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The 26th Conference on Laboratory Animals Science, organized by the Czech Laboratory Animal Science Association (Společnost pro vědu o laboratorních zvířatech), was held on April 23-25, 2025, at Hotel Dvořák, Tábor, Czech Republic. More than 130 scientists, veterinary experts, representatives of biomedical research organizations, universities and animal welfare authorities participated in the meeting. The presented lectures were focused on laboratory animal welfare and protection, new experimental methods and procedures, particularly utilizing laboratory pig models, and the current status of alternative *in vitro* methods as replacement of animal experimentation according to the 3Rs principle. Representatives of animal welfare authorities shared information on the implementation and amendments of regulations concerning the protection of animals used for scientific purposes.

HOME CAGE MONITORING – IMPROVING REPRODUCIBILITY AND ANIMAL WELFARE IN SCIENTIFIC RESEARCH

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Scientific communities have long debated how to improve the reproducibility and replicability of research involving laboratory animals. In 2021, European researchers working in biomedical, neurological, and behavioral sciences launched a COST Action project (CA20135 TEATIME) aimed at establishing procedures and methodologies for using Home Cage Monitoring (HCM) systems in studies on laboratory rodents. The ultimate goal was to enhance data reliability in both basic and translational research. HCM systems enable long-term, automated monitoring of animal activity, behavior, and metabolic parameters within the animals´ familiar home-cage environment, with minimal human interference. This approach significantly reduces stress related to handling and environmental changes, which are known sources of variability and poor reproducibility in behavioral studies. Moreover, HCM systems collect environmental data and track animal activity in real time, allowing timely intervention when animal welfare is at risk. They can provide insights ranging from basic parameters such as access to food and water, to more complex indicators like reduced activity suggesting declining health, increased activity potentially signaling aggression, or physiological data such as body weight, temperature, and heart rate. By providing continuous, high-resolution data, these systems not only improve scientific outcomes but also support the 3Rs principle by potentially reducing the number of animals required for research. As part of the TEATIME network, researchers have also established an online platform – The Behaviour Forum (www.TheBehaviourForum.org) – to share knowledge, experience, and best practices in HCM-based and behavioral research on animals. The forum is open to all interested researchers, offering the opportunity to join ongoing discussions, contribute insights, and actively participate in the development of methodologies and future directions in this evolving field.

LIGHT AND ITS IMPACT ON THE HEALTH AND WELLBEING OF LABORATORY ANIMALS

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Light is one of the most influential environmental factors affecting the physiology of all organisms, including laboratory animals. In addition to enabling visual perception, it regulates a wide range of biological processes – from circadian synchronization and hormonal secretion to immune function, metabolism, and neurodevelopment. Yet, lighting conditions in experimental facilities are often treated as a technical necessity designed primarily for the visual comfort of the staff. This approach has led to the widespread use of light intensities and spectra that fall well outside the natural range relevant to laboratory rodents. Inappropriate lighting can result in chronic stress and physiological dysregulation. Thanks to modern technologies, it is now possible to simulate natural day-night cycles, including spectral composition and temporal dynamics. Such biologically relevant lighting can improve ageing trajectories, behavior, vision, circadian function, and immune responses. Incorporating these findings can not only enhance animal welfare but also improve the validity and reproducibility of experimental outcomes while aligning housing conditions more closely with the principles of the 3Rs.

The work was supported by the Technology Agency of the Czech Republic, Programme: FW – TREND; Project ID: FW09020164; Nature-Like Lighting for Laboratory Animal Facilities (Czech Republic).

EFFECTIVENESS OF THE SELECTIVE NaV1.8 BLOCKER A-803467 IN SUPPRESSING COUGH IN A GUINEA PIG MODEL OF ALLERGIC RHINITIS VS. HEALTHY CONTROLS

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Chronic cough, commonly associated with conditions such as allergic rhinitis (AR), significantly affects the quality of life. It results from an overactive cough reflex due to inflammation-mediated sensory nerve irritation. Conventional antitussive treatments often lack efficacy and cause adverse effects. Recent advances in the understanding of voltage-gated sodium channels (NaVs) have revealed that subtype NaV1.8 is involved in initiation and conduction of cough and therefore presents a promising neural target. This study investigates the impact of the NaV1.8 blocker A-803467 on cough suppression in a guinea pig model of AR compared with control group. Dunkin-Hartley guinea pigs were sensitized with ovalbumin (OVA) and subjected to intranasal challenges at weekly intervals for six weeks, with AR symptoms monitored throughout. Cough was induced using citric acid aerosol (0.4 M) before and after nasal challenges (NCH). On the 4th and 6th NCH, a subset of OVA-sensitized animals was pre-treated with inhaled A-803467 (3 mM, 10 min) before tussigen exposure. Chronic AR led to a heightened cough response compared to controls (10.95±1.15 vs. 6.10±1.15, P=0.0147, n=10 per group). Pre-treatment with the NaV1.8 blocker significantly reduced coughing by approximately 73 % (3.05±0.36 vs. 10.95±1.15, P<0.0001) without altering the respiratory rate. Compared to the vehicle, the suppression of cough by the NaV1.8 blocker was more pronounced than in healthy animals pre-treated with the NaV1.8 inhibitor A-803467, where inhibition was approximately 50 % (5±0.47 vs. 2.5±0.35, n=13, P<0.01). These findings highlight NaV1.8 channels as a potential therapeutic target for developing more effective antitussive treatments.

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NEW TRENDS IN PAIN MANAGEMENT OF SMALL LABORATORY ANIMALS

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Recognizing and minimizing pain, distress, and suffering in laboratory animals is essential for both ethical and scientific reasons. Poor welfare can significantly alter physiological responses, increase variability in experimental results, and lead to complications such as infections or unexpected mortality. These issues not only compromise the research outcomes but also violate legal obligations under EU Directive 2010/63/EU, which mandates the implementation of the 3Rs – Replacement, Reduction, and Refinement. Systematic welfare assessment using standardized score sheets supports consistent monitoring of animal condition. Parameters such as coat condition, dehydration, tumor size, weight loss, behavior, and neurological signs are scored to evaluate health status and guide interventions. Clear thresholds help to determine when supportive care is needed or when humane endpoints - such as euthanasia – should be applied to prevent unnecessary suffering. Effective scoring systems are tailored to specific species and experimental models. Cognitive and neurological assessments are particularly important in studies involving neurodegeneration. Body condition scoring and tracking of weight trends provide additional insight into gradual welfare decline. Interventions may include fluid support, enriched housing, pain management, or refinement of procedures to reduce distress. The concept of cumulative severity is emphasized, highlighting that the total burden on an animal includes both procedural and environmental factors. Repeated or invasive procedures, when combined with inadequate housing or handling, can result in significant cumulative distress. Retrospective evaluation of the animal experience is encouraged to inform refinements in future studies. Real-life welfare indicators are illustrated through examples in common laboratory

species, such as skin abnormalities in rodents, pododermatitis in rabbits, and stress-induced infections in amphibians. These practical observations underscore the importance of regular and skilled monitoring. By applying structured assessments, refining experimental methods, and prioritizing humane care, it is possible to balance scientific goals with high standards of animal welfare throughout the course of a study.

This work was funded by the ISI-MR facility of the Czech-BioImaging infrastructure, supported by grant LM2023050 of the MEYS CR.

ANIMAL MODELS AS A PLATFORM FOR EVALUATING THE ANTICANCER PROPERTIES OF NEW ORGANOTIN COMPOUNDS

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Mouse cancer models are essential for elucidating the mechanisms underlying tumor development and the accompanying immune responses while also serving as a crucial platform for evaluating novel anti-cancer therapies. The clinical application of conventionally used chemotherapeutic agents is often restricted by their toxicity, highlighting the urgent need for the development of new therapeutics with fewer adverse effects. Previous studies have demonstrated that triorganotin compounds exhibit promising antitumor efficacy with reduced toxicity *in vivo*. In this study, we investigated the effects of tributyltin trichloroacetate (Cl_3CO), tributyltin trifluoromethanesulfonate (F_3SO_3), and N,N-dimethyldithiocarbamate triphenyltin (PhS_2) on a panel of breast cancer cell lines, both *in vitro* and *in vivo*. All tested compounds inhibited cell proliferation in a dose-dependent manner, with IC_{50} values ranging from 0.09 to 0.33 μM. Based on these promising *in vitro* results, we proceeded to evaluate their effects on tumor growth and metastasis. Before *in vivo* efficacy testing, acute and subacute toxicity assessments were conducted using a BALB/c mouse model. In acute toxicity testing, toxicity was not confirmed after 2 weeks of observation for any of the compounds. In the subacute toxicity study, BALB/c mice received Cl_3CO, F_3SO_3, and PhS_2 for four consecutive weeks, with no toxicity detected for Cl_3CO and F_3SO_3 at any dose. However, PhS_2 exhibited toxicity at 55 mg/kg and 25 mg/kg. The highest non-toxic concentrations of the compounds were selected for assessing their antitumor efficacy. BALB/c mice bearing orthotopic allografts from 4T1 cells were treated twice weekly for four weeks. Only PhS_2 exhibited a tumor volume reduction of 17 % and cisplatin by 34 % compared to the control group after treatment and a 2-week observation period. Metastatic lesions were observed in the lungs and liver across all the treatment groups. Subsequently, we assessed the effect of PhS_2 when administered five times per week for three weeks. However, this intensified dosing regimen did not enhance its efficacy, with tumor reduction remaining at 16 % compared to the untreated group. Histological analysis and qPCR confirmed the presence of lung and liver metastases in all the experimental groups, regardless of treatment. The promising results from *in vitro* experiments have not been confirmed *in vivo*, and the mechanism of action remains to be investigated.

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DEVELOPMENT OF A PORCINE MODEL FOR TWO TYPES OF DEEP WOUND INFECTIONS

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Multidrug resistance is a serious medical problem and represents a major challenge for the treatment of infectious diseases. There is an urgent need to develop new antimicrobial agents with diverse chemical structures and novel mechanisms of action to overcome resistance. For the implementation of new antimicrobial biomaterials and additives in human medicine, it is necessary to evaluate their potential not only *in vitro* but also *in vivo*. Porcine skin provides a comprehensive model for testing topical administration of antimicrobial biomaterials to simulate a real clinical situation – complicated skin and soft tissue infection (cSSTI). This poster presents the introduction of cSSTI caused by methicillin-resistant *Staphylococcus aureus* (MRSA) or *Pseudomonas aeruginosa* in fully immunocompetent pig models. Under general anesthesia, 10 full-thickness skin defects (5×5 cm) were surgically created on the dorsal surface of each pig after antiseptic preparation. Fascial incisions were made in the defects to induce complicated deep wound and soft tissue infection (fasciitis and myonecrosis) and to maintain the infection for the duration of the experiment. For wound infection, a bacterial inoculum of MRSA NRL/Atb 5922 strain (sequence type ST22) was diffusely applied to sterile gauze covering the skin defects in 10 pigs or the multidrug-resistant clinical strain *P. aeruginosa* FF2 (VIM-carbapenemase-producing) in 6 pigs. Subsequently, all wounds of each pig were covered with an occlusive dressing and sterile gauze, and finally the pigs were dressed in an elastic bandage. Analgesia was achieved with the use of meloxicam for three consecutive days. Preoperative antibiotic prophylaxis was used to reduce the risk of contaminating bacterial infections affecting the induced infections. On days 4, 7, 10 and 14, wounds were visually assessed and biopsies were taken for microbiological and histological examination as well as for gene expression. There was no significant deterioration in the overall health of the animals during the experimental period. The cSSTI infection model for both pathogens was effectively established and maintained for 14 days. Due to the immunocompetence of the model animals, all immunological responses to the infectious agent and wound healing processes are preserved.

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MINIATURE PIG – PRECLINICAL STUDIES

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From an economic and ethical point of view, the miniature pig currently represents a unique large animal model for biomedical research. Due to its size, organ arrangement and physiological similarity to humans in many aspects, the pig is very well suited for preclinical experiments and long-term safety studies. The anatomical similarity and the size of some organs and tissues allow various surgical procedures to be performed on a scale comparable to that of humans. A disease model created in a miniature pig can help to establish an optimal preclinical protocol that is surgically manageable and safe and allows for the prediction of complications associated with surgery and recovery in humans. The miniature pig is also an ideal animal for human surgeons to train classical and newly developed surgical approaches. The PIGMOD center has miniature pig models for various serious human diseases. Currently, hereditary neurodegenerative diseases, injuries of the nervous system, retinopathies and the development of new surgical approaches are the main topics of the Laboratory for Cell Regeneration and Plasticity, which is a part of the PIGMOD Centre. Using biochemical, proteomic and molecular biological methods, we investigate the mechanisms of these diseases and test possible treatments. The result of an extensive international collaboration are lines of transgenic minipigs for Huntington's disease (TgHD, KI-85Q-HD), Usher syndrome (USH1C, MYO7A), Stargardt disease and phospholamban dilated cardiomyopathy. International grants, foundations and pharmaceutical

companies have supported research on these unique models. Thanks to a long-standing collaboration with the University of California San Diego, we have developed a customizable computer-controlled compression model for spinal cord injury in minipigs to test cell and gene therapies and introduced a new surgical technique for subpial application of gene therapies. In addition, in collaboration with the ISCARE Centre, the Central Military Hospital and the General University Hospital in Prague, we have developed a novel, reproducible porcine model for anastomotic strictures with histologically proven changes mimicking Crohn´s disease, which has a stable diameter for more than 6 months. This model has been used for new treatment options or for training classical endoscopic approaches.

The work was supported by the Ministry of Health project No. NU22-08-00554 – Self-expandable metallic stent in the treatment of anastomotic stricture associated with Crohn's disease – an experimental randomized study and project No. NW24-10-00118 – Porous gyroid surface structures for improved implant stability in osteoporotic bone, Johannes Amos Comenius Programme (Research Excellence in Regenerative medicine, CZ.02.01.01/00/22_008/0004562), Usher 2020 Foundation, CHDI Foundation, Research Agreement (A-17193), Czech Academy of Sciences – Strategy AV21 (VP29) and Institutional support RVO: 67985904.

FIFTEEN YEARS OF LIFE WITH THE DOMESTIC PIG AS AN EXPERIMENTAL ANIMAL: PRACTICAL EXPERIENCE ON BREEDING, PERIOPERATIVE CARE AND ANAESTHESIA

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The pig is a highly valued *in vivo* model used in many fields of biomedical research due to its similarity to humans. On the other hand, it is also a very intelligent animal, not always willing to cooperate with experimenters and requiring a specific approach in many respects. This paper summarizes experiences and observations from many years of daily experimental work with pigs. Attention will be paid to practical issues such as the choice of housing technology for experimental pigs, animal handling and work safety, the possibilities and pitfalls of administering anaesthesia and providing pre- and post-operative care, as well as the collection of samples of biological material for analysis.

CREATING AN OPTIMAL LIGHTING ENVIRONMENT IN ANIMAL EXPERIMENTAL FACILITIES

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Scientific research has demonstrated the considerable impact of the day/night light cycle on physiological processes in organisms. The quality of electric lighting in current buildings, including animal experimental facilities, differs from natural lighting conditions. Light sources are typically optimised for the spectral sensitivity of human photoreceptors, which can differ from the visual sensitivity of laboratory animals such as rodents. Furthermore, visual perception differs in spectral sensitivity to non-image forming (melanopic, circadian) effects of light. In designing lighting systems for human spaces, the difference between visual and non-visual perception is gradually being considered in the concepts of so-called “integrative lighting”. However, lighting in animal experimental facilities has not yet reflected these findings, despite their known importance for the validity and reproducibility of research results. This presentation will introduce newly developed lighting systems for laboratory animal housing, designed to create an environment close to natural lighting conditions. Throughout the day, the light spectrally mimics daylight. Automatic control of light output ensures a three-order difference between day and night illumination, closely simulating nighttime conditions under the night sky. A 30-minute gradual transition between day and night mimics dawn/dusk conditions in Central Europe. In contrast to conventional light sources such as LED and fluorescent lighting, this innovative light source emits light with balanced representation of wavelengths in the central region of the optical radiation (450-650 nm). It also includes very short wavelengths up to the near-UV range and contains long wavelengths that are typically missing in usual light sources. The luminaire is available in several sizes, allowing it to be used as a central light source mounted on the laboratory ceiling or as local lighting directly at the breeding containers. The lighting settings and control are managed through a user-friendly application.

The work was supported by the Technology Agency of the Czech Republic, Programme: FW – TREND; Project ID: FW09020164; Nature-Like Lighting for Laboratory Animal Facilities (Czech Republic).

PHARMACOKINETIC STUDY TO OPTIMISE ANTIBIOTIC TREATMENT IN THE TARGET SPECIES AND ANIMAL CATEGORY – BROILER CHICKENS

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The inappropriate use or overuse of antibiotics to treat infections in human and veterinary medicine has led to the emergence and spread of antibiotic resistance in bacteria, which has become a global problem in recent years. Optimal dosing of antibiotics based on knowledge of the pharmacokinetic (PK) and pharmacodynamic (PD) properties of the antibiotic, which also determine the level of antimicrobial exposure required for maximum effect on bacteria, is an important factor in limiting the emergence and development of resistance. The current trend in veterinary medicine is to use the so-called 'old' antibiotic molecules, such as potentiated sulfonamides, for treatment. The use and dosage of such antimicrobials was mainly established in the 1970s and 1980s using less accurate methods than those available today. The main objective of the pharmacokinetic studies is to validate or update these established procedures or to design an experimental drug with these active ingredients in a different ratio for the treatment of bacterial infections in broilers. Based on the pharmacokinetic studies and mass spectrometry analysis, a new formulation of an experimental veterinary drug containing trimethoprim and sulfamethoxazole was proposed for the treatment of broilers. The efficacy of the newly designed formulation, including a drug dosage that allows reduced animal exposure to the antimicrobial agents in the drug, was verified in a bioassay by treating experimental infection of broilers with an avian pathogenic *Escherichia coli* (APEC) strain. The experiment compared the efficacy of treatment with a conventional registered veterinary drug and an experimental drug containing trimethoprim and sulfamethoxazole and found no statistically significant differences between the two drugs. The experimental results suggest that a significant reduction in the recommended daily dose of trimethoprim and sulfamethoxazole for the treatment of bacterial infections in broilers is feasible and may encourage the prudent use of antimicrobials, including limiting their overuse. All experiments were conducted in strict accordance with the European Medicines Agency Guideline on conduct of pharmacokinetic studies in target animal species, EMEA/CVMP/EWP/133/1999 - Rev 1, 2023.

The study was supported by the Ministry of Agriculture (project numbers QL24010174 and RO0523).

INCREASING THE EXPERIMENTAL REPRODUCIBILITY AND IMPLEMENTATION OF 3R PRINCIPLES NOT ONLY IN *DANIO RERIO*

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In recent years, there has been a significant increase in the use of *Danio rerio* in biomedical research. With this trend, there is a growing need to improve the design, transparency and reproducibility of experiments using the fish model. The lack of reproducibility of animal studies is

still a major problem in the scientific community, which leads to a waste of resources and animals and may compromise the validity of follow-up studies. High-quality experimental design, rigorous breeding and experimental recording, and systematic training of researchers and caretakers in the various tasks are also closely linked to the implementation of the 3Rs, in particular the principles of "Reduction" and "Refinement". In this talk, we focus on mapping the reasons for the lack of reproducibility in animal studies and how to remedy the situation. Emphasis is placed on the use of available tools in the planning and evaluation of experiments not only in the fish model or the establishment of standards of good practice in the management of breeding and experimental records. The procedures presented can help researchers and caretakers to think systematically about critical aspects of the experiment, thereby improving the quality of the data obtained. Increasing reproducibility and standardization is not only a matter of scientific integrity, but also an important step towards fulfilling the ethical principles of experimental research with animals, as one of the main ways to reduce their numbers. It can be demonstrated using the *Danio rerio* model that detailed planning, data sharing and standardization of experimental and husbandry procedures and record keeping can contribute significantly to reducing the number of animals used and refining of animal procedures in accordance with the 3Rs.

BRONCHIAL 3D HUMAN TISSUE MODELS FOR RESPIRATORY SENSITIZATION TESTING *IN VITRO*

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Sensitization in general is a specific immune response that may occur after contact with a sensitizing agent, leading up to asthma, rhinitis, contact dermatitis or other adverse systemic reactions. Chemicals, ingredients of cosmetics, medical devices and other consumer products, or environmental factors can trigger the process of sensitization in the sensitive population. Skin sensitization is described in detail and officially tested by many *in vivo* and *in vitro* methods included in the OECD guidelines. In contrast, very little is known about the principles, key factors and events involved in the respiratory sensitization process, where some features appear to be similar to skin sensitization. Further investigation of the respiratory sensitization mechanism and completion of the Adverse Outcome Pathway (AOP) is urgently needed. Epithelial human-derived 3D tissue models of the respiratory tract are being incorporated into respiratory sensitization and irritation tests providing more complex insights into the long-term and multiple exposure experiments essential for initiating the sensitization process without the use of animals. In this pilot study, a set of reference potential skin sensitizers and non-sensitizers was tested using validated alternative non-animal methods for skin sensitization (DPRA, OECD TG 442C; LuSens, OECD TG 442D, h-CLAT, OECD TG 442E) with the aim to develop a novel testing system able to distinguish not only between respiratory and skin sensitizers, but also between sensitizers and irritants. These chemicals were subsequently tested on two types of *in vitro* 3D bronchial epithelial models, EpiAirway (Mattek) and MucilAir (Epithelix) in single and multiple exposures mimicking human exposure in the air-liquid interface (ALI). Pilot data showed changing levels of the tested interleukins (namely IL-8, IL-6, IL-10 and TNF-α) after multiple exposures corresponding to the process of irritation and sensitization, with expression increasing for IL-6 and IL-8 and decreasing for IL-10 and TNF-α, as they are thought to activate different cell signaling pathways and interleukin expressions. Transepithelial electrical resistance (TEER) measurement, MTT and LDH assays were also performed to complement the results. A comparison of tissue models based on different parts of the respiratory tract (nasal, oral, tracheal, or alveolar) could also provide a more complex insight into sensitization of the respiratory tract. As the *in vitro* 3D tissue models appear to have great potential for testing respiratory sensitization and irritation on the tissue and molecular levels, we intend to continue and extend this research in the future, focusing on investigation of other predictive biomarkers and optimization of these methods for respiratory endpoints with the use of an innovative ExpoCube exposure system, designed for delivering droplet aerosols onto cells and tissues, in long-term inhalation exposure experiments.

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MYCOBACTERIUM TUBERCULOSIS INFECTION IN *GALLERIA MELLONELLA*

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The aim of this experiment is to test and evaluate the effects of *Mycobacterium tuberculosis* on *Galleria mellonella* larvae. *Galleria mellonella* are an established *in vivo* model for assessing the activity and toxicity of antimicrobials and for studying the immune response to pathogens and provide similar results to mammalian *in vivo* models.

PRETERM NEWBORNS AND PRIMARY MICROBIOTA: STUDY ON GNOTOBIOTIC PIGLETS

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Necrotizing enterocolitis (NEC) affects mainly preterm infants and facilitates bacterial translocation. It can result in sepsis and death. Attention is paid to Pathogen-Associated Molecular Patterns (PAMP), e.g., lipopolysaccharide (LPS), but Damage-Associated Molecular Patterns (DAMP), e.g., high mobility group box 1 (HMGB1), are neglected. Multiligand Toll-like receptor 4 (TLR4) plays an important role in developing NEC and recognizes as LPS, so HMGB1. Therefore, TLR4 participates in systemic inflammatory response both infectious and sterile origins. The aim of the study was to assess the impact of colonizing germ-free (GF) piglets with (i) undefined fecal infant microbiota (FIM) from breastfed infants, (ii) synthetic microbiota SM1 or (iii) SM2, and the influence of these colonizations on piglet health and immune response with focus on TLR4 mRNA signaling. Hysterectomy-derived preterm GF piglets were orally colonized with FIM, SM1, or SM2. Signs of enterocolitis - clinics, histopathology, intestinal barrier function, and inflammatory/sepsis changes were monitored by histology, TLR4, HMGB1, and calprotectin. Control GF piglets received bacteria cryoprotecting media only. The GF piglets were active for the two-week experiment, thrived, and showed no signs of enterocolitis, such as anorexia, fever, or diarrhea. FIM, SM1, and SM2 colonizations caused mild signs of enterocolitis but were exclusively lethal for the piglets. The micrographs of their ileum showed inflammatory changes. Compared to GF, acidomucin-producing goblet cell densities were decreased at SM1, SM2. TLR4 mRNA expression was upregulated in both the ileum and colon. Claudin-1 mRNA was unchanged, occludin mRNA expression was downregulated in the colon but not in the ileum. Ileal and colonic TLR4, CD14, and HMGB1 levels were increased, but calprotectin levels were unchanged. Preterm colostrum-free GF piglets are susceptible to sepsis. A suitable composition of SM can increase their colonization resistance to enteric pathogens. An SM has the potential to provide the initial bacterial community in the sterile gastrointestinal tract of an immunocompromised host (e.g., a Cesarean-born infant) in the first days of life.

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MEASUREMENT OF METHANE AND CO_2 EMISSIONS FROM PIGS IN EXPERIMENTAL STABLES

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Climate change and other environmental topics are a worldwide issue, where agriculture plays an important role. Reducing greenhouse gas (GHG) emissions is one of the effective approaches to addressing global warming. Agriculture is a large contributor to GHG emissions and livestock production contributes 10 % to 12 % of total global GHG emissions. Cattle, pigs, and poultry represent the top three sources of carbon emissions among various animal species, with pork production being the second contributor. The feed production followed by manure management are the largest contributors to emissions of GHG in the whole industry. In livestock systems, proteins used in animal feeds are one of the most expensive and limiting ingredients in diet formulations. The production and supply of feeds are critical steps due to their environmental impact such as land use, land change, land occupation, and energy and water use. Soybean is the most common feed ingredient to supply protein to pigs. However, its production is linked to several issues related to environment. In recent years, research has been focusing on finding alternatives (e.g. locally produced legumes, insects, microalgae) to replace the soybean in pig feeds. Different methods for quantifying GHG emissions are used with respiration chambers being the best-known and well-described. Here, we present a method for measurement of methane and CO_2 emissions in pigs fed with alternative sources of protein in experimental stables at the Veterinary Research Institute, Brno, without the use of respiration chambers. For these experiments, finishing pigs (*Sus scrofa domestica,* >70 kg) were divided into three groups – control group, 25 % replacement of soybean, and 50 % replacement of soybean. In two rounds of experiments, pigs were fed with two different feeds. In the first feed, soybean was replaced with insect protein (black soldier fly larvae), in the second feed, with legume protein (*Lupinus albus* seed). The experiment took one month, and feces, blood samples and animal weight were taken regularly. At the end of the experiment, pigs were sent to the slaughterhouse to determine the influence of alternative feeds on the meat quality. For the measurement of GHG emissions (methane and CO_2), two instruments were used – SENSIT® HXG-3P detector and TESTO CO_2 detector connected to TESTO 400 module with Bluetooth, respectively. Before measuring, the experimental room was cleaned, and pigs were fed. The local A/C system was switched off. After 2 hours, the measurement started – CO_2 detector was placed on a shelf (145 cm) and methane emissions were measured at three heights (10 cm, 110 cm and 240 cm). The aim of this study was to find a method to measure methane and CO_2 emissions in experimental stables as an alternative to well-described and used respiration chambers. The next step will be to evaluate and compare the obtained data regarding the different feeds used in this study to contribute to Life cycle assessment (LCA). This method is used to assess and calculate the environmental impact of products throughout their entire lifecycle.

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EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF MORUSIN IN A MODEL OF INDUCED PLANTAR EDEMA IN LABORATORY MICE

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Prenylated phenols are natural substances that are used in traditional medicine for their wide range of biological effects. Morusin is a prenylated flavonoid isolated from *Morus alba* with various biological activities, such as antitumor, antioxidant, and antibacterial properties. The anti-inflammatory activity of morusin has been described *in vitro*, and inhibition of NF-κB and STAT3 activity has been demonstrated. The next step in the investigation of the biological activity of these natural compounds is to verify their anti-inflammatory potential *in vivo*. The experiment follows the previous *in vitro* phases of the project, which confirmed the anti-inflammatory activity of the selected natural substances. The evaluation was performed in a mouse model of induced plantar edema. An animal model is essential at this stage of the evaluation of the activity of the substances, as the complexity of the mammalian organism cannot be replaced by an alternative method with the same reliability and predictive value. Forty 6-week-old male laboratory mice divided into four groups were included in the experiment. Two groups were orally administered morusin in two doses; indomethacin was used as a reference substance, and the control group was administered vehicle only. 0.1 ml of 1 % carrageenan solution was applied intraplantarly to induce experimental edema. Changes in volume parameters were monitored plethysmometrically at defined intervals. At the end of the experiment, the animals were sacrificed by cervical dislocation, and sampling (planta) was performed for histopathological examination. Compared to the control group, a statistically significantly lower increase in paw volume caused by the current inflammation, comparable to the reference substance, was demonstrated at several time intervals. The results achieved confirm, in the selected animal model, the expected antiphlogistic effect of morusin, which was also shown by the histopathological examination of the collected tissue.

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INCREASED VIABILITY OF TUMOR TISSUE OF PANCREATIC DUCTAL ADENOCARCINOMA IN A PATIENT-DERIVED XENOGRAFT MODEL

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Pancreatic cancer is a malignant disease with a continuously increasing incidence and dismal prognosis. The five-year overall survival rate of treated patients across all stages (I-IV) in the Czech Republic is approximately 12.6 %, primarily due to the absence of screening and a high degree of chemo- and radio-resistance. Therefore, the need to develop a research model aimed at overcoming these challenges has been clearly defined. The aim of this report is to demonstrate increase of tumor engraftment in *in vivo* patient-derived xenograft (PDX) models of pancreatic ductal adenocarcinoma (PDAC). We have introduced (PDX) into immunodeficient NOD/SCID and NU/NU mice as it encourages the study of the entire PC tissue (tumor and its microenvironment) of an operated patient. All experimental work with animals was carried out, implemented in compliance with and governed by the existing regulations and guidelines for the breeding and experimental use of animals in accordance with Act No. 246/1992 Coll. (CZ) and performed by persons with authorization to work with experimental animals in a specific pathogen-free environment of the Department for Welfare of Laboratory Animals, National Institute of Public Health. Procedures of the approved experimental project "Experimental model of an immunodeficient mouse with human pancreatic cancer" (MZDR 37099 /2021-5/OVZ) were followed. Animals were provided with immunodeficient mouse feeding and fluids *ad libitum*. The study was approved by the Ethics Committee of the UHKV. Tumor specimens from ten consecutive PDAC patients were implanted using our established methodology: five without Matrigel (Group A) and five after brief exposure to temperature-controlled (1-3°C) Matrigel (Group B). Preliminary data analysis suggests that the engraftment rate in the first (F0) generation was higher in Group A (62.5 %) compared to Group B (50 %). However, tumors in Group A exhibited very slow growth over several weeks or failed to grow entirely. In contrast, tumor growth in Group B was markedly faster. In the subsequent F1 generation, the engraftment rate was 0 % for Group A, while it reached

100 % for Group B. The use of Matrigel significantly enhanced tumor tissue viability and facilitated the establishment of additional generations of PDAC PDX models. Future objectives involve leveraging these PDX models to study diagnostic, prognostic, and therapeutic biomarkers of PDAC by performing comprehensive molecular profiling using next-generation sequencing techniques.

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EFFICACY OF TARGETED BIOTIN CONJUGATES OF NEW-GENERATION TAXANES SB-T-121605 AND SB-T-121606 IN RESISTANT OVARIAN CARCINOMA MODEL

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The resistance of aggressive tumor subtypes to currently available therapies, conventional cytostatics and their newer formulations represents the most serious obstacle in oncological therapies at present, which constitutes a significant socioeconomic problem. One of the possible solutions to overcome these obstacles is the development of new and highly potent analogs of conventional cytostatics, e.g. taxanes, as well as their tumor-targeted delivery. The aim of our study is to investigate the *in vitro* potency and *in vivo* antitumor activity of clinically used taxane paclitaxel (PTX) and newly developed tumor-targeted biotin-conjugates of experimental taxanes, BLT-SB-T-121605 and BLT-SB-T-121506. These drug conjugates target the biotin (vitamin B7) receptors overexpressed on the aggressive tumor cell surface. Upon binding the biotin receptors, the drug conjugates are efficiently internalized via receptor mediated endocytocis (RME) and release the highly potent payload, i.e., 3rd-generation Stony Brook taxanes, SB-T-121605 and BLT-SB-T-121506. The internalization via RME can circumvent ABC efflux pumps, which are responsible for multidrug resistence (MDR). In PTX-resistant ovarian carcinoma cell (NCI/ADR-RES and SKOV3/PTX-RES) *in vitro* models, the potency of BLT-SB-Ts was up to 50-times higher than PTX. BLT-SB-T-121605 and BLT-SB-T-121606 induced cell cycle arrest in the G2/M phase much more effectively than PTX. The most efficacious taxane conjugate, BLT-SB-T-121606, successfully reduced tumor growth *in vivo* in NCI/ADR-/RES xenografts in monotherapeutic regimen (doses 1-5 mg/kg). Furthermore, systemic toxicity of this BLT conjugate was lower than the parent payload, SB-T-121606. In conclusion, biotin-conjugates of SB-T-121605 and SB-T-121606, are highly promising candidates for further studies, that could potentially lead to the development of novel cancer therapeutics effective against aggressive tumors resistant to conventional taxanes.

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