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

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The Swiss Parliamentary Initiative Pa.Iv.19.475 on reducing the risk of pesticide usage calls for an appropriate reduction of nitrogen and phosphorus losses in the farming sector by 2030. As livestock husbandry is responsible for approximately 90% of agricultural ammonia losses, livestock management will need to make significant contributions to achieving the targets set on the reduction pathway. Feeding optimisation is particularly effective in this respect, as the potential for losses along the entire commodity chain can be automatically lowered by reducing nutrient inputs. In pig and poultry feeding, the use of nitrogen and phosphorus reduced (NPr) feed is widespread. In dairy cattle, however, the approach of feeding crude protein reduced diets has rarely been practised to date.

For this reason, the effects of protein-reduced rations on milk yield parameters were investigated in pilot trials on two Swiss commercial farms during two winter feeding periods. In crossover trials, experimental groups (V) and control groups (K) were paired for comparison. In the experimental groups, the rations' protein content was reduced by about 10 g/kg DM and the rations were supplemented with rumen-protected amino acids so that the amount of digestible lysine and methionine was at least equal to the amount contained in the control group's ration.

No significant effect on the energy-corrected milk yield and the milk fat and milk protein contents was detected. However, as in other studies, a slight numerical decrease in milk yield was observed and in one run, the daily milk protein amount was 0.05 kg lower in the experimental group ($p < 0.05$). A possible reason for this decrease could be the reduced nitrogen supply for the rumen microbes and their growth. It is possible that the supplementation with the amino acids lysine and methionine did not compensate for the difference in available protein in the intestine. There are indications in the literature that under certain conditions histidine may be a limiting amino acid in dairying. For further trials, therefore, histidine would have to be taken into consideration in addition to the amino acids methionine and lysine.

Given the correlation between nitrogen excretion and milk urea content, the latter can be used to assess the environmental impact of the experimental ration. For all experimental groups, a significantly lower value (-3.2 to -4.6 mg urea per dl milk) was observed compared to the control group. Other studies show that a reduction in ammonia emissions of between 8 and 14% can be expected for the protein-reduced ration at the observed milk urea levels.

The present study concords with other studies in showing that a reduction in protein intake in dairy cattle feeding can be achieved without a loss in milk yield if it is specifically balanced with rumen-protected amino acids. This approach can thus contribute to reducing ammonia emissions. A reduction of ammonia emissions by 10% by means of feeding dairy cattle a protein-reduced diet appears to be possible in future.

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

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Certain feed additives may reduce ammonia emissions in chicken stables through ammonia adsorption or promoting its utilization as a nitrogen source for microbial protein biosynthesis. We conducted a study to test different products on total nitrogen and ammonia excretion, as well as apparent nitrogen retention, during chicken fattening.

We used 120 slow-growing mix-sexed ROSS chickens and fed them the same organic diet (FORS2121; 13 MJ ME/kg, 200 g/kg CP) during a 10-day starter period and 5 weeks of fattening. We ensured excessive nitrogen intake by applying organic feeding in a one-phase system to create a worst-case scenario. Starter chickens were housed in five pens with 24 animals each, and during fattening, they were kept in metabolic cages with 2 birds per pen. The birds were randomly assigned to five feeding groups during fattening: Control, Klinofeed (5 g/kg), Bentonit (5 g/kg), BioChar (5 g/kg), and Arbocel (6 g/kg). These products were added on top of the control diet. In the last week of fattening, we collected excrements cage-wise without any losses, freeze-dried them, and analyzed the concentration of total nitrogen and ammonia in a commercial laboratory. We used the data to calculate the apparent retention of feed dry matter and nitrogen and analyzed it using mixed models with the fixed effect of "diet" and the random effect of the individual cage nested under the respective feeding group.

None of the tested products showed any significant differences in total and ammonia nitrogen excretion or the apparent retention of dry matter and nitrogen ($P > 0.3$). Therefore, we could not confirm that the products had any effects that would improve stable air quality. However, it is interesting to note that there were no differences between the control and treatment groups, despite the treatment groups receiving approximately 0.5% of materials with indigestible dry matter (Klinofeed, Bentonit, BioChar) or reduced levels of digestible dry matter (Arbocel), resulting in an overall reduction in digestible dry matter compared to the control. This suggests that the treatment groups may have utilized the diets more efficiently in terms of digestible dry matter conversion. This finding aligns with previous data from the same project, which showed comparable zootechnical performance of birds despite differences in the concentration of digestible dry matter in the treatment diets. Further research is warranted to investigate this phenomenon.

3 The ruminant sorting mechanism protects teeth from abrasives

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Dental wear due to ingestion of dust and grit has deleterious consequences. Herbivores that could not wash their food hence had to evolve particularly durable teeth, in parallel to the evolution dental chewing surface complexity to increase chewing efficacy. The rumen sorting mechanism increases chewing efficacy beyond that reached by any other mammal, and has been hypothesized to also offer an internal washing mechanism, which would be an outstanding example of an additional advantage by a physiological adaptation, but *in vivo* evidence is lacking so far. Here, we investigated four cannulated, live cows that received a diet to which sand was added. Whereas silica in swallowed food and feces reflected experimental dietary sand contamination, the regurgitate submitted to rumination remained close to silica levels of the basal food. This helps explain how ruminants are able to tolerate high levels of dust or grit in their diet, with less high-crowned teeth than nonruminants in the same habitat. Palaeo-reconstructions based on dental morphology and dental wear traces need to take the ruminants' wear-protection mechanism into account. The inadvertent advantage likely contributed to the ruminants' current success in terms of species diversity.

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

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Virtual Fencing (VF) can be a helpful technology in managing herds in pasture-based systems. In VF systems, animals wear a VF-GPS collar, and physical boundaries are replaced by virtual ones. Collars emit an acoustic signal when animal crosses the virtual fence, followed by a weak electrical pulse if the animal does not return into the defined area. The stimuli sequence is repeated up to 3 times if the animal continues to walk forward. Although has been demonstrated that animals learn the system quickly, is unknown if this learning capacity decreases with animal age. The study aimed to investigate the differences in the learning ability between young and old dairy cows; and whether VF impacts grazing activities and milk production, as well. The study was conducted in the Swiss lowlands on four comparable different strip-grazing paddocks. Twenty lactating Holstein-Friesian cows, divided into four groups of five animals, were equipped with VF collars (Nofence AS, Norway). Half of the animals (two groups) were young (first lactation) and old (more than one lactation). After a 7-day training, paddock's grazing size were increased by VF, during four consecutive grazing periods (P1-P4). Furthermore, each cow wore a pedometer (Peacock Technology Ltd., UK) to record daily step count, time spent standing, and time spent lying. Data were analyzed by generalized mixed-effect models demonstrating that age had no significant impact on animals' overall response to VF. The only difference was in the emission of acoustic signals registered in P4, in which young cows received more than old cows. Moreover, during training, the reduction in the acoustic signal duration, occurred faster for old than young cows, due to their overall fewer grazing activities, rather than a better learning capacity. Indeed, in training they performed less step per day, spent more time lying and less time standing. Finally, no changes in milk production were detected. In conclusion, results suggest that there was no age-related response to VF, and milk production was not affected as well.

5 Optimization of 16S ribosomal RNA V3/V4 amplicon generation from cytobrush samples for analysis of the uterine microbiome in the mare

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Studying the microbiome of the uterus can be quite challenging, particularly due to the very low microbial biomass present. In mares, collection of uterine samples as a source of DNA and RNA for microbiome analysis can be carried out during routine fertility assessments by using cytobrush and thus, avoiding animal distress. However, the unfavorable ratio of host DNA to bacterial DNA in these samples, together with the similarities among the 16S ribosomal RNA (rRNA), mitochondrial 12S rRNA, and the 18S rRNA, can impact the reproducibility of generating specific 16S rRNA PCR amplicons. Furthermore, it is worth noting that many PCR reagents contain contaminating bacterial DNA, which can introduce additional bias to the results of 16S rRNA sequencing.

In this study, we aimed to optimize the generation of 16S ribosomal RNA amplicon to analyze the mare uterine microbiome by using cytobrush samples. For this purpose**,** uterine cytobrush samples were collected from mares in estrus, and DNA extraction was performed using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen). Then, we amplified the V3-V4 hypervariable region of the 16S rRNA fragment, which spans approximately 465 bp, using universal prokaryotic primers Pro341F and Pro805R. To optimize the PCR reaction, we tested five different PCR kits and various PCR conditions, such as primer/template ratio, Mg^{2+} concentration, DMSO addition, and thermal cycling conditions. Among the five PCR kits tested, three yielded detectable products in the negative control and only two yielded clean negative controls (Hot MolTaq 16S/18S, Molzym, Bremen, Germany, and Platinum™ Taq DNA Polymerase, DNA-free, Invitrogen by ThermoFisher Scientific). Besides, the PCR kits yielded an additional non-specific 346 bp product when analyzing cytobrush DNA samples. Sanger sequencing confirmed that the 465 bp product corresponded to a fragment of the 16S rRNA gene, whereas the 346 bp product originated from the equine mitochondrial 12S rRNA gene. The ratio of specific 16S to non-specific 12S products varied across samples and reactions, possibly due to degenerated positions of the primers, which introduced a partially random bias during amplicon generation. To address this issue, two options were tested: 1) a competing oligonucleotide with a 3'-end amino modification was used to suppress the amplification of the 12S product in comparison to an oligonucleotide with a 3'-phosphate; and 2) a peptide nucleic acid (PNA) clamp. The PNA clamp did not provide satisfactory results in comparison to the oligonucleotides. The extent of suppression of the 12S product depended on the concentration of the oligonucleotide, with nearly complete suppression achieved at a final concentration of 0.4 µM.

The results of this study provided a reproducible and sensitive PCR protocol for generating 16S rRNA V3-V4 amplicons from low biomass uterine microbiome samples. This protocol utilized PCR reagents devoid of bacterial DNA together with a competing oligonucleotide with a 3'-end amino modification to effectively suppressed the amplification of non-specific equine mitochondrial 12S rRNA-derived PCR products.

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6 What are the effects of adapting dairy cows to a virtual fencing system on animal welfare and pasture management?

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Animals are fitted with a collar that emits an audio tone (**AT,** rising pitch, 5-20 s), followed by a weak electric pulse (**EP,** 0.2 Joule, 1 s) when they cross a digital boundary tracked by global positioning: virtual fencing (**VF**) opens up the possibility of replacing physical fences, thereby reducing manual labor for farmers. However, its application raises concerns on animal ethics. A VF-system was used on a Swiss dairy farm to follow the learning process of cows and to determine their stress responses compared to cows managed with electric fences (**EF**). Twenty lactating cows (3.7±1.5 lactations, 203±62 DIM), naïve to VF, were divided into 4 homogeneous groups of 5 individuals (2x VF; 2x EF). Each group grazed in a separate paddock during 3 days of acclimation (**P0**), followed by 4 periods (**P1-4**) of 21, 14, 14, and 7 days, respectively. All paddocks were electrically fenced, comparable in size and of similar topography and vegetation. During P0, all cows became accustomed to wearing an IceQube pedometer (Peacock Technology Ltd, Stirling, UK) and a deactivated VF collar (Nofence AS, Batnfjordsøra, Norway). During P1-P4, a virtual boundary was activated for the VF groups. Throughout the experiment, the sensors continuously tracked cow positions and activity behavior at 15-min intervals. From P1, the collars additionally recorded each AT and EP per cow. During P0-P4, individual feed intake was recorded daily in the barn, as well as milk yield and body weight twice per day. A total of 26 milk samples were collected per cow to examine milk cortisol after grazing. Observations were conducted for 2 h on 23 days to record agonistic behaviors, vocalizations, and excretions. The mean relative distances of the cows from the exclusion zones were determined depending on the maximum distance from the VF or EF (in %), respectively. During 56 days, each cow received a mean of 1.9±3.3 AT and 0.1±0.7 EP per day. The mean number of EP (n = 8) decreased by 74 % from Day 1 to 3 and remained below a mean daily level of 0.4 EP per cow for the remaining experiment. All cows learned to cope with the VF-system, as indicated by a decreasing ratio of mean EP/AT from 0.2 to 0.03, 0.02 and 0 in P1-4, respectively. Milk yield and cortisol, feed intake, body weight, and activity behaviors did not significantly differ between VF and EF groups. Overall, there were a mean of 11.2 vocalizations and 5.9 displacements more per cow in the VF groups ($p < 0.05$), but without differences between P0-P4. The mean relative distance of the cows to the exclusion zones was similar between EF (42±1.3 %) and VF groups (42±1.4 %) within the 59 days of experiment. The cows adapted to the VF-system without evidence of lasting adverse effects on animal welfare. Based on individual learning ability, sufficient training of the animals by at least 3 repetitions at a new virtual boundary, each maintained for at least 3 days, seem to be appropriate for the conditioning at herd level.

7 Identification and characterisation of social interactions using accelerometers in cattle

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Knowledge of social structure of cattle allows to ensure good welfare thereby reducing aggression and injuries, limiting disease transmission 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. Non-invasive electronic and biosensor devices are promising tools to provide a more efficient and reliable behavioural quantification and characterisation of social interactions. Yet, very limited research on identifying social interactions in farm animals using these technologies has been done so far. Hence, in this study we aimed at assessing whether the type and laterality of social interactions could be reliably inferred using accelerometers in cattle. Using video-recordings, we assessed the frequency and laterality of agonistic (headbutt) and affiliative (grooming) interactions expressed in a group of 10 suckler cows on pasture over 54 h of observations at the research station Früebüel (AgroVet Strickhof). Each cow was fitted with one tri-axial accelerometer fixed on the neck part of a halter. Acceleration data were first pre-processed (reformatted, standardised, and segmented in 1 or 2s windows), then labelled (matched to a behavioural category and laterality parameter) and finally acceleration features were calculated (mean, variance, max, min, ODBA). Mann Whitney U tests were used to compare features between social interactions (grooming vs. headbutt) or laterality parameters (left vs. right). We recorded 343 (214 left/129 right) and 529 (250 left/ 279 right) grooming and headbutt interactions, respectively. All acceleration features were found to differ ($P < 0.001$) between the two types of interactions on all three axes and for both segmentation windows, except for Max value on the x axis at a 2s segmentation window. Significant differences ($P < 0.001$) in the expression of left and right grooming interactions were observed on all axes and for both segmentation windows for all acceleration features. For headbutt interactions, significant differences ($P < 0.05$) were only found 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. By identifying differences in acceleration profile 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.

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

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The ultimate pH is an important indicator of quality and shelf life of meat. For a trial period of one year, ultimate pH was measured in the neck and over the course of 9 months, photos were taken from the left carcass side of the SUISAG testing animals. Routinely, the pH in the ham and loin is measured 90 min and 24 h post mortem using a pH-Star device (Matthäus, Eckelsheim, Germany). The photos were analysed visually regarding the skin lesions in four parts of the carcass (neck and shoulder, breast (front middle), flank (hind middle), and hind leg) using a scale from 1 to 4 (1: no lesions, 2: occasional lesions, 3: increased lesions, and 4: area-wide lesions). The final data set included 2'829 animals with pH measurements, of which 1'347 also had the skin lesion assessment. Different fixed effects on pH in the neck were tested using linear models. The results show highly significant effects of slaughter date and the combined sire*farm of origin effect; however, also significant effects from breed, breed of the sire, sire, farm of origin and sex were detected. The same analysis on the skin lesions showed significant effects of slaughter date on all body parts and sex on most. Correlations between pH in the neck and the different skin lesions are all highly significant, varying between 0.133 (hind leg) and 0.507 (neck and shoulder). In addition, heritabilities were estimated using animal models of the form: $\boldsymbol{y = Schlachttag + Geschlecht + Rasse + r(Tier) + \varepsilon}$. The heritability for pH in the neck is low with 0.004. The highest heritability was estimated for skin lesions on the breast (0.079) and the lowest for the skin lesion on the neck and shoulder (<0.0001). Using multi trait models of the same form, allowed to increase the heritabilities of pH in the neck to 0.096 and of skin lesions between 0.102 and 0.106.

Further analyses were performed using unsupervised learning approaches. These were supposed to aim at the classification and prediction of animals into aggressors, victims, and uninvolved individuals for selection purposes. Unfortunately, these results are not very promising for the practical application as the methods did not cluster each individual good enough.

Finally, the heritabilities are rather low for breeding purposes, especially the pH in the neck, which was the measurement that would be most interesting for practical implementation. These low heritabilities can be explained by the extremely high slaughter day effects, which dominate the hereditary component and prohibit reliable estimations.

9 Characterization of the Diurnal Pattern of Exhaled Volatile Fatty Acids and Enteric Me-thane Emissions of Dairy Cows

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The conventional methods used for the evaluation of rumen fermentation are invasive. Volatile organic compounds (VOCs) in a ruminant exhalome originate from the lungs and the rumen, and contain VOCs, such as ruminal methane (CH_4) and exhaled volatile fatty acids (eVFA). In this study, we aimed to use a non-invasive metabolomics approach using secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) platform to assess rumen fermentation parameters in dairy cows. Enteric CH_4 emission from 7 lactating cows was measured 8 times over 2 consecutive days using the GreenFeed system. Simultaneously, exhalome samples were collected and subsequently analyzed using the SESI-HRMS platform. In total, 1,298 features were detected, among them 3 targeted eVFA (i.e., acetate, propionate, butyrate), which were putatively annotated using their exact mass-to-charge ratio. Exhaled acetate had the greatest concentration among the eVFA, averaging (± SD) 21.3 ± 13.94 count-per-second (CPS) followed by propionate at 11.5 ± 3.10 CPS, and butyrate at 2.67 ± 2.495 CPS. Further, exhaled acetate was the most abundant at around 60% of the total eVFA captured, followed by 32% propionate and 8% butyrate, which correspond well with the previously reported ruminal VFA proportions. The diurnal patterns of CH_4 emission and individual eVFA were characterized using a linear mixed model with cosine function fit, and it demonstrated a daily pattern ($P < 0.05$) for ruminal CH_4 and H_2 emissions and eVFA concentrations over a 24-h period. Based on the model, the phase of total eVFA, and exhaled acetate, propionate, and butyrate was at 1354 h, 1348 h, 1448 h, and 1312 h (i.e., 4 to 6 h post-feeding), respectively, whereas the peak of CH_4 occurred at 1436 h and that of H_2 at 1600 h. This corresponds well with the expected relationship between rumen VFA and CH_4 formation. Results from the present study revealed a great potential to assess rumen fermentation and the daily pattern using exhaled metabolites as a non-invasive approach. Further validation and application of the proposed method are required.

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

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Agriculture, particularly the production of meat and dairy, contributes significantly to the global nitrogen surplus, posing environmental challenges. Sustainable dairy production relies on efficiently utilizing ingested protein, specifically converting it into milk protein. Excess protein leads to the excretion of nitrogen compounds, which adversely affect the environment, causing soil degradation and water body eutrophication. Furthermore, the application of manure to arable land can result in the emission of nitrous oxide, a greenhouse gas with a higher warming potential than methane. Previous studies have identified phenotypic and genetic differences in nitrogen use efficiency (NUE). However, the presence of genetic correlations between NUE and methane emissions, indicating trade-offs or opportunities for indirect selection, remains unexplored.

Starting in January 2022, the aim of this study is to investigate the genomic variation in NUE and methane emissions in dairy cows in Switzerland. Data will be collected over a four-year period, encompassing dairy cows from the Agroscope experimental farm in Posieux, as well as cantonal and private farms. The dietary regimen of the cows included herbage (grazed or ungrazed), hay, grass silage, maize silage, and partially concentrates, depending on the farm conditions and seasonal availability. In close collaboration with farmers, samples will be collected directly from cows between 90 and 250 days of lactation.

The reference method for phenotyping NUE and methane emissions is labour-intensive and costly, requiring specialised equipment such as weighing troughs with individual cow identification for recording feed intake, as well as the GreenFeed® system, which accurately measures methane emissions from the cow's breath. To enable higher throughput, predictive models developed using near-infrared (NIR) and mid-infrared (MIR) spectroscopy will be used. NIR spectra of lyophilized milk and faeces, along with MIR spectra of fresh milk, will be employed to estimate NUE and methane emissions. Continuously collecting reference data on NUE and methane emissions will facilitate the constant improvement of prediction accuracy and contribute to international consortia.

A genome-wide association study will be conducted to gain comprehensive knowledge of the gene variants associated with NUE variation, as well as other relevant traits like residual nitrogen intake in dairy cows. The insights gained from this study have the potential to enhance selection strategies, enabling the increase of NUE and reduction of methane emissions in dairy cows. Simultaneously, these findings can assist in reducing protein requirements, emissions, and losses in livestock production in the long term.

After the first year, over 400 cows have been sampled, with milk, faecal, and hair samples collected and subsequently genotyped using low-pass sequencing. In conclusion, this ongoing study aims to explore the genomic variation in NUE and methane emissions among Swiss dairy cows. By utilizing advanced spectroscopy techniques and predictive models, the research seeks to improve the accuracy of phenotyping, contributing to the understanding of genetic correlations between NUE and methane emissions. Ultimately, the knowledge gained from this study can inform selection strategies and drive sustainable practices in dairy farming, promoting increased NUE and reduced methane emissions while minimizing protein requirements, emissions, and losses in the livestock industry.

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

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The repercussions of climate change has already surfaced worldwide, with increasingly frequent and devastating natural disasters such as wildfires and floods ravages. By 2050, it is predicted that agricultural land degradation will decrease global crop yield by 10%, and 80% of world's cropland will experience water scarcity. Addressing both greenhouse gas (GHG) emissions and water usage in the agricultural sector is essential to sustaining our food production. Is there a way to achieve both of these objectives simultaneously?

Methane (CH_4) is a potent GHG that is 25 times more potent than carbon dioxide, moreover, it has also a considerable shorter atmospheric half-life of 8.6 years. Therefore, reducing CH_4 emission is an effective strategy to mitigate impacts of climate change, especially in the near-term. Enteric fermentation is responsible for 17% of global anthropogenic CH_4 emissions. The methanogenesis pathway requires hydrogen (H_2) to produce CH_4, and supplementation of malate, an intermediate substrate of methanogenesis, was found to reduce CH_4 emission by 16% in beef cattle, as it redirects H_2 away from methanogenesis by its conversion into propionate in the rumen.

This study introduced a new source of malate: hydroponic fodder. During the seed germination process, an organelle called glyoxysome transiently appears, catalyzing the conversion of stored fat to succinate, and thereby producing high levels of malate. Hydroponic fodder, or sprouted seeds has been adopted by farmers in drought-prone regions as a replacement for conventional grass, as it requires 90% less water. In this study, seven types of seeds - alfalfa, pea forage, Italian rye grass, rye, soybean, triticale and wheat - were grown in hydroponic system for 10 days to explore their malate and fumarate content chronologically during germination. Samples were harvested daily and frozen, and then, freeze-dried and grounded to 1 mm. The time point with the highest malate concentration were selected from each seedling for the Hohenheim gas test to assess the CH_4 mitigating capability. Individual rumen fluids were collected immediately prior to the morning feeding from 3 lactating cannulated Original Brown-Swiss cows and incubated for 24 h with total mixed ration (TMR, 40% grass silage, 40% maize silage, 15% hay and 5% concentrate, dry matter. 12.7% crude protein, 92.8% organic matter).

In terms of *in vitro* CH_4 mitigation, the most effective fodder was alfalfa. Using 10th day alfalfa, a 20% TMR replacement (18.7% crude protein, 92.2% organic matter) reduced CH_4 production by 6.6% ($P < 0.05$). Furthermore, a complete silage replacement (37.4% crude protein, 87.0% organic matter) reduced CH_4 production by 17.7% ($P < 0.05$), without significant difference in the *in vitro* organic matter digestibility. In conclusion, alfalfa fodder demonstrates potential as a dual solution, reducing both the agricultural CH_4 emission and water usage.

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

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The agricultural sector is responsible for a large part of methane emissions worldwide, mainly because of cattle detention. Methane is produced by fermentative processes in the digestive tract of ruminants. Hence, feeding strategies might lower methanogenesis.

Whey is a byproduct of caseation. It is available in large quantities, cheap and has a high nutritive value. Whey is rich in water-soluble carbohydrates since it mainly consists of lactose. Water-soluble carbohydrates do modify the fermentative processes in the rumen and might affect methanogenesis. A previous study from Agroscope (unpublished) on whey feeding in heifers observed a methane reduction of 37%. However, further research is needed to investigate the potential of whey as an inhibitory agent on methanogenesis.

To this aim, an *in vitro* study was conducted using the Hohenheim Gas Test (HGT) method. A basal diet, consisting of grass-silage and hay (66% : 33 %) was supplemented either with Emmentaler cheese whey, whey powder or pure lactose. The latter was added to test if the water-soluble carbohydrates in whey could be responsible for methane inhibition.

Ruminal fluid was taken from 3 rumen-canulated Original Brown Swiss cows and incubated in 4 consecutive runs. For every run the roughage-based diet served as basal diet. Whey, whey powder and lactose replaced the basal diet in 3 different dosages: 5, 15 and 30% of diet DM for whey and whey powder and 1, 10 and 20% of diet DM for lactose to test for an effect of dosage as well. In total, there were 20 replicates per treatment and dosage.

Against our expectations, preliminary results suggest that the addition of whey increased *in vitro* ruminal methanogenesis. The same effect, on a smaller scale, was found for whey powder and lactose. *In vitro* organic matter digestibility was also elevated with whey, whey powder and lactose. This finding is in line with previous studies which suggested that whey is an interesting option for replacing concentrate due to its high digestibility and feeding value.

13 Effect of 3-nitroxyproponol (3-NOP, BOVAER®10) and whole cottonseed on milk production, total-tract digestiblity, and enteric methane emissions in holstein and brown Swiss dairy cows

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The objective of this study was to determine the potential effect and interactions of 3-nitrooxypropol (3-NOP) and whole cottonseed (WCS) on lactational performance, and enteric methane (CH_4) emission of dairy cows. A total of 16 multiparous cows, including 8 Holstein Friesian (HF) and 8 Brown Swiss (BS) [224 ± 36 days in milk, 26 ± 3.7 kg milk yield], were used in a 4 x 4 Latin Square design with 2 x 2 factorial arrangement of treatments with 4, 24-d periods. The experimental treatments were: 1) Control (basal TMR), 2) 3-NOP (60 mg/kg TMR DM), 3) WCS (5% TMR DM), 4) 3-NOP + WCS. The treatment diets were balanced for ether extract, crude protein, and NDF content (4%, 16%, and 43% of TMR DM, respectively). The basal diets were fed twice daily at 0800 and 1800 h. Dry matter intake (DMI) and milk yield were measured daily, and enteric gas emissions were measured (using the GreenFeed system) during the last 3 d of each experimental period. There was an interaction between 3-NOP and WCS for DMI, being greatest for cows fed 3-NOP + WCS. The WCS treatment increased ECM yield and milk fat yield. There was no interaction of 3-NOP and WCS for any of the enteric gas emission parameters, but 3-NOP decreased CH_4 production (g/d), CH_4 yield (g/kg DMI), and CH_4 intensity (g/kg ECM) by 12, 15 and 13%, respectively. Further, a breed by 3-NOP interaction was observed for different enteric CH_4 emission metrics: HF cows had a greater CH_4 mitigation effect compared with BS cows for CH_4 production (g/d; 18 vs. 8%), CH_4 yield (g/kg DMI; 18 vs. 11%), and CH_4 intensity (g/kg ECM; 19 vs. 4%). Hydrogen production was increased by 285% in HF and 153% in BS cows receiving 3-NOP. Further, there was a 3-NOP x Time interaction for both breeds. In BS cows, 3-NOP tended to reduce CH_4 production by 18% at around 4 h after morning feeding but no effect was observed at other time points. In HF cows, the greatest mitigation effect of 3-NOP (29.6%) was observed immediately after morning feeding and it persisted at around 23% to 26% for 10 h until the second feed provision, and 3 h thereafter, in the evening. From midnight until morning feeding, there was no difference in CH_4 production between 3-NOP and control cows. In conclusion, supplementing WCS at 5% of DM improved ECM and milk fat yields but did not enhance CH_4 inhibition effect of 3-NOP of dairy cows. The low response to 3-NOP by Brown Swiss cows was unexpected and warrants further investigation and confirmation. Supplementing 3-NOP at 60 mg/kg DM to a high fiber diet resulted in 18 to 19% reduction in enteric CH_4 emission in Swiss Holstein Friesian cows.

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

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The compound 3-nitrooxypropanol (3-NOP) is one of the most consistent enteric methane (CH_4) inhibitors in ruminants. 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. Twelve multiparous lactating Holstein and 12 Swiss Brown cows, [232 ± 71 days in milk, 29.5 ± 4.63 kg milk yield (MY)], were used in a randomized complete block design study with a 25-d experimental period preceded by an 8-d covariate period (with no supplementation). Cows were assigned to 1 of the 6 treatments: 1) positive control (+CON: 3-NOP alone), 2) 3-NOP + TA, 3) 3-NOP + EO1, 4) 3-NOP + EO2, 5) 3-NOP + OS1 (oilseed, 5% of TMR DM), and 6) 3-NOP + OS2 (oilseed, 10% of TMR DM). Dry matter intake (DMI) and MY were recorded daily, and CH_4 emission measured (with GreenFeed System) and milk samples collected during the last 3 d of the covariate and experimental periods. Data were analyzed in a mixed model using the lmer procedure of R statistical language with treatment, period, breed and baseline covariate as fixed effects, and cow as a random effect. Compared with 3-NOP alone (+CON), 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 N was 32.0 mg/dL vs. 24.6 mg/dL for +CON and 3-NOP + TA, 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. For example, CH_4 yield was 16.3, 17.9 and 17.3 g/kg DMI, CH_4 intensity was 14.8, 14.4 and 13.7 g/kg FPCM for +CON, 3-NOP + OS1 and 3-NOP + TA, respectively. The results suggest that combining 3-NOP with TA or OS1 may enhance the efficacy of 3-NOP but warrants further investigation with a greater number of animals.

15 Comparison of dry matter intake, production, and enteric methane emissions in dairy cows housed in respiration chambers vs. a head-chamber system (GreenFeed)

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The open-circuit respiration chambers (RC) and GreenFeed System (GF; a head-chamber system) are commonly used techniques for measuring enteric methane (CH_4) emission from ruminants. This study compared the 2 techniques with 16 multiparous lactating dairy cows [(mean ± SD), 235 ± 68.4 days in milk, 29 ± 4.8 kg/d milk yield (MY)] receiving 1 of 4 dietary treatments: 1) positive control [CON: 3-nitrooxypropanol (3-NOP, Bovaer®10)], 2) 3-NOP + tannin extract, 3) 3-NOP + essential oil, 4) 3-NOP + oilseed for a 25-d experimental period preceded by a 8-d covariate phase. Data from both covariate and experimental periods were used for the analysis. Dry matter intake (DMI) and MY were recorded daily. Methane emissions were measured during the last 5 d of the covariate and experimental periods. In the first 3 d, gas emissions were measured, and milk samples collected, in a tie-stall barn using GF 8 times every 3-h, followed by 2-d RC period with 10-min measurement interval. The DMI (kg/d ± SE, 23.4 ± 1.89 vs. 24.6 ± 1.91), ECM yield (kg/d, 29.5 ± 3.75 vs. 30.3 ± 3.82), CH_4 production (g/d; 475 ± 90.4 vs. 455 ± 65.4), CH_4 yield (g/kg DMI; 20.4 ± 3.86 vs. 18.5 ± 2.32), and CH_4 intensity (g/kg ECM; 16.3 ± 3.15 vs. 15.2 ± 2.43) of cows in RC and GF were highly correlated, indicated by Pearson correlations: 0.55, 0.91, 0.85, 0.73, 0.82, respectively ($P < 0.01$). Prediction equations for CH_4 emissions in RC were developed in mixed models using the CH_4 measurements from GF as predictors. The interactions of treatment and CH_4 measurement techniques were removed from the model due to lack of significance. The final model included random effect of cow and treatment, and fixed effect of CH_4 emissions. For example, the CH_4 production equation $Y_{RC} = 248 + 0.4 * X_{GF}$ was fitted with $R^2 = 0.82$, but further cross validation is needed. Overall, the results showed that the measurement of CH_4 parameters using RC and GF are highly correlated. In conclusion, the GF system can be considered a suitable alternative to the RC system and allows for a higher throughput by measuring more animals.

16 Dairy cow differential somatic cells and precision livestock farming

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Mastitis is one of the major diseases that affects dairy farms. An early recognition of symptoms could prevent pain in cows and economic losses due to low milk production and waste. Somatic Cell Count (SCC) is the most economic tool for the detection of mastitis in farms, but in the first stages of inflammation and the presence of some photogenes don't induce an increase of SCC. 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 during lactation and data obtained from automatic sensors used in milking systems (milking time and milk electrical conductivity, EC). Six farms were involved and 205 cows were monitored during the entire lactation with the collection of 5 milk samples per cow (60 days on the average from one sample and another). Milk was analysed with Vetscan DC-Q Milk Analyzer (AAD - Advanced Animal Diagnostics, NC, USA), that detects TLC (cells/ml) and the concentration of all the three single fractions of differential somatic cells (cells/ml and %), and with DeLaval Cell Couter (DeLaval, Tumba, Sweden) for the detection of SCC (cells/ml). Four of the six farms had milking sensors (Afimilk, Kibbutz Afikim, Israel) for the detection of EC and milking time per cow (129 cows). Average values of differential somatic cells during lactation were calculated with Global Linear Model, with time as repeated value, (SAS 9.4). Data about EC (mS), milking time (min), TLC (log10 cells/ml), SCC (log10 cells/ml), parity and lactation stages were divided in classes and analysed with multiple correspondence analysis. During lactation, an increasing trend was observed for SCC and TLC from 4.8 log10 cells/ml to 5.1 log10 cells/ml ($P<0.001$). NEU (%) was higher (65%) in the first 60 days of lactation following by a decrease during the 120-240 days in milk (55%) and an increase in the last 60 days of lactation (61%) ($P <0.0001$). MAC (%) had the opposite trend of NEU (%), with a higher percentage in the 120-240 days in milk (31%) compared to the first and last days of lactation (19%) ($P <0.0001$). The results of the multiple correspondence analysis underlined a close relationship, for primiparous cows, between low conductivity (≤ 8.8 mS) and fast milking (≤ 4 min) during 120-240 days in milk. On the opposite, there was a good correlation between high EC (> 8.8 mS), high milking time (> 4 min) for multiparous cows in the first 120 days in milk. High NEU (≥ 63 %) was related with high SCC (>5 log10 cells/ml) and high TLC (>5 log10 cells/ml). The results suggest that the first days of lactation are the critical ones, especially for high concentration of NEU (%) and low concentration of MAC (%), indicating the suspicious of mastitis. Moreover, EC and milking time could be used as supplementary parameters for mastitis identification.

17 Communicating knowledge on grassland management using videos and online fact sheets

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Digital information is of increasing interest to farmers and land managers. Scientists do a lot of research providing advice and solutions for farmers. However, these results are too often not accessible to non-scientists. A severe gap in knowledge transfer was identified for weed control on alpine summer pastures. These areas are under pressure of problematic plants reducing the forage quality and ecological value of the pastures. Therefore, careful pastures management and weed control is a precondition of direct payments to alpine summer farms in order to maintain biodiversity, openness and beauty. Due to its seasonal nature, work on alpine pastures is frequently carried out by external staff without formal training and advisory documents concerning pasture management and weed control are scattered in different formats and from different organisations. Therefore, we set up a project to synthetize existing knowledge and to make this information easily accessible through online fact sheets and educational videos.

The videos of around ten minutes duration always featured a farmer and an expert, presenting for about half of the time. Combining peer and expert teaching facilitates knowledge transfer to the heterogeneous community of alpine staff. For the production, we established a standardized procedure: Before creating videos and web content, we gathered all available knowledge and elaborated take-home messages. These were discussed in a group of experts (scientists and farm advisors) and together with farmers to elaborate a coherent and understandable scenario. Special emphasis was placed on adapting pasture management and mechanical intervention rather than the use of herbicides. The videos were subtitled in German, French, Italian and English, and released on YouTube. Alongside the video, detailed information was prepared for release on the website www.patura-alpina.ch. As for the videos, a standardized format was established. The primary sections were (A) occurrence and distribution of the problematic plant, (B) situation analysis, (C) regulation measures, (D) adjustment of management, (E) mechanical intervention and (F) chemical regulation. After consultation within the editorial team and with additional experts and practitioners, the information was published online.

The website was released in summer 2019 and is freely available in three languages (German, French and Italian). In 2023, it contained information about eight plant species groups, commonly perceived problematic on alpine pastures: alpine dockweed (Rumex alpinus), white helleborum (Veratrum album), rush (Juncus effusus and Juncus inflexus), alpine ragwort (Senecio alpinum), bracken (Pteridium aquilinum and Dryopteris filix-mas), dwarf shrubs (Juniperus communis and Rhododendron hirsutum), thistles (Cirsium palustre and Cirsium arvense) and green alder (Alnus viridis). Since the website was released, the videos were viewed nearly 30 000 times in total. Direct feedback received from farmers and farm advisors was very positive.

Communicating to farmers by means of video and web contents is a contemporary means of knowledge transfer. The standardized participatory procedure involving scientists, extension services and farmers, to produce educational videos and web contents has proven to be cost and time-efficient. We are convinced that communicating scientific knowledge in an easy-accessible and low-threshold way improves the adoption in agricultural practice.

18 M2M networking of devices in the dairy barn

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In dairy barns, robots are increasingly taking over routine tasks such as feeding, feed supplementation, and manure removal with a high degree of automation. These devices capture a large amount of data in the barn, including the operational status of the devices and information about barn climate parameters. Typically, this data is used to control the respective devices or for herd management, providing the farmer with information about the current situation or as a basis for decision-making. Intelligent barn technology allows automated workflows to be adapted to the current conditions in the barn. For example, a feeding robot can detect the feed needs on the feed table and independently initiate the next feeding cycle. The intelligence of these devices is primarily based on the data collected by their own sensors, enabling them to flexibly adjust their workflows to the current needs in the barn. However, the autonomy throughout the barn depends on the data flow between the devices or machines. Machine-to-machine (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. So far, the farmer regulates the different devices in the barn and makes manual settings such as when to feed, what to feed, and where to remove manure. However, to increase the autonomy of all devices in the barn, it is necessary to establish a solid foundation through M2M networking. Smaller automated devices are often overlooked in M2M communication. However, when intelligent devices change their own workflows, they may collide with the pre-set workflows of smaller devices. Currently, data and information exchange between the devices is not possible, but it is necessary to ensure the autonomy of the entire barn operation. This study investigates the expectations, experiences, and barriers of farmers regarding digital and connected barn technology in dairy barns. Due to a lack of practical examples, the collaboration between two or more devices and direct coordination of workflows in the barn is not yet tangible enough to clearly recognize the benefits. Through personal exchanges and online surveys, the farmers' attitudes towards digital and connected barn technology were determined to better align the development process of barn technology manufacturers with the end-users. The farmers with experience in digital technologies see the M2M networking of barn technology as advantageous. Attitudes towards M2M networking in the barn vary depending on the existing experience with the technology, which can lead to different expectations and perceptions of the advantages and disadvantages of networking. Overall, M2M networking in dairy barns offers promising potential for increasing automation and enhancing the autonomy, flexibility, and interaction between robots.

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19 Effects of 3-nitrooxypropanol (3-NOP, Bovaer®10) and whole cottonseed on enteric emissions, milk production and total-tract digestibility, in holstein and brown swiss dairy cows

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The objective of this study was to determine the potential effect and interactions of 3-nitrooxypropanol (3-NOP) and whole cottonseed (WCS) on lactational performance, and enteric methane (CH_4) emission of dairy cows. A total of 16 multiparous cows, including 8 Holstein Friesian (HF) and 8 Brown Swiss (BS) [224 ± 36 days in milk, 26 ± 3.7 kg milk yield], were used in a 4 x 4 Latin Square design with 2 x 2 factorial arrangement of treatments with 4, 24-d periods. The experimental treatments were: 1) Control (basal TMR), 2) 3-NOP (60 mg/kg TMR DM), 3) WCS (5% TMR DM), 4) 3-NOP + WCS. The treatment diets were balanced for ether extract, crude protein, and NDF content (4%, 16%, and 43% of TMR DM, respectively). The basal diets were fed twice daily at 0800 and 1800 h. Dry matter intake (DMI) and milk yield were measured daily, and enteric gas emissions were measured (using the GreenFeed system) during the last 3 d of each experimental period. There was an interaction between 3-NOP and WCS for DMI, being greatest for cows fed 3-NOP + WCS. The WCS treatment increased ECM yield and milk fat yield. There was no interaction of 3-NOP and WCS for any of the enteric gas emission parameters, but 3-NOP decreased CH_4 production (g/d), CH_4 yield (g/kg DMI), and CH_4 intensity (g/kg ECM) by 12, 15 and 13%, respectively. Further, a breed by 3-NOP interaction was observed for different enteric CH_4 emission metrics: HF cows had a greater CH_4 mitigation effect compared with BS cows for CH_4 production (g/d; 18 vs. 8%), CH_4 yield (g/kg DMI; 18 vs. 11%), and CH_4 intensity (g/kg ECM; 19 vs. 4%). Hydrogen production was increased by 285% in HF and 153% in BS cows receiving 3-NOP. Further, there was a 3-NOP x Time interaction for both breeds. In BS cows, 3-NOP tended to reduce CH_4 production by 18% at around 4 h after morning feeding but no effect was observed at other time points. In HF cows, the greatest mitigation effect of 3-NOP (29.6%) was observed immediately after morning feeding and it persisted at around 23% to 26% for 10 h until the second feed provision, and 3 h thereafter, in the evening. From midnight until morning feeding, there was no difference in CH_4 production between 3-NOP and control cows. In conclusion, supplementing 3-NOP at 60 mg/kg DM to a high fiber diet resulted in 18 to 19% reduction in enteric CH_4 emission in Swiss Holstein Friesian cows. Supplementing WCS at 5% of DM improved ECM and milk fat yields but did not enhance CH_4 inhibition effect of 3-NOP of dairy cows. The lower response to 3-NOP by Brown Swiss cows was unexpected and warrants further investigation and confirmation.

20 First detection of systemic porcine circovirus 3 associated disease in Switzerland

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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, the knowledge on clinicopathological and epidemiological aspects of PCV-3 infection and the pathogenic effect of the virus is limited.

Materials and Methods: In March 2023, a Swiss breeding farm reported an increase in spinal malformations, thickened ribs and facial oedema with otherwise unremarkable clinical condition in suckling piglets and weaners. Three 4- to 6-week-old piglets were euthanised and submitted for diagnostic examination. A full necropsy and histological examination as well as a qPCR and in situ hybridisation (ISH) for PCV-3 were performed.

Results: Grossly, all animals exhibited multiple rib fractures with callus formation; one pig also showed facial oedema. Histologically, the costal periosteal arteries exhibited lymphoplasmacytic and histiocytic infiltrates. Similar peri- and arterial changes were observed in several organs including facial skin, heart, kidney, mesentery, spleen, lymph nodes, liver, lung, nasal mucosa and skeletal muscles. The qPCR for PCV-3, advised by the histological features, revealed a systemic PCV-3 infection with high viral loads. ISH detected abundant viral genome expression in multiple organs, including the bones.

Conclusions: This is the first report on PCV-3 infections in Switzerland. It is also the first to detect the virus in bone lesions. In future, pathomorphological investigations and possible detection of PCV-3 is advisable in pigs displaying bone lesions similar to those reported here.

21 Effects of follicular superstimulation on OPU-IVP outcome in German Fleckvieh heifers

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Follicular wave synchronization and follicular superstimulation by FSH are both routinely used nowadays to maximize results in OPU-IVP programs. Studies conducted so far analyzed either dairy or beef cattle and results were sometimes inconsistent. Herein we investigated the effect of follicular superstimulation preceded by follicular wave synchronization on OPU-IVP outcomes in German Fleckvieh, a dual-purpose breed with high milk- and beef performance. Twelve German Fleckvieh heifers (mean age ± SD: 484±62 days) were enrolled and underwent routine OPU-IVP twice seven weeks apart in a cross-over design (control cycle and superstimulated cycle). Starting on a random day of the cycle, all heifers received 0.5 mg cloprostenol (day 0) and 10 ug buserelin (day 2), respectively, for follicular wave synchronization. Then, six heifers were randomly chosen for follicular superstimulation before the first OPU, while the others were superstimulated before the second OPU. Follicular superstimulation was done using four injections of each 75 IU FSHp and 75 IU LHp (1.5 mL i.m. Pluset, Calier, Barcelona, Spain) 12 hours apart, on days 4 and 5, and a coasting period of 40 hours was allowed before OPU. Statistical analysis was performed using SPSS Software and the nonparametric Mann-Whitney test was employed to test for the effect of treatment (control vs. follicular superstimulation) on recovery rate, number of oocytes, oocyte quality (grade 1-4), cleavage rate, blastocyst rate and blastocyst quality. Results are presented as mean±SEM. A similar oocyte recovery rate was achieved in both groups (58.9% control, 59.1% superstimulated, P=0.98) resulting in 13.2 oocytes/control cycle and 14.8 oocytes/stimulated cycle (P=0.71), respectively. There was a tendency (P=0.07) for more excellent quality oocytes in the superstimulated versus control group (7.9±2.4 vs. 2.9±0.6), but no difference regarding their cleavage (83.6±3.8% vs. 84.6±4.2%, P=0.80) or blastocyst (40.3±4.5% vs. 42.9±6.8%, P=0.93) rates. The total number of transferable embryos was numerically but not statistically higher (4.8±1.1 vs. 4.1±0.9, P=0.67) in the superstimulated group compared to the control. In conclusion, our results demonstrate no positive effect of follicular superstimulation in addition to follicular wave synchronization on OPU-IVP outcomes in the dual-purpose breed German Fleckvieh. Although the number of animals enrolled in this study was relatively small, these results suggest limited beneficial effects of superstimulation before OPU-IVP in German Fleckvieh heifers.

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

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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 (IVF). Thus, 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 cells. For this, single ejaculates were collected from three Holstein-Friesian and one Jersey bull, and split in two equal aliquots: the SS aliquot was subjected to flow cytometric sorting for X-chromosome sperm (90% purity; 4×106 sperm/0.25-ml straw), and the CON aliquot was processed using standard methods for conventional semen (15×106 sperm/0.25-ml straw). Both SS and CON straws were cryopreserved and stored in liquid nitrogen until use. Cumulus-oocyte complexes from slaughterhouse ovaries were used for IVF with SS or CON sperm. In total, 136 presumptive zygotes were cultured in microwell dishes and imaged every 10 min until day 9 using a time-lapse monitoring system. The times when key developmental stages occurred were recorded: time of the first (t1) and second (t2) cleavage; the last cleavage before entering the lag-phase (t3); cleavage resumption after lag phase (tRCl); the onset of blastocyst expansion (tSB); and the time of blastocyst hatching (tHB). The cleavage rate and blastocyst rate (both expressed as % of initial number of oocytes placed in culture), and the hatched blastocyst rate (expressed as % of blastocysts) were compared between treatments using the Mann-Whitney U test. Treatment differences for embryo transitions between states were explored with multi-state Cox proportional hazards regression models. The SS treatment had lesser cleavage and hatching rates compared with the CON treatment (82.8%±8.0% vs. 88.9%±4.2%, P = 0.541, and 61.1%±8.0% vs. 74.2%±26.5%, P = 0.442, respectively), but the blastocyst rate was greater (51.6%±8.0% vs. 41.7%±19.8%, P = 0.321). The oocytes fertilized with SS sperm had 35.1% less likelihood of reaching t1 (P = 0.046) and needed more time to reach state t1 compared with oocytes fertilized with CON semen (31.05 vs. 29.25 hours). Having completed the t1 state, there were no differences between treatments in the ability to achieve subsequent developmental stages. In conclusion, our results indicated that oocytes fertilized with SS sperm had compromised ability to complete the first cleavage, but thereafter developmental potential was not affected.

23 Effect of ambient temperature on reticulorumen temperature, feed and water intake, and milk yield of dairy cows during colder seasons

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Colder seasons in temperate countries can often reach ambient temperatures (AT) much lower than the body temperature maintained by cows. Moreover, free water temperature (FWT) and feed temperature (FT) are highly dependent on AT and can be critical for maintaining body and reticulorumen temperature (RT) especially in open barn systems during these seasons. The objective of this study was to determine the effects of FWT and FT on RT fluctuations, and of AT on RT, dry matter intake (DMI), free water intake (FWI), and milk yield (MY) of lactating dairy cows during cold exposure (i.e., when the environment has a much colder temperature than the body). Measurements and data of 16 multiparous lactating dairy cows were collected for four 6-d periods throughout the autumn and winter seasons. The daily average AT during the experiment averaged 12.5 ± 3.99°C (mean ± SD) and ranged from 4.38 to 17.25°C which were lower than the average RT of the cows (38.84 ± 0.163°C). Individual, independent drinking (n = 490) and eating (n = 618) events were identified and selected from raw events. Generalized additive mixed model (GAMM) framework was used to analyze how the temperature and amount of the ingested water or feed, respectively, influenced RT change and recovery time. Effect of the daily AT on RT, DMI, FWI, and MY were also analyzed using GAMM. Both RT change and recovery time were affected by the FWT (+0.0596°C/°C and -1.27 min/°C, respectively; $P < 0.01$), but not by the FT ($P > 0.05$). Both the amount of the ingested free water and feed affected RT change (+0.108°C/kg drink size and +0.150°C/kg meal size, respectively; $P < 0.01$), and RT recovery time (-2.13 min/kg drink size and -3.71 min/kg meal size, respectively; $P < 0.01$). Colder AT decreased RT of the cows by 0.0151°C/°C when AT was between 9.93 to 17.25°C ($P < 0.01$). Meanwhile, cows increased their DMI by +0.365kg/d with every 1°C drop in AT below 10.6°C ($P < 0.01$), but with no significant change in MY ($P = 0.06$). In fact, MY: DMI decreased by -0.0106 with every 1°C decrease in AT from 17.25 to 4.38°C ($P < 0.01$). Colder AT also negatively affected FWI with a decreased of 0.0856 FWI:DMI/°C as AT decreased from 17.25 to 8.27°C. In conclusion, exposure to low AT increased thermoregulatory responses, leading to greater feed intake, reduced water intake, and reduced feed efficiency in dairy cows. Additionally, the consequences of cold exposure on dairy cows may be aggravated by the ingestion of feed and free water at temperatures much lower than body temperatures, potentially impacting feed efficiency due to the extra energetic cost to the animal.

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

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In recent years, the 3R concept (replace, reduce, refine) has gained momentum as a practical guideline for animal experimentation both in the scientific community and the public throughout Switzerland. The optimization of animal husbandry is an essential aspect of applied 3R. In this regard, sperm cryopreservation became a vital pillar for experimental animal husbandries for keeping the number of genetically modified or even constrained lines in breeding at a minimum (REDUCE). However, before a particular line can be discontinued, it must be ensured that rederivation from the cryopreserved gametes is possible. Up to now, no animal-free methods have been scientifically validated to provide sufficient information for that decision. Therefore, many researchers and facilities use in vitro fertilization (IVF) to verify the fertility of frozen sperm. Thus, large numbers of females are bred, super-ovulated, and ultimately killed simply for oocyte collection and subsequent testing of frozen-thawed sperm – a strategy far from being considered as an optimal application of the 3R principles. Hence, there is an urgent need to replace the current standard that involves animals and the animal-free assessment of cryopreserved sperm quality. To achieve this exact goal, we suggest a fertility predicting scheme based on sperm quality measures obtained through up-to-date methods like multicolor flow cytometry and computer-assisted sperm analysis; assays that have been well established in our research teams and shown to have a strong relation with male fertility in farm animals. The projected study is driven by and aims to solve a troubling practical issue, namely the decision-making about the cryopreservation and further verification of the male gametes of valuable mouse lines. Thus, it focuses on developing a practicable and user-friendly algorithm for sperm fertility prognostics using machine learning. Upon successful conclusion of the project, we will make the methodology and predictive models of the project publicly available. Given the worldwide use of cryopreserved mouse sperm, we estimate that our sperm fertility prediction scheme will help save up to 300,000 oocyte donors per year, making this a very advantageous REPLACEMENT approach in experimental animal facilities.

25 Nanobodies, highly specific antibody fragments as an alternative to classical antibodies for use in research, diagnostics, and therapy

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All camelid species (camel, dromedary, llama, alpaca, vicuña, guanaco) and some cartilaginous fish (e.g. nurse shark, rays) naturally produce a unique class of antibodies that lack light chains, termed heavy chain-only antibodies. Those antibodies are fully functional part of the humoral immune system of those animals, and their variable domains represent the smallest (~15 kDa) naturally occurring antigen-binding domains (single-domain antibodies [sdAb], nanobody, VHH). Nanobodies, having only one variable domain, lack the complexity of the antigen-binding fragments of classical antibodies (scFv), but retain the same specificities and affinities of the antibodies they originate from. Nanobodies recognise a single epitope, but are more stable and easier to genetically manipulate and produce in recombinant expression systems than the classical antibodies, or their fragments. In addition, because of their small size nanobodies can recognise epitopes and bind protein pockets which are not accessible, or not recognised by the classical antibodies. Their small size, excellent solubility and high affinity enables nanobodies to efficiently cross biological barriers (gut, blood-brain barrier) making them ideally suited for non-invasive cancer imaging or immunotherapy. To date many nanobodies were identified with potential to inhibit tumour growth, viral and bacterial infections, and neutralize toxins. The first nanobody was EMA and FDA approved for human therapy (Caplacizumab, Ablynx), and many more are currently in the late phase of clinical trials. Versatility of nanobodies enables them to be used in many diagnostic applications where high affinity and specificity are imperative. In research, nanobodies have been used with success for diverse immunoassays (pull-down, microscopy applications, ELISA), and they are especially proven to be valuable as co-crystallization chaperones that helped stabilize and determine 3D structure of many important membrane proteins like GPCRs, SLCs and other proteins involved in cellular signaling and homeostasis.

The Nanobody Service Facility of the University of Zurich has specialised in the production of *in vivo* matured nanobodies by immunization of alpacas with the target proteins, followed by the generation of a phage display libraries and in vitro selection of target-specific binders. Through our service we advance research by making the nanobody technology available to the Life Science Community.

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

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Nanobodies are recombinantly produced antigen binding domains derived from heavy-chain-only antibodies that naturally occur in camelid species and some cartilaginous fish. Physicochemical properties of these small, single-domain antibodies made them a useful in various disciplines of biology. The main advantages over conventional antibodies is in much smaller size and solubility that reflects in superior tissue penetration, excellent thermal stability, and ease of expression and selection in vitro, whilst maintaining the same binding affinities as conventional antibodies. In addition, nanobodies are often able to recognise epitopes which are not accessible to conventional antibodies.

The Nanobody Service Facility of the University of Zurich is the core technology platform for production, screening and identification of nanobodies for many academic and industry research projects.

Our immunisation strategy includes 4 injections of antigens administered in a two week interval. The success of raising a specific immune response depends greatly on the nature and the quality of the target antigen and it is serologically monitored for the increasing titre of IgG2 and IgG3 subclass (heavy-chain-only antibody). Following immunisation, lymphocyte mRNA is obtained from 60-80 ml blood and the genetic material of the nanobody repertoire is then cloned into a phage display vector. Routinely, phage libraries of $1x10^8$ - $1x10^9$ individual colonies are obtained and screened in consecutive rounds of binding, elution and amplification of phages which specifically bind to the target. After successful enrichment the nanobodies are then validated in ELISA against the target. We use the advantage of phage display that allows for the identification the coding sequence of the positive binders. Nanobodies can be easily genetically modified and recombinantly produced in unlimited amounts using any expression system (bacteria, yeast, insect cells) and further characterized in analytical and functional assay of choice.

Applications range from immunohistochemistry, live imaging, pull-down assays, functional analysis of protein-protein interactions, modulation of protein functions in the cell, or as mediators of specific protein knock-out. Highly diverse nanobody libraries also offer a powerful high-throughput tool for development of diagnostic tests.

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

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Tail biting in pig farming is a well-known problem that can be caused by a variety of environmental effects and genetic predisposition. It is always a welfare problem and can cause an increase in animal losses in severe cases (Bavarian State Office for Health and Food Safety, 2018). The goal of this project was to design and validate different evaluation keys that can describe tail lesions at the Mast- und Schlachtleistungsprüfanstalt (MLP) in Sempach and that could be used in the field during linear description to find out more about the genetic background of tail biting.

Data was collected since 2013 where pigs are assessed before slaughter, with an evaluation key that has 3 levels. The scale was defined from 0 to 2, where 0 - is an intact tail, 1 - is a tail with a slight shortage and/or little damage, and 2 - is a severely shortened and/or damaged tail. This dataset included 27’882 rated animals and was analyzed regarding the influence of sex, breed, barn, expert, and farm of origin on tail condition. Striking negative effects showed the breed (PREMO and Piétrain), and the sex (males and castrates). Further the newly renovated barn has a positive effect on intact tails and the experts have very varying results.

To test a novel evaluation key, a schema was implemented that differed between shortened and injured tails. Both factors were set up with 4 levels. For shortage: 0 - intact; 1 - slight shortening; 2 - medium shortening; 3 - heavy shortening. For damage: 0 - intact; 1 - small superficial injury; 2 - small deep injury; 3 - large deep injury. This key was tested on 57 animals with 4 experts simultaneously. While the concordance was quite well (84.2% for injured and 80.7% for shortage), the experts perceived the schema as too complicated and taking too much time to apply during the linear description.

Therefore, a second key was implemented with 4 levels describing the shortage and 2 levels describing the lesions of the tail. For shortage: 0 - intact; 1 - slight shortage, >20 cm; 2 - medium shortage, between 5-20 cm; 3 - severe shortage, <5 cm tails left. For damage: YES - there is an injury, with wet or dried blood; NO - intact tail without any wound. This key was tested on 60 animals with 4 experts simultaneously. Unfortunately, the concordance dropped for the shortage (65%) and for the injuries (81.7%). Nevertheless, the experts applying the evaluation keys agreed on the better applicability of the second key. Finally, a combination of the original key and the last tested key will be implemented for a trial period of a year at the MLP. The key shall include 3 levels for shortage and 2 levels for the damage of tails.

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

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The objective was to characterize the time budget and circadian rhythm of lying behavior in dairy cows during heat stress (HS) and to assess the effect of dietary supplementation of vitamin D3 and Ca (+D3/Ca). Twelve multiparous Holstein cows were used in a split-plot design with the level of dietary vitamin E and Se as main plots (LESe: 11 IU/kg and 0.55 ppm; HESe: 223 IU/kg and 1.8 ppm). Within each plot, cows were randomly assigned to 1) HS (D3: 1012 IU/kg; Ca: 0.73%), 2) HS+D3/Ca (D3: 3,764 IU/kg; Ca: 0.97%), or 3) thermoneutral pair-fed to HS (TNPF) in a Latin Square design with 14-d periods and 7-d washouts. Lying time was measured with HOBO® Loggers in 15-min intervals. Difference in daily lying time (DLT) and hourly lying time (HLT) between treatments were analyzed using mixed model. Cows in HS had reduced DLT compared with TNPF in both LESe (487 vs. 677 min/d) and HESe (546 vs. 780 min/d), whereas HS+D3/Ca increased DLT by 64 min/d relative to HS in LESe ($P < 0.05$). A treatment by time interaction ($P < 0.05$) was observed for HLT: hourly lying time was reduced in HS relative to TNPF during early morning (0000-0600 h) and night period (1800-2400 h). The diurnal patterns of lying behavior were characterized by fitting a cosine function of time in linear mixed model. Daily rhythmicity of lying was detected ($P < 0.05$) for cows in TNPF and HS+D3/Ca, but not in HS ($P = 0.06$). Cows in TNPF had the highest mesor (the average level of diurnal fluctuations; 34.2 min/h) and amplitude (the distance between the peak and mesor; 17.9 min/h). The acrophase (time of the peak) occurred after midnight in all treatments. In conclusion, reduced daily lying time and disrupted circadian rhythms in dairy cows under HS can be partially restored by +D3/Ca. Lastly, restricted feeding might alter cows' natural behavior, as shown by increased ($P < 0.05$) lying time in TNPF during the first 4 d of each period as DMI was gradually restricted. Pair-feeding might be a good model to investigate production and physiology under HS, but it may introduce biases when used for animal behavior research.

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

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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. Traditional methods of RR monitoring, e.g., counting the flank movements, is labor intensive and not applicable in on-farm settings. There are growing interests in developing contactless video-based RR monitoring techniques, however, prior studies have primarily focused on estimations using short-term video clips, which limits the accuracy and utilization of these techniques given farm environment. This study aimed to develop computer vision-based models to estimate RR of dairy cows using contactless video for extended periods. The experiment involved video recording of 3 cows for 24 hours each, with RR measurements collected through Embla XactTrace Respiration Belt, serving as the ground truth (GT). A digital camera (DH-SD1A404XB-GNR) was positioned at the side of the cow to capture the region around abdomen. To automatically monitor the RR by video, a Transformer model called VideoMAE (https://github.com/MCG-NJU/VideoMAE) was fined tuned on the collected dataset. Specifically, the collected video was segmented to short videos with length one minute each, and the mean RR of each short video was computed. The short videos were split to training, validation and test sets, with number of video are about 200, 50 and 100 respectively. The training and validation set were used to train the model, and the test set was used to test the effectiveness of the trained model. The GT measurements for the selected videos of the 3 cows had a mean RR (± SD) of 23.4 ± 2.77 breaths per minute (bpm). The comparison between the RR from the test set vs. GT showed the effectiveness of the computer vision-based method, with mean absolute error (MAE), root mean squared error (RMSE) and root mean square percentage error (RMSPE) values of 2.47, 3.14 and 18.03% respectively. Future investigations will aim to enhance the performance of the model by including more videos from diverse occasions to increase the sensitivity.