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1 **Christian Hofer, Director of the Federal Office for Agriculture**

«Future agriculture and nutrition: which trends are emerging?»

Numerous factors that have characterized the agricultural and food industry in the past will continue to be important in the future as well. These include natural conditions such as topography and climate, and technological progress. Key trends include i) the continuing pressure on natural resources, especially on soil, as a result of a growing population and a probable further increase in prosperity, ii) the changing conditions for production in Switzerland and abroad as a result of climate change, and iii) technological developments in the areas of digitalization, gene technology and production of food outside the agricultural land (such as laboratory meat or integrated vertical farming methods in the city). The extent to which technological progress will prevail is also a question of social acceptance. Other important trends are iv) the (renewed) growing interest of the population in how food is produced and in nutritional topics in general, and v) the increasing demand for regionally and sustainably produced food. This is generally positive, because it is clear that in addition to the actors in the value chain and the agricultural and food knowledge system, also consumers need to contribute to overcome the challenges and thus improve the sustainability of the agricultural and food system. Policymakers are challenged to further develop the corresponding framework conditions in the various policy fields in a coherent manner, especially in the areas of agriculture, food and the environment. The Federal Council has already laid the foundations with its long-term climate strategy and the Sustainable Development Strategy 2030 and more will follow in the coming years, among others with the report in response to the postulate of the Economic Commission of the Council of States "Future orientation of agricultural policy".

2 **Martin Keller, CEO fenaco**

«New technologies – reliable values»

fenaco cooperative was established in 1993 as a rural self-help organisation and supports farmers in the economic development of their businesses. It is owned by some 170 local LANDI cooperatives and their 43,000 members, mainly farmers. It operates in four strategic business fields (agro, food industry, retail trade and energy) and pursues three main priorities (innovation, sustainability and international competence). fenaco seeks proximity to the consumers in order to understand and fulfil their requirements, in accordance with its slogan “from farm to table”. fenaco promotes the USPs of Swiss foodstuffs: a high standard of product quality and safety, proximity and local products, animal welfare and protection of natural resources, transparency and traceability, and rural family structures. In order to achieve these goals together with the farmers, fenaco has started several research cooperations and strategic partnerships in the last few years, among others with Agroscope, ETH Zurich, FiBL, crop.zone and Yasai. In 2020, fenaco spent more than CHF 2 Mio. for such kind of partnerships. We are convinced that this enhances our own capability to innovate and makes us more attractive for young professionals.

3 Thomas Echtermann, UZH

«Influence of management on lying behavior in free farrowing – and in all the nests is rest?!»

Free farrowing of the sow has been fully established as an important feature of species-appropriate pig management promoting animal welfare in Switzerland since 2007. Other countries within Europe are also discussing to ban confinement of the sow during birth and suckling. The main point of discussion in this context is the mortality of the suckling piglets, which is evaluated differently in free farrowing depending on the study. Therefore, the aim of our study was to investigate whether the behavior of the suckling piglets and the suckling piglet mortality divided into crushed, underdeveloped and other losses in free farrowing in Switzerland can be influenced and improved with simple management measures.

For this purpose, in a case-control study, 15 litters were locked-up in the piglet nest for two hours twice a day, while no management measures were applied to 13 litters. All piglets were weighed at birth and several times afterwards, individually marked and their lying, suckling and activity behavior was monitored and evaluated using video technology.

Our study showed that locking-up had a significant effect on the number of crushed piglets (p -value=0.04), but did not significantly influence total mortality. Similarly, no significant influence on an improvement in daily weight gain was observed. The sow had a greater influence on the behavior of the suckling piglets than the management measure confinement and general conclusions between both experimental groups regarding behavior were not possible.

Therefore, it seems to be important to observe the litters individually and to decide specifically when locking-up is useful. For this purpose, observing the sows over several litters should provide clarity in the future.

1 **Are the uterine extracellular vesicles key players in embryo-maternal interactions in the mare?**

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Extracellular vesicles (EVs) present in different reproductive fluids have emerged as key players in the early reproductive events leading to successful pregnancy. By transferring their molecular cargo from the uterus to the embryo and vice versa, they act as multi-signal messengers and might be crucial for the first embryo-maternal interactions or maternal recognition of pregnancy (MRP). The identification of the uterine EVs cargo of different species has revealed key molecules involved in these events, which could be of great value as diagnostic tools for fertility status. In the mare, little is known about the molecular composition of the uterine EVs. Moreover, the embryonic signals or mechanisms for MRP remain to be elucidated compared to other mammals. We hypothesize that the signals of MRP in the mare are packed in the uterine EVs, consisting of maternal and embryonic origin in pregnant mares, while only of maternal origin in cyclic mares. Therefore, the objective of our study was to determine the RNA and protein cargo of uterine EVs collected from pregnant and cyclic mares in order to identify molecules involved in MRP.

For this purpose, uterine lavage (UL) (100 ml PBS) was performed in pregnant mares on day 10 (P10, n=4), 11 (P11, n=4), 12 (P12, n=4) and 13 (P13, n=5) after insemination (pregnancy con-firmed by embryo recovery) and in cyclic mares (controls) on day 10 (C10, n=4) and 13 (C13, n=4) of the estrous cycle. Isolation of EVs from UL was conducted by combining Centricon ultrafiltration, size-exclusion chromatography and ultracentrifugation to obtain EVs samples with good purity and yield. Characterization of equine uterine EVs was performed by electron microscopy, Western blot and flow cytometry. These analyses confirmed the presence of a population of mainly exosomes (40-100nm), positive for known exosome markers (CD9, CD81, Alix and TSG101) and a smaller population of microvesicles (100-250nm). Preliminary analysis of the EVs RNA cargo by RNA-sequencing (including short and long RNAs) across all samples showed a dynamic transcriptome profile, with marked differences between C13 and C10. Interestingly, differences were less evident between P13 and P10, suggesting changes in cyclic samples which are prevented in early pregnancy. When pregnant and cyclic samples were compared, significant differences were found for P11 vs. C10, P12 vs. C13, and with the highest number of differential transcripts for P13 vs. C13. By contrast, almost no differences were found for P10 vs. C10. Mass spectrometry revealed also differences for the EVs' proteome, mainly between day 10, regardless of pregnant or control, and the rest of the samples. This analysis identified EVs proteins which were also found as highly abundant in the embryos in a parallel analysis, suggesting that these components can have an embryo origin or are transferred from the mare to the embryo. Ongoing analysis of the EVs RNA components are identifying specific mRNAs, miRNAs, and other small non-coding RNAs with significant differences between pregnant and control mares. The integration of uterine EVs, embryo, and endometrium datasets will reveal molecules potentially involved in signaling of MRP in the mare.

2 Stable isotopes of oxygen: the key to understand the soil fate and cycling of fertilizer phosphorus?

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Sourcing phosphorus (P) locally from bio-wastes produced in urban or agricultural environments is gaining significance. Conventional fertilization with mined mineral phosphates raises sustainability concerns. However, mineral fertilizers are manufactured with specific P formulations, whereas bio-wastes contain a wide diversity of P forms in variable amounts, and a complex matrix that influences soil properties. Understanding the soil P transformation processes triggered by using bio-wastes is important to improve P fertilization strategies. In the long-term field experiment CRUCIAL⁽¹⁾ plots have been fertilized for almost two decades with one of five bio-based fertilizers (human urine, composted organic household waste, sewage sludge, cattle slurry, deep litter) or a mineral NPK, in doses that fulfil (normal) or triplicate (accelerated) the yearly nitrogen demand of crops. Both doses exceed crop P requirements. We sampled: soils one month both before and after the yearly fertilization, and archived fertilizers applied in previous years, and determined total P concentration and P distribution in operationally defined fractions using an adapted sequential extraction protocol⁽²⁾. Those fractions are, with extractant given in brackets: readily soluble P (anion exchange resin), microbial P (hexanol and resin), P sorbed to soil Al & Fe or organic matter (0.1M NaOH + 0.025M EDTA) and recalcitrant P (1M HCl). We have calculated the yearly average input by fertilizers to each soil P fraction. We use a stable isotopic approach using oxygen isotopes in phosphate (¹⁸O-P) as indicator of specific enzyme-driven soil microbial P turnover processes. We are currently determining the ¹⁸O-P composition of fertilizer P and in the soil P fractions from the plots treated with sewage sludge and compost in both normal and accelerated doses. We are testing two hypotheses: a) soils do not keep the fertilizer ¹⁸O-P signal in soluble P fractions and maintain it in more recalcitrant ones as they are not easily accessible to microbial processes. b) Microbial processes will not be able to process all applied P in the accelerated dosed soils, allowing P with unaltered fertilizer ¹⁸O-P composition to associate with soil components, causing a shift in the detection of the fertilizer ¹⁸O-P signal to more soluble P fractions. Results so far indicate that most fertilizers contain a high proportion of soluble P forms. Soil data suggests that these forms settle into recalcitrant soil P pools after fertilization, and/or are transported to deeper soil horizons⁽³⁾. This effect is particularly evident in accelerated doses. Results will provide insights on the microbial processes stimulated by the application of bio-wastes, and a better understanding the soil cycling of bio-waste P.

Keywords

Bio-waste, phosphorus, fertilization, stable isotopes, microbial processes, ¹⁸O-P

Literature

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3 The future is now – Delphi forecasts for the adoption of precision agriculture enabling technologies in Swiss outdoor vegetable production

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Precision farming technologies are a promising means to tackle the increasing global challenges such as climate change, water pollution or soil degradation. These technologies can also help revolutionising agricultural production, thereby turning it more sustainable. However, despite the fact that these technologies having been in development for more than 20 years now, penetration in Switzerland is still low. Building on this knowledge, we conducted a two-stage Delphi study with 34 experts who have expertise in the fields of vegetable production and digital technologies to identify the key drivers and barriers, the most promising technologies and possible measures to support technology adoption in Swiss outdoor vegetable production. The Delphi method is an established tool for scientific forecasting consisting of a series of survey rounds, offering the experts the possibility to change or modify their opinions. The experts are anonymous and their identity remains unknown. With that, group interactions such as group pressure or domination of the process by few individuals can be avoided. In Round 1 of the Delphi survey, we used open-ended questions to collect expert opinions. We then transformed these into close-ended questions for Round 2, where controlled feedback about the results obtained in the first round was provided to the experts. A total of 26 experts participated in both rounds, resulting in an overall response rate that was comparably high with 76%. The results show that experts see great potential in the use of electronic measuring systems in irrigation and hoeing. The adoption rates in 10 years were estimated to be between 50 and 60%. In 2018, The adoption rates were around 10%. We believe that these technologies are driven by climate change and societal and political pressure limiting the use of pesticides. The experts, on the other hand, saw little potential in the use of spray drones for vegetable production. Most experts estimated the adoption rates for spray drones in 10 years to be no higher than 10%. We further found that economic factors were important drivers and barriers of adoption. While resource saving can motivate technology adoption, high technology costs are a substantial barrier. Consequently, experts recommended financial measures to support technology adoption. Practical relevance provided through communication or training holds further potential to promote new technologies. These findings are valuable beyond research. Educators and policy makers can build on these insights to tailor their efforts to optimally support technology adoption and contribute to a more efficient and sustainable food production.

4 The endometrial luminal epithelium shows the main response to conceptus signals during the period of maternal recognition of pregnancy in the mare

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Maternal recognition of pregnancy (MRP) is essential in numerous mammalian species to establish and maintain pregnancy. In the equine species, the conceptus needs to prevent the endometrial release of PGF2 α into the blood circulation before day 14 post-ovulation in order to prevent luteolysis and guarantee the continuation of progesterone secretion by the corpus luteum, which is crucial for maintenance of pregnancy. In contrast to other domestic species, the mechanisms leading to MRP in the mare are still not fully understood. During the period of MRP the equine conceptus is propelled through the uterus of the mare, producing a mechanical stimulus on the uterus, and is secreting several molecules including estradiol, PGE2, and 17 alpha-hydroxyprogesterone which induce changes in the endometrium. Based on our previous studies, we hypothesized that the endometrial changes during MRP in response to the presence of a conceptus are highly cell type-specific. Therefore, laser capture microdissection and low-input-RNA sequencing were combined to study differential gene expression in luminal and glandular epithelium (LE, GE), and in stroma of endometrial biopsies collected on days 10, 11, 12, and 13 of pregnancy and 10 and 13 of the estrous cycle (n=5 per group).

The data analysis revealed that the main changes in gene expression were located in the LE starting already on day 11 of pregnancy. Expression patterns of LE samples from pregnant mares showed dependence on the number and size of recovered conceptuses. In total, 1251 differentially expressed genes (DEGs) were identified in LE (FDR<1%), 248 (FDR<5%) in GE, and 103 (FDR<13%) in stroma. The overlaps of DEGs among the three endometrial compartments were very low indicating a highly cell type-specific response to embryo signaling.

The functional analysis of the DEGs in LE showed overrepresentation of terms related to secretion and vesicle transport, signal transduction, cytoskeleton, cell adhesion, blood vessel development, and lipid metabolism. Particularly, ErbB, Rho family GTPases, Wnt/ β -catenin, and TGF- β signaling were identified as activated, whereas cholesterol biosynthesis, RhoGDI signaling, and PPAR α /RXR α activation were inhibited. Upstream regulator analysis suggested beta-estradiol, oleic acid, EGF, and IL1B as causal for differential gene expression in LE. Furthermore, a search for genes related to prostaglandin metabolism and signaling found two prostaglandin transporters as upregulated in LE that could be involved in regulation of PGF2 α release from LE. The detailed analysis of the gene expression profiles in LE over pregnancy and cycle stages identified two interesting sets of genes which were either upregulated from day 10 to day 13 of the estrous cycle or downregulated but stayed unchanged in samples derived from pregnant mares. These genes contained a strikingly high number of transcription factors including members of the SRY-box transcription family, KLF family, and nuclear receptor family.

Our study revealed interesting genes with potential roles in the process of MRP, mainly at the LE level. The integration of these endometrial transcriptome results with the proteome analysis and together with the corresponding conceptus and uterine extracellular vesicle data will bring key insights to fully understand the embryo-maternal crosstalk during MRP in the mare.

5 Molecular characteristics of the bovine embryo during the preimplantation phase

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In mammals, pregnancy establishment is a delicate process that requires a precise balance between the mother and the embryo to produce healthy offspring. It is known that the embryo develops autonomously during the first days, which in cattle accounts until approximately day 8 after fertilization. After the embryo has reached this stage, embryo transfer to a recipient mother is needed to allow implantation and further development to term. To better understand the process of preimplantation development, we aim to evaluate the embryo molecular characteristics using a bovine *in vivo* and *in vitro* approach. To obtain *in vivo* embryos from healthy bovine donors, the estrus cycle of cows was synchronized and hormonal stimulation using gonadotropins was implemented to induce multiple follicular growth. Thereafter, ovulation was induced, and artificial insemination performed (day 0). Embryos were recovered non-surgically from the uterine horns on days 6, 8, 10, 12 and 14 using *in vivo* uterine flushing. After retrieval, embryos were classified according to their quality and stage of development and frozen for RNA library preparation and sequencing. A total of 86 embryos including quality I, II, and III were obtained from 14 donors for days 6, 8, and 10. For days 12 and 14, a total of 102 quality I embryos were retrieved from 19 donors. Additionally, embryos were produced *in vitro* and collected on days 2 to 8 of development post fertilization (2, 4, 8, 16-cell embryo, morula, and blastocyst stages). Both groups of embryos are currently subjected to single embryo RNA sequencing. Next to enhancing our knowledge on bovine embryo development, the findings of the *in vivo* versus *in vitro* produced embryos will allow to deduce new strategies of improving *in vitro* culture conditions.

6 Vine leaves and early grass cutting decrease methane emissions in dairy cows

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Milk and dairy products are widely consumed and provide valuable nutrients for human nutrition. However, dairy cows are increasingly vilified for their contribution to global warming caused by enteric methane emission. Nutritional strategies targeting methane mitigation in dairy cows is therefore an essential component in a united front against global warming. Additionally, governments and consumers are increasingly concerned about sustainable, locally produced dairy production and are more willing to pay a premium for these types of products. Therefore, grassbased feeding is of major interest in grassland dominated regions and mountainous communities with a scarcity of arable land. However, grass-fed dairy cows are thought to produce more methane per unit of feed or milk than those fed maize- or concentrate-based diets, which in turn competes with human food production. Therefore, we attempted to define grass-based diets with a low methane emission potential. Tannins, a group of plant secondary compounds, have demonstrated great potential in reducing noxious emissions from dairy cows. At moderate to low concentrations they provide a double benefit by specifically inhibiting key rumen microbiota, thus reducing enteric methane production, and their protein binding ability decelerates ruminal protein degradation, allowing digestion of these proteins in the small intestine. Vine leaves (*Vitis vinifera*) are rich in tannins and vines are essentially pruned of all growth annually. Our study aimed to evaluate differences between two grass cutting regimes, 21 days vs 42 days (early (ES) vs late stage (LS); 2nd cut onwards) and the addition of dried vine leaves on methane emissions in dairy cows. Twenty-four cows were randomised to four dietary groups (n = 6). Each diet consisted of 70% fresh grass, 10% grass hay and 5% concentrate (DM, dry matter). Two diets comprised ES and two LS grass, ES diets contained early-stage hay and LS diets, late-stage hay. Of these, one group received 15% vine leaves (+V) and the other an additional 15% hay (DM). No significant differences were observed in feed DM intake and milk yield, energy-corrected milk yield (ECM) was numerically higher for ES vs LS diets. Methane emissions were impacted by cutting regime and vine leaf addition. Absolute methane production decreased by 10% with the addition of vine leaves (ES vs ES+V, 334 vs 301 g/day). Methane yield decreased by 17% when comparing dry matter intake (DMI) and digested organic matter (dOM) for the ES+V vs LS (DMI, 17.9 vs 21.6; dOM, 20.2 vs 24.3 g/kg) diets and by 15% for digested neutral detergent fibre with the addition of vine leaves (ES+V vs ES, 32.4 vs 38.2 g/kg). Methane emission intensity decreased by 28% for milk yield and ECM in the ES+V vs. LS diets (milk yield, 21.0 vs 29.2; ECM, 19.1 vs 26.5 g/kg). Our results reveal that a well-maintained grass cutting regime combined with the addition of vine leaves to dairy cow diets clearly allows decreased methane emissions with limited competition for human food production.

7 Optimization of the amino acid supply for beef fattening calves

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In bull fattening systems, the change in housing of calves is challenging due to diseases, stress and changes in feed. Moreover, the growth performance of fattening animals continues to increase, due to the progresses in breeding. To optimize the amino acid supply during the youth stage, a project on the fattening animals of AgroVet-Strickhof was done in collaboration with the feed company Meliofeed AG, Herzogenbuchsee. Animals were fed an amino acid supplement in the fore-fattening phase, with the aim of proving that an improved amino-acid supply of young animals leads to a higher fattening performance.

The Melior 2380 Amino Booster product is a soy-free protein concentrate with a high content of essential amino acids such as lysine, methionine and leucine. The amino acids are available both in rumen-available form and as by-pass protein.

Amino Booster was fed with an amount of 150g per animal and day, up to a live weight of 200kg. The aim of this study was to investigate the young animals' development whilst fed with the additive and to know if a positive effect can be noticed up until slaughter.

For this purpose, two groups of 8 animals were put in housing, with an average body weight of 76.5kg weight for the experimental Melior group and 79.2kg body weight for the control group.

Feeding regimen:

The calves were weaned with whole milk and milk powder within 42 days. In addition, the animals were given a rearing mash made from alfalfa hay and concentrate at their disposal and received calf rearing feed. After weaning, animals received an ad lib basic feed ration of hay (4%), grass silage (21%), corn silage (72%) and straw (3%). Protein was supplemented through 1.6kg concentrate. The test group received 150g of additional concentrate. The Amino Booster was administered with the milk during the weaning phase and later on top of the 150 g ration.

Results and discussion

After 139 days, at the end of the experiment, the test group achieved an average daily gain of 1084 g with a live weight of 227 kg compared to 963 g gain per day with a live weight of 213 kg in the control group.

The test group achieved an average daily gain of 1289g over the entire fattening period, a good 70g more than the control group. Interestingly, variation in the test group is smaller. Whether this is only related to the amino booster or if there is an influence of genetics cannot be conclusively assessed in this study.

However, it seems that the good development in youth due to the Amino Booster remains visible until slaughter.

Thanks to Meliofeed AG for their support during this project and to the whole stable team from AgroVet-Strickhof for their animal care.

8 Agroscope - 'Alpine and Mountain Farming' Experimental Station

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In November 2018, the Swiss Federal Council delivered a landmark decision, ruling that the Swiss Federal Centre of Excellence for Agricultural Research would in future consist of central headquarters in Posieux (canton of Fribourg), two regional research centres in Changins (canton of Vaud) and Reckenholz (canton of Zurich), as well as 14 experimental stations (satellites). This article introduces the 'Alpine and Mountain Farming' Experimental Station, which has begun its research activities in 2021.

Agroscope will establish and run the 'Alpine and Mountain Farming' Experimental Station in partnership with the cantons of Bern, Grisons, Ticino, Uri and Valais as well as with Agridea. The practice-oriented research activities of this experimental station will be defined in a project-oriented manner. The cantons participating in the experimental station will provide and support the cross-cantonal network of experimental plots and farms necessary for the experiments and projects. In addition, they will contribute their existing infrastructure (including livestock herds), and will support trials and projects on a technical level. Agroscope will be responsible for the design of the research projects as well as for the scientific support and evaluation of the trials. Knowledge transfer and exchange will be conducted jointly with all partners, in particular via Agridea and the cantonal education and extension organisations as well as via the sector.

The added-value of the experimental and farm network is created by the investigation and answering of alpine and mountain farming questions throughout the alpine region, with inter alia structural, organisational, economic and climatic factors which differ between the cantons being taken into account. Hence, the network is also accorded a high importance from the point of view of research, and forms the basis for the development of applied research into a topic of exceptional relevance for the Swiss agricultural sector. At the same time, the network ensures the inclusion of all important partners over the entire alpine region.

The cantons have prioritised the following topics for the start of the experimental station's research activities:

1. Site-adapted management for changing climatic conditions
2. Dairy technology
3. Farm management and social matters

The aim is to develop effective approaches to the following topics, among others: the sustainable management of alpine meadows and grassland; the use of small ruminants in the mountain region (e.g. to control scrub encroachment); increasing the value-added from small-ruminant husbandry; comparisons of economic systems with different farm sizes and strategies, as well as new forms of cooperation and types of farms. Further questions on the topic of alpine and mountain farming will be defined in the coming years.

9 Screening of commercial Swiss mixed pig feeds for particle size distribution

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The grinding intensity of the diet is important in pig production systems. Cereals and other ingredients are finely ground to achieve a higher digestibility, but a disadvantage of a high grinding intensity is the risk of stomach ulcers. So far, the concrete etiopathology of feed-related stomach ulcers remains to be investigated. Additionally, the particle size distribution of typical mixed pig feeds in Switzerland has not been assessed recently.

We collected 53 different commercial mixed pig feeds (40 meals, 13 pellets/granulates) and submitted them to sieve analysis. Of these, 22 were for fattening pigs, 23 for sows and 2 for piglets. The particle size distribution was always determined by parallel dry and wet sieving for meals, and by wet sieving only for pellets/granulates. Sieve sizes were 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.063, 0.040 and 0.025 mm. In wet sieving, water and particles passing the finest sieve or being soluble were lost. Their amount was estimated by the difference to the total dry matter weighed prior to sieve analysis. To analyze the effect of processing (pelleting), we compared the results for two meals with 3 mm and 6 mm sized pellets produced at AgroVet-Strickhof with a pellet press model RP18 (Ecokraft AG, Deggendorf, Germany) from the same meal. Mean particle size (MPS) was calculated according to Fritz et al. (2012) and does, by definition, not include the particles passing the finest sieve. For one meal, the water passing the finest sieve was retained and additionally analyzed using a series of centrifugation and filtration steps to separate very fine particles from solutes.

Dry sieve analysis of all commercial meals yielded a MPS of 0.58 to 2.90 mm. Only 0.02 to 2.71% of the dry matter was not retained on the finest sieve. By contrast, wet sieve analysis of all commercial meals and pellets yielded a MPS of 0.59 to 1.66 mm, and between 34.9 and 66.5% of the dry matter was not retained on the sieves. Our own pellets had a lower MPS than the two meals from which they were produced, and they also had a lower MPS at 3 mm compared to 6 mm pellet diameter. In the one meal submitted to special analysis, about 30% of DM had a particle size between 0.22 and 2.5 μm , and about 20% were either smaller or soluble.

To our knowledge, the difference in the very fine particle fraction identified between dry and wet sieving is little discussed in feed science, and is most likely the consequence of adhesion forces between very fine and larger particles that are not overcome during dry sieving. Identifying production steps responsible for this fraction, and its consequences for animal health, are important future research aims.

Fritz J, Streich WJ, Schwarm A, Clauss M (2012) Condensing results of wet sieving analyses into a single data: a comparison of methods for particle size description. *Journal of Animal Physiology and Animal Nutrition* 96: 783-797

10 Methane emissions of poultry - a pilot study with broiler chicken

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It is widely accepted that the contribution of poultry to greenhouse gas emissions is negligible, inspite of reports on methane production from poultry litter, the presence of methanogens in poultry caecal content, and measurable methane production *in vitro* in poultry caecal content. In order to quantify the enteric methane emission of live chickens in a pilot study we performed gas exchanged measurements in 12 organic-type broilers (Hubbard I 657; age at start 65 days, 1.76 ± 0.28 kg) in open circuit respiration chambers after an adaptation period of 10 days and 5 days of feed intake recording. Animals were kept as six pairs. Three pairs received a standard diet (UFA 637 Poulet Mastfutter, Herzogbuchensee, CH; mean daily intake 106 ± 4 g dry matter (DM/animal)); three pairs received the same basal diet and extra soybean meal in a ratio of 0.7:0.3 (mean daily intake 106 ± 16 g DM/animal). The purpose of this treatment was to test the assumption that uric acid present in urine, which should be increased at higher protein intake and is transported from the cloaca into the caeca in poultry, would be fermented there, which might be a trigger for methane production.

The broilers emitted 92 ± 16 ml methane/animal and day on the standard diet and 102 ± 23 ml methane/animal and day with the addition of the protein component. This corresponded to 0.87 ± 0.16 and 0.96 ± 0.15 ml/g DM intake, respectively. The CH₄:CO₂-ratios for the two groups were 0.0025 ± 0.0004 and 0.0029 ± 0.0007 .

The results do not suggest that there is a very strong effect of the protein addition. Nevertheless, they document a measurable methane emission of broilers in this age group, even on a diet that is comparably low in fibre. Compared to other birds, where methane emission was measured on more fibrous diets, the emissions were low. If the contribution of chicken - both broilers and layers - to global greenhouse gas budgets should be quantified and if they should be implemented in national inventories, more measurements in animals of different age groups and production types on different diets are required.

11 The forestomach washing mechanism in llamas (*Lama glama*)

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Dust and grit are ingested by herbivores in their natural habitats along with the plants that represent their selected diet. Among the functions of the rumen, a washing of ingesta from adhering dust and grit has recently been demonstrated. The putative consequence is a less strenuous wear on ruminant teeth by external abrasives during rumination. The same function should theoretically apply to camelids, but has not been investigated so far. We fed six llamas (*Lama glama*) a diet of grass hay and a lucerne-based pelleted food in which fine sand had been included at about 8% of ingredients, for ad libitum consumption for six weeks. Subsequently, animals were slaughtered and content of the different sections of the gastrointestinal tract was sampled for the analysis of dry matter (DM), total ash, and acid detergent insoluble ash (ADIA, a measure for silica). Additionally, two of the animals were subjected to whole-body computer tomography (CT) after death in the natural sternal resting position. No clinical problems or macroscopic changes in the faeces were observed during the experimental period. The results indicate an accumulation of ADIA in the C3 compartment of the stomach complex, in particular in the posterior portion that is the equivalent of the abomasum in ruminants. By contrast, contents of the C1, from which material is recruited for regurgitation and rumination, were depleted of ADIA, indicating that the contents had largely been washed free of sand. The washing effect is an unavoidable side-effect of the flotation- and sedimentation-based sorting mechanisms in the ruminant and the camelid forestomaches. In theory, this should allow ruminants and camelids to live in similar habitats as nonruminant herbivores at lower degrees of hypsodonty.

12 Evaluation of the prevalence of stomach ulcers in slaughtered pigs

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Domestic pigs develop gastric ulcers due to various reasons, among them unfavorable feed particle size composition and infectious agents, even though the etiopathology still remains to be completely resolved. In Switzerland, the last survey on the prevalence of gastric ulcers and possible risk factors was performed in 2005 (Van Den Berg et al., 2005).

The present study aimed to reassess the prevalence of gastric ulcer and its risk factors today. Therefore, 1005 stomachs of fattening pigs from 136 slaughter groups and 87 producers were evaluated in a local slaughterhouse. The Pars oesophagea of the stomachs was scored (from 0 = healthy to 10 = severe ulceration and strictures). Differences between the labels 'Integrierte Produktion Schweiz' (IPS; n = 242 stomachs from 18 farms) and 'Qualitätsmanagement-Schweizerfleisch' (QM; n = 649 stomachs from 58 farms) and others (n = 114 stomachs from 11 farms) were compared.

The results showed a prevalence of 26.8 % slight mucosal changes (scores 1-3), 14.7 % moderate mucosal changes (scores 4-6) and 19.1 % severe mucosal changes (scores 7-10). Only 39.4 % of the stomachs were rated 0 and thus considered as healthy. Compared to the results from 2005, there is no difference concerning the lower values (0-2). However, there was a clear shift from middle values to higher extremes. The comparison of the three groups showed significant differences ($p < 0.001$), indicating that pigs from IPS farms had healthier stomachs. Generally, stomachs that were empty at slaughter had higher scores.

Our results indicate that there is still much room for improvement in pig feeding and handling to ensure animal welfare. The reasons for gastric ulcer development have to be clarified in order to allow targeted countermeasures.

Reference:

Van Den Berg, A., Brülisauer, F., & Regula, G. (2005). Prävalenz von Veränderungen der kutanen Magenschleimhaut bei Schlachtschweinen in der Schweiz. *Schweizer Archiv Für Tierheilkunde*, 147(7), 297–303. <https://doi.org/10.1024/0036-7281.147.07.297>

13 Effect of pilocarpine, a saliva-stimulating compound, on characteristics of intake, digestion and methane emission: a comparative study in cattle and sheep

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Methane emitted by livestock animals has gained increasing attention in the past decades. Many researchers focused on feeding strategies and diet manipulation to mitigate methane emission. However, relatively few studies have focused on physiological aspects. For example, a decreased ruminal mean retention time (MRT) of the ingested feed is related to a decreased methane production per unit of dry matter intake (DMI). Existing *in vitro* studies also showed that a shorter fluid retention time is related to a decrease in methane production and an increased microbial yield. Our study aimed to test whether methane production and ruminal microbial yield were affected by fluid retention time in live cattle and sheep.

We stimulated saliva production by orally dosing pilocarpine at 0, 1, 2.5 and 5 mg/kg body mass with the purpose to enhance fluid flow (i.e., shorten fluid retention) in four non-lactating cows and three sheep fed grass hay. Measurements included chewing behavior, nutrient digestibility, particle, and fluid mean retention time (MRT), ruminal microbial protein production estimated *via* urinary nitrogen parameters, and methane emission. The data were analyzed using a linear mixed model approach with the fixed effects of treatment (different dosages of pilocarpine) and the random effect of animal and experiment run.

We observed a significant effect of pilocarpine on fluid MRT for both cattle ($p=0.002$) and sheep ($p=0.025$), but no significant effect on particle MRT. Fluid MRT decreased by up to 8.6% in cattle and 15.3% in sheep as daily pilocarpine increased from 0 to 5 mg/kg body mass. Pilocarpine tended to reduce methane emission per unit of DMI for cattle ($p=0.08$) but not for sheep ($p=0.32$). Between the lowest and highest pilocarpine dose (0 vs. 5 mg/kg BW), the average methane emission per unit of DMI decreased by 6.8% for cattle. No significant effects of pilocarpine were found on nutrient intake, chewing activity, estimated rumen microbial protein synthesis, and apparent nutrient digestibility.

The treatments of orally given pilocarpine reduced fluid retention (i.e., increased fluid flow) in the gastrointestinal tract, though only to a limited extent, with no significant effect on particle retention and no measurable effect on microbial synthesis, which was not consistent with previous *in vitro* or *in vivo* studies. Further microbial analyses are needed to explain the trend in methane emission.

14 Video analysis of lying and suckling behaviour of piglets in free farrowing systems

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The behaviour of piglets can be an expression of welfare, but also of lacking care or an ongoing disease. In addition, it influences the development of healthy piglets as a base for weaning and fattening period.

The goal of the present study was to observe the individual behaviour of piglets within their litter. It was examined if an individual and litter-independent lying behaviour in piglets can be identified and to what extent it can be associated with suckling behaviour, weight gain and mortality.

69 piglets from five litters have been recorded for an hour, four times a day, during the first three days postpartum. The behaviours "lying TOTAL" differentiated into "resting in piglet nest", "lying at the udder", "resting in a group", "resting alone" as well as "suckling total" differentiated into "suckling cranially" for the cranial half of the udder and "suckling caudally" for the caudal half of the udder and "activity" were registered for each piglet individually. "Lying at the udder" as well as "suckling cranially" and "suckling caudally" were added to "time at the udder". For every behaviour and every piglet, a mean minutes per piglet and hour was calculated. Associations between the individual behaviours as well as behaviour and weight were analysed via linear regression and Pearson-Correlation. The Mann-Whitney U test was used to analyse differences of behaviour in relation to mortality of the individual piglets. A level of significance was determined at $p < 0.05$.

The number of shown described behaviours differed between the individual piglets and varied between four to eight shown behaviours. "Lying at the udder" (18 minutes), "resting in a group" (10 minutes) and "resting in piglet nest" (8 minutes) were the behaviours with the highest mean per hour. The total time spent lying ($p < 0.01$, $R^2 = 0.3$), "resting in a group" ($p = 0.01$, $R^2 = 0.1$) and "lying at the udder" ($p = 0.01$, $R^2 = 0.1$) showed a linear relationship to the time piglets spent suckling. Lying at the udder ($p < 0.01$, $R^2 = 0.1$) as well as time spent at the udder in general ($p < 0.01$, $R^2 = 0.3$) were associated to elevated weight gains during the first three days postpartum. No association between individual lying behaviour and mortality could be found in this study ($p > 0.1$).

The results of the present study indicate an individual lying and suckling behaviour of piglets. Suckling and lying were associated, so that piglets who consumed enough milk, rest more. The association between frequent lying and a higher weight gain is underlining this aspect. Piglets that spent a lot of time at the udder in total also showed elevated weight gains, which is underlined by the finding, that piglets that frequently lie at the udder also were suckling more often. The missing link between behaviour and mortality could be attributed to the small number of deceased piglets in this study and remains a subject of future research.

15 Oviductal extracellular vesicles: What do they tell us about early embryo quality?

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In recent years there has been an increasing interest in the role of the extracellular vesicles (EVs) as mediators of the early cross-talk between the oviduct and the embryo. Extracellular vesicles (EVs) are nanosized membrane vesicles with diverse molecular cargo, which can be transferred between the mother and the embryo. It has been shown that the oviductal EVs (oEVs) cargo changes across the estrous cycle. Moreover, poor quality embryos seem to release different EVs compared to good quality embryos. This led us to hypothesize whether the oviduct could recognize the quality of the embryo and secrete EVs with different cargo in response to good or poor-quality embryos.

To trace and decode the nanomessages packaged inside EVs released from the oviduct towards the embryo and vice versa, an *in vitro* co-culture model of bovine oviduct epithelial cells (BOEC) with early embryos was established. Ovaries and oviducts were collected from a slaughterhouse. Retrieved oocytes were matured, fertilized, and cultured *in vitro*, to obtain early embryos (at 53 h after fertilization). At this point, embryos were classified according to their morphological aspect in good quality embryos (GE \geq 8 cell embryos), poor quality embryos (PE < 8 cell embryo). Both GE and PE were co-cultured with BOEC, which were previously cultured until monolayer formation. Additionally, embryo and BOEC were cultured separately and as well as media alone (control groups). After 24 h of co-culture, conditioned media (CM), embryos and BOEC from the different experimental groups were collected for further analysis. Blastocyst rates and cell viability were also assessed. Isolation of EVs from CM of each experimental group was performed by a combination of serial centrifugation, size exclusion chromatography, and ultracentrifugation. Characterization of isolated EVs is being performed by transmission electron microscopy (TEM), flow cytometry, Western blot and Nanosight. Protein and RNA cargo of EVs will be analyzed by mass spectrometry and RNA-sequencing, respectively.

Up to now, our study showed that blastocyst rates (regardless culture with or without BOEC) were significantly higher for GE compared to PE (~ 50% vs ~10% at Day 8, $P \leq 0.001$) confirming that the embryo morphological selection was appropriately performed before embryo-BOEC co-culture. The viability of the BOEC was not affected by the co-culture with GE or PE. Regarding EVs characterization, TEM observations displayed nanoparticles resembling exosomes with size around 50 to 100 nm in all experimental groups, except for the control media. Flow cytometry analysis confirmed that EVs were positive for known exosome markers CD9 and CD63. Oviductal EVs RNA profile was analyzed by Agilent Bioanalyzer, showing the typical EVs profile with short RNA fragments and absence of rRNA peaks, and a low RNA yield but sufficient for RNA-sequencing.

The identification and integration of all RNA and protein components of the EVs from all experimental groups will show if the oviduct can distinguish between good and poor-quality embryos by releasing different EVs cargo, which might be implicated in continuation of pregnancy. Our results may help to identify biomarkers with potential clinical use in assisted reproductive technologies.

16 Substitution of soy components – Does feeding diets based on *Hermetia illucens* larvae impair growth performance, carcass and meat quality of organic broilers?

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The increasing global demand for animal protein produced in an environmentally friendly manner is resulting in the search for more sustainable feeds that offer alternatives to imported soy. Soy is currently used in large quantities in poultry nutrition. The larvae of the black soldier fly (BSFL, *Hermetia illucens*) have been discussed in the past years as such a possible alternative. Especially in the nutrition of laying hens, they have already shown great potential. However, the feeding value of the entire larvae material instead of only the defatted protein meal when used in broiler diets has so far been less intensively investigated. To extend the knowledge in this regard, partially defatted BSFL meal and fat of two different production origins were added together to the diets of organic broilers instead of soy. It was also aimed to determine if the nutritional value of the larvae is equivalent or even superior to that of soy, allowing to use lower amounts of BSFL in the diets. For this purpose, 80 organic day-old chicks (Hubbard S757) were fattened for 9 weeks, feeding the last 7 weeks one of five experimental diets. Two of these diets, based on soybean cake and oil (SS, SS-), were compared with three diets based on the two different partially defatted BSFL meals and BSFL fats (AA-, AB-, BB-). SS- and the BSFL-based diets were designed with a deficiency in limiting amino acids. The birds were kept in pairs and individually marked by rings. Body weight of each broiler was measured once per week. Feed and water intake were measured daily. The birds were slaughtered after 24h of fasting. One broiler of each pair was used for carcass and meat quality assessment and the other for whole body analysis to determine protein and energy retention. The final body weight of the birds of groups SS- and BB- ($P < 0.05$) differed from birds on diet SS, while AA- and AB- resulted in birds of comparable weight to diet SS. Broilers from groups SS, AA- and AB- approached the maximum gain of 27.5 g/day specified for organic fattening in Switzerland. Concomitantly, the birds of these three groups showed higher feed intake than the birds fed SS- and BB- ($P < 0.05$). The AA- and AB-broilers retained more protein in the body than the BB- and SS- broilers. Breast meat yield was higher with AA- and AB- than with BB- and SS-, but still lower than with SS. While there were no significant diet effects in thawing loss, the breast meat of group BB- showed higher cooking losses compared to SS ($P < 0.05$). The breast meat reflected in its fatty acid profile the high lauric acid content of the BSFL lipids, which was up to 80 times higher than when the soybean-based diets were fed. Overall, the results of this study indicate that diets based on *Hermetia illucens* larvae can have a comparable or higher nutritional value to soybean and are therefore suitable to provide a full substitute for soybean in organic broiler feeding.

17 Effects of increased dietary grass silage proportions on performance, nitrogen and energy balance as well as methane emission of beef cattle

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Grass silage is only scarcely used in intensive beef production in Switzerland as a lower performance is expected due to the lower energy supply compared to that provided by the commonly fed maize silage. This contradicts governmental strategies for increasing the proportion of grassland-based feeds in meat production. To investigate the effects on performance, nitrogen (N) and energy utilization as well as methane emission, we replaced part of the maize silage and concentrate by grass silage, with or without corn-cob mix (CCM) supplementation. Three diets consisted of grass silage, maize silage and concentrate in ratios of 0.1:0.6:0.3 (G10; control), 0.3:0.5:0.2 (G30) and 0.5:0.3:0.2 (G50), and two diets consisted of grass silage, CCM and concentrate at 0.5:0.3:0.2 (G50_{CCM}) and 0.75:0.15:0.1 (G75_{CCM}), respectively. These diets were tested in 5×6 Limousin-sired bulls with 164±18 kg body weight. A high-protein concentrate was fed to G10 (27% crude protein (CP)), whereas a low-protein concentrate (14% CP) was included in the remaining diets. At 521±2.0 kg BW, bulls were slaughtered. Between experimental week 6 and 22, each animal was subject to a balance trial in which total faeces and urine were collected and sampled for 7 days, followed by measuring gaseous exchange in respiration chambers during 48 h. Animals fed G10 and G50_{CCM} had higher ($P < 0.001$) average daily gains (ADG; 1.43 and 1.34 kg, respectively) than the remaining groups. Although silage and concentrate dry matter intake (DMI) varied (both $P < 0.001$) in response to the experimental design, total DMI was not affected. Nitrogen intake was higher ($P < 0.001$) for G10 and G75_{CCM} than for G30. Higher ($P < 0.001$) urinary N losses were observed in G10 and G75_{CCM} than in G30, G50 and G50_{CCM}. Total N loss was higher ($P < 0.01$) in G75_{CCM} than in G30 and G50_{CCM}. An increased ($P < 0.001$) urinary N proportion of total N excretion was found when feeding the diet with the high-protein concentrate (G10) or the diet with the highest proportion of grass silage (G75_{CCM}), whereas it was lowest in G50_{CCM}. Energy utilization was mostly unaffected, except for higher ($P < 0.01$) urinary energy losses found in G75_{CCM}. Absolute methane emission per animal and day was not affected by the diet. However, at the time point of the balance trial, methane emission intensity (g methane/kg ADG) was lower in G10 than in G30 and G75_{CCM}, whereas it was not different from G50 and G50_{CCM}. In conclusion, strategic combination with energy-rich forages allows increasing grass-land-based feeds in the diet at maintained performance and limited nitrogen emissions.

18 A comparative trial with the rumen simulation technique RUSITEC

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Animal experiments are increasingly questioned, and scientists are urged to develop and apply alternative methods for their research. An alternative method for use in ruminant nutrition studies is the rumen simulation technique (RUSITEC), originating from the 1970s which has been used by several research groups worldwide. This system consists of a varying number of fermenters, each simulating the rumen microbial metabolism, including saliva flow and ruminal gas production. In order to inoculate the RUSITEC fermenters with the original ruminal microflora, ruminally cannulated donor animals are needed. The RUSITEC has not been commercially available for buying until recently, thus the systems in use are mostly self-made and differ in sizes and implemented materials. In addition, varying operating procedures are applied. However, so far, no comparative study between different laboratories has been conducted. The ration of the donor animals, time of sampling the ruminal fluid from the donor animals, chemical composition of the artificial saliva buffer and buffer flow rate through the RUSITEC fermenters are just a few of the varying parameters known to affect the study outcome. Thus, the German Federal Institute for Risk Assessment (BfR) together with its committee for alternatives to animal testing in animal nutrition research initiated a comparative RUSITEC trial with in total nine participating research groups from Germany and Switzerland.

For a first experiment, to record the interlaboratory variability, all groups received the same feedstuffs (concentrate and hay) for incubation in their own RUSITEC systems according to their inhouse operating procedures. Relevant information of the individual systems and procedures were collected in surveys and analytical results included traits of the fermenter fluid (pH, redox potential, concentration of NH₃ and short chain fatty acids (SCFA)) as well as gas production and nutrient degradation (DM, OM, CP and NDF).

As expected, the variability in RUSITEC constructions and operating procedures was large and several effects on the results were found. Fermenter volumes ranged from 650 to 1100 mL. Buffer composition varied, and buffer turnover ranged 0.44 to 1.0 per day. Although pH could be kept relatively constant in the different groups and anaerobic conditions were maintained, the concentrations of NH₃ (10.2 mmol/L \pm 2.9 SD) and SCFA (63.4 mmol/L \pm 20.6 SD) varied considerably. The degradability of DM and NDF averaged 53% \pm 10 SD and 41% \pm 18, respectively.

A second experiment is currently prepared with unified operating procedures in order to further decrease the variability of results. All groups from the first experiment have agreed to participate in this second round.

19 Maternal spirulina supplementation during pregnancy and lactation affects the meat fatty acids profile of fattening pigs

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Evidence from the literature, including studies with livestock, shows that maternal nutrition during pregnancy and lactation is a major factor that influences offspring health and performance. Particularly, maternal dietary fatty acid (FA) intake during these two critical periods is important for an adequate pre- and postnatal development. Although the effects of maternal dietary FA proportions on the offspring's metabolism shortly after weaning are well established, little is known if these effects persist for a longer period. Spirulina (Sp) is a microalga, known for its specific FA profile, as it has a relatively high proportion of γ -linolenic acid (GLA). This FA is assumed to possess health promoting properties. Studies in pigs and sheep showed that a direct intake of Sp can enhance proportions of FA that are beneficial for human health in meat. Indeed, Sp seems to influence a differential incorporation of GLA, α -linolenic acid (ALA; n3), eicosapentaenoic acid (EPA), total mono- and polyunsaturated fatty acids (MUFA and PUFA, respectively) into muscle tissue. The aim of the present study was to investigate if maternal Sp supplementation during gestation and lactation may positively affect the FA profile in the offspring's muscle. In the context of a large experiment where pigs were used as models for human nutrition, 19 gilts (Landrace \times Large White, same sire) were fed either a commercial control (CTR) diet or a diet rich in saturated FA, sugars and cholesterol (WES). Two months afterwards, the gilts were artificially inseminated with sperm of the same boar and Sp supplementation was started for half of both dietary groups (20 g/day) and continued throughout gestation and lactation. Four experimental groups resulted (CTR, CTR+Sp, WES, and WES+Sp). At weaning, two males and two females per litter, with birth weights closest to the average of their respective litter, were selected and fattened until reaching a body weight of approximately 100 kg. Throughout the fattening period, the animals were fed with commercial diets. The offspring was never offered Sp. At slaughter, samples of the *Longissimus thoracis* muscle were collected and the FA profile was analyzed by gas chromatography. Maternal Sp supplementation significantly affected the FA profile. The meat lipids from pigs from Sp supplemented mothers showed significantly higher proportions of C18:2 n6 (linoleic acid), C20:0 (arachidic acid), C20:2 n6, C20:4 n3 and C20:5 n3 (EPA), and lower proportions of C18:1 trans9, C18:1 cis9 (oleic acid), C18:3 n3 (ALA), C18:2 and C22:4 n6. Moreover, the proportions of dihomo- γ -linolenic acid (C20:3 n6), total MUFA and PUFA, n3 and n6 FA showed a trend to be higher in animals from sows fed Sp. Unexpectedly, the proportion of GLA, the FA characteristic for Sp, was not affected. These results show that supplementing the mothers with Sp may influence the FA profile of the meat of fattening pigs, a change which persists even months after weaning. Consequently, improving the sow's diet during pregnancy and lactation might improve the FA composition of the meat of fattening pigs in a direction of an improved nutritional value for human consumption.

20 Multicolor flow cytometry for quality control of frozen bovine sperm and bull fertility prognostics

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Our study aimed a) to evaluate the use of multicolor flow cytometry for monitoring the quality of commercially produced frozen bovine sperm, and b) to explore the value of flow cytometrically assessed sperm functional traits for the prediction of sire fertility after artificial insemination (AI). Thus, the annual fertility index (FI) of 151 sires with more than 300 first AI was recorded between November 2018 and November 2019. Sires lying at the extremes of the FI distribution (outside the range of mean FI \pm SD) were classified as low- (LF; n=16 bulls) or high-fertile (HF; n=17 bulls), showing a non-return rate of 61.7 \pm 6.8% and 73.1 \pm 1.2%, respectively. At least four cryopreserved batches per bull (31 \pm 13 batches per bull) were examined immediately after thawing. The percentage of sperm with high esterase activity (C_{pos}), intact plasma membrane (PI_{neg}), unstained acrosome (PNA_{neg}), low intracellular Ca²⁺ levels (F_{neg}) and high mitochondrial membrane potential (M_{pos}) was quantified using a five-color flow cytometric panel that included calcein violet, propidium iodide, peanut agglutinin, Fluo-4 AM and MitoProbe™ DiIC₁(5), respectively. The Sperm Chromatin Structure Assay™ was performed to assess the percentage of sperm with high DNA fragmentation index (%DFI). Between-group differences of sperm functional traits were assessed through mixed-effects linear models, that included the random effect of batch (nested within bull) and the fixed effects of production month, sire fertility group and their interaction term. Both HF and LF bulls showed similar PI_{neg}PNA_{neg} (56.7 \pm 11.1% and 57.8 \pm 9.9%, respectively) and %DFI values (3.7 \pm 1.7% and 3.7 \pm 3.4%, respectively), but the fraction of PI_{neg}F_{neg} sperm within the C_{pos}M_{pos} (viable) subpopulation was significantly higher in HF than in LF bulls (74.2% vs. 66.7%, respectively; b=7.45, P=0.008). Concluding, flow cytometric analysis revealed a heterogeneous ability of viable sperm to control their Ca²⁺ homeostasis; this ability appeared relevant to sire fertilizing potential.

21 Effect of bull exposure to high temperature-humidity index levels on the quality of sperm selected through density gradient centrifugation for *in vitro* fertilization

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Bulls exposed to hot and humid conditions experience adverse effects on sperm quality and *in vitro* embryo production outcomes. Commonly, a sperm sub-population with favorable functional characteristics is selected by means of a sperm separation method and thereafter used for *in vitro* fertilization (IVF). Therefore, in order to study the effect of sperm modifications on *in vitro* embryo production, it is essential to focus on the detailed functional analysis of the sperm fraction selected for IVF. This study aimed to evaluate the functional status of frozen-thawed bovine sperm produced under high or low temperature-humidity index (THI) selected through a density gradient centrifugation (DGC), using computer-assisted sperm analysis (CASA) and flow cytometry. On this regard, we examined 12 ejaculates collected from six mature Simmental bulls (aged 6 ± 1 years), housed in an artificial insemination center in Southern Germany and exposed to high ($n=6$) and low ($n=6$) THI during epididymal maturation phase (seven days prior to sperm collection). For each bull, two 0.25-ml straws per ejaculate were pooled post-thaw and motile sperm were separated using the BoviPure® density gradient system. Samples of the selected sperm population were examined after 0 (0h) and 3 (3h) hours of incubation at 38 °C in HEPES-TALP medium. The percentage (%) of total and rapidly motile sperm was evaluated using CASA. Flow cytometry was employed to determine the percentage (%) of sperm with intact plasma membrane and acrosome (PMAI), with high DNA fragmentation index (%DFI) as well as the size of the sperm sub-population with high esterase activity, intact plasma membrane and acrosome, low Ca^{2+} levels and high mitochondrial membrane potential ($C_{pos}PI_{neg}PNA_{neg}F_{neg}M_{pos}$ sperm). Differences in sperm parameters were explored using the Least Squares Means procedure and Tukey's HSD pairwise comparisons were established. Sperm of the high-THI group showed a profound deterioration of their %DFI values between 0h and 3h (5.9 ± 6 and 11.5 ± 9 , respectively; $P < 0.05$), while such deterioration was not observed for the low-THI group (5.2 ± 6 and 9.2 ± 8 , respectively at 0h and 3h; $P > 0.05$). Selected sperm of both THI groups showed similar values of CASA traits in both 0h and 3h ($P > 0.05$), while PMAI and $C_{pos}PI_{neg}PNA_{neg}F_{neg}M_{pos}$ values decreased after 3h incubation in both high- and low-THI groups ($P < 0.05$). In conclusion, our results suggest a relation between bulls' high-THI exposure and the chromatin integrity of frozen-thawed sperm selected for IVF; however, the observed chromatin instability appears to be of subtle nature and only evident after incubation-induced stress.

22 Validation of a new measurement method to determine ammonia concentrations in calf fattening barns

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Calf rearing and fattening is challenged by high disease and mortality rates. Obviously, this contributes to the widespread prophylactic use of antibiotics in livestock farming. Barn climate is a major factor influencing the health and welfare of calves. Harmful gases such as ammonia have a negative impact on the barn climate, as well as on the health of the calves. Currently, there is a lack of suitable and validated measurement methods to assess the barn climate in practice.

The present study developed a method to assess ammonia concentrations in the direct environment of the calves. In a first attempt, two types of electrochemical ammonia sensors were compared: the stationary Dräger Polytron 8100 and a mobile prototype of Dräger x-am 5100. The two sensor types will be validated for their reliability and repeatability of measurements. Additionally, the new measurement method should provide measurements of ammonia concentrations in the macroclimatic (stationary sensors) and microclimatic (mobile sensors) range.

Therefore, we investigated the ammonia sensors under close-to-practice conditions in a single two-compartment calf fattening barn. In each of the two compartments one stationary sensor was installed at animal height, approximately 25 cm above the floor. To test whether mobile and stationary sensors provide comparable measurement values, we installed one mobile sensor beside each stationary sensor. Additionally, one calf per compartment was randomly chosen and fitted with a mobile sensor. With the aid of a halter we attached the mobile sensors to the calves' heads. This allowed to take measurements in the calves' respiration area. In total we installed four mobile ammonia sensors and two stationary sensors. Within each compartment, the mobile sensors were exchanged with each other on a two-day cycle. This enabled the comparison and validation of all four mobile sensors with the stationary sensors. Ammonia was measured continuously. Measurement intervals were set to 10 min. and peak values were recorded for each interval. The measurements lasted over a period of two weeks during a normal fattening cycle.

The first measurements were successfully completed. Data from stationary and mobile sensors will be validated in a descriptive analysis. Measurements of stationary and mobile sensors will be compared to determine the agreement between the two different measurement systems. In addition, the first measurements in the microclimatic area of the calves were generated. Descriptive analysis will also be performed on these data.

23 Relationships between antral follicle count, blood serum concentration of Anti-Müllerian hormone and fertility in dairy cows

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In cattle, size of ovarian reserve can be estimated by means of antral follicle count (AFC) or peripheral Anti-Müllerian hormone (AMH) concentration. Low AFC and low AMH concentration have been associated independently from each other rather with poor than with high fertility. The aim of this study was to analyze the potential of a single AFC and/or AMH determination during postpartum period to predict subsequent fertility of dairy cows. For this purpose, 121 primiparous (31±4 months), 112 biparous (45±6 months) and 280 pluriparous (75±22 months) cows were enrolled in the study. The cows belonged to four different breeds: Holstein Frisian (n=241), Red Holstein (n=128), Brown Swiss (n=112) and others (n=32, mostly Montbéliard, except for one Jersey and two crossbreeds) and had a predicted 305 days milk yield of 8359.1±2260.9 kg. A single AFC determination was performed at 28-56 days after parturition by transrectal ultrasonographic evaluation. At the same time, blood was collected, and serum stored frozen (-20°C) until analysis for AMH, using a bovine AMH ELISA kit (A79765, AMH Gen II ELISA, Beckman Coulter, Brea, CA, USA). Cows were subjected either to artificial insemination or bred by bulls and the number of insemination and last date of breeding were recorded. Pregnancy diagnosis was performed using transrectal ultrasonography at least twice in every cow, on days 28-35 and days 50-70 after insemination, respectively. If cows were open for more than 200 days after parturition, they were classified as not pregnant. Based on number of antral follicles, cows were classified as having low (≤ 15 follicles, mean 10.5±3.0, n=246), intermediate (16-24 follicles, mean 19.3±2.8, n=202), or high (≥ 25 follicles, mean 28.7±3.4, n=65) AFC, respectively. Primiparous cows had lower AFC ($P < 0.001$) than biparous and pluriparous cows (13.6±6.2 vs. 17.4±6.9 vs. 17.0±7.0), but there was no difference between animals with two and three or more lactations. AFC was not affected by breed of the cows or by milk yield. In 204 (39.8%) cows, AMH concentration was below detection limit of the assay (0.05 ng/ml), but it was not affected ($P > 0.05$) by parity. Cows with AMH concentration below detection limit had higher ($P < 0.05$) milk yield than those over detection limit (8635.2±2120.4 kg vs. 8177.2±2334.4 kg). Furthermore, a shorter ($P < 0.05$) calving to first service interval (84.0±36.7 days vs. 93.8 vs. 43.8 days) and a tendentially shorter ($P = 0.07$) calving to conception interval (122.4±52.5 days vs. 131.3±53.3 days) were observed in cows with AMH concentration above detection limit. There was no effect of AMH concentration on first service conception rate and pregnancy rate. Results of this study showed that AMH concentration affected calving to first service interval and calving to conception interval, but also milk yield, suggesting its association with productivity in dairy cattle.

24 Survey on Salmonella occurrence in meat producing rabbitries in Switzerland in the context of the husbandry system

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A recent outbreak of salmonellosis in a large Swiss meat rabbit farm was detected coincidentally by routinely performed necropsies, since there is no mandatory surveillance of *Salmonella* sp. in meat rabbits. Although *Salmonella* Typhimurium occasionally causes severe outbreaks of salmonellosis with clinical signs, such as fever, enteritis, abortion and high mortality, rabbits may be subclinical carriers and shedders of several *Salmonella* serovars. Thus, it was assumed that the actual extent of *Salmonella* carriage in commercial rabbits in Switzerland might be underestimated.

Therefore, faecal pool samples of fattening and breeding rabbits of 50 farms – representing approximately 90% of the Swiss commercial rabbitries – were tested for the presence of *Salmonella* sp. Further, 236 perished rabbits showing clinical signs compatible with salmonellosis were necropsied and pathologically suspicious organs were tested microbiologically for *Salmonella* sp.

A single serovar – *Salmonella* Typhimurium – was detected in the faecal samples of three farms and in tissues of rabbits of one of these farms. This corresponds to an occurrence of 6.0%.

A direct connection between the three affected farms could be proved by the trading of animals. This epidemiological link could be further corroborated by whole genome sequencing data showing that all *Salmonella* Typhimurium isolates belonged to a single clone.

Thus, it was stated that salmonellosis in rabbits is rather a local hazard and that the three cases are to be attributed to one single source of infection.

Although the Swiss standards of husbandry may harbour several risk factors for the infection with *Salmonella* sp. (e.g. ground covering litter, group housing), an increased occurrence of salmonellosis could not be detected when compared to previous studies in different countries. However, as the infection with *Salmonella* sp. is not necessarily accompanied by obvious clinical symptoms, its prevalence in Swiss rabbitries can only be assessed reliably by regular monitoring.

25 Influence of Negative Energy Balance and Periparturient Diseases on Follicular Dynamics, Oocyte Quality and Subsequent *in vitro* Embryo Development in Dairy Cows

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During transition period, high-yielding dairy cows suffer from profound negative energy balance (NEB) which leads to reduced milk yield and predisposes to production diseases. The changes associated with NEB affect follicular environment and likely result in the ovulation of a developmentally incompetent oocyte. The objective of this study was to determine the influence of NEB and periparturient diseases on oocyte competence and embryonic development. For this purpose, 30 multiparous lactating dairy cows from AgroVet-Strickhof herd in Switzerland were enrolled in the study. Cows were clinically and gynecologically examined immediately after calving and in each of the following eight weeks in order to detect the occurrence of metabolic or inflammatory diseases. During examination, blood and milk samples were collected to determine β -hydroxybutyrate acid (BHBA) concentration and somatic cell count (SCC), respectively. Cumulus oocyte-complexes (COCs) were recovered from all follicles larger than 3 mm in diameter by Ovum Pick-up (OPU) on a biweekly schedule from the 5th to the 8th week postpartum. All COCs collected during first OPU of every week (four in total) subsequently underwent *in vitro* maturation followed by fertilization and embryo culture to the blastocyst stage. Cows were assigned to three groups: healthy (C, n=8), metabolic disorders (MD, BHBA levels > 1.2 mmol/L, n=14), or inflammatory disease (ID, milk SCC >200000 cells/mL and/or with vaginal discharge, n=8). In total, 154, 232 and 163 COCs were collected in group C, MD and ID, respectively. There was no significant difference in the cleavage rate among groups: C: 84.0 \pm 20.5%, MD: 81.8 \pm 18.1%, and ID: 82.6 \pm 14.4%. Blastocyst rates were also not statistically different between C, MD, and ID cows: 58.6 \pm 26.5% vs. 56.9 \pm 26.5% vs. 64.1 \pm 22.6%. Hatching rate of blastocysts was neither affected by metabolic disorders, nor by inflammatory diseases (C vs. MD vs. ID: 63.7 \pm 28.1% vs. 76.1 \pm 22.0% vs. 72.9 \pm 31.4%). In conclusion, results of this study showed no effect of postpartum diseases on developmental competence of bovine oocytes, as assessed by cleavage, blastocyst, and hatching rates. This suggests that *in vitro* embryo production can successfully be performed early after parturition, regardless of periparturient disorders. However, the limited sample size included in this study should be taken into consideration. Further investigations on the transcriptome and miRNA profile of oocytes, cumulus cells and embryos collected in this study should shed light on molecular changes associated with metabolic disorders and periparturient diseases and the chances to establish a pregnancy.

26 Practice-oriented research in the field of pigs at Agrovet-Strickhof

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The generous infrastructure at Agrovet-Strickhof as well as the joint use with ETH, university and agricultural school offer optimal conditions for practice-oriented and interdisciplinary research. Despite the restriction as a core breeding pig farm, very interesting projects could be realized in the last years, especially in the fields of disease prophylaxis, disease diagnostics and on the epidemiology and control of MRSA. In the following, 3 lighthouse projects will be described in more detail.

In a very time consuming experiment it could be shown the colonization status of the sow changes several times during the suckling period and MRSA negative sows can also be colonized without direct contact to MRSA-positive, if they are kept in the same airspace as MRSA-positive carriers. Furthermore, it was shown that the increase in MRSA prevalence in slaughter pigs is mainly due to the transport of animals and the waiting room in the slaughterhouse. An attempt to rinse the sow's pharynx and vagina with a staphylococcus-specific bacteriophage cocktail, as well as the weekly washing of the piglets and the administration of the MRSA cocktail via feed, drinking water and room fogging led "only" to a reduction, but not to an eradication.

Societal demands to reduce antibiotics, pesticides and fungicides and the research into alternatives, are opening many new research fields for Agrovet. It is suggested that pig manure is a reservoir for the transfer of resistance gene into the feed and food chain. In an SNF-funded project we are currently investigating resistance gene transfer from pig manure to plants and soil bacteria. Therefore, a clover-grass mixture as well as spring wheat are manured with a) fresh, b) three months stored and c) anaerobic fermented slurry (four weeks at 38°C) from a pig farm with high antibiotic consumption, to investigate the effect of storage on antibiotic residues as well as on the presence and transfer of resistance genes to soil and plant bacteria. Additionally, slurry application (spray method versus drag hose method) will also be investigated.

Since in many countries the use of antibiotics in veterinary medicine has to be recorded and high consumers are forced to implement measures to reduce their antibiotic consumption, producers often switch to zinc oxide for diarrhea prophylaxis and to stabilize the intestinal flora. However, it is known that zinc in high doses can co-select antibiotic resistance in addition to environmental damage. Currently, dose-response trials are conducted as part of an Innosuisse project to register a titanium core, coated with zinc oxide, as a feed additive. In-vitro experiments have shown that thanks to the surface enlargement of a zinc-coated titanium core, 50 ppm of titanium zinc oxide has the same effect as conventional 3000 ppm zinc oxide, which makes the new zinc compound an interesting alternative product for the control and prophylaxis of weaning diarrhea.

Increasing importance is being given to the pig as a model for human medicine, although the current orientation of the pig farm as a core breeding farm and the lack of infrastructure still somewhat limits this research direction.

27 Establishing an air liquid interphase (ALI) culture model of the bovine mammary gland to study extracellular vesicles

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Extracellular vesicles (EV) take part in mediating the communication between cells. In cattle, mammary gland epithelial cells (MEC) release EV into milk, and we aim at studying their physiological role in the mammary gland *in vitro*. To model the tissue, we established a culture of primary MEC in an air liquid interphase (ALI) system and with a bioprinting approach. Primary MEC were bioprinted on top of or embedded in Geltrex™ at 7,8 or 9 mg/mL. In all conditions, MEC proliferated and after confluency formed aggregates and duct-like structures stable for more than 20 days. The permeability of the cells layer did not differ among seeding conditions. 3-4 days after the apical medium removal, MEC were covered by a liquid. Cells seeded on top of Geltrex™ were covered by 1-18 µL of liquid, with a 1.7-fold protein concentration compared to the basolateral medium concentration. In contrast, cells embedded in Geltrex™ were covered by 13-100 µL liquid, with a concentration of 1.0-1.2-fold the basal medium. EV were isolated from the apical liquid by differential centrifugation followed by size exclusion chromatography and ultracentrifugation. Transmission electron microscopy confirmed that EV were present in the apical liquid. Particle mean diameter size and amount, measured by tunable resistive pulse sensing, were: 100 ± 34 nm and 5.25E+10 particles (on top of Geltrex 7.8 mg/mL), 95 ± 38.2 nm and 4.14E+10 (on top of Geltrex 9 mg/mL), 96 ± 31 nm and 1.54E+10 particles (embedded in Geltrex 7.8 mg/mL), 84 ± 31.7 nm 4.59E+10 particles (embedded in Geltrex in 9 mg/mL). These preliminary results show for the first time that bovine MEC tolerate the bioprinting procedure, the ALI culture and produce EV under these condition. Similarly to ALI cultures of other epithelia, the liquid on the apical side is likely a secretion (here a milk surrogate) produced by MEC. We will further investigate if this liquid is diluted to different degrees by the basolateral medium, and characterize the bioprinted ALI culture in more detail.

28 Effect of tannins in *Onobrychis* on *in vitro* ruminal gas and ammonia formation and digestibility

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Mitigation of methane and ammonia contribute to greenhouse gas abatement objectives and can have a positive effect on animal production efficiency. Reducing the production of methane and ammonia can take place by influencing ruminal methanogenesis through feedstuff. In this regard, the secondary plant metabolites tannins, which are polyphenolic compounds, have shown promising results. Numerous studies demonstrated mitigating effects of tannins on *in vitro* and *in vivo* methane and ammonia formation. In the present study the potential of the Swiss indigenous tannin-containing plant sainfoin (*Onobrychis*) was explored. Sainfoin can be produced on Swiss soil. This presents the possibility for farmers to orientate their cultivation in this direction and helps to diminish emission due to transportation to a minimum. Hence, this study has the potential to propose a practical and cost-efficient solution for the reduction of greenhouse gas emissions in the Swiss dairy production sector.

Method: Four different cuts of sainfoin from three different agricultural sites in Switzerland were tested *in vitro* using the Hohenheim Gas test (HGT) method. Ruminal fluid from four Original Brown Swiss cows was taken and tested separately in four consecutive runs. For every run the same mixed ration, containing maize silage, grass silage, hay, alfalfa, concentrate mixture and UFA 249 (32 : 60 : 2 : 2 : 2.6 : 1.5) was incubated and served as control. Every sainfoin cut was tested in different dosages (sainfoin: 20, 30, 40, 50 and 60 mg DM) which replaced the respective amount of the mixed ration. The dosages were tested twice per run to evaluate the consistency of their effect resulting in a total of 8 replicates per treatment.

Linear mixed model investigated if tannins in indigenous sainfoin have the ability to affect *in vitro* methane and ammonia formation as well as digestibility.

Results/ Discussion: Analysis revealed no effect on methane emission for cut or dosage. The results with regard to methane emission are in contrast to other studies which found that tannins in sainfoin had a mitigating effect on methane emission. There are different explanations for this lack of effect. Laboratory analysis revealed that tannin content in our sainfoin samples was very low. Hence, the amount of tannins in the rations tested could have been simply too low to have an effect on methanogenesis and ammonia production. Tannin content in plants are influenced by harvest point, agronomic factors during cultivation and conservation processes. In this study we used sainfoin pellets from different harvest points which were produced under commercially customary conditions, i.e. dried with a temperature above 100°C. Pelleting reduces tannin content by 33% and alters their chemical structure which renders them less efficient in the rumen. Hence, in future studies, plants should be preserved with different conservation methods in order to investigate their impact on tannin content and their mode of action on methanogenesis and ammonia formation. In addition, harvest points of sainfoin should be chosen in a manner that maximizes tannin content in the plant, i.e. after maturity, since flowers are known to contain more tannin. In conclusion, further studies are necessary to evaluate the mitigation potential of tannins in sainfoin on methane emission and ammonia formation.

29 Long-term study of the development of early osteochondrosis lesions by computed tomography (CT), pathology, histology, and blood analysis and their association with the development of osteochondrosis dissecans and lameness

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Healthy limbs are a prerequisite for rearing productive pigs. Osteochondrosis (OC) is considered to be the dominant noninfectious cause of lameness. It is estimated that as many as 80% of all slaughter pigs show at least mild signs of OC (Bertholle et al., 2016). On the other hand, the presence of OC lesions alone does not provide sufficient information concerning the occurrence of lameness, which usually becomes apparent with 4-18 months of age. Twenty Swiss fattening pigs (F2) were observed over a period of ten months. During this period, three CT's (160th, 210th and 280th day of life), three descriptions of the limb position, weekly lameness examinations and monthly blood tests (Ctx, osteocalcin, CP2) were performed and weight gain was determined on a weekly basis. At the end of the study, all animals were slaughtered, and the legs were examined for lesions - both macroscopically and histologically. The aim of this pilot study was to monitor the occurrence and progression of OC lesions by CT examinations and to identify possible correlations between the collected data.

There were no pigs or legs without lesions. This shows that osteochondrosis is also an important topic in Switzerland. The MHK was the most interesting location showing the most pronounced lesions in both CT and pathological examination. The MHK and MFK showed first indications of an association with lameness, so that the question arises whether in future these localizations should be weighted more in the breeding evaluation. Since most pigs had lesions on the DU and talus, which also showed a high healing tendency, clinical relevance of these findings is questionable. The lateral condyles also seemed to be less importance. Other results still have to be evaluated. Ideally, the new knowledge gained from the study will allow a better risk assessment for lameness development, which could improve animal welfare as well as economic losses in the long term (breeding hygiene).