Protein-reduced feeding of dairy cows supplemented with rumen-protected amino acids

Wasem Daniela, Stettler-Abraham Andrina & Probst Stefan

Berner Fachhochschule BFH, Hochschule für Agrar-, Forst- und Lebensmittelwissenschaften HAFL, 3052 Zollikofen Contact: stefan.probst@bfh.ch

Optimizations in feeding are particularly effective for reducing nitrogen losses, since a reduction in nutrient input can automatically lower the potential for losses along the entire production chain. The aim of this study was to demonstrate the effects of a protein-reduced ration in practical dairy cattle feeding in a pilot trial.

Material and methods

- Two farms for two winter feeding periods, total 141 animals
- Crossover experimental design with experimental and control group
- Reduction of the crude protein (CP) content of the ration by 10 g/kg DM in the experimental groups (table 1)
- Supplementation of the experimental rations with rumen-protected lysine and methionine to the level of the control group
- 3 weeks adaptation phase, 1 week survey phase, then change of group

Table 1: Overview of the rations in the trial (K = control group, V = experimental group)

	Rütti 2019/20		Rütti 2020/21		Wallierhof 2019/20		Wallierhof 2020/21	
NEL MJ/kg DM	6.1	6.1	6.0	6.0	6.7	6.7	6.6	6.6
CP g/kg DM	152	141	151	139	168	160	170	160
APDE g/kg DM	86	82	93	87	112	108	101	95
APDN g/kg DM	98	90	100	90	114	108	113	106

Statistical analysis was performed using R software and the ImerTest package (Kuznetsova et al. 2017) in a mixed linear model with fixed and random effects.

Model:

$y_{ijklmn} = u + Animal_i\ (random) + Farm/Year_j + Group_k + (Farm/Year \times Group)_{jk} + Lactation_l + Days\ in\ Milk_m + Rest_{ijklm}\ (random)$

Selected results

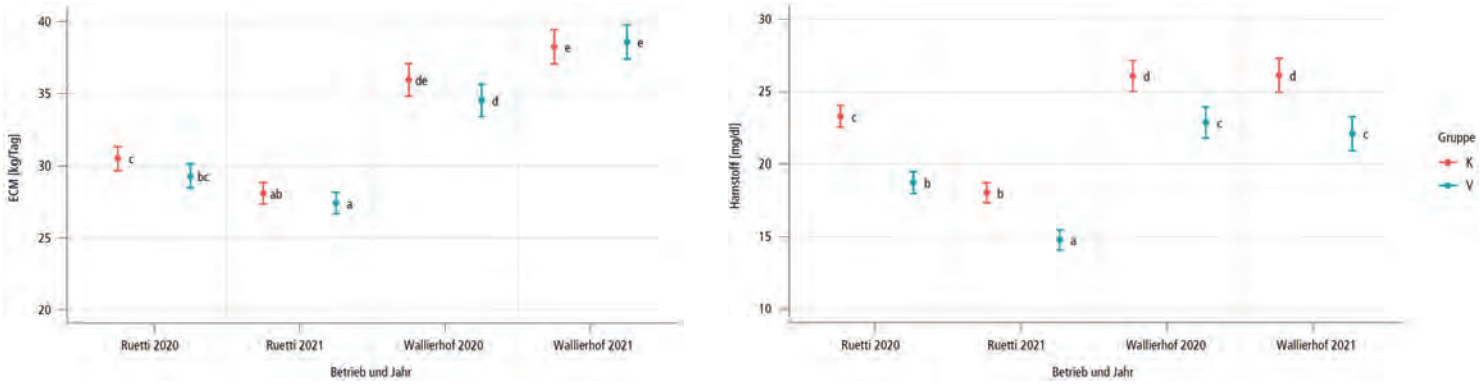

Figure 1: Energy corrected milk yield (ECM) and milk urea content (K = control group, V = experimental group). Estimated means ± standard error

Conclusions

- A reduction of crude protein levels in dairy cattle rations is possible without negative effects on milk yield when compensated by rumen-protected amino acids.
- Reducing protein intake leads to significantly lower milk urea levels and nitrogen excretion.
- A reduction of ammonia emissions by 10% seems to be realistic in the future with the help of protein-reduced dairy cattle feeding.
- Consideration of amino acids other than lysine and methionine, especially histidine, is likely to be necessary.

Excretion and apparent retention of nitrogen in slow-growing ROSS fattening chickens as affected by different feed additives

Daniel Brugger[1], Stefan Grossmann[2], Annette Liesegang[1], Matthias Schick[3], Roger Bolt[3]

[1]Institute of Animal Nutrition and Dietetics, Vetsuisse-Faculty, University of Zurich, Switzerland
[2]Unipoint AG, Ossingen, Zürich, Switzerland
[3]Agrovet Strickhof, Fachbereich Tierhaltung, Lindau, Switzerland

Background

Increased microbial ammonia production in the hindgut associated with high dietary crude protein may pose a burden for animal health and the stabling environment[1]. Some additives are claimed to adsorb ammonia or to bind its nitrogen in microbial protein.

How do these additives affect the excretion of total and NH_3-nitrogen as well as apparent retention of nitrogen and dry matter?

Conclusion

- Treatment groups received less digstible dry matter due to the nature of the respective feed supplements.
- Yet, no difference to control was evident.
- This, in combination with earlier reported performance data[2], suggests improved endogenous nutrient utilization in the presence of the additives.
- We currently estimate the endogenous N utilization via the quantification of uric acid in faeces.

Results

	Control	Klinofeed	Bentonit	BioChar	Arbocel	*P*
DM intake, kg	2.33 *±0.26*	2.19 *±0.34*	2.32 *±0.25*	2.22 *±0.20*	2.26 *±0.17*	*0.59*
DM excretion, kg	0.30 *±0.03*	0.27 *±0.04*	0.29 *±0.03*	0.28 *±0.03*	0.28 *±0.03*	*0.40*
DM retention, %	87.3 *±1.01*	87.6 *±1.33*	87.5 *±1.24*	87.1 *±1.17*	87.7 *±0.94*	*0,74*
N intake, g	73.8 *±8.11*	69.8 *±10.7*	72.3 *±7.75*	69.2 *±6.21*	71.3 *±5.31*	*0.61*
N excretion, g	12.7 *±1.86*	11.4 *±2.00*	11.8 *±1.44*	12.0 *±1.29*	11.6 *±1.14*	*0.28*
N retention, %	82.7 *±2.43*	83.6 *±2.30*	83.6 *±2.20*	82.6 *±1.65*	83.7 *±1.67*	*0.49*
NH_3-N conc., g/kg DM	1.26 *±0.25*	1.18 *±0.27*	1.27 *±0.25*	1.15 *±0.51*	1.32 *±0.25*	*0.72*
NH_3-N excretion, g/kg N	29.5 *±6.40*	28.0 *±5.55*	30.9 *±5.28*	27.1 *±11.8*	31.7 *±6.34*	*0.53*

- **In the end of fattening, no significant difference in any measure was observed between groups.**
- **Treatment groups received lower digestible dry matter due to the nature of the additives, therefore, absence of any difference to control suggests superior endogenous utilization of energy and nutrients irrespective of the feed supplement.**

Methods

120 slow-growing mix-sex ROSS broilers, 60 cages, n=12 cages. Trial from hatch to slaughter (6.5 w).

Basal diet: FORS 2121, 13 MJ ME/kg, 200 g/kg crude protein.

Treatments: Klinofeed, Bentonit, BioChar, at 5g/kg and Arbocel at 6 g/kg.

Cage-wise quantitative collection of excreta during the last week of fattening.

Data analysis comprised mixed-models comprising “feeding group” as fixed effect and “cage” as random factor nested under the respective feeding group.

References

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Der Sortier-Mechanismus im Wiederkäuer-Vormagen schützt die Zähne vor abrasivem Material

Sarah O. Valerio[1], Jürgen Hummel[2], Daryl Codron[3], Jean-Michel Hatt[1], Marcus Clauss[1,4]

[1]Clinic for Zoo Animals, Exotic Pets & Wildlife, Vetsuisse Faculty, University of Zurich, Switzerland; [2]Ruminant Nutrition, Department of Animal Sciences, University of Göttingen, Germany; [3]Zoology & Entomology, Natural & Agricultural Sciences, University of the Free State Bloemfontein, South Africa; [4]AgroVet-Strickhof, Lindau, Switzerland

Wenn Pflanzenfresser Nahrung aufnehmen, ist immer Erde, Sand, Staub, Dreck mit dabei …

- Belegt in zahlreichen Studien mit Wild- und Haustieren.
- Hinweis, dass die Aufnahme von Erde nicht nur ein unvermeidliches Übel ist: Hält man Ferkel oder Fohlen ohne Zugang zu Weide mit Erde, dann muss man besonders auf ihre Eisen-Versorgung achten.
- Um trotz des Zahnabriebs, der beim Kauen von Erde/Sand/Staub/Dreck ausgelöst wird, lange leben zu können, haben viele Pflanzenfresser im Zuge der Evolution besonders hochkronige Zähne entwickelt.

Fragen zur vergleichenden Biologie von Pflanzenfressern

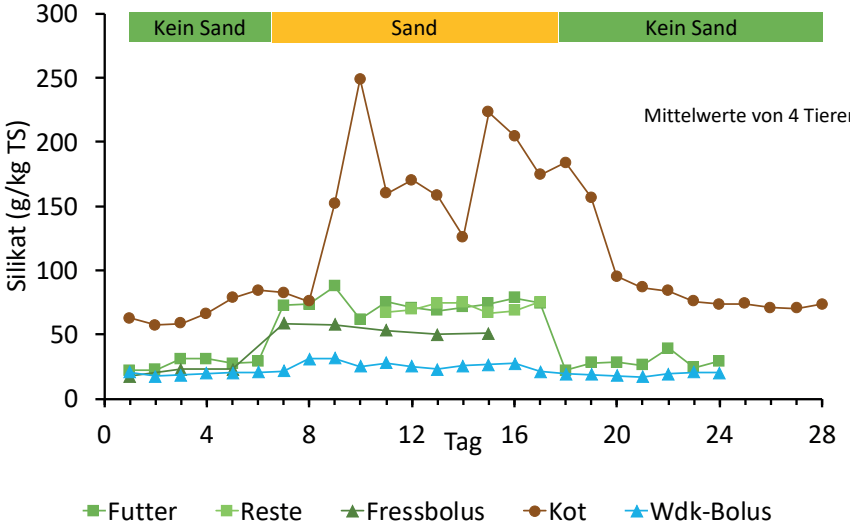
- Warum haben Wiederkäuer nicht so hohe Zähne wie Pferde, auch wenn sie genauso Grasäser sind?
- Warum kauen Wiederkäuer beim Fressen wenig gründlich, obwohl sie das – wie beim Wiederkäuen bewiesen – ja prinzipiell könnten?

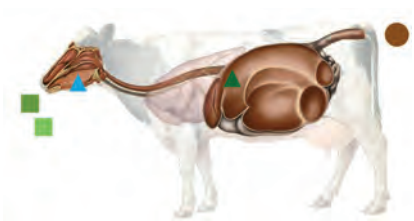
Hypothese

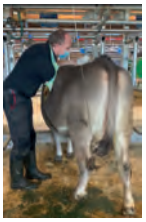
- Der Sortiermechanismus im Vormagen von Wiederkäuern (in Pansen und Netzmagen) sortiert nicht nur das Material, das zum Wiederkäuen wieder hochgewürgt wird – er wäscht es auch, so dass die Tiere beim Wiederkauen auf weniger abrasivem Nahrungsbrei kauen. Für Wiederkäuer lohnt es sich also, erst beim Wiederkäuen so richtig intensiv zu kauen.

Unsere Studie auf AgroVet Strickhof

- 4 Kühe mit Pansenfistel; Fütterung mit und ohne Sand; Beprobung verschiedener Materialien u. Analyse auf Silikat (=Sand)







Sand **im Futter** findet sich im **Fressbolus** wieder sowie v.a. im unteren Pansenbereich (hier nicht gezeigt), und mit etwas Zeitverzögerung **im Kot**.
Sand in **Futter** und **Futterresten** ist gleich – die Tiere konnten nicht selektiv fressen.

Der Sand-Gehalt **im Wiederkau-Bolus** (aus dem Maul entnommen) ist dramatisch niedriger als der im **im Futter** und im **Fressbolus** .

- Nebenbefunde:
  - keine klinischen Störungen der Tiere (passt zu Berichten, dass freilebende Tiere regelmässig Erde/Sand aufnehmen)
  - Keine Reduktion der Futteraufnahme bei Sand-Beimengung, kein Einfluss auf Tränke-Aufnahme
  - Kein Unterschiedliches Kauverhalten (gemessen mit Kauhalftern in Zeiten mit und ohne Sand-Beimengung)


PNAS
The Ruminant sorting mechanism protects teeth from abrasives

=> Hypothese des «Waschens» bestätigt

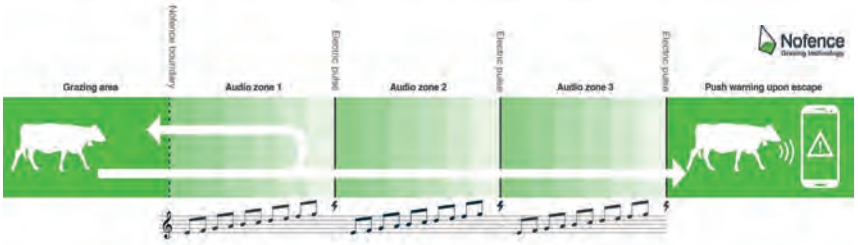
<= unsere publizierte Studie … wird im Editorial gut aufgenommen =>


PNAS
Reassessing assumptions about the evolution of herbivore teeth

Contact : Marcus Clauss mclauss@vetclinics.uzh.ch

Does age affect learning capacity and grazing activities of dairy cows managed with a virtual fencing system?

Andrea Confessore, Manuel K. Schneider, Caren Pauler, Chiara Aquilani, Patricia Fuchs, Carolina Pugliese, Camilla Dibari, Giovanni Argenti, Massimiliano Probo
andrea.confessore@unifi.it



Grazing experiment

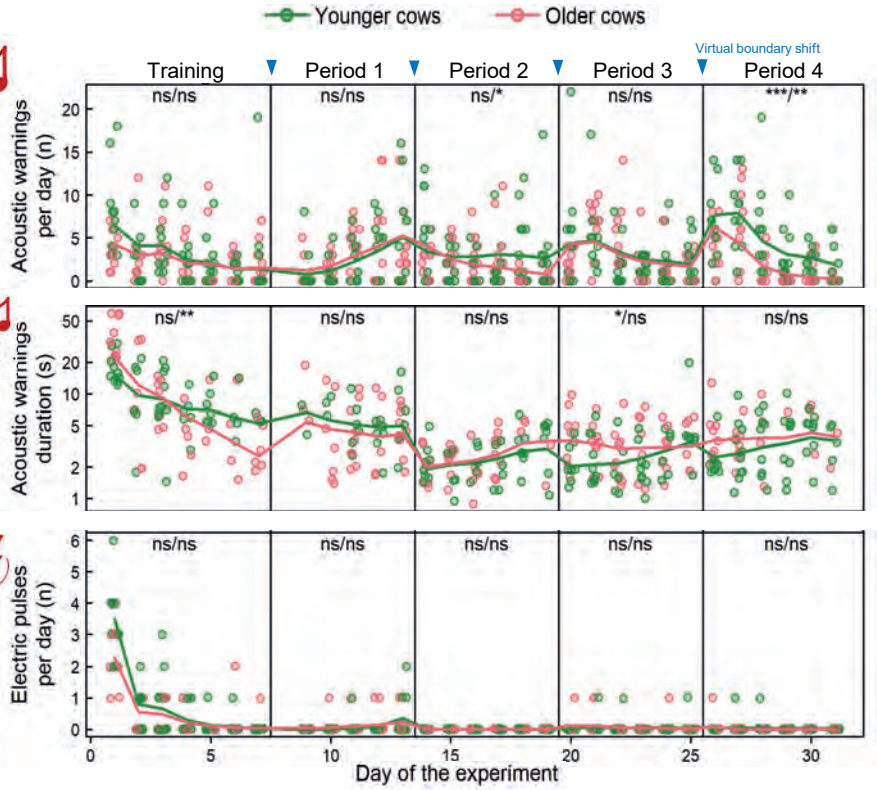
- 2 Age groups (younger and older dairy cows)
- 5 grazing periods (4 virtual boundary shifts)
- Recording of acoustic warnings and electric pulses

Results

No significant difference in acoustic warnings between younger and older cows. Only in Period 4 younger cows received more warnings.

Overall acoustic warning duration strongly decreased during training and stabilized in the following period for both age groups.

No differences in the number of electric pulses between age groups.



Data points represent values for individual cows per day, colored lines are predicted average values from the fitted generalized linear mixed-effects models. Labels within each panel show the significance of the effects of the age and within period effects. First label: age effect (younger vs older cows) within period; second label: temporal trend within each period.

Conclusion

- Cows learned virtual fencing fast.
- Age does not affect learning capacity.

Acknowledgements
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The uterine bacterial microbiome and fertility in the mare

Antonia Dyroff[1], Igor Canisso[2], Giorgia Podico[2], Carmen Almiñana[1,3], Stefan Bauersachs[1]

[1]Functional Genomics Group, Institute of Veterinary Anatomy, Vetsuisse Faculty, University of Zurich, Lindau (ZH), Switzerland
[2]Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana Champaign, Urbana, IL, USA
[3]Department of Reproductive Endocrinology, University Hospital Zurich, Zurich, Switzerland

antoniaisabelle.dyroff@uzh.ch

Swiss National Science Foundation

Introduction

Subfertility in the mare is a major issue for horse breeding and for animal welfare, due to repeated ineffective treatments. One of the main causes for subfertility is reduced uterine receptivity. Compared to humans, the uterine microbiome, transcriptome, and the molecular content of uterine extracellular vesicles (EVs) remain poorly understood in the mare. Few studies exist on the uterine microbiome of mares, indicating changes in non-pathogenic microbiota leading to inflammation and subfertility. A small number of studies analyzed changes in the endometrial transcriptome during the estrous cycle and early pregnancy to get insights into gene expression related to uterine receptivity and maternal recognition of pregnancy (MRP). Furthermore, gene expression in uterine samples collected from subfertile and healthy fertile mares have been compared. Uterine extracellular vesicles (uEVs) have been pointed to play a major role in embryo-maternal interactions and recognition of pregnancy in various mammals including the horse, with changing content during the estrus cycle and pregnancy. To further investigate the altered uterine environment of subfertile mares, combining approaches is needed, e.g., exploring the uterine microbiome composition using 16S rRNA sequencing, spatial transcriptomics of the endometrium, together with the analysis of changes in the uEVs molecular content. This thesis aims to study the uterine microbiome during the estrous cycle, early pregnancy, in mares of different age, and in mares presented at the clinic. Additionally, the study will assess how microbiome alterations affect the uterine environment, i.e., gene expression and histological changes in the endometrium, and the content of uEVs.

Hypothesis

- Bacterial composition in the uterus changes with different physiological conditions and is altered in subfertile mares.
- In subfertile mares, the altered microbiota, along with uterine extracellular vesicles (EVs), play a significant role in reduced uterine receptivity.
- Each of those initiates cellular and transcriptomic changes in the endometrium, resulting in diminished fertility.

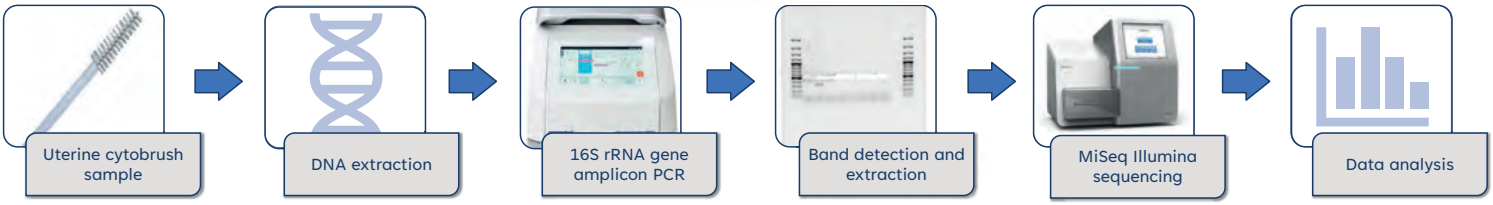
Experimental design

Physiological conditions	Subfertility
estrus - diestrus	fertile
pregnant - non pregnant	subfertile, explained
young - old	subfertile, unexplained

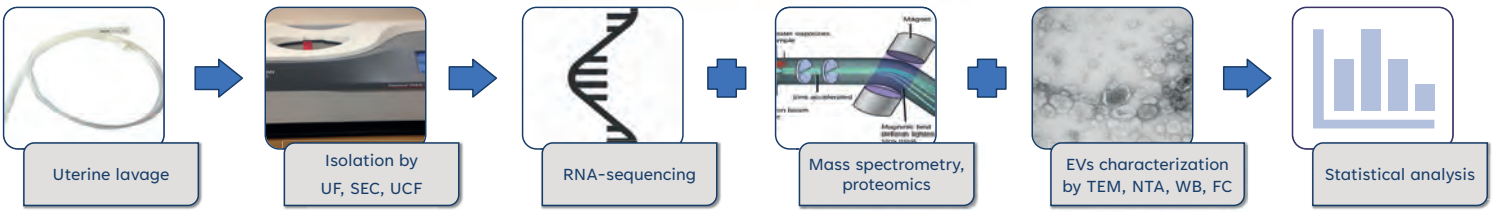
Material and methods

Sample collection at Urbana Champaign, University of Illinois, Illinois USA

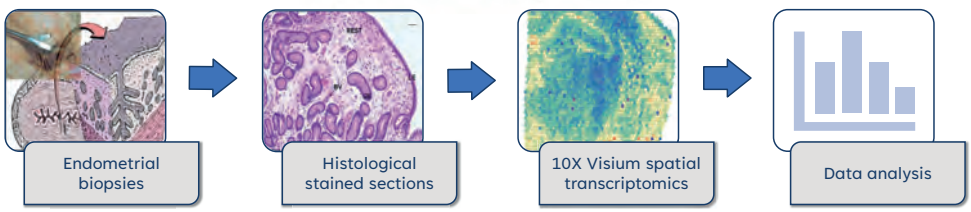
Uterine microbiome analysis



Uterine extracellular vesicles



Spatial transcriptomics



Expected results

- **Microbiome:** changes during different physiological conditions, alterations of microbiome composition in subfertile compared to fertile mares, identification of a healthy microbiome supporting uterine receptivity
- **Extracellular vesicles:** have a cargo function and are involved in uterine receptivity, alterations of EVs cargo in subfertile compared to fertile mares
- **Spatial transcriptomics:** spatial alterations of gene expression in the endometrium of subfertile compared to fertile mares in the context of histological alterations

Identification and characterisation of social interactions using accelerometers in cattle

Sébastien Goumon*, Konradin Messmer, Susanne E. Ulbrich
ETH Zürich, Animal Physiology, Institute of Agricultural Sciences
*sebastien.goumon@usys.ethz.ch

Introduction

Background: Knowledge of social structure (i.e. agonistic and affiliative relationships) of groups of farm animals allows to ensure good welfare thereby reducing aggression and injuries and promoting socio-positive behaviour (e.g. stress buffering). While monitoring of social interactions can be achieved by relying on visual live observations or video recordings, it remains very time-consuming, labour intensive, and subject to human error. Precision Livestock Farming technologies may be a suitable solution. These include RFID tags, computer vision and sensor technologies such as proximity or acceleration sensors. All of them show challenges in identifying social interactions, especially under extensive housing conditions[1,2]. Acceleration sensors provide promising results in detecting social behaviours in farm animals[3]. However, research remains limited, and no accelerometer-based monitoring system has been validated or commercialised yet[4].

Aim: This study aimed at assessing whether the type and laterality of social interactions could be differentiated using accelerometers in cattle.

Material and methods

Data collection

- Animal trial at the research station Früebüel.
- Experimental animals: 10 suckler cows.
- Assessment of frequency and laterality of headbutt and grooming interactions via video-recording and collection of acceleration data (triaxial; sampling rate: 10Hz) on pasture over 54h on 9 non-consecutive days.
- Total of 343 grooming (214 left/129 right) and 529 headbutt (250 left/ 279 right) interactions recorded.

Figure 1 : placement of the accelerometer

Data processing and analysis workflow

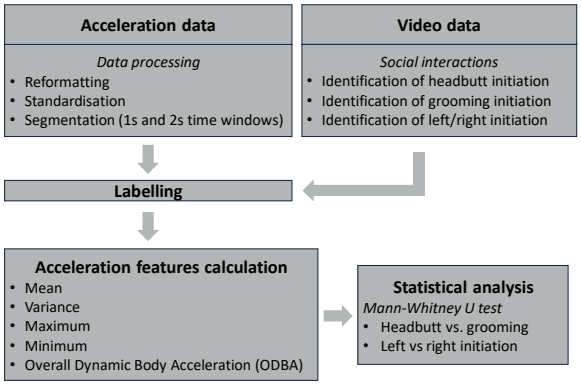


Figure 2: Schematic workflow of the data processing of raw acceleration and video data

Results and Discussion

Table 1: Comparison of acceleration features between headbutt and grooming interactions.

All acceleration variables differed between the 2 types of interactions on all 3 axes and for both segmentation windows, except for Max value on the x axis at a 2s segmentation window.

Axes	Window size	Mean	Variance	Max value	Min value	ODBA	
x	1s	***	***	***	***	1s	***
	2s	***	***	NS	***		
y	1s	***	***	***	***	2s	***
	2s	**	***	***	***		
z	1s	***	***	***	***		
	2s	***	***	***	***		

NS p>0.05 ; * p < 0.05 ; ** p < 0.01 ; *** p < 0.001

Behaviour	Axes	Window size	Mean	Variance	Max value	Min value	ODBA	
Grooming	x	1s	***	***	***	***	1s	***
		2s	***	***	***	***		
	y	1s	***	***	***	***	2s	***
		2s	***	***	***	***		
	z	1s	***	***	***	***		
		2s	***	***	***	***		
Headbutt	x	1s	NS	NS	NS	NS	1s	NS
		2s	NS	NS	NS	NS		
	y	1s	NS	NS	NS	NS	2s	*
		2s	NS	NS	NS	NS		
	z	1s	NS	NS	NS	NS		
		2s	NS	NS	NS	NS		

Table 2: Comparison of acceleration features between left and right initiations for grooming and headbutt interactions.

While the expression of left and right grooming interactions differed on all axes and for both segmentation windows across all acceleration features, the expression of left and right headbutt interactions showed significant differences only for OBDA at a 2s segmentation window.

Grooming and headbutt interactions could be differentiated based on their acceleration profiles. The laterality of behavioural expression could clearly be identified via acceleration data for grooming interactions. It remains unclear why this was not the case for headbutt interactions. Additional acceleration features in the time and frequency domains, and comparisons to other similar behaviours should be considered in future research to provide a more comprehensive and reliable characterisation of the 2 behaviours.

Conclusion

By identifying differences in acceleration profiles between the expression of agonistic and affiliative interactions, this study is a first step towards providing insights into the feasibility of using acceleration data. The future aim is to develop an automated assessment of the social structure and dynamics of farm herds, which could be a valuable tool for better management of cattle.

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Exhausted hooligans – genetic analysis of the correlations between ultimate pH in the neck and skin lesions

Research questions

The ultimate pH 24 hours after slaughter (pH24) is an indicator for meat quality.
(1) Does pH24 in the neck correlate with skin lesions?
(2) Are the pH24 in the neck and skin lesions heritable?
(3) Are skin lesions classifiers for aggressors, victims and aggressive victims?

Data collection

Measurements 24 hours after slaughter:

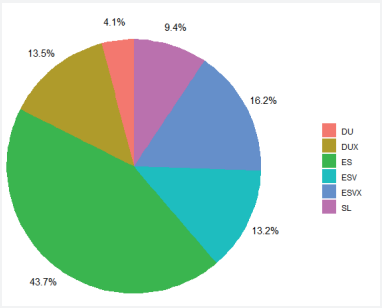
pH24 in the neck	Skin lesions
– Duration: over 9 months – Device: pH-Star	– Photos taken of the left carcass in the slaughterhouse – Assessment at 4 body parts – Skin lesion scores (sls): • 1: no lesions • 2: occasional lesions • 3: increased lesions • 4: area-wide lesions
→ **2'829 measurements**	→ **1'347 animals**

Body parts that are assessed:

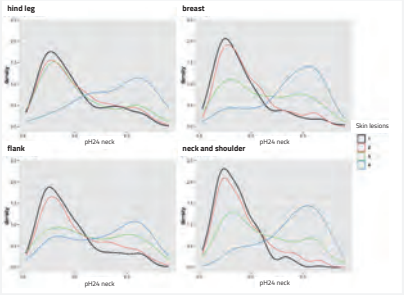
Results

(1) Phenotype analysis:

Breed distribution:



pH24 distribution in association with skin lesions:



Correlations calculated in R:

skin lesions	pH24
hind leg	0.133***
flank	0.224***
breast	0.349***
neck and shoulder	**0.507***

(2) Statistical model:

$$y = slaughter\ day + sex + breed + r(animal) + \varepsilon$$

Fixed effects analysis:

trait		sex	breed	slaughter day
pH24	DF	2	10	44
	F-value	8.28	7.20	4.73
	p-value	**0.004**	**2.2E-10**	**<2.2E-16**
hind leg	DF	2	10	21
	F-value	11.31	1.35	3.82
	p-value	**0.0008**	0.205	**2.9E-08**
flank	DF	2	10	21
	F-value	0.046	0.46	3.48
	p-value	0.830	0.903	**3.4E-07**
breast	DF	2	10	21
	F-value	1.59	1.65	2.72
	p-value	0.208	0.097	**6.6E-05**
neck and shoulder	DF	2	10	21
	F-value	6.15	1.04	3.79
	p-value	**0.0130**	0.402	**3.7E-08**

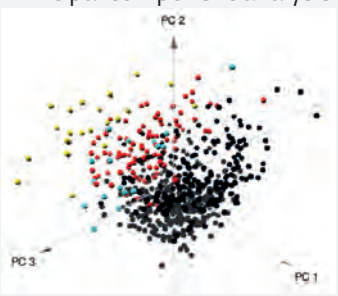
Heritability estimations:

	pH24	hind leg	flank	breast	neck and shoulder
genetic variance	0.0004	0.0037	0.008	0.060	3.1E-6
residual variance	0.099	0.567	0.548	0.695	0.834
heritability	0.004	0.007	0.015	**0.080**	3.7E-6

(3) Clustering:

– aggressors (sls shoulder ≥3)
– victims (sls hind leg ≥3)
– aggressive victims (sls shoulder ≥3 and hind leg ≥3)

Principal component analysis:



→ **No clear division**

K-means with k=3:

→ **41% in the correct cluster**

Hierarchical clustering:

→ **80% in the correct cluster**

Conclusions

(1) There are significant correlations between the pH24 and skin lesions.
(2) Heritabilities are low for all analysed phenotypes. Extremely high slaughter day effects keep these low.
(3) Classification into aggressors, victims and aggressive victims does not work good enough.

Contact information

Irene Häfliger, iha@suisag.ch, Allmend 10, 6204 Sempach

Characterization of the diurnal pattern of exhaled volatile fatty acids and enteric methane emission of dairy cows

Md Zakirul Islam[1], Stamatios Giannoukos[2], Susanna Räisänen[1], Kai Wang[1], X. Ma[1], Fabian Wahl[3], Renato Zenobi[2], Mutian Niu[1]

[1]Animal Nutrition, Department of Environmental Systems Science, ETH Zürich
[2]Analytical Chemistry, Department of Chemistry and Applied Biosciences, ETH Zürich
[3]Food Microbial Systems Research Division, Agroscope, Bern, Switzerland

Abstract #: **1772W**

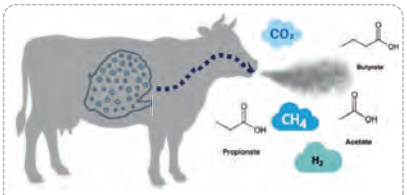
Background

Context

- The exhalome (all exhaled volatiles) contains volatile organic compounds that can reflect animal physiological processes.
- The commonly used methods to assess rumen functions are invasive.

Objective

- To explore the breath metabolome of dairy cows as a non-invasive technique, and to characterize the diurnal patterns of rumen fermentation.

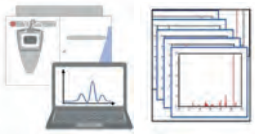


Exhalome of cows contains hundreds of volatile organic compounds

Bovine exhalome = lungs + rumen

Exhalome sampling from GreenFeed system

Enteric gas emissions were measured using the GreenFeed system

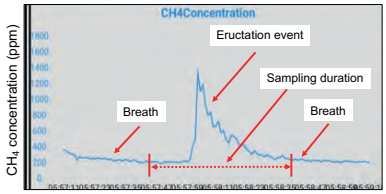


Exhalome analysis in SESI-HRMS

Materials and Methods

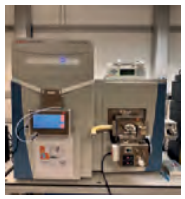
- 7 lactating cows were fed the same basal diet once per day.
- Enteric methane (CH_4) was measured using the GreenFeed system 8 times over 2 days to represent every 3-h of a day, simultaneously exhalome samples were collected using Tedlar gas sampling bags (1L).
- Exhalome samples were analyzed using secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) analytical platform.
- Short-chain volatile fatty acids (VFA) were annotated using their exact *m/z* ratios.

GreenFeed measurement

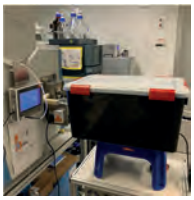


Real-time eructation monitoring

Exhalome sampling



SESI-HRMS



De-sampling unit

Results

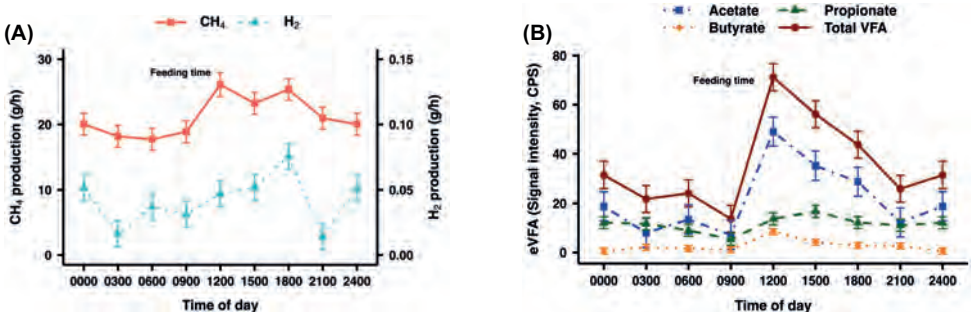


Fig 1. (A) Methane and hydrogen emission (g/h), and (B) exhaled VFA (CPS = count-per-second) of dairy cows measured in 3-h intervals

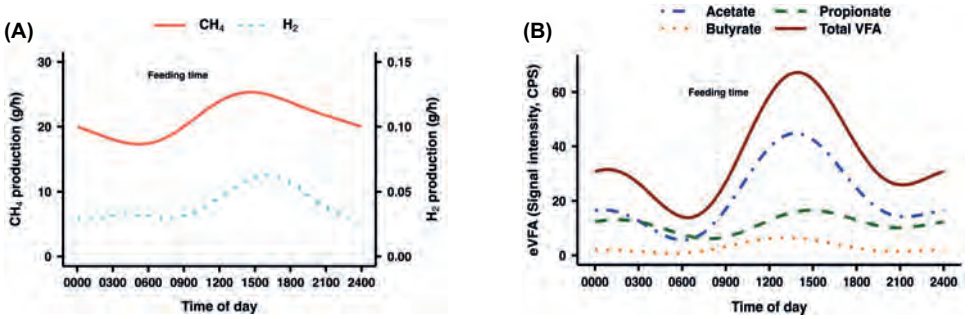


Fig 2. Fitted daily patterns of (A) enteric CH_4 and H_2 emission, and (B) exhaled VFA of dairy cows using cosine function with a linear mixed model

Conclusions

- The present study revealed a great potential for using exhalomics as a proxy for the assessment of rumen fermentation and its daily pattern.
- Further research is needed to validate the method and its establishment as a non-invasive tool for the assessment of the rumen and metabolic health of dairy cows.

For details (JDS article in press):

Contact:
Zakirul Islam
zakirul.islam@usys.ethz.ch

The genetic background of nitrogen use efficiency and methane emissions in Swiss dairy cows: Ongoing activities at Agroscope

Claudia Kasper[1], Fredy Schori[2], Silvia Ampuero Kragten[3], Bastien Hayoz[4], Raphael Siegenthaler[4] und Lukas Eggerschwiler[4]

Agroscope, [1]Animal GenoPhenomics, [2]Ruminant Nutrition and Emissions, [3]Methods Development and Analytics, [4]Research Contracts Animals, CH-1725 Posieux; www.agroscope.ch

Background

Breeding dairy cows with increased nitrogen use efficiency (NUE) can help **reduce nitrogen emissions from agriculture** in the long term. Individual differences in NUE between cows on the same ration suggest genetic differences. The aim of this study is to determine the **genomic variation in NUE of dairy cows** in relation to methane emissions (CH_4) and other traits.

Animals, Material and Methods

- **Duration of experiment:**

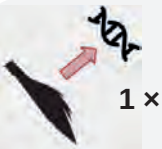
- **Holstein cows**

× 1'500 – 2'000
Lactation day 90 - 250

- **Participation of cantonal and private farms**
- **Ration depending on farm and season**

- **1 measuring period/cow**

1 × / cow
Blood (for physiology)



1 × / cow
Hair (for DNA)

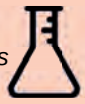
Feed analysis
1 × / farm and measuring period

Phenotypes

Infrared spectroscopy is a **cost-effective alternative to chemical analysis** for the detection of NUE and CH_4 with higher throughput. **Algorithms** that «translate» infrared (IR) spectra of milk or faeces into NUE or CH_4 are developed based on reference data *and* IR spectra. Existing algorithms will be **further developed** in international collaboration. Once the algorithms have reached a high level of accuracy, **IR spectra will be sufficient for the determination of NUE or CH_4.**

★ *Reference methods*

Nitrogen use efficiency
Weighing feed intake, chem. analysis of milk and feed



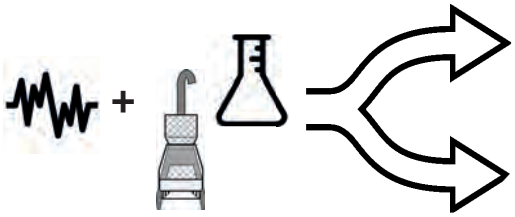
NUE
measured

Methane emissions
via GreenFeed®

CH_4
measured

★ *Infrared spectroscopy*

★ *Algorithms (artificial intelligence)*



NUE
estimated

CH_4
estimated

Status/situation as of October 31, 2023

- > 4,200 samples collected (of milk, faeces, hair, blood each)
- 1,010 different individuals
- Gold standard measurements of 83 individuals for feed intake and 282 individuals for methane emissions
- 21 farms (Experimental Farm Agroscope Posieux, farm of the Penitentiary Facility of canton Fribourg in Bellechasse, Grangeneuve School Farm, Sorens Organic School Farm and 17 private farms within a radius of about 30 km)

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra
Swiss Confederation
Federal Department of Economic Affairs, Education and Research EAER
Agroscope

Agroscope good food, healthy environment

ETH zürich

AgroVet Strickhof

Institute of Agricultural Sciences
Animal Nutrition

Hydroponic fodder as alternative feeds to reduce methane emission and water usage: an *in vitro* study

Yang Li[1*], Rong Peng[1], Carmen Kunz[1], Melissa Terranova[3], Yixin Zhang[1], Monika Macsai[2], Emmanuel Frossard[2], Mutian Niu[1]

[1] Group of Animal Nutrition, Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland
[2] Group of Plant Nutrition, Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland
[3] AgroVet-Strickhof, ETH Zürich, 8315 Lindau, Switzerland
*corresponding author. Email: yang.li@usys.ethz.ch

1 Introduction

2050

2 billion people

Water scarcity 80% cropland

Productivity 12-14%

- Malate (7.5% dry matter, DM) reduced CH_4 production by 16% in beef cattle (Foley et al., 2009)
- Germinating seedling can be an alternative source of malate?
- Hydroponic fodder requires 90% less water
- **Objective 1:** explore the chronological malate content of hydroponic fodders
- **Objective 2:** Assess their CH_4 mitigation capability *in vitro*

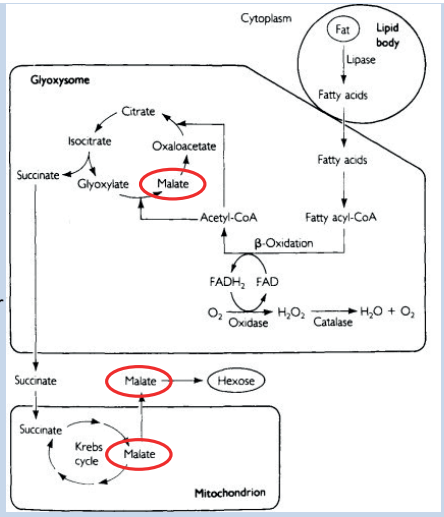


Figure 1. Glyoxylate cycle (Sadava 1993)

2 Method Overview

Alfalfa | Wheat | Triticale | Rye | Italian Ryegrass

Forage beans | Soybeans

- Grow hydroponic fodder
- Test malate content by day (0-10)
- Test in Hohenheim Gas Test (HGT) according to Menke (1988), Soliva and Hess (2007)

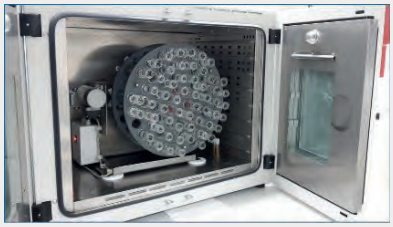


Figure 2. Hohenheim gas test

- **Basal diet:** 40% grass silage, 40% maize silage, 15% hay, 5% concentrate (DM)
- **Treatment:** 20% replacement and/or 80% silage replacement
- **Positive control:** Pure malate (12 mM)

3 Results and Discussion

The timepoint(s) with highest malate content were chosen for HGT.

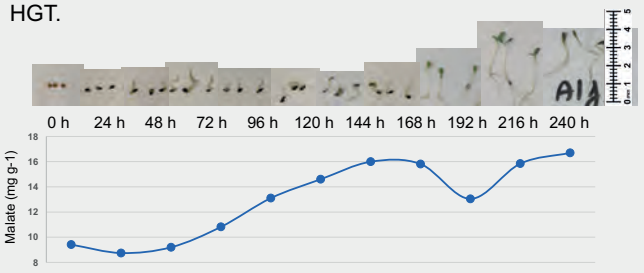


Figure 3. Chronological malate content of alfalfa

Table 1. Nutritional parameters of selected hydroponic fodder

Hydroponic fodder	DM (%)	Malate (mg g^{-1})	CF (%)	RF (%)	NDF (%)	ADF (%)	CP (%)	TA (%)	Starch (%)	OM (%)	Nfe (%)
Alfalfa 144 h	92.7	14.82	7.53	15.40	25.3	18.6	39.9	3.70	1.86	89.0	49.8
Alfalfa 240 h	94.0	15.69	4.85	14.59	28.6	21.2	42.7	4.29	0.71	89.7	50.7
Wheat 168 h	92.8	1.30	1.52	3.69	15.6	3.55	13.9	2.24	56.6	90.5	58.8
Triticale 240 h	93.9	5.26	2.13	3.16	21.2	3.31	14.0	2.04	58.5	91.9	59.1
Rye 216 h	93.8	**26.0**	1.09	3.72	34.5	3.86	10.5	2.11	**54.2**	91.7	59.9
Italian Ryegrass 216 h	94.1	1.10	1.20	12.65	24.3	12.2	13.9	3.78	18.9	90.4	57.1
Forage bean 240 h	93.6	20.1	1.30	9.45	32.6	11.7	24.8	4.71	41.3	88.9	55.2
Soybean 240 h	95.3	1.02	12.38	6.44	14.3	11.0	42.2	6.73	4.71	88.5	50.1

DM = Dry matter; CF = Crude fat; RF = Raw fibre; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; TA = Total ash; CP = Crude protein; OM = Organic matter; Nfe = Nitrogen free extract.

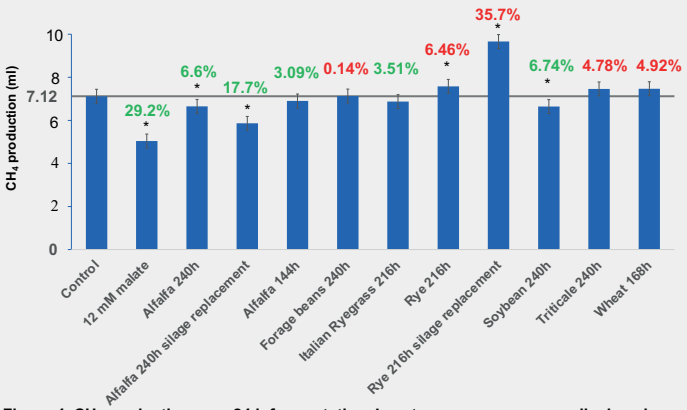


Figure 4. CH_4 production over 24 h fermentation. Least square means were displayed with error bars as standard errors. * = *P-value* of the contrast between hydroponic fodder vs. control <0.05.

4 Conclusion

- Alfalfa hydroponic fodder **240 h** has high malate content. It may serve as animal feed to reduce CH_4 emission and water usage concomitantly
- High malate content alone does not warrant CH_4 mitigation

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Effect of whey on *in vitro* ruminal methane formation and digestibility in cows

Luisier-Sutter, H.[1, 3], Isele, L.[3], Terranova, M.[2], Amelchanka, S.L.[2],Schick, M.[1, 3]

[1] Strickhof, Division Animal Husbandry& Dairy Production, Lindau; [2] ETH Zurich, AgroVet-Strickhof, Lindau; [3] University of Hohenheim, Institute for Agricultural Engineering, Stuttgart

Contact: *helene.luisier@strickhof.ch*

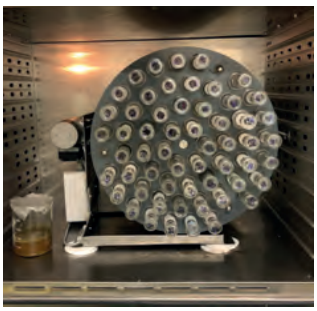
1. Introduction

- 14.5 % of anthropogenic methane emissions are caused by livestock supply chain (Gerber et al., 2013)
- Dietary modification impacts on ruminal fermentation processes and can reduce methanogenesis
- Whey is a by-product of cheese manufacturing process, which is high in water-soluble carbohydrates, with approx. 70% lactose (De Frain et al. 2004)
- In a preliminary study addition of whey reduced methanogenesis by 37% in beef heifers (Cerf and Dufey, 2014)

→ Aim: investigate the methane inhibiting potential of whey in different forms and dosages

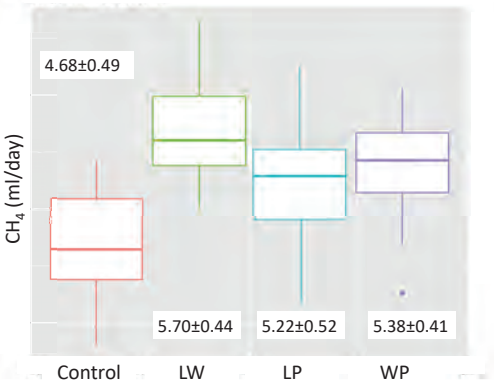
2. Method

- Hohenheim Gas Test (Menke & Steingass, 1988)
- Rumen fluid from three Original Brown Swiss dairy cows
- Basal and control diet: 33% hay, 66% grass silage
- Supplemented with liquid whey (LW) from Emmentaler cheese production, whey powder (WP) and lactose powder (LP)
- 2 doses per supplement: high (30% dry matter) and low (15% dry matter)
- 3 Runs, 5 replicates per treatment & dose
- Gas measurements and ruminal fermentation parameters measured after 24 h



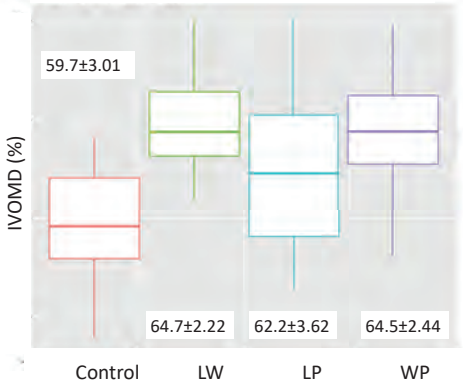
3. Results & Discussion

Daily Methane Production



- LW, WP and LP addition increased methanogenesis significantly ($p < 0.001$) by 21.8%, 15% and 11.5% respectively
- LW, WP and LP increased *in vitro* organic matter digestibility significantly ($p < 0.001$) by 8.6%, 8% and 4.4% respectively

In vitro organic matter digestibility (IVOMD)



→ No methane inhibition

Possible explanations:

- Different types of whey: Emmentaler vs. Gruyère cheese whey
- Rumen microbiome not adapted

→ Increased IVOMD

Possible explanation:

- Favourable effect of sugar on fibre digestion

4. Conclusion

- Liquid Emmentaler whey, whey powder and lactose powder increased *in vitro* methanogenesis in Swiss Original Brown dairy cows
- Significant increase in *in vitro* organic matter digestibility = might increase animal production parameters *in vivo* = potential for reduction in methane per unit of animal production

→ **Further studies needed to investigate the potential of whey in cattle feeding**

References: Cerf and Dufey,(2014) in Dufey et al., (2014) "Journée thématique Lactobeef", *Documents de cours, Alpage de Corison, Rossinière,* 26.08.2014 | De Frain et al., (2004) "Feeding Lactose Increased Ruminal Butyrate and Plasma ß-hydroxybutyrate in Lactating Dairy Cows", *JDS* | Gerber et al. (2013) "Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities", *FAO* | Menke and Steingass (1988) "Estimation of energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid", *Animal Research and Development*.

Combined effects of 3-nitrooxypropanol (3-NOP, Bovaer®10) and whole cottonseed on production and enteric methane emissions of dairy cows

X. Ma[1], S. E. Räisänen[1], T. He[1], M.Z. Islam[1], Y. Li[1], R. Peng[1], M, Reichenbach[1], X. Sun[1], K. Wang[1], S. Yang[1], Z. Zeng[1], I. Müller[2], M. Niu[1]

[1]Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich 8092, Switzerland
[2]Department of Animal Nutrition, DSM Nutritional Products, Wurmisweg 576, 4303 Kaiseraugst, Switzerland

Introduction

- 3-nitrooxyproponal (3-NOP) is a proven inhibitor for enteric methane (CH_4) mitigation.
- Dietary inclusion of whole cottonseed (WCS) decreased protozoa count and relative abundance of archaea (Castro Veloz, 2023).
- Gossypol, derived from WCS, have shown antimicrobial effects on some bacteria and yeasts (Wang et al., 2009), but unclear on its effect on rumen methanogens.
- **Objective**: to evaluate the effects of WCS and 3-NOP combined on dry matter intake, production, total-tract digestibility and enteric CH_4 emissions in dairy cows.

GreenFeed

Total collection of excreta

Material and Methods

- Sixteen dairy cows were arranged in a split-plot design, where the main plot was the breed of cows [8 Holstein Friesian (HF), 8 Brown Swiss (BS)]. Within each block, cows were used in a 4 × 4 Latin Square design with 2 × 2 factorial arrangement of treatments with 4, 24-d periods.
- Treatments were: CON (basal diet); 3-NOP (Bovaer®10, 600 mg/kg DM); WCS (50 g/kg DM); 3-NOP + WCS.
- The diets were balanced for ether extract and NDF content (4.0 and 43% of DM, respectively) and fed 2x/day at 0800 and 1800 h.
- Data were analyzed using mixed models in R.

Results

Table 1. Dry matter intake, ECM, and enteric gas emissions of lactating dairy cows from two breeds receiving dietary treatments.

	3-NOP			WCS			Breed			*P*-value						
Items	**No**	**Yes**	**SE[3]**	**No**	**Yes**	**SE**	**BS**	**HF**	**SE**	**3-NOP**	**WCS**	**Breed**	**3-NOP × WCS**	**3-NOP × Breed**	**WCS × Breed**	**3-NOP × WCS × Breed**
DMI, kg/d	24.6	24.8	0.51	24.7	24.7	0.51	23.6	25.8	0.68	0.42	0.89	0.03	0.03	0.10	0.77	0.65
ECM, kg/d	28.2	28.5	1.43	27.7	28.9	1.43	28.0	28.6	1.99	0.60	0.03	0.83	0.60	0.93	0.07	0.23
CH_4 production, g/d	460	400	9.1	422	438	9.1	437	422	11.8	<0.01	0.20	0.39	0.11	**0.05**	0.82	0.30
CH_4 yield, g/kg DMI	18.9	16.3	0.39	17.2	17.9	0.35	18.7	16.4	0.39	<0.01	0.26	<0.01	0.54	0.24	0.75	0.22
CH_4 intensity, g/kg ECM	16.3	14.1	0.60	14.9	15.6	0.61	15.4	15.1	0.77	<0.01	0.34	0.99	0.39	**<0.01**	0.97	0.79
H_2 production, g/d	1.26	3.75	0.236	2.70	2.32	0.236	2.17	2.85	0.268	<0.01	0.01	0.01	0.03	**<0.01**	0.16	0.11

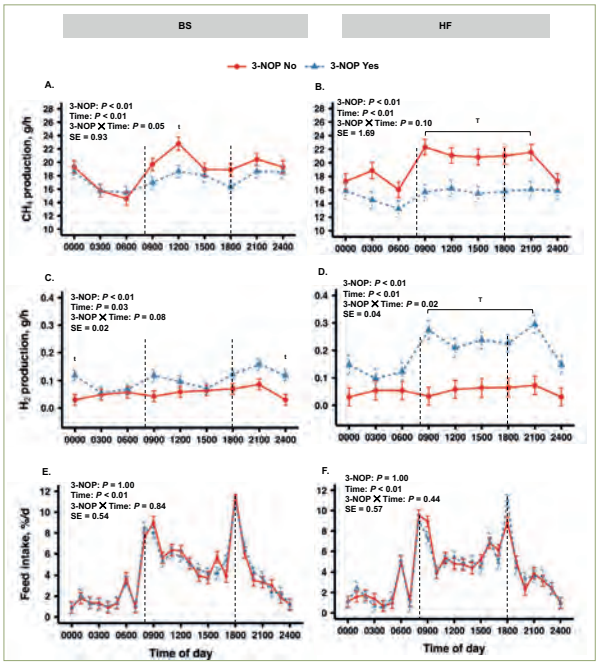


Figure 2. Effect of supplementing 3-NOP to Brown Swiss cows (Left: BS) and Holstein Friesian cows (Right: HF) on the intraday pattern of CH_4 production (A, B), H_2 production (C, D), and feed intake (E, F). Dashed line: morning and afternoon feeding.

- There was WCS × breed interaction for milk fat yield ($P = 0.04$) and ECM ($P = 0.07$): WCS increased milk fat yield by 12.2% and ECM by 8.2% in BS.
- There was 3-NOP × breed interaction in CH_4 production ($P = 0.05$), CH_4 intensity ($P < 0.01$), H_2 production ($P < 0.01$).
- Brown Swiss had lower intake than HF; apparent total-tract digestibility of all nutrients, expect for CP and starch, were decreased ($P < 0.01$) in cows fed WCS.

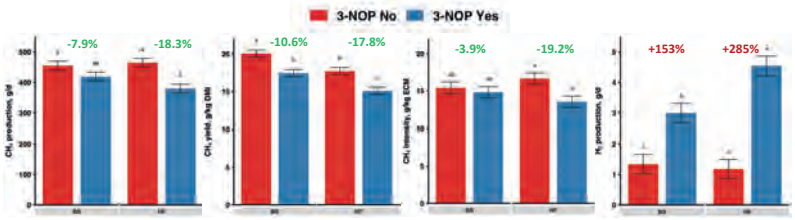


Figure 1. Effect of supplementing 3-NOP to Brown Swiss cows (BS) and Holstein Friesian cows (HF) on enteric gas emissions.

Conclusions

- Combining WCS with 3-NOP improved ECM and milk fat yield but did not have additional enteric CH_4 emission inhibition in dairy cows.
- Further investigations are necessary to effectively assess dietary CH_4 mitigation strategies related to various animal breeds.

Reference

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Contact: Xiaoqi Ma, xiaoqi.ma@usys.ethz.ch

Effects of combining plant-based compounds with 3-nitrooxypropanol (3-NOP, Bovaer®10) on methane emissions and lactational performance of dairy cows

X. Ma[1], S. E. Räisänen[1], K. Giller[1], MZ. Islam[1], Y. Li[1], R. Peng[1], M. Reichenbach[1], X. Sun[1], I. Müller[2], M. Niu[1]

[1]Animal Nutrition, Institute of Agricultural Sciences, D-USYS, ETH Zurich
[2]Department of Animal Nutrition, DSM Nutritional Products, Wurmisweg 576, 4303 Kaiseraugst, Switzerland

Introduction

- The compound 3-nitrooxypropanol (3-NOP) is one of the most consistent enteric methane (CH_4) inhibitors in ruminants.
- **Objective**: This pilot *in vivo* study aimed to investigate the effect of combining 3-NOP with 4 different plant-based compounds [tannin extract (TA), 2 essential oils (EO1 and EO2), and oilseed (OS)] compared with the CH_4 mitigating potential of 3-NOP alone.

GreenFeed

Material and Methods

Baseline (basal diet; CON)		Experimental period (treatment diets)	
Adaptation	CH_4 using GF	Adaptation	CH_4 using GF
2-d	3-d	21-d	3-d

- Sixteen dairy cows (8 Holstein, 8 Brown Swiss) cows were randomly assigned to 1 of 6 dietary treatments:
  1) 3-nitrooxypropanol [3-NOP through Bovaer®10; as the positive control (+CON)]
  2) 3-NOP + tannin extract (3-NOP + AMT)
  3) 3-NOP + essential oil 1 (3-NOP + EO1)
  4) 3-NOP + essential oil 2 (3-NOP + EO2)
  5) 3-NOP + oilseed with low inclusion rate (3-NOP + OSL)
  6) 3-NOP + oilseed with high inclusion rate (3-NOP + OSH)
- Daily milk yield and milk composition were collected for 3 d.
- Gas emissions measured with GF 8 times to represent every 3-h across the day.
- Data were analyzed using mixed models in R.

Table 1. Nutrient composition (DM basis) of experimental diets

Item	CON	3-NOP	3-NOP + AMT	3-NOP + EO	3-NOP + EO	3-NOP + OSL	3-NOP + OSH
CP, %	14.4	14.3	13.6	14.5	14.5	14.4	14.9
NDF, %	43.0	41.8	40.0	42.1	42.4	41.5	44.6
EE, %	2.40	2.40	2.40	2.60	2.46	3.23	4.36
Starch, %	14.8	13.5	13.9	13.6	14.2	14.1	14.5
Ash, %	7.40	7.60	7.30	7.70	7.59	7.59	7.22
GE, MJ/kg	18.1	18.2	18.3	18.2	18.2	18.3	18.6
NE_L, MJ/kg	5.30	5.30	5.30	5.30	5.30	5.40	5.80

Results

- Compared with 3-NOP alone (positive control), none of the tested combinations affected DMI (23.3 kg/d; SE = 0.60), MY (28.1 kg/d; SE = 1.15), or milk components.
- Milk urea was 32.0 mg/dL vs. 24.6 mg/dL for +CON and 3-NOP + AMT, respectively.
- Methane production, yield, and intensity were similar between the combinations vs. +CON , averaging 408.3 g/d, 17.6 g/kg DMI and 20.4 g/kg fat and protein-corrected milk (FPCM), respectively.

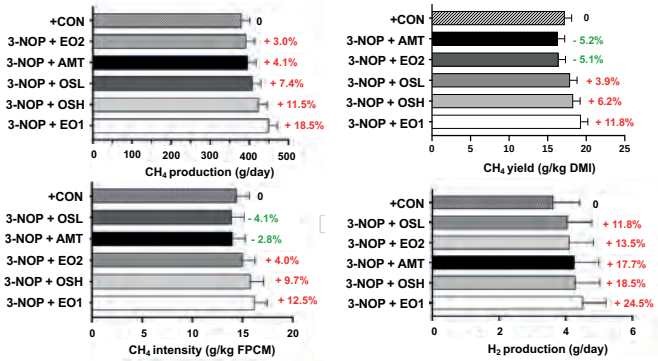


Figure 1. The effect of dietary treatment on CH_4 production, CH_4 yield, CH_4 intensity and H_2 production. The comparison was made on the least squares mean between 3-NOP positive control and one of the five combinations.

Conclusion

The results suggest that combining 3-NOP with AMT, EO2, or OSL may enhance the efficacy of 3-NOP but warrants further investigation with a greater number of animals.

Partner/Sponsor:

Contact: Xiaoqi Ma, xiaoqi.ma@usys.ethz.ch

Comparison of gaseous exchange in dairy cows housed in respiration chambers vs. in tie-stall measured by a head-chamber system (GreenFeed)

X. Ma[1], S. E. Räisänen[1], K. Wang[1], S. Amelchanka[2], A. M. Serviento[1], K. Giller[1], MZ. Islam[1], Y. Li[1], R. Peng[1], M, Reichenbach[1], X. Sun[1], and M. Niu[1]

[1]Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich 8092, Switzerland
[2]AgroVet-Strickhof, ETH Zürich, Eschikon 27, 8315 Lindau, Switzerland

Introduction

- Respiration chambers (RC): the "gold standard" for precise gaseous exchange measurement, providing **high-resolution** gas emission data.
- The GreenFeed system (GF; C-Lock Inc.): a head-chamber system for ruminants, capable of measuring **multiple animals within a short time frame**.
- **Objective**: to compare gaseous exchange of dairy cows based on the above-mentioned widely used chamber systems.

Material and methods

- Data were collected from an animal experiment that screened 4 dietary treatments for their methane (CH_4) mitigating potentials.
- Data set contained the information of 16 multiparous lactating dairy cows housed in tie stall (TS), measured by GF, and later moved to the RC.
- Cows were arranged in complete randomized design with a baseline period (receiving basal TMR) established for each cow.
- Data were analyzed using mixed models in R.

Respiration chamber

Baseline period (basal TMR; CON)			Experimental period (treatment diets)			
TS	GF in TS	RC	Diet adaptation in free-stall	TS	GF in TS	RC
2-d	3-d	2-d	21-d	2-d	3-d	2-d

GreenFeed

Results

- There was **no method × diet interaction** for daily measurements.
- There was **method × time of day interaction** for intraday measurements of CH_4 ($P < 0.01$), O_2 ($P = 0.02$), and metabolic heat production (MHP, $P = 0.08$).
- Daily lying time of cows was 21.6% greater ($P < 0.01$) when they were housed in RC compared to the TS during the sampling days of GF measurements. However, there was no difference between RC and TS (when cows were not interrupted by GF measurements).

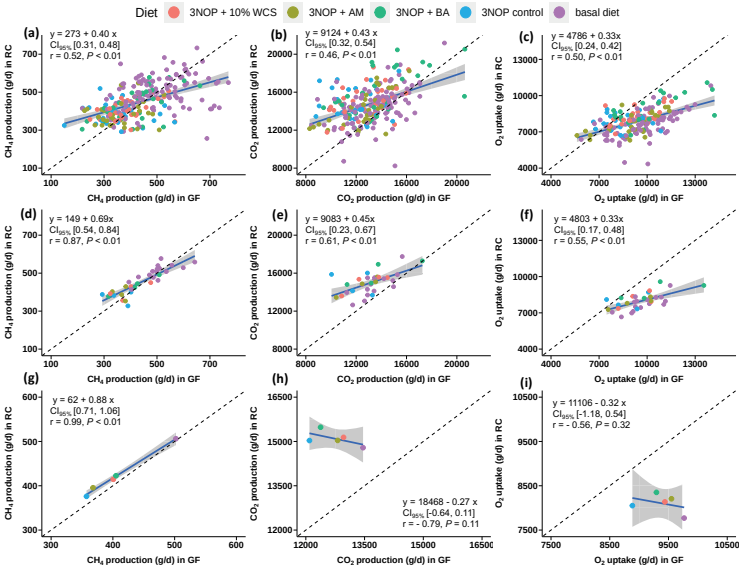


Fig 1. Comparison of CH_4, CO_2 emission, and O_2 uptake measured by RC and GF
(a),(b), (c) comparison by cow, diet, and by each time point of spot sampling
(d), (e), (f) comparison by cow and diet for the daily measurement
(g), (h), (i) comparison the diet-specific daily measurement

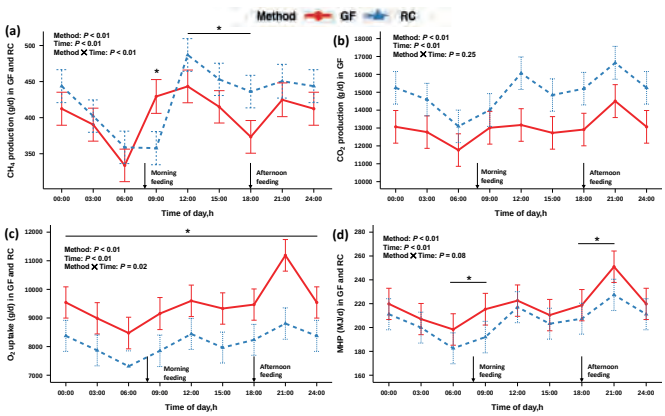


Fig 2. Intraday emissions of (a) CH_4, g/d , (b) CO_2, g/d, (c) uptake of O_2, g/d, and (d) MHP[1], MJ/d from RC and GF displayed in 3-h intervals
* denotes significant difference ($P < 0.05$) between RC and GF measurement at the same time point
[1]MHP was calculated as 16.18 × O_2 uptake + 5.02 × CO_2 production - 2.17 × CH_4 production) /1000 (Brouwer (1965)) without correcting for urinary nitrogen

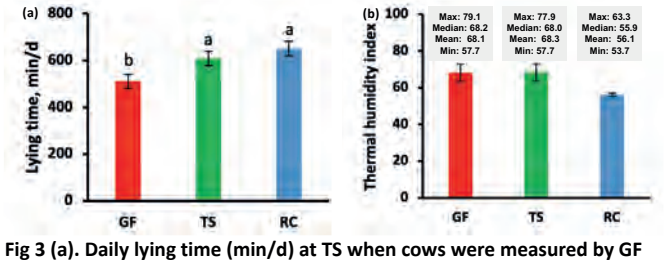


Fig 3 (a). Daily lying time (min/d) at TS when cows were measured by GF (red), at TS without interruption (green), and in RC (blue)
Fig 4 (b). Average daily thermal humidity index (THI)[1] at TS when cows were measured by GF, at TS without interruption, and in RC
[1]THI = (1.8 × Temperature + 32) - (0.55 - 0.0055 × Humidity) × (1.8 × Temperature - 26) (NRC, 1971)

Conclusions

- Methane emissions measured by GF and RC were very similar, especially on daily- and treatment-level.
- The measurements of CO_2 emission and O_2 uptake was not correlate very well between the two methods, which is likely due to the metabolic changes of cows under heat stress (during GF measurements in the tie-stall), as indicated by the elevated THI and reduced lying time.

References

NRC, A. Guide to Environmental Research on Animals. 1971. National Academy of Sciences, Washington, DC.

Brouwer, E. (1965). Report of sub-committee on constants and factors. In Energy Metabolism. Proc. 3rd Symp., 1965. Academic Press.

Contact: Xiaoqi Ma, xiaoqi.ma@usys.ethz.ch

DAIRY COW DIFFERENTIAL SOMATIC CELLS AND PRECISION LIVESTOCK FARMING: AN ITALIAN CASE

Sara Mondini*, Maddalena Zucali, Giulia Gislon, Luciana Bava

Department of Agriculture and Environmental Science, University of Milan, Via Celoria 2, 20133, Milan, Italy

**mail: sara.mondini1@unimi.it*

Introduction

Somatic Cell Count (SCC) is the most economic tool for the detection of mastitis in farms but, during the first stages of inflammation, and in presence of some pathogens, the increase of SCC could not occur. For these reasons, differential somatic cells (neutrophils NEU, lymphocytes LYM and macrophages MAC), that compose the leucocyte fraction (Total milk Leucocyte Count-TLC) of SCC, were investigated as indicator of mastitis.

The objective of this study was to detect the relationships between TLC, the three fractions of differential somatic cells and data obtained from automatic sensors used in milking systems (milking time and milk electrical conductivity).

Materials and methods

6 farms in Northern Italy

179 cows

5 milk samples per cow

One sample per cow every 60 days for the entire lactation

>850 milk samples analysed with:

Vetscan DC-Q Milk Analyzer for the detection of:

- TLC (cells/ml) = NEU + MAC +LYM
- NEU (%, cells/ml)
- MAC (%, cells/ml)
- LYM (%, cells/ml)

DeLaval Cell Counter for the detection of:

- SCC

Milking parameters collected by AfiMilk system:

- Milking time
- Milk electrical conductivity

Mixed model with repeated values

Effects: parity, stage of lactation, farm, stage*parity, TLC classes during lactation

Multiple correspondence analysis

Between: parity, stage, TLC, SCC, NEU, milk electrical conductivity, milking time

Results

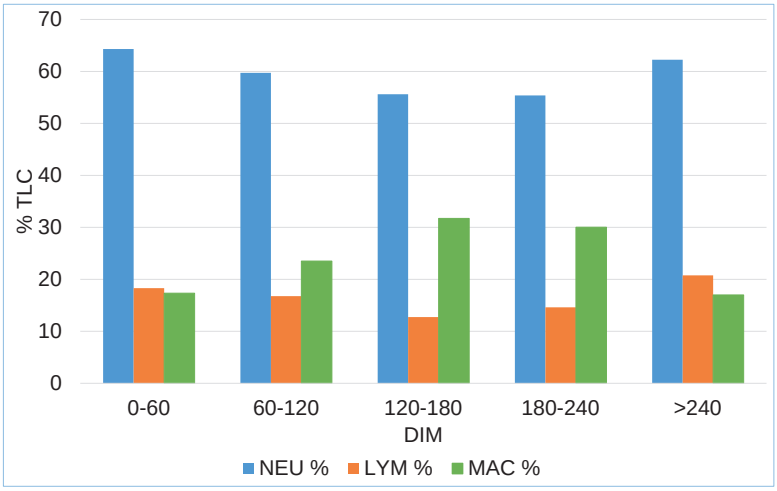


Figure 1: Differential somatic cells during lactation

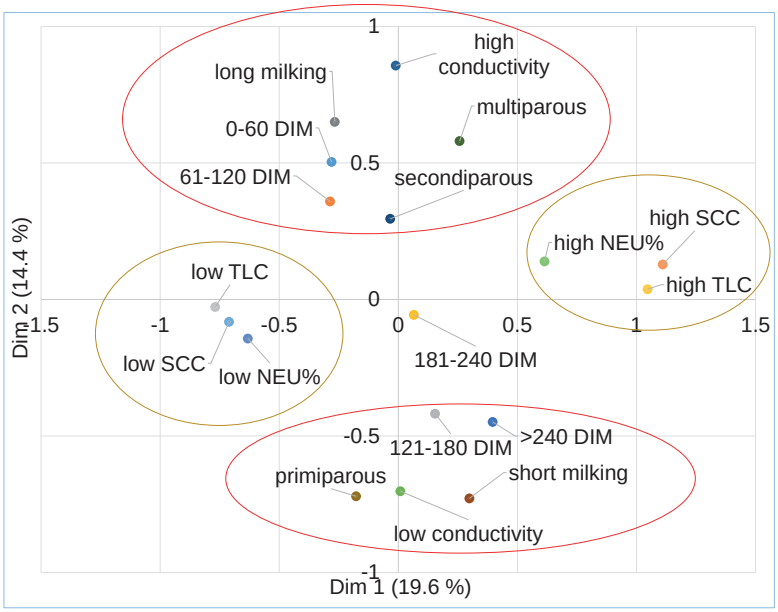


Figure 2: Multiple correspondence analysis. *DIM=Days in Milk*

Conclusions

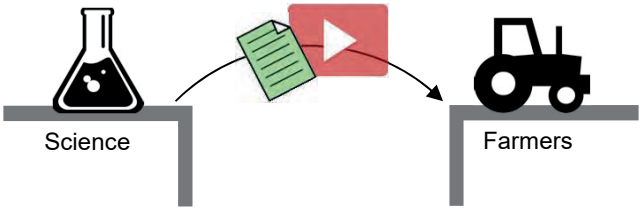
- **The first days of lactation are the critical ones, especially for high concentration of neutrophils (%) and low concentration of macrophages (%), indicating the suspicious of udder problems.**
- **Milk Electrical conductivity and milking time could be used as supplementary parameters for udder problem identification**

Communicating knowledge on grassland management using videos and online fact sheets

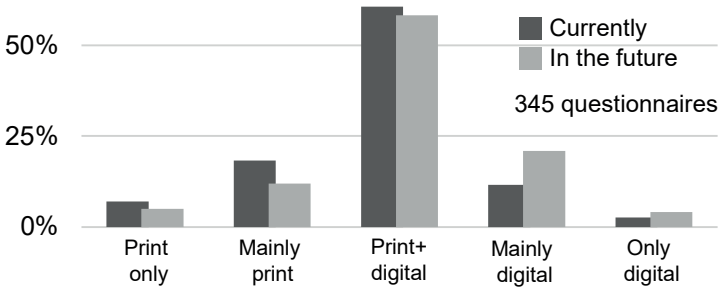
Caren M. Pauler[1,2], Daniel Mettler[3], Thomas Alföldi[4], Helen Willems[5], Cornel Werder[5], Manuel K. Schneider[1,2]
[1] Agroscope, [2] Swiss Grassland Society, [3] Agridea, [4] Research Institute of Organic Agriculture (FiBL), [5] Büro Alpe; Switzerland.
caren.pauler@agroscope.admin.ch

Aim

Summarising existing grassland knowledge and make it accessible to farmers → Closing the gap between science and practice.



Motivation: Where do farmers look for information?



Example: Weed control in alpine summer pastures

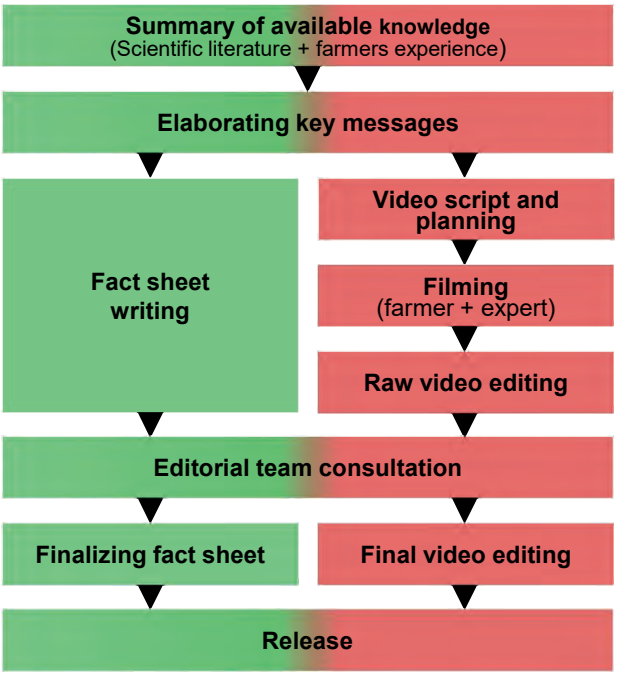
- Fact sheets and videos about 8 typical alpine weeds created
- Content: plant biology and control measures
- Standardised production and content structure: reduces production workload + enhances usability for farmers
- Release: **www.patura-alpina.ch**
  YouTube, smartphone app of Agridea

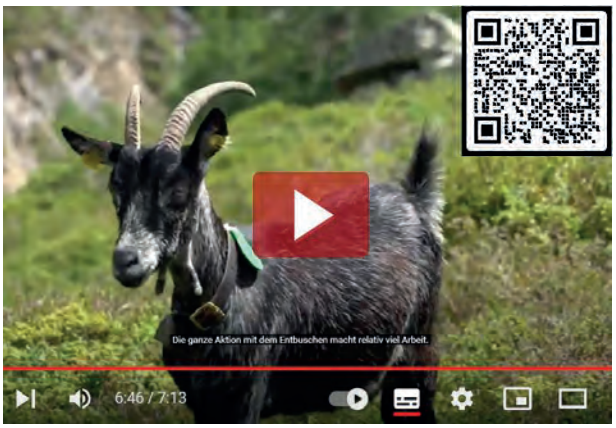
~ 16 500 YouTube views

Lessons learned

- Featuring farmer AND expert is most convincing.
- Video and fact sheet are not alternatives, but complements (attention + background information).
- A rough script (including all key messages) speeds up filming, but allows freedom for authenticity of speakers.
- Synthesis and discussions within the editorial team are fruitful.

Key steps to a successful fact sheet and video





Agroscope good food, healthy environment

agridea FiBL Alpe AGFF IWMPRAISE Agroscope

M2M networking of devices in the dairy barn

J. Poteko, J. Harms

Background

- **The interest of dairy farmers in digital technology is increasing** ➔ A rising number of installed automatic milking systems (AMS) and investment interest in digital technologies such as feeding systems and herd management programs (HMP).
- **Automated devices in dairy barns generate a variety of data** ➔ Increased data availability during their operation. However, there is a lack of information exchange between these devices (machine-to-machine communication, M2M).
- **The farmer regulates the different devices in the barn and makes manual settings** ➔ M2M communication between devices would enable them to consider not only their own data but also the data from other devices in the planning and execution of rule-based workflows and to adapt the barn devices/technology to the current conditions/needs in the barn without immediate intervention from the farmer.

Research and practice networking

Aim of project ➔ To present the **expectations, experiences, and obstacles** regarding M2M networking of barn technology **from the perspective of farmers** and to encourage barn technology manufacturers to **initiate the development process of M2M** communication.

About Experimental field DigiMilch

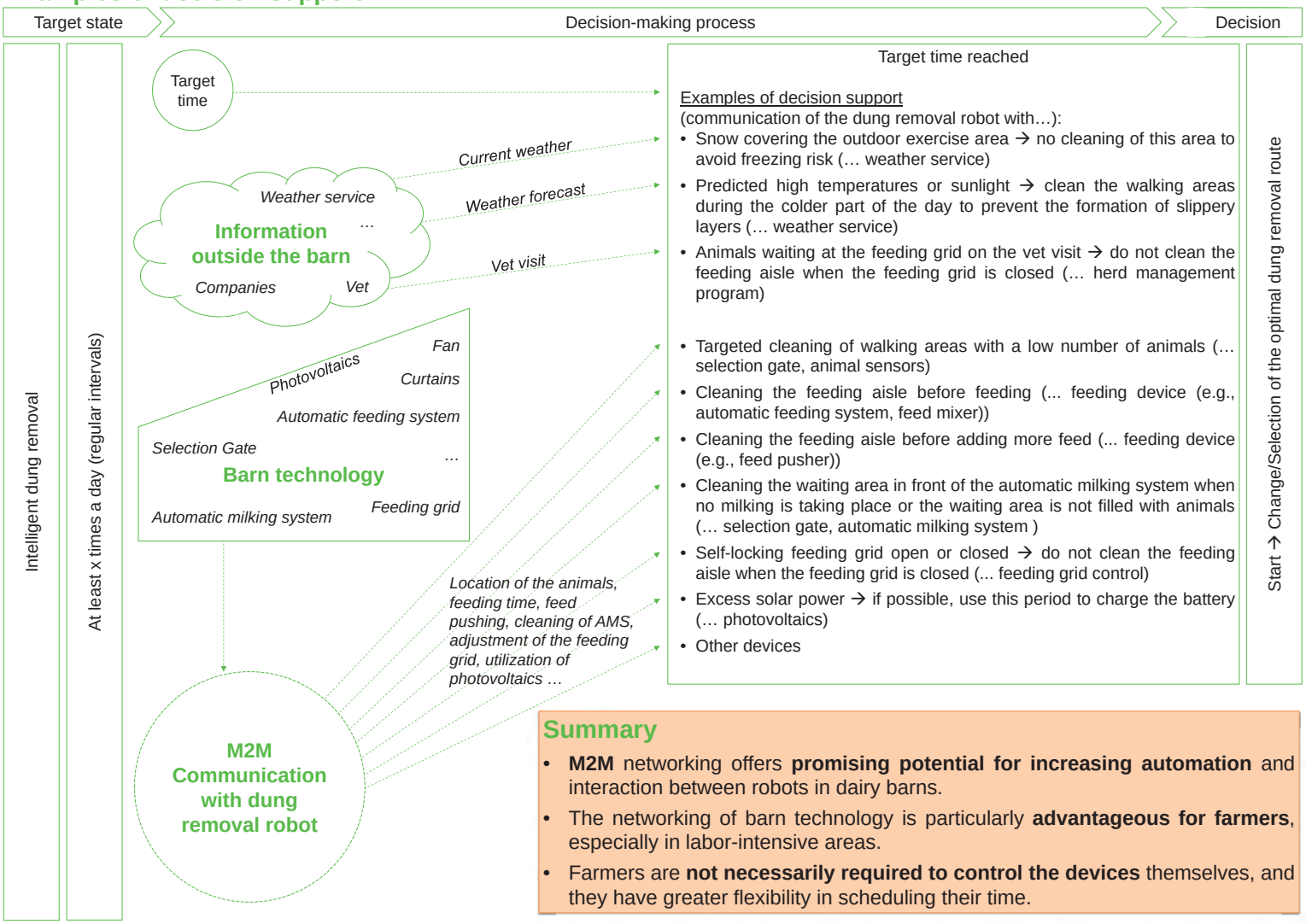
The **Experimental field DigiMilch** focuses on existing digital solutions within the milk production process, capturing farmers' experiences in their implementation, and identifying gaps or deficiencies in smart solutions.

Within the one of the sub-projects, the needs, requirements, and benefits of farmers regarding the **M2M networking of devices in dairy barns** are investigated

M2M communication in barn technology ➔ Example with a dung removal robot

- **Example of M2M from the perspective of a small device** ➔ Approach of intelligent dung removal and the requirements on an autonomous dung removal robot in the future to adapt to the needs of animals, farmers, and the environment.
- **An exemplary demonstration of the M2M and decision support** ➔ An overview of the devices/data sources (selection gate, online weather service, etc.) with which a dung removal robot could exchange information or consider when planning its own routes.

Examples of decision support



Summary

- **M2M** networking offers **promising potential for increasing automation** and interaction between robots in dairy barns.
- The networking of barn technology is particularly **advantageous for farmers**, especially in labor-intensive areas.
- Farmers are **not necessarily required to control the devices** themselves, and they have greater flexibility in scheduling their time.

Bavarian State Research Center for Agriculture,
Institute for Agricultural Engineering and Animal Husbandry
Prof.-Dürrwaechter-Platz 2, 85586 Poing, www.LfL.bayern.de

Combined effects of 3-nitrooxypropanol (3-NOP, Bovaer®10) and whole cottonseed on production and enteric methane emissions of dairy cows

X. Ma, **S. E. Räisänen**, T. He, M.Z. Islam, Y. Li, R. Peng, M, Reichenbach, X. Sun, K. Wang, S. Yang, Z. Zeng, I. Müller, M. Niu

Animal Nutrition, Institute of Agricultural Sciences, D-USYS, ETH Zurich

Abstract #: 1442T

Introduction

- 3-nitrooxyproponal (3-NOP) can persistently inhibit enteric methane (CH4) emission without adverse effect on production performance
- Whole cottonseed (WCS) is commonly fed to dairy cows since it contains high proportions of protein and effective fiber, along with a high energy content which may also contribute to CH4 reduction
- Hypothesis: WCS enhances lactational performance while reducing CH4 emissions, and would have an additive CH4 mitigation effect when combine with 3-NOP

Material and Methods

Experimental period	
Adaptation	Sample collection
21-d	3-d

- Sixteen dairy cows (8 Holstein Friesian [HF], 8 Brown Swiss [BS]) cows were used in a 4 × 4 Latin Square design and randomly assigned to 1 of 4 dietary treatments:
  - CON (basal diet)
  - 3-NOP (Bovaer®10, 60 mg/kg DM)
  - WCS (50 g/kg DM)
  - 3-NOP + WCS
- The diets were balanced for ether extract and NDF content (4.0% and 43% of DM) and fed 2x/day at 0800 and 1800 h

GreenFeed

Total collection of excreta

- Daily milk yield and composition were recorded for 3 d
- Emissions of CH_4, H_2, and CO_2 measured with GF 8 times to represent every 3-h across a day.
- Feed weight was recorded every 10 sec to plot intraday feed intake pattern
- Total fecal output was recorded for 3 d to estimate apparent total-tract digestibility

Conclusions

- Combining cottonseed with 3-NOP improved ECM and milk fat yield but did not enhance CH4 inhibition effect of 3-NOP of dairy cows.
- The lack of interactions between 3-NOP and WCS might be a result of an unexpected low response of BS cows to 3-NOP and requires further investigation.

Results

- There was WCS × breed interaction for milk fat yield (P = 0.04) and ECM (P = 0.07): WCS increased milk fat yield by 12.2% and ECM by 8.2% in BS
- There was 3-NOP × breed interaction in CH4 production (P = 0.04), CH_4 intensity (P < 0.01), H_2 production (P < 0.01): CH_4 production was reduced by 11.8% and CH_4 intensity was reduced by 19.2% while H_2 production was increased by 285% in HF
- Brown Swiss had lower intake than HF; Apparent total-tract digestibility of all nutrients, expect for CP and starch, were decreased (P < 0.01) in cows fed WCS

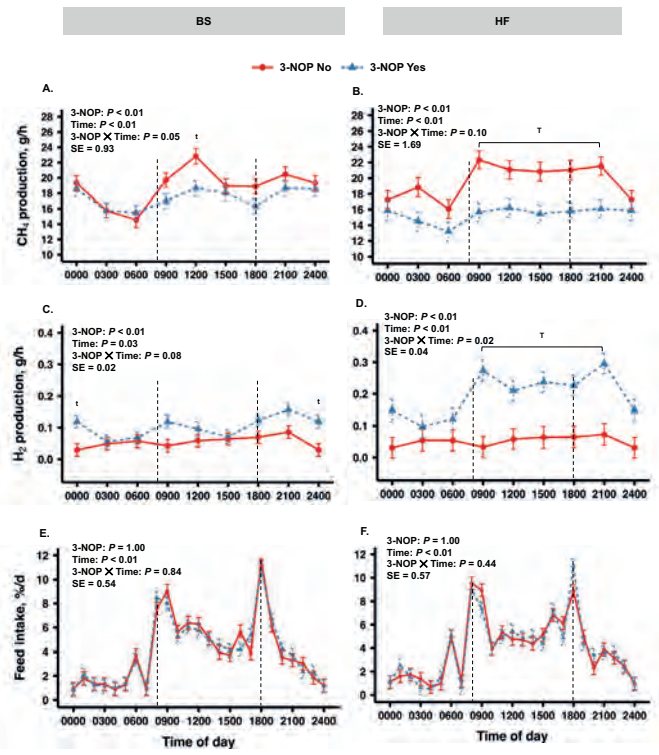


Figure 2. Effect of supplementing 3-NOP to Brown Swiss cows (Left: BS) and Holstein Friesian cows (Right: HF) on the intraday pattern of CH4 production, H2 production, and feed intake.

Contact:

Susanna Räisänen, susanna.raeisaenen@usys.ethz.ch

Xiaoqi Ma, xiaoqi.ma@usys.ethz.ch

FIRST DETECTION OF SYSTEMIC PORCINE CIRCOVIRUS 3 ASSOCIATED DISEASE IN SWITZERLAND

G. Rosato*, F. Seehusen*, M. Hilbe*, J. Segalés†§, M. Sibila†Δ, À. Cobos †Δ, and R. Graage +

*Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
†Unitat mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Barcelona, Spain
§ Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain
Δ IRTA Programa de Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain
+ Qualiporc Cooperative, Appenzell, Switzerland

Introduction

Circoviruses are single-stranded DNA viruses that infect a wide spectrum of animals. In pigs, four different circoviruses have been identified. Porcine circovirus 3 (PCV-3), first reported in 2015, has been detected worldwide in pigs with or without clinical signs. However, knowledge of clinicopathological and epidemiological aspects of PCV-3 infection and the pathogenic effect of the virus is limited.

Figure 1: 6-week-old piglet with «humpy-back syndrome» (lumbar kyphosis and thoracic lordosis).

Material and Methods

In March 2023, an increase in spinal deformities, characterised by an upward curvature of the lumbar spine (kyphosis) and downward curvature of the thoracic spine (lordosis), a condition known as “humpy-back syndrome” (Fig. 1) was reported in a Swiss breeding farm. Additionally, suckling and weaners piglets showed thickened ribs and facial oedema in otherwise unremarkable clinical condition.

Three 4- to 6-week-old piglets were euthanized and submitted for diagnostic examinations.

Full necropsy and histological examination were performed. Based on the findings of the histological examination, a qPCR and *in situ* hybridisation (ISH) were performed to detect PCV-3 DNA and RNA, respectively, in multiple organs.

Results

- In macroscopic examination, the animals showed multiple differently pronounced rib fractures with prominent callus formation. Histologically, callus formations were composed mainly of cartilage (Fig. 2).
- Histologically, several internal organs (including vascular mesenteric plexus, kidney, nasal turbinate, heart and spinal cord) and costal periosteal arteries exhibited (peri-)arterial lymphohistiocytic and plasmacytic infiltrates (Fig. 3).
- ISH detected abundant PCV-3 RNA in rib (periosteal arterial wall, osteocytes and osteoblasts), mesenteric arteries (intima and media; Fig. 3B, 3F) and renal arteries.
- The qPCR for PCV-3 revealed high viral loads (Ct values 18 - 24) in kidney, heart and mesenteric lymph node.

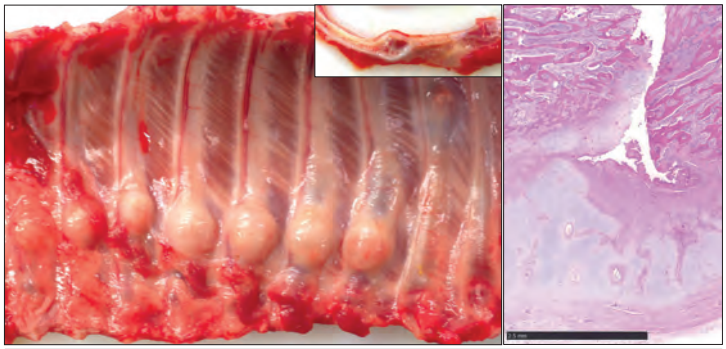


Figure 2: Left thoracic rib cage showing seven adjacent rib with prominent callus formation (left) and corresponding histological image (right). Insert: longitudinal section through callus revealing the rib fracture.

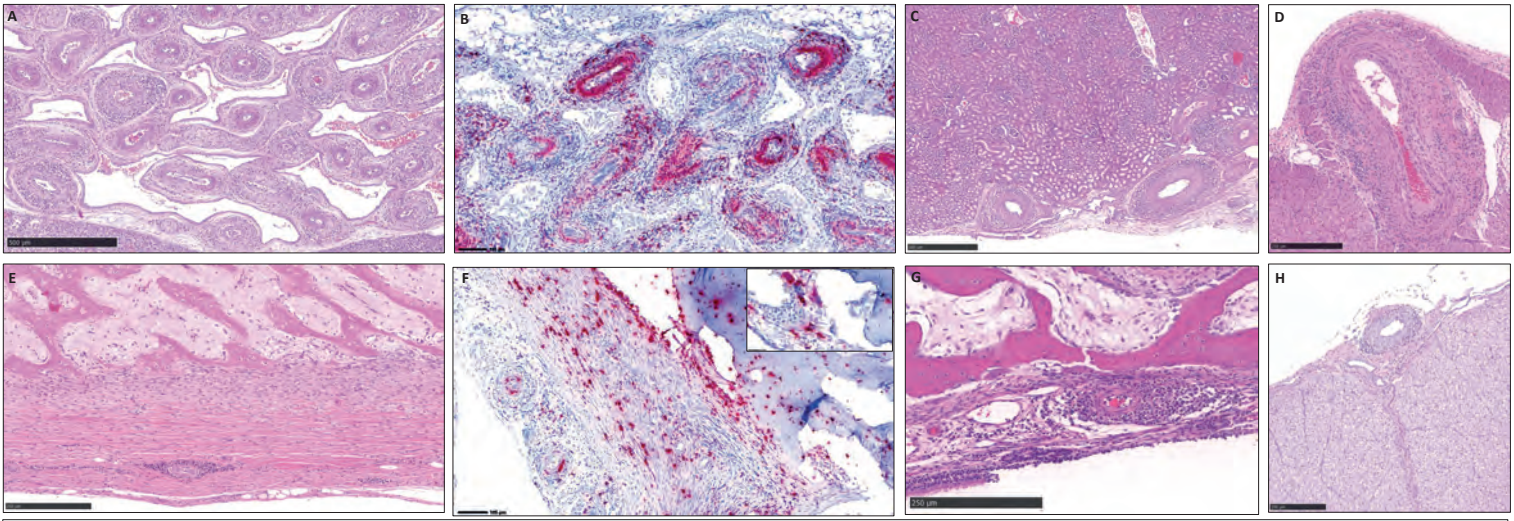


Figure 3: Histology and ISH. Vascular mesenteric plexus (A), kidney (C), myocardium (D), costal periosteum (E), nasal turbinate (G) and spinal meninges (H) showing lymphohistiocytic and plasmacytic (peri-)arterial infiltrates mainly involving the tunica adventitia. H&E. Vascular mesenteric plexus (B) with strong PCV-3 ISH positive signals of the arterial tunica intima and media as well as rib (F) with multifocal signals in the intima of the periosteal arteries, the periosteum and within osteocytes and osteoclasts (insert). PCV3-ISH, red-chromogenic staining counterstained with hematoxylin..

Conclusions

This is the first report on PCV-3 infections in Switzerland. The histological lesions in this case match the descriptions of PCV-3 systemic disease, but for the first time the virus has been detected in bone lesions. In the last decade, authors reported cases of “humpy-back" pigs exhibiting histologically inflammatory vascular lesions comparable to those reported here. Therefore, pathomorphological investigations and possible detection of PCV-3 is recommended in pigs displaying bone lesions and “humpy-back” posture.

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EFFECTS OF FOLLICULAR SUPERSTIMULATION ON OPU-IVP OUTCOME IN GERMAN FLECKVIEH HEIFERS

Dragos Scarlet[1,2], Idil Serbetci[1], Matthias Lautner[3], Heinrich Bollwein[1]

[1]Clinic of Reproductive Medicine, Vetsuisse Faculty Zurich
[2]Institute of Veterinary Anatomy, Vetsuisse Faculty Zurich
[3]Besamungsverein Neustadt a. d. Aisch e. V., Neustadt a. d. Aisch, Germany

dragos.scarlet@uzh.ch

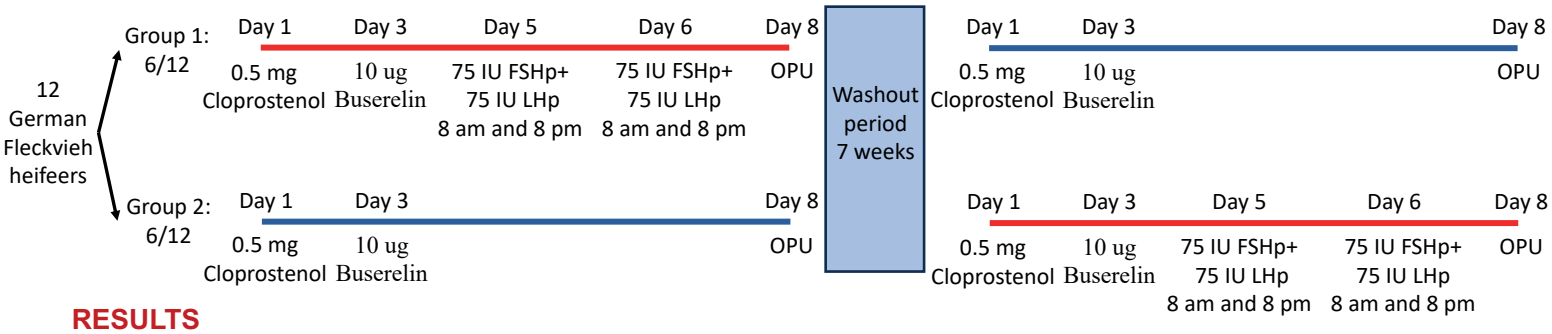
BACKGROUND

- Follicular wave synchronization and follicular superstimulation with FSH/LH are both routinely used nowadays to maximize results in OPU-IVP programs.
- Results of studies conducted so far either in milk or in beef cows provided sometimes inconsistent results.

AIM OF THE STUDY

Follicular superstimulation preceded by follicular wave synchronization improves in vitro embryo production outcomes in German Fleckvieh, a dual-purpose breed with high milk- and beef performance.

MATERIALS AND METHODS



RESULTS

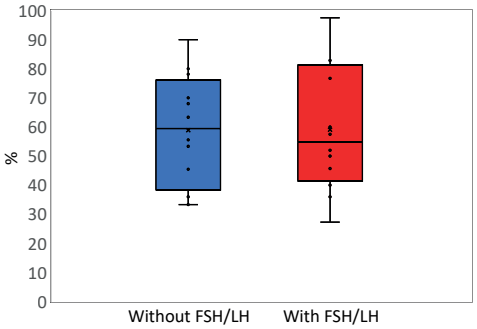


Figure 1. Recovery rate (%) of oocytes retrieved by OPU from heifers with or without follicular superstimulation before oocyte collection.

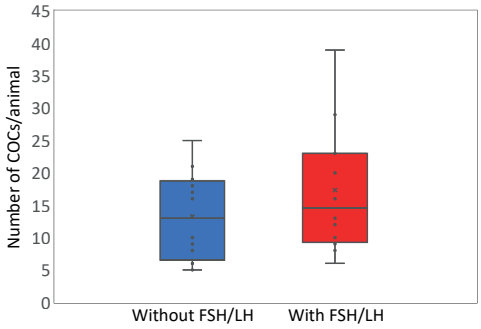


Figure 2. Average total number of oocytes recovered by OPU from heifers with or without follicular superstimulation before oocyte collection.

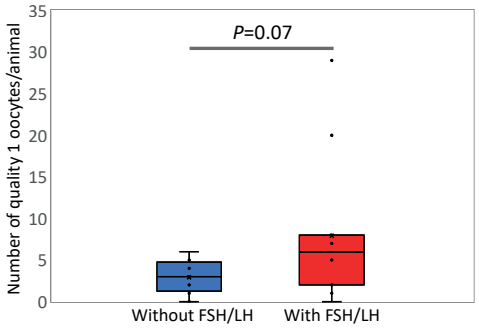


Figure 3. Average number of quality 1 (excellent) oocytes recovered by OPU from heifers with or without follicular superstimulation before oocyte collection.

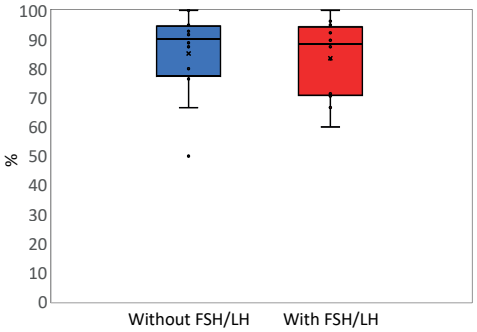


Figure 4. Cleavage rate (%) after in vitro fertilization of oocytes from heifers with or without follicular superstimulation before oocyte collection.

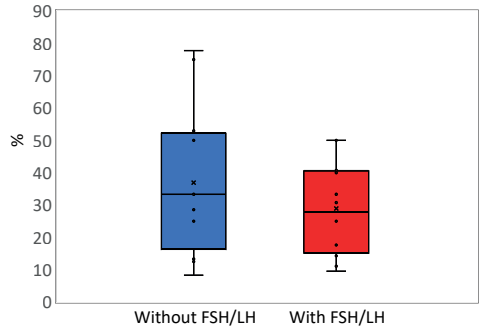


Figure 4. Blastocyst rate (%) after in vitro fertilization of oocytes from heifers with or without follicular superstimulation before oocyte collection.

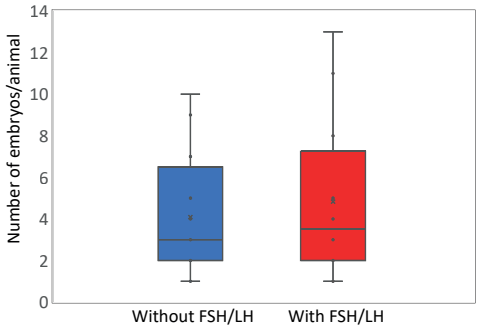


Figure 5. Number of transferable embryos produced by in vitro fertilization of oocytes from heifers with or without follicular superstimulation before oocyte collection.

CONCLUSION

Results of this study demonstrate a limited positive effect of follicular superstimulation in addition to follicular wave synchronization on OPU-IVP outcomes in the dual-purpose breed German Fleckvieh.

Comparative study of the morphokinetic parameters of embryos produced in vitro with sex-sorted vs. unsorted bovine sperm

Idil Serbetci[1]*, Carolina Herrera[1], Manuel Melean[1], Marianne Steiner[1], Mathias Siuda[1], Shauna Holden[2], Eleni Malama[1], Stephen Butler[2], Heinrich Bollwein[1]

[1]Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, CH-8057 Zurich, Switzerland
[2]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy Co. Cork. P61 C997, Ireland

Background and Aims

The protocols used for flow cytometric sex-sorting of sperm have been substantially improved in the last decade, but the use of sex-sorted bull sperm can result in variable blastocyst rates after *in vitro* fertilization. We aimed to perform a **comparative study of the morphokinetics of embryos that were *in vitro* produced with either cryopreserved sex-sorted (SS) or unsorted (control, CON) bovine sperm**.

Materials & Methods

- Single ejaculates collected from three Holstein-Friesian and one Jersey bull
- Ejaculates split in two aliquots
  - **SS aliquot** → flow cytometric sorting for X-chromosome sperm and cryopreservation (90% purity; 4×10^6 sperm/0.25-ml straw)
  - **CON aliquot** → conventional cryopreservation (15×10^6 sperm/0.25-ml straw)
- *In vitro* embryo production with SS and CON sperm samples (64 and 72 presumptive zygotes produced, respectively)

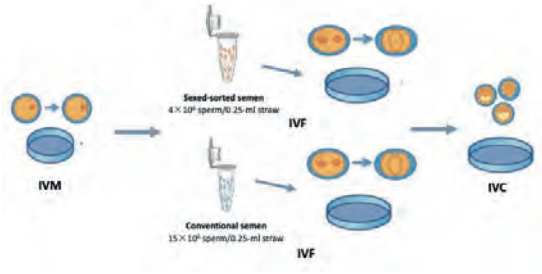
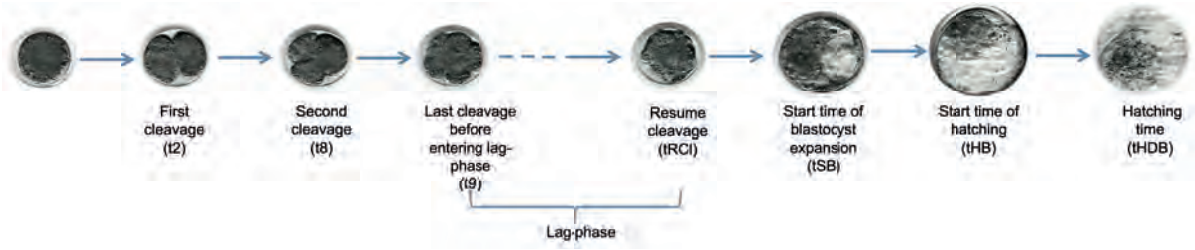


Figure adapted from Ferré et al. (2020), doi:10.1017/S1751731119002775

Time-lapse monitoring of *in vitro*-produced embryos



Results

✓ SS zygotes were 35.1% less likely (P = 0.046) and needed more time to enter the first cleavage compared with CON zygotes (31.05 vs. 29.25 hours).

✓ Having completed the first cleavage, SS and CON embryos showed the same ability to achieve subsequent developmental stages (Table).

Table: Developmental rates (cleavage, blastocyst and hatching rate) of 64 and 72 presumptive zygotes produced after *in vitro* fertilization with sex-sorted vs. unsorted bovine sperm, respectively.

Rate	Conventional (*n*=72)	Sexed-sorted (*n*=64)
Cleavage (%)	88.9±4.2 (64/72)	82.8±8.0 (53/64)
Blastocyst (%)	41.7±19.8 (30/72)	51.6±8.0 (33/64)
Hatching (%)	74.2±26.5 (23/30)	61.1±8.0 (21/33)

Conclusions

✓ Oocytes fertilized with sex-sorted sperm had compromised ability to complete the first cleavage, but their developmental potential thereafter was not affected.

**Corresponding author: Idil Serbetci, idil.serbetci@uzh.ch*

Modeling the effect of ambient temperature on reticulo-rumen temperature, feed intake, and milk yield of dairy cows during colder seasons

Aira Maye Serviento, Tengfei He, Xiaoqi Ma, Susanna E. Räisänen, Mutian Niu
Animal Nutrition, Institute of Agricultural Sciences, D-USYS, ETH Zurich

Introduction

- Maintaining reticulo-rumen temperature (RT) in an optimal range is important for proper rumen functions and thus for cow productivity.
- Free water and feed temperatures, which are intrinsically linked to AT, can be critical in open barn systems during autumn and winter when the ambient temperature (AT) is substantially lower than RT.
- **Objectives**: To determine the effects of FWT and FT on RT fluctuations, and to evaluate how AT affects RT and the drinking and eating behaviors of lactating dairy cows during colder seasons

Material and Methods

Animals and data collection

- 16 lactating dairy cows (224 ± 36 days in milk; 732 ± 51.9 BW, 25.2 ± 2.64 kg DMI/d, 85.3 ±12.77 kg WI/d).
- Four 6-d data collection periods 18 d apart from Sep to Dec 2022. Average AT during the periods: 15.0 ± 1.41, 14.3 ± 1.50, 10.0 ± 0.97, and 6.40 ± 2.36 °C, respectively.
- Daily recordings of FI, FWI and milk yield (MY)
- Continuous measures of RT(smaXtec bolus), AT (Neatmo smart system), FWI (GWF technology) and FI (Mettler Toledo), FWT, (i-Button) and FT (based on AT)

Calculations

- Data of non-overlapping ingestion events were selected:
  - Drinking (n= 490)
  - Eating or meal (n = 618)
- Variables of interest (Fig. 1):
  - RT change (°C)
  - RT recovery time (min)

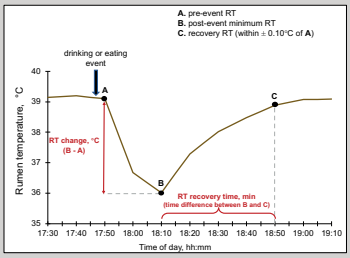


Figure 1. Illustration of RT change and recovery time calculations

Statistical analyses: Generalized additive mixed model

- RT fluctuations in relation to ingestion events
  - Fixed smooth term of the FWT or FT and of the drink or meal size
  - Random effect of cow
- Daily RT, feed (dry matter) and free water intake, and MY
  - Fixed smooth term of the average AT of the day
  - Fixed effect period, breed, and diet
  - Random effect of cow

Conclusions

- Drinking and eating events contribute to RT fluctuations.
- At temperatures colder than RT, amount of FWI and FI affected the magnitude of RT change and length of RT recovery time.
- Cold exposure increased thermoregulation responses, i.e., decreased RT and FWI, and increased DMI of dairy cows.
- Cold exposure can have consequences on feed efficiency (MY:DMI) likely due to additional energetic demands of the animal for thermoregulation rather than for production functions.

Results

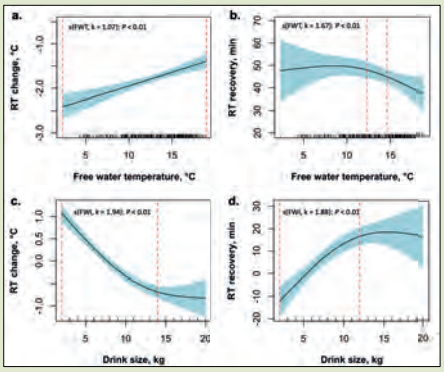
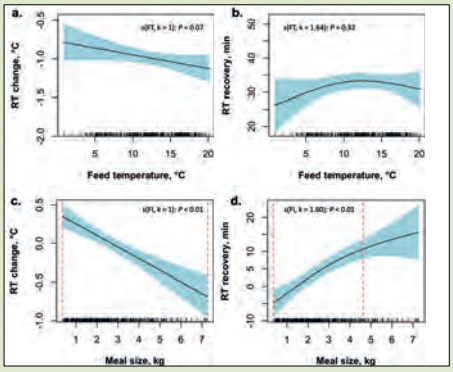


Figure 2. Effect FWT (°C; a and b) and of drink size (kg; c and d) on RT change (°C; a and c) and RT recovery time (min; b and d). Temperatures or drink sizes between the 2 red vertical lines represent the range of FWT or drink size with significant effect ($P < 0.05$).

- For every -1°C in FWT:
  - **-0.0596°C** RT change
  - **+1.27 min** RT recovery time
- For every +1kg in drink size:
  - **-0.108°C** RT change
  - **+2.13 min** RT recovery time

Figure 3. Effect of FT (°C; a and b) and of meal size (kg; c and d) on RT change (°C; a and c) and RT recovery time (min; b and d). Temperatures or meal sizes between the 2 red vertical lines represent the range of FT or meal size with significant effect ($P < 0.05$).

- For every +1kg in meal size:
  - **-0.150°C** RT change
  - **+3.71 min** RT recovery time



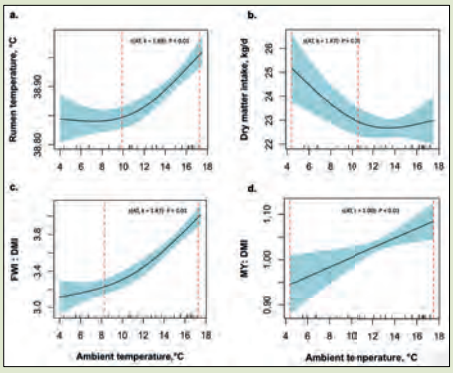


Figure 4. Effect of AT (°C) on a) RT (°C); b) dry matter intake (DMI, kg/d); c) FWI:DMI (kg FWI/kg DMI); d) MY:DMI (kg MY/kg DMI). The 2 red horizontal lines represent AT range with significant effect ($P < 0.05$).

- For every -1°C in AT:
  - **-0.0151°C** RT
  - **+0.365 kg/d** DMI
  - **- 0.0862** FWI: DMI
  - **-0.0106** MY: DMI

Contact: AM Serviento
airamaye.serviento@usys.ethz.ch

An alternative to the use of live mouse oocytes for the quality control of cryopreserved mouse sperm

Nataliia Shapovalova[1,2*], Johannes vom Berg[1], Heinrich Bollwein[2], Eleni Malama[2]
[1]Institute of Laboratory Animal Science, University of Zurich, 8952 Schlieren, Switzerland
[2]Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland

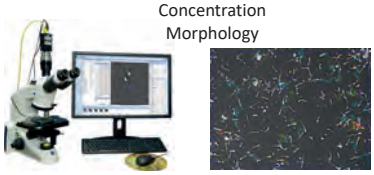
Background and Aim

Sperm freezing is a widely spread way to preserve the genetic material of genetically modified mouse lines without breeding animals unnecessarily. However, before a particular line can be discontinued, it must be ensured that rederivation from cryopreserved sperm is possible. Up to now, no animal-free methods have been validated to provide sufficient information for that decision. Many researchers and breeders use *in vitro* fertilization to verify the fertility of frozen sperm. Thus, a large number of female mice are bred, hormonally treated, and ultimately sacrificed for oocyte collection and subsequent testing of frozen-thawed sperm – a strategy far from being considered as an optimal application of the 3R principles. **Our aim is to develop an animal-free fertility predicting algorithm based on measures of mouse sperm quality obtained through up-to-date methods.**

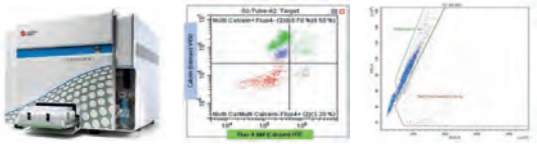
Project outline

Sperm quality analysis

Computer-assisted sperm analysis
Sperm motility
Concentration
Morphology



Flow cytometry
Sperm functional status
DNA integrity



Omics technologies
Transcriptomics
Proteomics

Fertility prediction

- Identification of sperm characteristics with high fertility predictive value
- Development of machine learning algorithms & routine protocols for mouse sperm analysis

Validation

- Multi-center validation study
- Knowledge dissemination
- Practical application

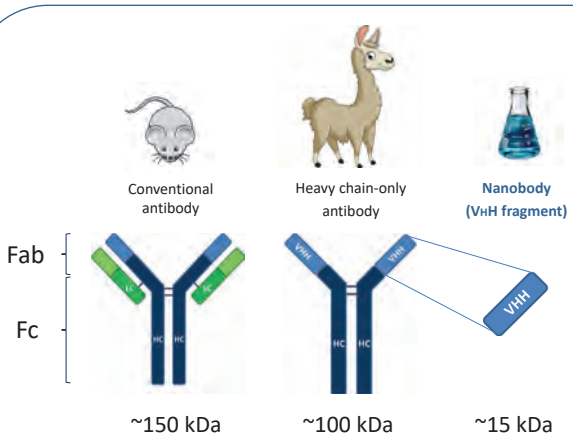
Future outlook

- **Replace** IVF-based quality control of cryopreserved mouse sperm with an animal-free test
- **Reduce** number of female mice used for sperm fertility testing

The project is funded by the SNSF NRP 79 «Advancing 3R – Animals, research and society»
**Corresponding author: Nataliia Shapovalova, nataliia.shapovalova@uzh.ch*

University of Zurich[UZH]

Nanobodies from alpaca immune libraries

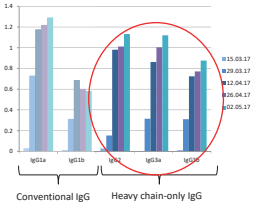


Why making nanobodies?

- Same affinities and specificities, but much smaller than an antibody
- More stable to temperature, pH, reducing conditions
- More soluble (easier crossing of biological barriers)
- Superb tisue penetration (therapeutic potential)
- Recognition of cryptic epiptopes (inhibitors)
- Easy to express recombinantly
- Amenable to bioengeneering
- Intracellular expression!

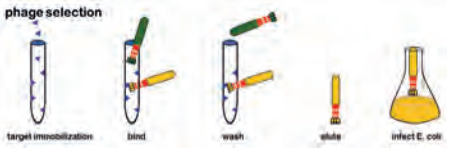
- Poor binding to linear epitopes (reducing western blot)
- Short serum life (but easily solved by constructing larger fusion proteins)

Nanobody generation process



- Immunisation of an alpaca with the antigen of choice
- Subcutaneously in shoulder/neck region
- Serological monitoring of immune response by ELISA for heavy chain-only antibodies (IgG2 and IgG3)

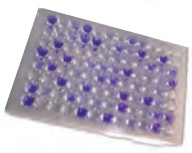
- Isolate RNA from lymphocytes and reverse transcribe
- Amplify VHH repertoire
- Clone into a phagemid vector
- Nanobody library displayed on the tip of filamentous phages



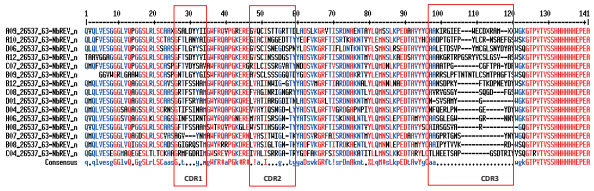
http://2011.igem.org/wiki/images/d/d1/UP_Procedere.png

- Enrichment of desired phages through rounds of selection and amplification

- Positive enrichment

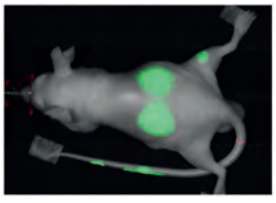


- Confirm reactivity of nanobodies from individual clones by ELISA



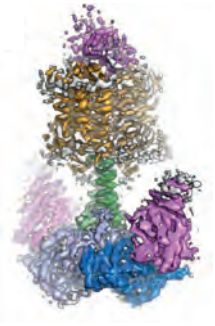
- Sequence, align and determine unique nanobodies

Applications

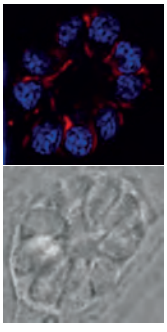


Methods Mol Biol. 2012;911:559-67. doi: 10.1007/978-1-61779-968-6_35.

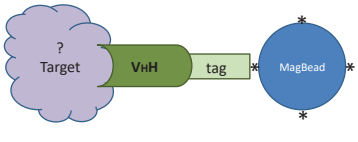
- Non-invasive *in vivo* imaging
- Structural biology of proteins
- Immunofluorescence microscopy



Elife. 2022 Aug 18;11:e77032. doi: 10.7554/eLife.77032.



R. Winiger / S. Stefanic, IPZ, UZH



- Immunoprecipitation
- Protein-proten interactions
- Field diagnostic tests

Therapy!

Nanobody Service Facility - NSF

https://www.nsf.uzh.ch/en.html

University of Zurich[UZH]

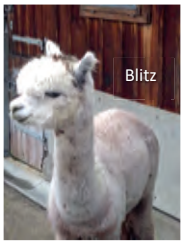
Nanobody Service Facility

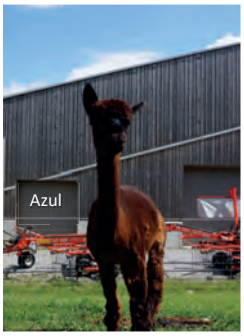
Production, screening and identification of *in vivo* matured camelid antigen binding fragments (nanobodies) generated against protein of interest

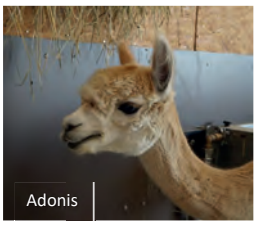
We require:
- 0.6 – 1.0 mg target antigen
- ~4-5 months time

We deliver:
- Alpaca hyperimmune serum
- Peripheral blood lymphocytes
- Phage-displayed nanobody libraries
- ELISA-screened bacterial clones
- Plasmids encoding amino acid sequences of the target-specific nanobodies

We make your nanobodies!







- 12 year-long experience in nanobody production and screening
- Since 2017 one of strategic technology platforms of the University of Zurich (https://www.research.uzh.ch/en/platforms.html)
- 64 nanobody projects to date, including more than 150 target antigens
- Success of immunisation procedure is around 75%
- Success of nanobody generation when desired immune response is obtained is >90%

References citing NSF:

Structure of Mycobacterium tuberculosis Cya, an evolutionary ancestor of the mammalian membrane adenylyl cyclases
Elife. 2022 Aug 18;11:e77032. doi: 10.7554/eLife.77032. PMID: 35980026; PMCID: PMC9433096.

Structural and functional properties of a magnesium transporter of the SLC11/NRAMP family
Elife. 2022 Jan 10;11:e74589. doi: 10.7554/eLife.74589. PMID: 35001872; PMCID: PMC8806188.

Structural basis for ion selectivity in TMEM175 K+ channels.
Elife. 2020 Apr 8;9. pii: e53683. doi: 10.7554/eLife.53683.

Conformation space of a heterodimeric ABC exporter under turnover conditions.
Nature. 2019 Jul;571(7766):580-583. doi: 10.1038/s41586-019-1391-0. Epub 2019 Jul 17.

Identification of conformation-selective nanobodies against the membrane protein insertase BamA by an integrated structural biology approach.
J Biomol NMR 73, 375–384 (2019). https://doi.org/10.1007/s10858-019-00250-8

The extracellular gate shapes the energy profile of an ABC exporter.
Nat Commun. 2019 May 21;10(1):2260. doi: 10.1038/s41467-019-09892-6.

Generation and Characterization of Anti-VGLUT Nanobodies Acting as Inhibitors of Transport.
Biochemistry. 2017 Aug 1;56(30):3962-3971.

Structural basis of nanobody-mediated blocking of BtuF, the cognate substrate-binding protein of the Escherichia coli vitamin B12 transporter BtuCD.
Sci Rep. 2017 Oct 30;7(1):14296.

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ETH Eidgenössische Technische Hochschule Zürich Swiss Federal Institute of Technology Zurich · UNIVERSITÄTS-KINDERSPITAL ZÜRICH · Das Spital der Eleonorenstiftung

RESOLUTE Research Empowerment on Solute Carriers · UNIVERSITY OF OXFORD

https://www.nsf.uzh.ch/en.html

Evolution of a rating key - how to evaluate tail lesions in fattening pigs?

SUISAG
LBBZ Schluechthof Cham

Research questions

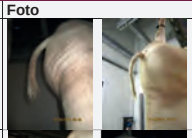
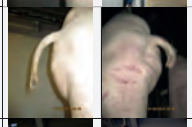
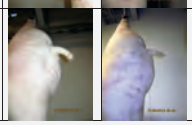
Tail biting is an abnormal behaviour where a pig bites another one's tail.

- Which factors influence tail biting?
- Which rating key is best suited for Swiss pig breeding?

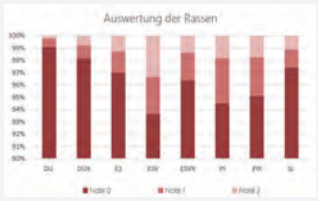
Results

Main factors increasing the occurrence of tail biting:

Previous rating key (since 2015 at LB)

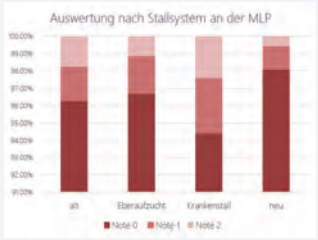
Stufe	Schwanz	Foto	Umschreibung
0	intakt		Keine Verletzung, normale Länge
1	leicht verletzt/ verkürzt		Normal lang, aber verletzt oder leicht verkürzt (mehr als halber Schwanz noch vorhanden)
2	stark verkürzt		Stark verkürzter Schwanz (weniger als Hälfte vorhanden) Wunde oder bereits abgeheilt

25,696 observations



→ **Breed**
(ESV: Large White sire line)

→ **Technicians**



→ **Available space**
(smaller pens)

→ **Sex (male and castrates)**

Conclusions

Measures to prevent tail biting:

- **No overcrowding in the pens**
- **Good environment**
  (e.g., climate, temperature and airflow)
- **Good health conditions of the animals**
  (e.g., no diarrhoea, lameness and inflammations)
- **Good management**
  (e.g., regular feeding times, sufficient feeding places and good hygiene)
- No mycotoxins in feed as well as in straw
- Sensitized animal observation for early detection

Contact information

Nina Stöckli, nst@suisag.ch, Allmend 10, 6204 Sempach

Evolution of the rating key

First tested rating key

Simplification

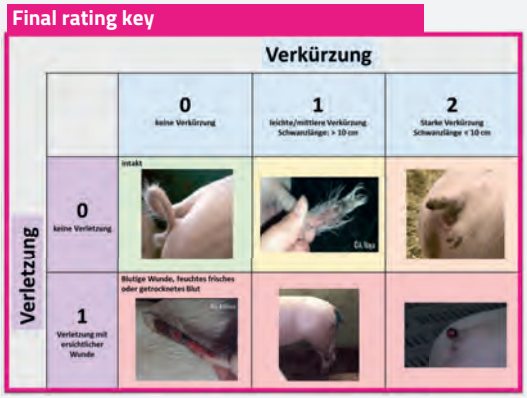
Second tested rating key

117 observations

Insights in the evolution of the rating key

- Split in shortening and injury is helpful
- Shortened tails can have no lesions
- A short tail does not necessarily indicate tail biting behaviour; it might just be born with a short tail
- Less is more: many subgroups make rating difficult
- Concordances between 4 technicians
  → can be improved by trainings

Final rating key



Dietary supplementation of vitamin D3 and Ca partially recover compromised lying behavior and its circadian rhythm in lactating cows under heat stress

K. Wang[1], A. Ruiz González[2,3], S. E. Räisänen[1], V. Ouellet[3], A. Boucher[3], D. E. Rico[2], M. Niu[1]

[1]Department of Environmental Systems Science, ETH Zurich, Zurich, Switzerland
[2]Centre de Recherche en Sciences Animales de Deschambault (CRSAD), Quebec, QC, Canada
[3]Department of animal science, Université Laval, Quebec, QC, Canada

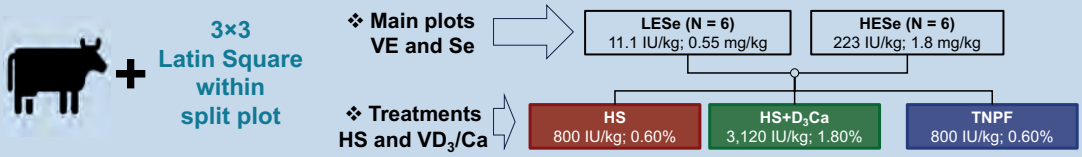
Background

- Heat stress (HS) influences animal welfare and sustainable production.
- Dietary supplementation of antioxidants and immune modulators may alleviate symptoms of HS.

Objective

- To characterize the time budget and circadian rhythm of lying behavior in dairy cows during HS.
- To assess the alleviating effect of dietary supplementation of vitamin D3 and Ca (+D3Ca) on HS.

Experimental design



THI inside chambers
- **HS**: 72 ~ 82
- **HS+D_3Ca**: 72 ~ 82
- **TNPF**: 61 ~ 64

* **TNPF**: Thermoneutral pair-feeding

Results

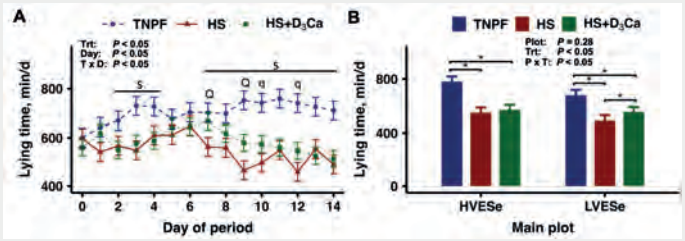


Fig 1. Effects of HS and diet treatments on daily lying time
A) Daily lying time across experimental period
B) Average daily lying time by main plot

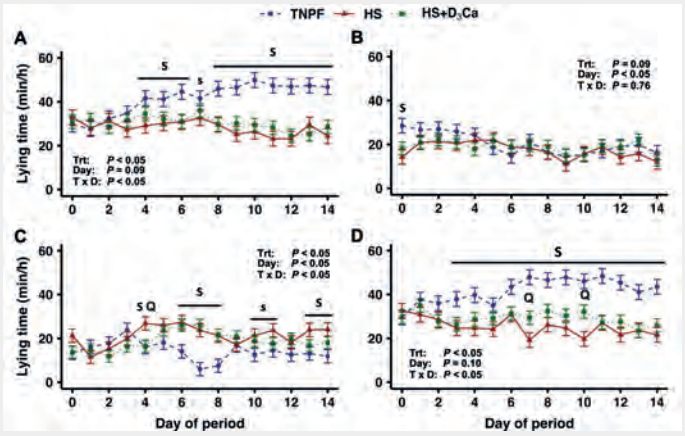


Fig 2. Lying time during different time frames of day
A) 0000 – 0600 h; **B)** 0600 – 1200 h
C) 1200 – 1800 h; **D)** 1800 – 2400 h

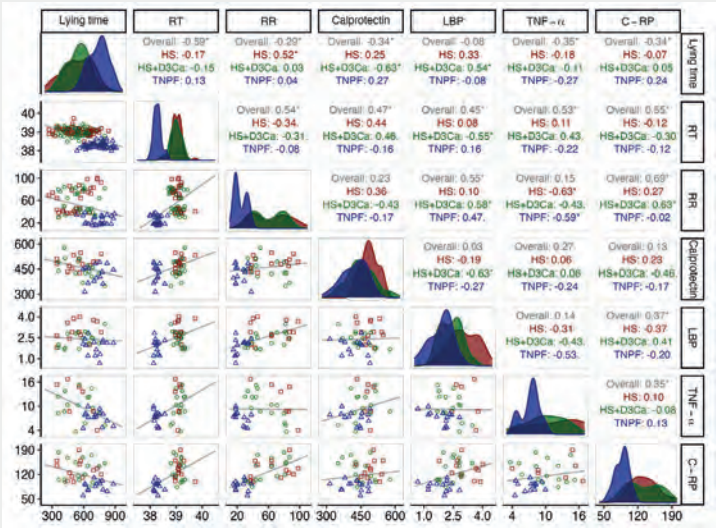


Fig 3. Correlations between daily lying time, rectal temperature (RT), respiratory rate (RR), and inflammation markers. Markers include fecal calprotectin, lipopolysaccharide-binding protein (LBP), tumor necrosis factor-α (TNF-α), and C-reactive protein (C-RP).

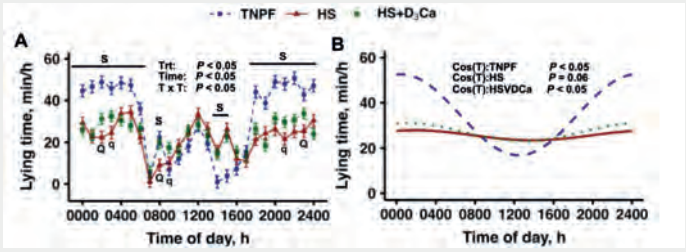


Fig 4. Daily patterns of lying behavior
A) Least squares means
B) Circadian curves fitted by cosine functions

Conclusion

- ❖ Lying behavior was compromised in dairy cows under HS.
- ❖ The compromised lying behavior can be partially restored by supplementation of VD3 and Ca.
- ❖ Intake restrictions may shift feeding behavior and introduce biases in the lying behavior of animals.

Contact:
Kai Wang
kai.wang@usys.ethz.ch

Computer vision-based models for estimation of respiratory rate of dairy cows using contactless videos

M. Wang[1], S. Li[1], R. Peng[1], S. E. Räisänen[1], A. M. Serviento[1], X. Sun[1], K. Wang[1], F. Yu[2], M. Niu[1]

[1]Animal Nutrition, Institution of Agricultural Sciences, D-USYS, ETH Zurich

[2] Computer Vision Lab, D-ITET, ETH Zurich

Introduction and Objective

Physiological ecology

- Respiratory rate (RR) serves as an important physiological indicator of animal health and welfare, for example, for dairy cows experiencing heat stress and respiratory illness.
- Non-invasive and less labor-intensive contactless methods are especially attractive for RR monitoring.

RR monitoring

- When cow breath, the abdomen will show regular fluctuations.
- Analyzing the video and extracting the motion changes of the abdomen automatically can help monitor the RR directly from the video.

Main Objective

To develop an end-to-end model that can directly monitor the cow's RR from contactless videos.

Material and Methods

Animal experiment

- Six cows were video recorded for 24 hours each by using digital cameras (DH-SD1A404XB-GNR) positioned at the side and top of the cow to capture the region around abdomen.
- Each cow wore an Embla XactTrace respiration belt for RR measurements to serve as the ground truth (GT).

Results

Implementation

- Seventeen videos with a mean length (± SD) of 16 min 9 s ± 11 min including both day and night occasions, were selected. The selected videos were segmented to shorter segments, each lasting 1 minute, with an overlap of 30 seconds between consecutive clips.
- The RR extraction task was reformulated as a video classification problem, and the RR for each minute served as the label for that minute's video segment. A state-of-the-art computer vision model, videoMAE, was utilized to extract features for monitoring the RR directly from the video.

Comparison with GS

- Mean absolute error (MAE), root mean square error (RMSE) and root mean squared prediction error (RMSPE) were used to compare the results with GS. The unit of MAE and RMSE is breaths per minute (bpm).

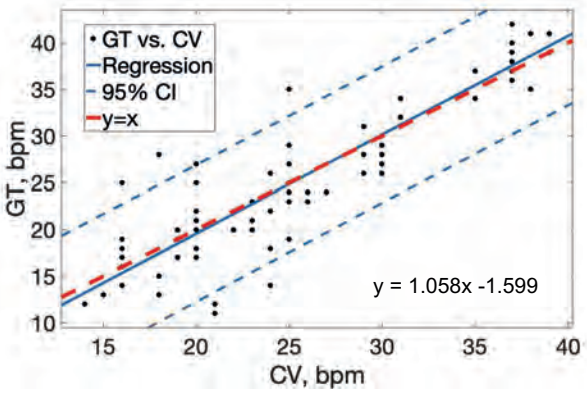


Fig 1. Regression analysis: computed RR and GT

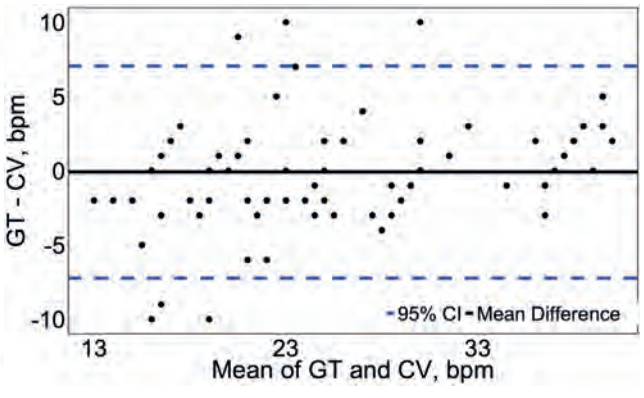


Fig 2. Bland Altman plot: difference between RR and GT

MAE(bpm): 2.60
RMSE(bpm): 3.62
RMSPE(%): 14.02

Scan me for video demos

Conclusions & Future Research

Conclusion

- RR can be monitored directly from contactless videos, and it is possible to continuously monitor the RR of a cow when the animal is relatively still.

Future research

- The model could be combined with lying behaviour detection and identification algorithms for monitoring respiration rate of multiple cows in free-stall barns.
- This approach could be a potential tool to detect heat stress as cows show increase in RR in such case.

Contact: meiqing.wang@usys.ethz.ch