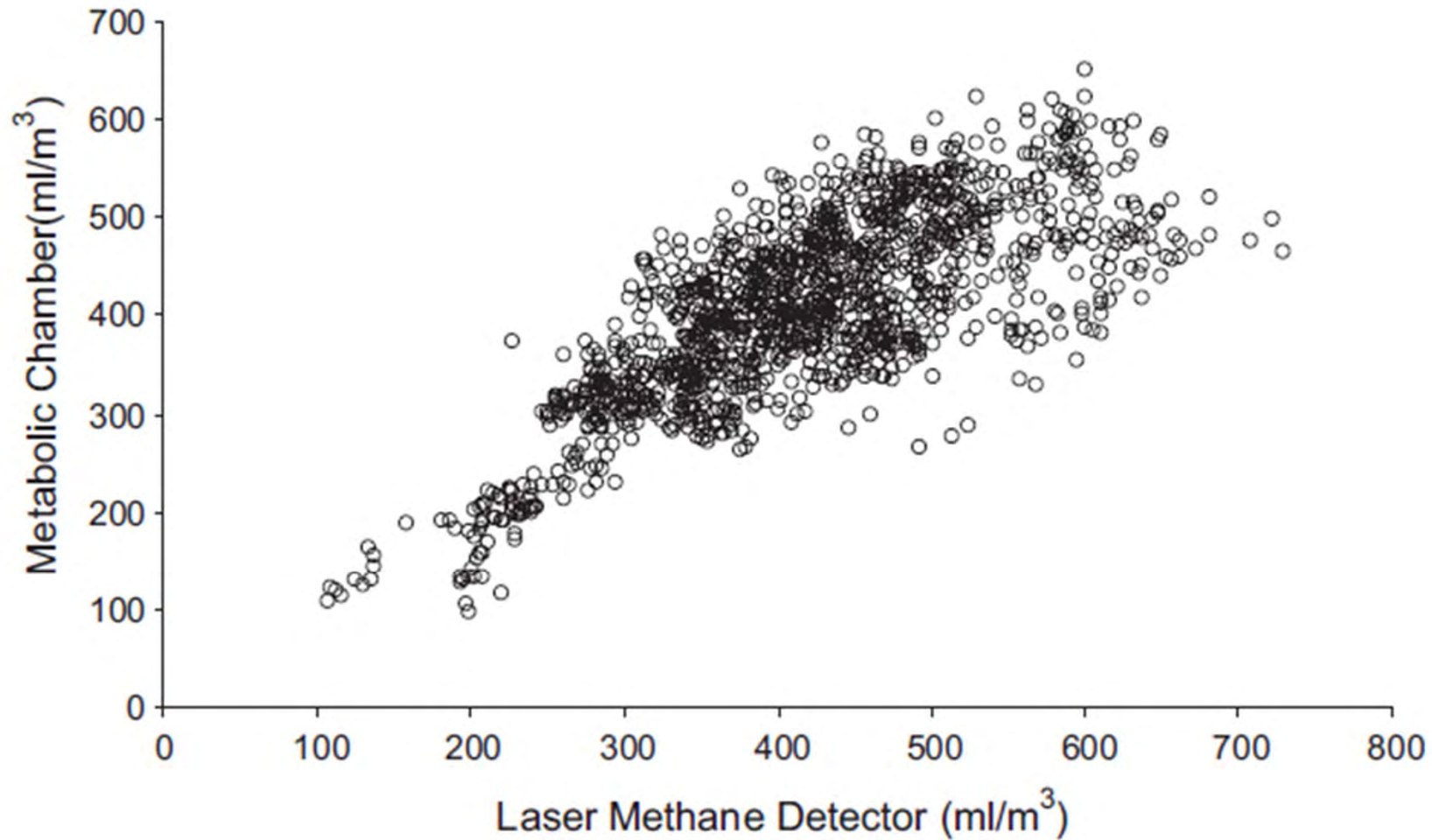

GreenFeeder

Laser

FTIR
(pictures by Jan Lassen)

LIVESTOCK RESEARCH
WAGENINGEN UR

Measuring methane (Halle team; Diana Sorg et al.; Feb 2015)

Measuring point, marked by laser

Two laser devices, BT-connected with smartphones

Comparing methane emissions
Respiration chamber – Laser-Methane-Detector

Chagunda und Yan (2011)

Spectral data: Relationship with methane emissions

Dehareng et al. (2011)
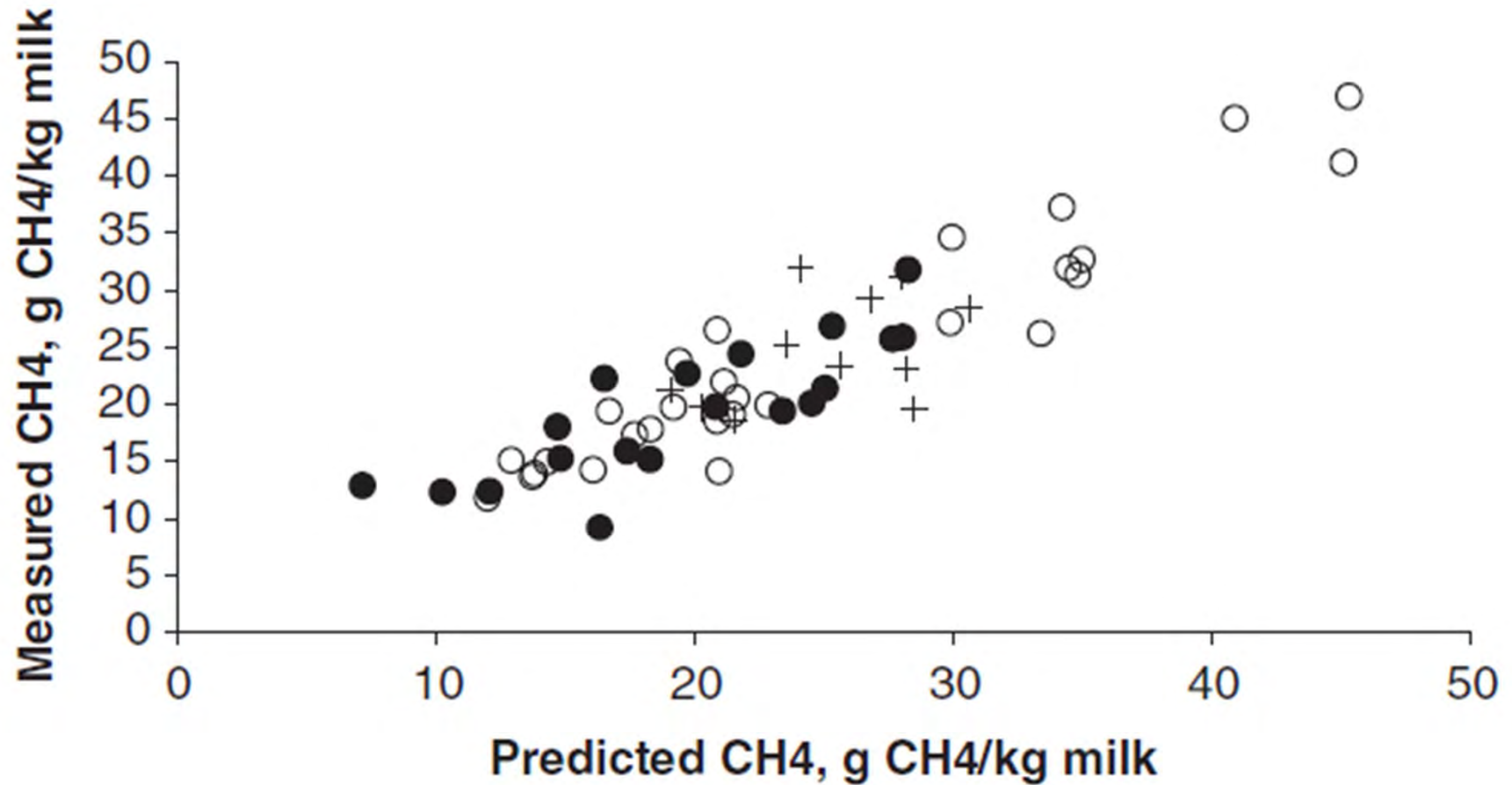
Spectral data in routine laboratories of milk recording agencies:

Here: Determination of fatty acids
(Gengler, 2009)

Fatty acids (g/dl)	Mean	SD	SEC	R^2C	SEcv	R^2cv	RPDcv
C4:0						0.86	2.69
C6:0						0.91	3.41
C8:0						0.87	2.80
C10:0						0.84	2.49
C12:0						0.84	2.48
C14:0						0.90	3.14
C14:1						0.36	1.25
C16:0						0.83	2.46
C16:1						0.32	1.22
C18:0						0.85	2.62
C18:1 trans						0.88	2.83

CombiFoss™ FT+

Already a few studies have been published that established relationships between spectral data and:
- Feed intake – feed efficiency
  – Fatty acids composition
- Methane emissions of individual cows
- The microbiom of individual cows
- Reproductive traits

Possibilities for breeding to improve resource efficiency

❖ Genetic improvement of health, reproduction, and longevity
- not of utmost importance for resource efficiency, but also of importance for animal welfare
- Methods: <u>Genomic Selection</u>, phenotyping in test herds
  - ➔ KUH-L project

❖ Genetic improvement of feed efficiency / Reducing green house gas
- many scientific questions yet to be answered
- Large team effort of nutritionists and geneticists
  - ➔ New, upcoming project
    - ➔ Recording of feed intake, respiration chambers, mobile measurements of methane, relationships with health ➔ test herds
- Application through <u>Genomic Selection</u>

Conclusions

❖ The breeding organizations of German Holsteins have
   applied Genomic Selection with great success

❖ Now it is the era of precise phenotyping

❖ Highly innovative projects are underway that will help
   to continue to breed the robust and functional dairy cow