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MIA EECKHOUT

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SHORT BIOGRAPHY

Mia Eeckhout is a Senior Full Professor at Ghent University. She is teaching Feed Technology for M.Sc. students since 1989 and performs research in this domain for over 30 years. She conducts applied scientific research in the field of compound feed production at the request of and in close collaboration with the Belgian animal feed industry. She coordinated numerous research projects in a Belgian, European and global context on innovative feed ingredients, feed technology and feed safety. From 2015 to 2020 she was chair of the public-private platform Feed Design Lab, Wanssum, the Netherlands.



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Catalin Dragomir is an animal science engineer by training, with a strong scientific background in ruminant nutrition and related disciplines. With over 20 years of experience in research management and technology transfer, he has coordinated more than 20 national projects and programs and has served as partner or national representative in over 10 European projects. He is also the Editor-in-Chief of the Archiva Zootechnica journal and actively organizes scientific events, trainings, and workshops.



MIAN RIAZ

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SHORT BIOGRAPHY

Dr. Mian N. Riaz is Associate Dept. Head in the Department of Food Science and Technology at Texas A&M University, USA. His first academic appointment was in 1992 at Food Protein R&D Center (FPRDC), where he was put in charge of the Food and Feed Extrusion Program and went on to become the head of the Extrusion Program. He served as Director FPRDC from 2005 to March 31, 2020. Currently, he offers 5 courses every year in food and feed extrusion. He has published seven books (three of them on extrusion technology) 28 chapters and more than 163 papers.



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SHORT BIOGRAPHY

Christos Rumbos is Assistant Professor in the Department of Agriculture, School of Agricultural Sciences, University of Patras. His main research interests include integrated plant protection, post-harvest insect biology and ecology, and more recently, the use of insects as food and feed, with a focus on the optimization of mealworm mass rearing. He has actively participated as researcher in numerous research projects and has published more than 110 scientific papers in peer-reviewed journals, along with four book chapters. Christos has taken part in many international congresses and conferences, whereas he serves on the editorial board of several international journals.



ANDREJA RAJKOVIĆ
ICFMH president
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Research field: food safety

SHORT BIOGRAPHY

Prof. Dr. Andreja Rajkovic is a Full Professor of food safety and microbiology at Ghent University, Belgium. His research bridges the fields of microbial food hazards, natural and emerging toxins, and the impact of contaminants such as micro- and nanoplastics on human health. He is internationally recognized for his interdisciplinary work combining molecular microbiology, toxicology, and risk assessment, often using advanced tools such as 3D cell models. Prof. Rajkovic plays an active role in several international research consortia and expert groups, contributing to both scientific excellence and the development of evidence-based food safety policies.



Arnaud Bouxin
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SHORT BIOGRAPHY

Arnaud Bouxin is agronomist by education and graduated from the Institut National Agronomique Paris-Grignon. He started his carrier as policy advisor in the French Association of feed manufacturers, SNIA, in 1990 and joined FEFAC in 1998 where he holds the position of Director for feed safety and regulatory affairs. He is busy primarily with feed legislation and the drafting of tools to support its implementation, such as the FEFAC Guide to Good Hygiene Practice for compound feed and premixture manufacturing (EFMC) and the Code of practice for compound feed labeling drafted in cooperation with Copa-Cogeca. He is also coordinating the EU Feed Chain Task Force gathering 43 EU organisations of the feed chain.

ORAL PRESENTATIONS

CIRCULAR FEED: EXPLORING NEW OPPORTUNITIES

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Compound feed is a balanced mixture of various plant- and animal-based raw materials, minerals, and vitamins. Its composition depends on the nutritional needs of the target animal, the availability and cost of raw materials, as well as certain anti-nutritional properties, technological aspects, and local legislation. In Europe, the annual production of feed for farm animals, fish feed, and pet food is estimated at approximately 150, 1, and 10 million tons respectively.

The European feed industry originated from the reuse of wheat bran from grain mills—a by-product not suitable for human consumption. This forms the basis of the sector’s circular character. Eventually, the sector positioned itself as a user of grains and protein-rich seeds alongside by-products from the agro-food and bio-industries. It was only since 2010 that sustainability became an explicit focus within the sector. The beginning of an explicit focus on circular feed dates from this decade (2020) and has driven business decisions, innovations, and of course research within the themes of resource efficiency and nutrient recovery.

However, circularity is not the only driver. Research is also being conducted into the added value of the resources that fall within this scope. What do they mean for animal health? What is their potential environmental impact?

Several striking examples of ongoing research within circularity in a broader context are highlighted:

- The use of whole insects or insect fractions appears to have positive effects on the gut health of animals. These insects can be used to valorize residual streams and potentially neutralize toxic components (1).
- Duckweed can be used for nutrient recovery from agro-industrial effluents, serve as a protein source, and possibly reduce greenhouse gas emissions (2).
- Side streams can serve as a basis for fermentation processes, converting them into a pre- and probiotic mixture with a positive effect on gut health (3).

Keywords: resource efficiency, gut health, insects, duckweed,

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REDUCING ENERGY COST & CARBON FOOTPRINT IN PET FOOD FACILITY

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The global pet food industry is experiencing significant growth, driven by rising pet ownership and increasing consumer demand for high-quality, nutritious products. However, this growth comes with heightened scrutiny of environmental impacts, particularly concerning energy consumption and greenhouse gas emissions. Pet food manufacturing facilities are energy-intensive operations, relying heavily on thermal and electrical energy for processes such as extrusion, drying, cooling, packaging, and refrigeration. Consequently, reducing energy costs and the overall carbon footprint is not only an environmental imperative but also a strategic economic priority.

This presentation will explore innovative approaches and best practices for reducing energy consumption and carbon emissions in pet food facilities. Key strategies include optimizing process efficiency, integrating renewable energy sources, upgrading equipment, and implementing advanced energy management systems. For instance, waste heat recovery systems can capture and repurpose thermal energy from extrusion and drying processes, reducing the demand for new thermal inputs. Similarly, transitioning to high-efficiency motors and variable frequency drives (VFDs) can significantly cut electricity usage in pumps, fans, and conveyors.

Reducing the carbon footprint also involves examining the broader supply chain, including sourcing locally produced raw materials to minimize transportation emissions and adopting eco-friendly packaging solutions. Furthermore, employee engagement and training programs play a critical role in fostering a culture of sustainability within the facility.

The combined benefits of these strategies include substantial reductions in operating costs, improved compliance with increasingly stringent environmental regulations, enhanced brand reputation, and alignment with global sustainability goals. As consumers and stakeholders demand greater transparency and environmental stewardship, proactive measures to minimize energy use and emissions become essential for long-term competitiveness.

In summary, the transition towards energy-efficient and low-carbon operations in pet food facilities is both an environmental necessity and a business opportunity. Through technological innovation, operational excellence, and a commitment to sustainable practices, pet food manufacturers can achieve significant progress toward a greener future while maintaining profitability and meeting consumer expectations.

CIRCULAR FEED INGREDIENTS FOR THE APPLICATION IN COMPOUND FEED FOR RUMINANTS, PIGS AND POULTRY: A BELGIAN PERSPECTIVE

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To enhance the sustainability of animal production and reduce competition between food and feed, it is crucial to increase the use of circular ingredients and waste streams (CI&W) in animal feed. This study is part of a broader research project aimed at the integration of CI&W into compound feed for ruminants, pigs, and poultry. In the first phase, a market analysis was conducted in Belgium to identify currently available CI&W. This inventory was compiled through direct contact with producers, a survey of Belgian feed manufacturers, and supplementary internet research. A total of 44 ingredients were identified and summarized in an overview table including relevant properties such as availability, composition, and digestibility. In the second phase, a selection of CI&W with high potential for further research was made based on input from the feed industry. A follow-up survey and a dedicated workshop with Belgian feed manufacturers helped refine the selection and formulate relevant research questions. CI&W identified as promising across all three animal categories included press cakes from alternative oilseeds (e.g., marigold, false flax), cookie mixes, and distillers dried grains with solubles (DDGS) and wheat yeast concentrate. For pigs and poultry in particular, processed animal proteins (PAPs) drew considerable interest. A key research question raised across all CI&W was the variability in product composition. To address this, five batches of each selected CI&W were sampled and subjected to proximate analysis to obtain a preliminary assessment of variability. The findings indicate that batch-to-batch variation is highly dependent on both the product type and the producer.

Keywords: circular ingredients, poultry, pigs, ruminants, compound feed

Acknowledgements: The study was funded by the Flanders Innovation & Entrepreneurship (VLAIO).

CIRCULAR FEED PRODUCTION USING MARINE SIDE STREAMS – WILL CONTAMINANTS UP-CONCENTRATE?

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The reuse of by-products from the food chain is a key aspect of the European Union's (EU) strategy to transition towards a more circular food production system. Consequently, there is a need for alternative feed raw materials. Low-trophic organisms, such as certain species of insects, i.e. black soldier fly (BSF) larvae (*Hermetica illucens*) are allowed as feed for farmed animals, including farmed salmon, pig and poultry. However, there are regulations restricting which feed materials are allowed.

Marine side-streams, such as fish sludge from aquaculture (feed spillage and feces) and category 2 animal by-products of fish are resources that may have the potential to become future feed materials for producing invertebrates. These materials are currently not permitted for feed production, and several EU regulations prevent their use. Prior to any regulatory changes, scientific documentation on the risks is needed. The aim of this work was to study potential risks related to the potential accumulation of contaminants from such growth substrates in invertebrates.

Data from two experimental feeding trials with BSF larvae will be presented. The first trial focused on BSF larvae fed on fish sludge from land-based salmon aquaculture in Norway. A 15-day trial with BSF larvae fed 100% fish sludge, showed good growth, with larvae retaining fat, protein and marine fatty acids from the sludge. However, cadmium, mercury, dioxins- and dioxin-like PCBs accumulated in BSF larvae, showing bioaccumulation factors above 1, resulting in concentrations which exceed EU maximum limits for animal feed. To further investigate, a trial using ensilage from Atlantic salmon (category 2 animal by-product) was conducted to assess the risk of contaminant accumulation when insects are fed alternative marine raw materials.

Overall, these studies show that insects can be raised on alternative marine side streams; however, some contaminants may bioaccumulate in the feed chain when a circular feed production approach is taken.

Keywords: insects, animal by-products, metals, dioxins, minerals

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TOWARD SUSTAINABLE ANIMAL FEED: THE NORWEGIAN GOVERNMENT’S APPROACH AND BLUE MUSSEL OPPORTUNITIES

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As global demand for animal protein continues to rise, the need for sustainable, locally produced feed ingredients is becoming increasingly urgent. In response, the Norwegian government has outlined strategies to ensure that feed for farmed fish and livestock shall be sourced from renewable and environmentally sustainable sources. One promising candidate is the blue mussel, a local species of high nutritional value, low environmental impact, and big potential production as a novel feed ingredient in Norway.

Currently, only 0.4% of feed ingredients used in Norwegian aquaculture are derived from alternative sources such as insect meal or microalgae. With feed demand in the farmed fish industry projected to increase from 1.8 to 2.8 million tonnes by 2030, scalable and innovative solutions are urgently required. Blue mussels could play a key role in closing this gap.

This research explores the feasibility of scaling up blue mussel production in Norwegian waters under the Ecosystem Approach to Aquaculture. Specifically, it aims to assess the production potential and ecological carrying capacity across varied coastal environments, by identifying the optimal cultivation areas and production cycles. The study also evaluates processing methods to determine the most suitable techniques for incorporating blue mussels into animal feed without compromising animal health or welfare. By providing integrated data on mussel-environment interactions, processing, and feed inclusion, the results will support both economic feasibility and evidence-based policy development.

Findings from this project will help determine the viability of blue mussels as a sustainable feed ingredient and support Norway’s efforts to expand aquaculture industry while reducing its environmental footprint.

Keywords: Fish feed, Production carrying capacity, Alternative feed ingredients, *Mytilus edulis*.

Acknowledgements: This study is supported by FHF-Norwegian Seafood Research Fund (Mussel up 901895).

FEEDING THE FUTURE: ADVANCES AND CHALLENGES IN SUSTAINABLE INSECT PRODUCTION SYSTEMS

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The growing demand for alternative nutrient sources in animal feeds has positioned insects as a key component of sustainable feed production systems. Insects such as mealworms, flies, crickets or grasshoppers represent a promising alternative to traditional feedstocks like soymeal and fishmeal in poultry, pig and fish diets. This growing interest is well justified, as insects provide numerous benefits, including high nutritional value, excellent feed conversion efficiency, and a minimal environmental impact (such as lower land and water requirements and reduced greenhouse gas emissions). However, given that the insect sector is still in its early stages there are still several open questions and challenges that should be addressed to support its development. Among these, insect nutrition plays a fundamental role in the efficiency, sustainability, and overall success of insect production systems. However, the nutritional and dietary requirements of major farmed insect species are still not fully understood. Properly defining the nutritional needs of farmed insects could ensure optimal growth, reproduction, and health of insects, directly influencing their productivity and the quality of insect-derived products. Under current EU regulations, farmed insects may only be fed materials of plant origin, with few exceptions. Broadening the range of authorized substrates for insect feed could further enhance the resilience and sustainability of the sector. In addition to nutrition, insect breeding also influences the long-term productivity and efficiency of insect farming systems. Through the selection and propagation of insects with desirable genetic traits, insect breeding programs can enhance performance traits such as growth rate, feed efficiency, reproductive capacity, and product quality. However, insect breeding programs are still limited, highlighting the need for standardized yet adaptable methodologies to support their development. Future research should address these challenges to unlock the full potential of insects as sustainable feed ingredients and support the further development of the insect sector.

Keywords: alternative proteins, circular economy, insect farming, insects as feed, sustainability

Acknowledgements: This paper has been partly financed by the funding programme “MEDICUS” of the University of Patras.

INSECT MEAL IN RUMINANT NUTRITION: *IN VITRO* FERMENTATION CHARACTERISTICS AND COMPARISON WITH CONVENTIONAL PROTEIN SOURCE

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This research aimed to evaluate the nutritional composition, *in vitro* digestibility, and gas production kinetics of *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) processed proteins compared with soybean meal f.e. as a reference raw material. Nutritional characterization and *in vitro* ruminal fermentation tests were performed to determine *in vitro* short-chain fatty acids production, the gas production profile and methane production. HI and TM were both full fat meal with a mean protein content of 41 and 61 % respectively and the soybean meal had 45 % of crude protein. Total gas production (value expressed in mL as mean $\pm$ SD) was lower for both HI (27 $\pm$ 4.5) and TM (23 $\pm$ 3.3) processed proteins compared to soybean meal (77 $\pm$ 2.1). Similarly, differences have been observed in the case of short-chain fatty acids production which was higher when soybean meal was used as substrate than with HI and TM. However, when considering fatty acid molar proportion differences were observed only in case of propionate, isobutyrate, isovalerate, and valerate. Interestingly methane production (value expressed in μ mol as mean $\pm$ SD) was lower for insect processed proteins (HI 70 $\pm$ 15; TM 59 $\pm$ 4.5) compared to soybean meal (282 $\pm$ 7.6). Obtained results indicated that insect processed proteins hereby tested were characterized by low fermentation profile, thus it might speculate that they could represent a valid by pass protein source.

Keywords: insect proteins, soybean meal, nutritional composition, *In vitro* digestibility, gas production kinetics, ruminal fermentation

BSF INSECT MEAL AS A SUSTAINABLE SOYBEAN SUBSTITUTE IN BROILER DIETS

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The application of circular economy and resource efficiency principles in animal nutrition offer a significant framework for promoting innovative economically viable, sustainable and eco-friendly practices in the livestock feeding sector. In this context, vigorous research activity is initiated towards the reuse, redesign and recovery of materials previously regarded as low-value waste. Final goal is to optimize feed formulations, reduce environmental burden and minimize resource input through innovative valorization strategies, aiming to align the animal production systems with sustainability goals.

Herein, a novel insect meal produced by rearing the Black Soldier Fly (BSF) Larvae with potato plant cultivation waste biomass was evaluated as dietary ingredient in broiler chickens feeding. Prior utilization, the insect meal was subjected to a thorough chemical and toxicological evaluation to reveal the absence of toxic alkaloids. Then, insect meal was incorporated into 200 one-day-old broiler diets mainly as soybean replacement (0%, 5%, 10%, and 15% insect meal inclusion), with the 15% level corresponding to a 40% replacement of soybean meal on a weight to weight basis. Each treatment consisted of five replicates, comprised by ten chicks. The feeding trial was conducted during the first 10 days of their growth, under floor-rearing conditions using a 50:50 mixture of rice husk and wheat straw bedding.

At the end of the trial, a series of health and growth indicators were determined and indicated that potato plant-based rearing of BSF is capable to provide insect meal which may be considered as a promising future alternative protein source for functional poultry nutrition.

Keywords: circular economy, livestock feeding, broiler nutrition, soybean meal replacement, black soldier fly

ZINC AND SELENIUM ENRICHED BY-PRODUCT FOR INSECT MEAL PRODUCTION: EFFECT ON GROWTH PERFORMANCE, CHEMICAL PROFILE, AND MICROELEMENTS BIO ACCUMULATION

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Black soldier fly larvae are converters of wide variety of rearing substrates, including agricultural by-products, into edible biomass. It has been demonstrated that these larvae possess the capacity to accumulate microelements when present in the rearing substrate, including heavy metals and pollutants. On the first hand this ability poses a risk in the selection of alternative substrates, on the other hand it could be exploited to produce insect meal (feed specialties) enriched with selected micronutrients. Thus, the objective of this experimental study was to assess the impact of an alternative substrate composed of okara and potato waste, as well as its potential enrichment with two selected micronutrients, zinc (Zn) and selenium (Se). An evaluation of the larval performances has been conducted, assessing their growth performance, chemical composition, and micronutrient, metal, and metalloids content. Five different substrates were prepared: I) a control diet composed of Gainesville diet (CTR); II) a substrate composed by 50% okara and 50% potato waste (OP); III) OP enriched with 150 mg/kg of inorganic Zn (OPZn); IV) OP enriched with 0.3 mg/kg of inorganic Se; V) OP- ZnSe enriched with both 150 mg/kg and 0.3 mg/kg. The experiment was conducted with five replicates for each diet. A total of 500 4-day-old larvae were selected for each replicate. The results indicated that the CTR group exhibited a higher total biomass. In contrast, the OP-based larvae demonstrated higher levels of lipid but lower ash content. The ingestion of Zn and Se resulted in the production of enriched larvae. Furthermore, the micronutrient levels observed indicated the presence of an interaction between these two elements. The metal and metalloids content resulted lower in the OP-based substrates. These findings suggest that okara and potato-based substrates, with and without Zn and Se supplementation, are suitable for BSFL rearing.

Keywords: *Hermetia illucens*, feed, microminerals, larval production, ICP-MS

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RECENT DEVELOPMENTS IN THE IDENTIFICATION OF INSECTS IN FEED

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In recent years, insects used for food and feed have attracted increasing attention from the media and the scientific community, both for their high protein content and their low environmental impact. To date, the use of insects in feed for aquaculture, pig and poultry farming is regulated by European Regulations 999/2001 and 142/2011, which specify which species may be used: These are the yellow mealworm *Tenebrio molitor*, the buffaloworm *Alphitobius diaperinus*, the black soldier fly *Hermetia illucens*, the house cricket *Acheta domesticus*, the banded cricket *Gryllodes sigillatus*, the silent cricket *Gryllus assimilis*, the house fly *Musca domestica* and the silkworm *Bombyx mori*. These regulations have required the development of appropriate analytical methods for the specific detection of insects. Although there is currently no certified and universally accepted method, several groups have published different molecular methods based on DNA or protein detection. In particular, the National Reference Laboratory for Animal Proteins in Feed has published several validated real time PCR-based methods for the specific detection of approved and non-approved insect species. An overview of the group's most recent publications and unpublished results is presented. In particular, the discrimination of the species *G. sigillatus* and *M. domestica*, of the giant mealworm (*Zophobas atratus*) and the two-spotted cricket *Gryllus bimaculatus*, as well as the strange case of the crazy red cricket *Gryllus locorojo*, a species not authorized for animal feed but often confused with the authorized species *G. assimilis*. All these insect species are present and available on the European market, and among them, insects not yet authorized as feed are used as pet food. Correct species identification is necessary to comply with European regulations and to ensure protection for both the producer and the final consumer.

Keywords: insects, real time PCR, species identification

FORMULATION OF FEED ADDITIVES

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A wide range of bioactive ingredients is used in animal feed to promote growth, enhance health, improve digestion, neutralize or absorb mycotoxins, and serve as natural alternatives to pharmaceutical products. These ingredients are typically in liquid form, such as fermentation broths containing live microorganisms or enzymes, phytogenic extracts, organic acids, etc. However, in this state they may be too concentrated, too diluted, unstable, unsafe or otherwise unsuitable for direct feed application. To address these challenges, formulation is essential. The goal is to create a product that is safe, stable over time, and has appropriate form and concentration for use by feed millers or farmers.

In most cases, a powdered product (granulate) is preferred due to its superior stability, as well as ease of transport and application, compared to a product in liquid form. Granulation typically includes a drying step that encapsulates the active ingredient within a matrix of selected additives. An example is matrix encapsulation of our mycotoxin degrading enzymes that shields the enzyme from heat during granulation, preserves its stability under ambient conditions during storage, protects it from thermal degradation during feed processing and from acidic environment in the animal's stomach.

Depending on desired particle size, matrix encapsulation could be achieved through spray drying or fluid bed processing. For highly heat-sensitive active ingredients, such as microbial products, freeze-drying may be more appropriate.

In some cases, matrix encapsulated ingredients require further protection by additional coating layer(s) to enhance protection or to achieve specific release profiles. This process, known as core encapsulation, involves applying coatings made from salts, polymers, or fats. Core encapsulation is typically used to enable targeted release in the gastrointestinal tract, mask unpleasant flavours, and to provide additional heat stability during feed processing. One example of combining different formulation methods is our phytogenic product that combines two essential oil mixtures within single formulation but in two distinct phases of the powder particles. Core contains matrix-encapsulated blend of essential oils with antioxidative and anti-inflammatory effect. These spherical, uniform particles are produced via a prilling process. To address the strong odour of some compounds, a second step involves applying an outer coating using fluidized bed processing. This coating contains essential oils that are palatable and have an appetizing effect for animals.

In addition to powder-based products, market demands sometimes require the development of liquid formulations, for example products for post pellet liquid application, or direct addition to drinking water.

The formulation team at the R&D Center in Tulln has extensive experience in formulating a wide range of active ingredients using mentioned but also some other techniques. Besides product formulation, a significant part of team's work involves testing of the formulated products to ensure that target characteristics are met. These include particle size distribution, particle shape, flowability, apparent density, compressibility, recovery of active ingredients, storage stability, stability in feed, etc. This approach ensures that each product not only meets performance expectations but also maintains quality and stability throughout its lifecycle.

Keywords: feed additives, formulation, granulation, coating

GREEN FEED LABELLING TO SUPPORT TRANSITION TO MORE ENVIRONMENTALLY FRIENDLY LIVESTOCK FARMING

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Since the publication of the FAO report on Livestock Long Shadow 20 years ago, the livestock sector has been finger pointed for its negative impact on the environment. Although more emphasis is put nowadays on the positive contribution that the livestock sector brings to sustainable and circular food systems, the reduction of livestock emissions, in particular linked to feed production and digestion, remain a key societal & political priority.

Feed formulation is the key lever to achieve this objective, whether through selection of feed ingredients with low environmental footprint or via feed related abatement techniques (nutrients, feed additives, feed materials). Being able to provide reliable information on the environmental footprint of feed production and expected impact on digestion-related emissions is a prerequisite to an uptake of these improved diets by farmers.

FEFAC representing the EU compound feed & premix industry and Copa-Cogeca representing the EU farmers, with the support of FEFANA, the EU feed additives association, developed guidelines for communication of information on the environment footprint of feed at production stage and on claims related to the use stage. These guidelines are based on the EU developed methodology Product Environmental Footprint (PEF) and more specifically the PEF Category Rules (PEFCR) for feed for food producing animals, as well as the FAO LEAP Guidelines for the claims. They also refer to the Global Feed LCA Initiative (GFLI) as preferential LCA database for the performance of the PEF study. The guidance on the nature of the information being communicated and its carrier was aligned with the approach followed by the EU Commission in its draft directive on Green Claims for B2C communication. Indeed, although the main purpose of communication of information is to help farmers improving their performance, it may also be used by farmers to perform their own environmental footprint assessment in the objective of a B2C communication on the environmental footprint of livestock products.

The guidelines were included in the Copa-Cogeca/FEFAC Code of Good Labelling Practice endorsed by the EU Commission and the Standing Committee on Plants, Animals, Food and Feed in December 2024.

GHG REDUCTION THROUGH HIGHER EFFICIENCY OF NUTRITION

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Reducing the net GHG emissions (or the Carbon footprint) of the livestock farming while maintaining its sustainability represent a major challenge of the European agriculture. Animal nutrition represents a determinant factor for influencing the farms' competitiveness and the quality of animal products but also the Carbon footprint of animal production, expressed in kg CO₂ equivalent / kg of milk or meat.

Small ruminants sector represents a good example of using animal nutrition as a tool for reducing Carbon footprint. While accounting for only 6.5% of the total emissions related to the animal husbandry, its characteristics lay the premises for important reductions, especially within low-input production systems. LIFE Green Sheep project (LIFE19 CCM/FR/001245) comprised a large-scale program of assessing and reducing Carbon footprint of sheep farms from five European countries (France, Spain, Italy, Romania, Ireland) was implemented.

This led, for the first time in Romania, to a campaign of assessment of the Carbon footprint of 90+ sheep farms, using the French CAP2'ER tool, followed by guided implementation of mitigation measures in a set of selected farms and the assessment of the magnitude of mitigations.

First round of assessments retrieved an average of 5.8 kg CO₂ equivalent / kg of milk; following corrections for differences between French and Romanian sheep production systems, the average was adjusted at 3.7 kg CO₂ equivalent / kg of milk. Details of these results and their implications are further discussed.

Of the selected mitigation measures, those specific to animal nutrition are depicted, focusing on their potential influence on the magnitude of mitigation. Also, the second assessment of a selected set of farms is analysed, by highlighting the importance of various factors that can influence Carbon footprint of livestock farms. This led to the conclusion that nutrition has an important role in mitigating Carbon footprint through higher efficiency.

Keywords: GHG, Carbon footprint, sheep, nutrition

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WATER-SOLUBLE OREGANO SUPPLEMENTATION IMPROVES ANTIOXIDANT CAPACITY AND INTESTINAL HISTOMORPHOLOGY IN BROILERS CHALLENGED WITH *EIMERIA* SPP.

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Supplemental phytobiotics may mitigate the effects of intestinal problems, strengthening antioxidant defense and improve the quality of poultry meat.

This study evaluated the efficacy of a water-soluble oregano-based supplement (0.5 ml/L) through an experimental trial in broiler chicken. 252-day old-male broilers were assigned to three groups (six replicates/group): an unchallenged negative control (UNC), a challenged positive control (POC), and a challenged group supplemented with oregano (ORG). Challenged birds were raised on reused litter and received a 10-fold dose of an anticoccidial vaccine at day 7, while UNC birds were kept on fresh litter. Performance metrics including body weight (BW), body weight gain (BWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were evaluated. Lipid peroxidation (MDA), total phenolic content (TPC), and total antioxidant capacity (TAC) were measured in the breast, thigh, liver, and ileal digesta of 12 birds per group at day 42. Additionally, intestinal samples were taken for histological analysis.

The ORG group had significantly increased BW from d14 onwards, ADFI (d7-d14) and similar weekly FCR. Performance was similar between UNC and ORG birds ($p>0.05$). MDA was higher in the breast and thigh tissues, TPC and TAC levels were lower in all tissues studied in the POC group. In comparison to the POC group, the ORG group had significantly reduced intestinal lesion scores and oocysts per gram ($p\leq 0.05$). Although there were no differences in villus length (VL) and goblet cells between groups, crypt depth (CD) was significantly reduced and duodenal and ileal VL: CD ratio was significantly increased in the ORG group compared to POC group ($p\leq 0.05$).

These results demonstrate that water supplementation with oregano improves the antioxidant profiles of meat, boosts broiler performance, and provides a natural way to meet customer needs while also improving poultry health.

Keywords: *Eimeria* spp., histomorphology, VL: CD ratio, goblet cells

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FROM INNOVATION TO APPLICATION: *IN VITRO* AND *IN VIVO* EFFICACY ASSESSMENT OF A PATENTED BENTONITE-BASED MATERIAL

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Bentonites preferentially adsorb aflatoxins but exhibit low adsorption efficacy for other mycotoxins. To address this limitation, a bentonite based-adsorbent, called bio-organoclay, was developed and patented as a multi-mycotoxin adsorbent through an acid activation using 15% w/w sulfuric acid and a functionalization with 70 meq of choline chloride/100g of bentonite on a selected sodium smectite. The process was optimized at laboratory and industrial scales. At low dosages (0.25-0.5% w/v), the bio-organoclay effectively sequestered more than 95% of aflatoxin B1 (AFB₁), fumonisin B1 (FB₁), ochratoxin A (OTA), and zearalenone (ZEA) across a wide pH range (3-9). Mycotoxin adsorption occurred simultaneously with high capacity and affinity, as determined by equilibrium isotherms. The thermodynamic studies evaluating Gibbs free energy on the adsorption process provided insights into the strength and nature of binding interactions. The efficacy of the bio-organoclay in reducing systemic exposure to AFB₁, FB₁, OTA, and ZEA was further assessed in rats and piglets using a biomarker-based exposure approach. Mycotoxins were administered via intragastric oral bolus, individually in rats and, as a mixture, in piglets. Control animals received the mycotoxins without the detoxifier, while treated animals were given the mycotoxins along with bio-organoclay at 0.5% w/w of feed consumption. Urine samples from rats and blood samples from piglets were collected at various time points (4-72 hours) and analyzed for mycotoxin content using UPLC-FLD/PDA and UPLC-MS/MS methods, respectively. Toxicokinetic parameters, including the area under the curve and maximum mycotoxin concentration, were calculated to compare control and treated groups. The bio-organoclay significantly reduced urinary excretion of aflatoxin M1 (AFM₁), ZEA, FB₁, and OTA in rats. In piglets, it was highly effective in decreasing systemic exposure to AFB₁ OTA and FB₁, while the reduction of ZEA exposure was not statistically significant. In conclusion, the high efficacy of the bio-organoclay in sequestering AFB₁, ZEA, OTA, and FB₁, as observed in vitro adsorption studies, was fully confirmed in rats and partially confirmed in piglets.

Keywords: bio-organoclay, mycotoxins, adsorption, detoxification, toxicokinetics

HIGH-PROTEIN ALFALFA CONCENTRATE (*MEDICAGO SATIVA*) IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) DIETS: GROWTH, DIGESTIBILITY, HEALTH, AND PELLET QUALITY ASPECTS

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Aquaculture is rapidly expanding to meet global protein demands and holds significant potential for improving food security. However, conventional protein sources in fish feed, fish meal and soybean concentrate, pose serious environmental challenges. Sustainable growth of fed aquaculture depends on identifying novel, environmentally friendly protein alternatives. This study evaluates white protein (WP) concentrate extracted from alfalfa (*Medicago sativa*) via green biorefinery as a potential substitute. A 47-day feeding trial was conducted on rainbow trout (*Oncorhynchus mykiss*) to assess effects on apparent digestibility, pellet quality, growth performance, and fish health. Four diets were formulated: a fish meal-based control (CTRL) and three experimental diets replacing fish meal with WP at 5%, 10%, and 20% inclusion levels (WP5, WP10, WP20). The WP20 diet was pre-extruded. Pellet quality indicators included water stability, bulk density, expansion ratio, and hardness. WP20 exhibited significantly higher water stability across all time points (30, 90, 180 min), likely due to pre-extrusion. No significant differences were found in bulk density or expansion ratio, though higher WP levels correlated with reduced expansion and pellet hardness, indicating more compact and softer pellets. Growth performance showed no significant differences between CTRL, WP5, and WP10. However, increasing WP levels led to gradual declines in weight gain, feed intake, specific growth rate (SGR), and Fulton's condition factor, with WP20 showing the poorest results and highest feed conversion ratio (FCR). Apparent digestibility coefficients (ADCs) for dry matter and crude protein also decreased with higher WP inclusion. Histological assessment of the distal intestine revealed no histopathological alterations in the WP diets compared to the control. These findings suggest WP can be included at 5–10% in rainbow trout diets without adverse effects. Further research should explore long-term impacts, effects across life stages, and the role of pre-extrusion in improving pellet quality.

Keywords: alfalfa white protein extract, rainbow trout, physical pellet quality, growth performance

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INNOVATIONS IN PET TREAT EXTRUSION: EMERGING TRENDS AND TECHNIQUES

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The global pet food market has witnessed rapid growth in recent years, driven by increasing pet ownership, humanization of pets, and growing demand for high-quality, functional products. Within this landscape, extrusion technology has become a cornerstone for the manufacturing of pet treats, offering versatility, efficiency, and cost-effectiveness. Recent innovations in pet treat extrusion are redefining product quality, nutritional value, and consumer appeal, while also addressing sustainability and production challenges.

One key trend is the development of advanced twin-screw extrusion systems, which provide superior control over temperature, pressure, and shear forces. This enhanced control allows manufacturers to incorporate novel ingredients—such as high-protein insect meals, alternative plant proteins, and functional additives like probiotics and omega fatty acids—without compromising product texture or palatability. Additionally, these systems enable the creation of complex shapes and multi-textured treats, meeting growing consumer demand for premium and engaging products. Another significant advancement is the application of precision moisture and thermal management during extrusion. Innovations such as in-line moisture sensors and advanced die design have led to improved consistency in treat texture and expanded shelf-life stability. The integration of real-time monitoring and feedback controls enables manufacturers to reduce batch-to-batch variability and optimize energy and water use, aligning with broader sustainability goals. In parallel, there is a growing emphasis on clean label formulations and minimal processing, driven by consumer preferences for transparent and health-conscious pet foods. Innovations in low-temperature extrusion and gentle cooking methods are being explored to preserve bioactive compounds and natural flavors, further enhancing the nutritional profile of treats.

Overall, the convergence of ingredient innovation, precision processing, and customization technologies is reshaping the pet treat extrusion landscape. Future advancements are expected to focus on smart manufacturing systems, increased use of alternative proteins, and enhanced sustainability practices. These trends promise to deliver not only high-quality and diverse treats for pets but also environmentally responsible and economically viable solutions for manufacturers.

FROM PROCESSING TO SAFETY: HOW TECHNOLOGY SHAPES FEED QUALITY

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Ensuring global food security increasingly relies on the sustainable production of compound feed for all animal species. As demand grows, the feed industry must adapt through improved technologies, efficiency, and safety measures. Feed technology encompasses the processing of ingredients and the production of animal feeds, playing a crucial role in a sustainable animal production system aimed at providing high-quality and nutritious food. Many scientists have mostly analyzed the influence of thermal processes on grain / feed quality. However, it is important to summarize the influence of all processes in the feed mill on animal feed safety. The work was conducted to provide an overview of all technological processes, starting from the reception of animal feed components that influence the presence and concentration of mycotoxins, pathogenic microorganisms, and pesticides in compound feeds. The feed production processes discussed encompassed raw material storage, cleaning, dehulling, batching, mixing, pelleting, extrusion, cooling, application of liquids, and storage of compound feeds.

A holistic approach to monitoring and managing feed processing technologies is essential for ensuring feed safety and, consequently, animal and human health. Each stage of compound feed production presents both risks and opportunities for mitigating contamination. Technological optimization, along with systematic quality control and hygiene measures, can significantly reduce the presence of harmful substances in feeds. This integrated view emphasizes the need for interdisciplinary collaboration among feed technologists, microbiologists, toxicologists, and quality assurance professionals. Ultimately, strengthening safety at all stages of feed production contributes directly to sustainable farming practices and global food security.

Keywords: animal feed technology, feed safety, feed mill.

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OPTIMIZING PELLET PRESS PARAMETERS TO ENHANCE FEED QUALITY AND ENERGY EFFICIENCY IN POULTRY FEED PRODUCTION

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Feed quality plays a crucial role in commercial poultry production, impacting both economic outcomes and animal welfare. Pelleting, the predominant method of feed processing, involves thermal and mechanical treatment to improve feed handling and nutritional uniformity. This study aimed to evaluate the influence of key pelleting parameters on physical properties of the pellets and energy efficiency.

A factorial experimental design was employed to assess the effects of die diameter (3 mm and 5 mm), die thickness (24, 36, 51 mm for 3 mm dies; 40, 60, 85 mm for 5 mm dies), roller type (corrugated vs. dimpled), roller–die gap (0.1 mm and 1.0 mm), production capacity (400 kg/h and 800 kg/h), and conditioning temperature (75°C, 80°C, 85°C). The feed formulation used contained 21.58% crude protein, 3.15% crude fiber, 4.0% acid detergent fiber (ADF), 12.63% neutral detergent fiber (NDF), 50.3% starch, and 4.43% crude fat.

Pellet samples were collected post-processing, cooled, and analyzed for hardness and pellet durability index (PDI). Results indicated a strong positive correlation between die diameter and both pellet hardness and PDI. Die thickness, roller type, and roller–die gap exhibited weak or negative correlations with pellet quality. Conditioning temperature and production capacity showed no significant correlation with pellet hardness or PDI. Notably, energy consumption was inversely correlated with PDI, suggesting that improved pellet durability may be achieved with reduced energy input under optimized conditions.

These findings highlight die diameter as a critical parameter for enhancing pellet quality and energy efficiency in poultry feed manufacturing.

Keywords: feed pelleting, PDI, hardness, energy consumption, poultry feed

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CHALLENGES IN RABBIT PRODUCTION IN MALTA: FEEDING PRACTICES, ADVISORY SERVICES, AND FARMERS’ KNOWLEDGE ON ANIMAL WELFARE

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Intensive rabbit meat production in Malta faces several structural challenges, including spatial limitations, dependency on imported feedstuffs, and a lack of formal advisory frameworks. This study explores the knowledge, feeding practices and animal welfare awareness of local rabbit producers through on-farm visits, semi-structured interviews, and direct observations. Data were collected from a representative sample of 9 out of 11 farms having more than 50 breeding does across Malta, focusing on feeding strategies, sources of advisory support, and compliance with welfare standards and feed labelling regulations. The findings reveal a heavy reliance on nutrition advice from feed mills and importers of ready-formulated feeds. In the absence of formal extension services, producers often seek guidance from animal health product retailers, peers, online platforms, and social media, while veterinarians remain underutilized in routine management. Feeding strategies are not homogenous across the nine farms. Six producers use three different feed formulations to meet the requirements of the breeding does, weaner and grower/finisher rabbits, whilst the rest all implement a unique feeding program different from others. Medicated feeds, particularly those containing coccidiostats, are commonly used at weaning and often administered up to the point of slaughter. Field observations and supporting documentation revealed instances of mislabeled compound feeds imported from other EU Member States, sometimes bearing solely in a foreign language—unintelligible to many Maltese producers—thus breaching EU labelling regulations requiring information in both English and Maltese. This mislabeling poses risks to food safety, animal welfare, and legal compliance. The findings underscore the need for improved regulatory oversight, targeted nutritional guidance, and capacity-building initiatives to support sustainable growth and welfare standards in Malta’s rabbit farming sector.

Keywords: Malta, animal health and welfare, production performance, ecological footprint

RESOLVING THE CREEP FEED DILEMMA: A TWO-STAGE EXTRUSION-PELLETING PROCESS ENHANCES STARCH DIGESTIBILITY WHILE PRESERVING HEAT-SENSITIVE NUTRIENTS

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To address the core conflict in creep feed production—namely, the need for high temperatures to achieve efficient starch gelatinization versus the preservation of heat-sensitive nutrients such as vitamins and enzymes—this paper systematically analyzes the evolution, practical application, and scientific mechanisms of the “low temperature pelleting with all material extruded” process.

In the first stage, heat-resistant ingredients such as corn and soybean meal are subjected to high-temperature extrusion, achieving starch gelatinization and partial protein denaturation. In the second stage, these extruded materials are blended with heat-sensitive components such as whey powder, vitamins, and enzymes, and then pelleted under low-temperature conditioning to maximize nutrient retention.

The study combines production parameter analysis with in vitro digestion simulations to validate the process. Results show that: (1) This process raises starch gelatinization levels in the final product to an ideal 60%–70%, while significantly improving the retention rates of vitamin C, phytase, and probiotics; (2) Feeding trials confirm that the resulting creep feed increases feed intake and reduces feed conversion ratio, with optimal values reaching 1.07; (3) In vitro digestion experiments further verify that extrusion is a critical step in improving the digestibility of crude protein, dry matter, and metabolizable energy.

In conclusion, the “low temperature pelleting with all material extruded” process effectively resolves the technical challenges in creep feed production by tailoring thermal treatment to the properties of different raw materials. Its advantages have been validated through both industrial practice and scientific research, making it a leading technology for producing high-quality, highly digestible feeds for suckling piglets. This approach is of great significance in promoting the precision and premium development of creep feed products.

Keywords: creep feed, low-temperature pelleting, heat-sensitive nutrients, starch gelatinization

WHEN MICRO- AND NANOPLASTICS COLLIDE WITH CO-CONTAMINANTS: A PERFECT STORM FOR TOXICITY, ANTIMICROBIAL RESISTANCE, AND PATHOGEN PERSISTENCE IN THE FOOD CHAIN

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Microplastics (MPs) and nanoplastics (NPs) are emerging contaminants raising growing concerns for food safety and human health. These particles can adsorb and transport environmental toxins, such as microcystin-LR (MC-LR), while also exerting direct cellular effects. Here, we systematically investigated their uptake by plants and their bioenergetic and cellular consequences in human models relevant to major exposure routes.

Using labeled NPs, we demonstrated uptake and translocation in basil (*Ocimum basilicum*), highlighting dietary exposure as a realistic risk. Independently, we confirmed MC-LR accumulation in basil, showing that irrigation water contamination represents an additional hazard. Notably, MPs altered the dynamics of MC-LR uptake.

To evaluate human health effects, four cell lines representing lung, colon, and liver were exposed under acute and prolonged conditions. Undifferentiated Caco-2 cells showed the highest initial uptake of MPs, though no immediate stress responses were detected. In contrast, long-term exposure induced significant oxidative stress and mitochondrial dysfunction, particularly in Caco-2 cells that retained MPs. Differentiation state did not markedly change the biological response, although differentiated cells internalized fewer MPs.

We further examined combined effects of polystyrene NPs and MC-LR on mitochondrial function in Caco-2 and HepG2 cells. Short-term exposure to polystyrene NPs (PSNPs) inhibited respiration in Caco-2 cells, reducing ATP-linked and maximal respiration and spare capacity, while shifting energy production toward glycolysis. MC-LR alone had minimal effects, but co-exposure with PSNPs caused dose-dependent hepatotoxicity in HepG2 cells, indicating synergistic interactions.

Finally, we explored the role of MPs in pathogen transmission. Our data show that certain foodborne pathogens, including *Listeria monocytogenes* and *Campylobacter jejuni*, can form biofilms on MPs and display altered virulence, though this effect is not universal (e.g. with *Staphylococcus aureus*) and whole genome sequencing did not show genetic traits that would explain this.

Together, our findings underscore the dual role of MPs as vectors for environmental toxins and as bioactive contaminants capable of disrupting cellular metabolism. These results reinforce the need for comprehensive risk assessment of microplastic exposure at the interface of food safety and toxicology.

Keywords: microplastics, nanoplastics, toxicity, pathogens, uptake, food safety, health

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SCREENING FOR DIOXINS USING A BIOASSAY: IDENTIFYING AN UNKNOWN CONTAMINANT IN EGGS AND CHICKEN

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Food authorities safeguard our food. This not only requires sensitive analyses to guarantee detection of both known banned and regulated substances at low concentrations, but also broad screening methods to identify new emerging risks. For this purpose, effect-based bioassays offer an advantage. Several cases where bioassays have proven their added value for monitoring food and feed will be shown, e.g. for steroids in feed and supplements and sildenafil-analogues in supplements, eventually focusing on a case where during the regular monitoring of dioxins in agricultural products, a discrepancy was observed between the results of the DR CALUX (Dioxin-Responsive Chemical Activated Luciferase gene Expression) bioassay and the confirmatory gas chromatographic high resolution mass spectrometric (GC-HRMS) analysis in egg and broiler fat samples. The response in the bioassay was high, suggesting a clear exceedance of the maximum limits of dioxins in these samples. However, regulated dioxins or dl-PCBs were not detected by confirmational GC/HRMS analysis. Following an expert opinion that this was not a false positive in the bioassay, a broad screening analysis using GC-HRMS ultimately resulted in the identification of 2,3,7,8-tetrabromodibenzofuran (2,3,7,8-TBDF) in both egg and broiler fat samples. The potential source of this brominated furan contamination was investigated by analyzing different samples: bedding material, poultry feed, feed additives (choline chloride and L-lysine), and seaweed. A batch of poultry feed and several feed additives tested turned out to contain 2,3,7,8-TBDF. Using a feed-to-food transfer model, it became clear that the poultry feed was probably the source of 2,3,7,8-TBDF in broilers and eggs through a feed additive like L-lysine or choline chloride. This study and the other cases mentioned, underline the importance of using a combination of effect-based screening assays with sensitive analytical methods (LC- or GC-HRMS) to detect potential new and emerging risks.

Keywords: bioassays, screening, unknown actives, confirmation, GC- and LC-HRMS

Acknowledgements: This research was financially supported by the Dutch Ministry of Agriculture, Nature and Food Quality (KB37-002-0009 and WOT-002-001-004).

AFLATOXINS IN THE DAIRY CHAIN: RISKS FOR MILK SAFETY AND QUALITY

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Milk safety is a key component of food security, especially due to contamination with aflatoxin M₁ (AFM₁), a hydroxylated metabolite of aflatoxin B₁ (AFB₁). After ingestion of contaminated feed, AFB₁ is absorbed in the gastrointestinal tract of dairy cows, converted into AFM₁, and secreted into milk. Both are classified as Group 1 carcinogens by the IARC, posing serious public health and economic concerns due to the rejection of milk and dairy products.

This study evaluated AFM₁ contamination in milk and chesse products from cows fed a Total Mixed Ration containing AFB₁ and AFB₂ at levels below the EU legal limit (5 µg/kg; Directive 2002/32/EC). A 14-day *in vivo* trial was conducted using two authorized feed additives: additive 1 (smectite and lignin) and additive 2 (humic acid and plant extracts), to assess their ability to reduce aflatoxin carry-over into milk and dairy products.

Although the feed complied with EU limits, AFM₁ levels in milk exceeded the maximum allowed concentration of 0.05 µg/kg (Regulation 2023/915), raising concerns about the adequacy of current standards. Trace amounts of AFB₁ (0.003 µg/kg) were also detected in milk—low, yet significant due to its toxicity. Both additives significantly reduced AFM₁ in milk by about 30%, lowering the AFB₁ carry-over to under 2%. They also reduced AFM₁ levels in mozzarella and grana-type cheeses, keeping concentrations below recommended thresholds for soft and hard cheeses.

Animal feed also impaired milk quality, lowering total protein and casein content. Both parameters were restored by the additives. Mineral imbalances (higher sodium and chloride, lower potassium contents) suggested mild mammary inflammation, which was reversed with additive use. No negative effects on milk yield or cheese quality were observed.

In conclusion, stricter control of AFB₁ in feed and preventive strategies are essential to ensure milk safety and consumer protection.

Keywords: aflatoxins, dairy cows, milk safety, feed additives, food security

ANTIMICROBIAL CROSS-CONTAMINATION IN FEED: CHALLENGES AND ANALYTICAL ADVANCES UNDER EU REGULATION 2024/1229

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Antimicrobial cross-contamination during feed production is a critical issue with implications for animal health, food safety, and the development of antimicrobial resistance (AMR). Residual traces of veterinary antibiotics from medicated feed batches can be unintentionally carried over into subsequent non-target feed, even at very low concentrations. This contamination, though often below therapeutic levels, may contribute to selective pressure on intestinal microbiota and the emergence of AMR in both animals and humans.

In response to these risks, the European Union has introduced Regulation 2024/1229, which establishes significantly lower Maximum Levels for antimicrobials listed in ANNEX II to Regulation (EU) 2019/4 in non-target feed. To ensure compliance with these thresholds, the adoption of highly sensitive, reliable analytical techniques capable of detecting residues at sub-ppb levels is required.

Within this context, the analytical strategies adopted by II.ZZ.SS. network to face the challenges encountered in method development is discussed as a critical step forward.

This communication presents the new guidelines introduced by the Italian Ministry of Health and the National Reference Centre on Animal Feed (C.Re.A.A.) with the analytical strategies adopted by II.ZZ.SS. network to face the challenges encountered in the development and validation of LC-MS/MS-based screening and confirmatory methods for the detection of 24 antibiotics in feed matrices.

This work will support the Official Control in the field of National Monitoring Control Plans and contribute to the broader goals of antimicrobial stewardship and One Health policy.

Keywords: Antibiotic residues, Cross-contamination, feed, LC-MS/MS, EU Regulation 2024/1229.

Acknowledgements: The authors wish to thank the National Reference Centre for Surveillance and Control of Feed for Animals (C.Re.A.A.) for their support in the ongoing development and validation of the analytical methods. We also acknowledge the valuable contribution of the laboratories within the Istituti Zooprofilattici Sperimentali (II.ZZ.SS.) network. This work is funded by the Italian Ministry of Health within the framework of the National Monitoring Control Plans.

HIGHER PREVALENCE OF EMERGING MYCOTOXINS DETECTED IN 2025 HARVESTED WHEAT AND BARLEY FROM DIFFERENT REGIONS OF SERBIA

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The aim of the present study was to quantify mycotoxins in 2025 harvested barley and wheat samples received from different regions of Serbia using LC-MS/MS triple quadrupole (Agilent 6460 series) based multi-mycotoxin method for 34 mycotoxins. These wheat and barley samples were analysed using LC-MS/MS for European Union (EU) regulated mycotoxins: aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂), deoxynivalenol (DON), fumonisins (FB₁, FB₂, FB₃), HT-2 toxin, ochratoxin A (OTA), T-2 toxin, and zearalenone (ZEN); emerging mycotoxins: beauvericin (BEA), enniatin (ENNA, ENNA₁, ENNB, ENNB₁), fusaric acid (FA), and moniliformin (MON); and other mycotoxins: 15-acetyl deoxynivalenol (15-ADON), 3-acetyl deoxynivalenol (3-ADON), deoxynivalenol 3-glucoside (D-3-G), citrinin (CIT), alternariol (AOH), diacetoxyscirpenol (DAS), ergosine (ES), ergocryptine (ECR), neosolaniol (NEO), nivalenol (NIV), patulin (PAT), fusarenon x (FX), zearalanone (ZAN), α – zearalenol (α -ZEL) and β – zearalenol (β -ZEL). The results showed that 6% barley samples were not contaminated with any of the tested mycotoxins whereas 12% samples had 1 mycotoxin and 82% samples were contaminated with more than 1 mycotoxin. BEA, FA, ENNA, ENNA₁, ENNB, ENNB₁, and T-2/HT-2, NEO, DON were the main mycotoxins detected in majority of barley samples. The mycotoxin results of wheat samples showed that 30% wheat samples were not contaminated with any of the tested mycotoxins whereas 13% samples have 1 mycotoxin and 57% samples showed the presence of more than 1 mycotoxin. BEA, ENNA, ENNA₁, ENNB, ENNB₁, and DON were the main mycotoxins detected in majority of wheat samples. Co-occurrence has significant implications for food and feed safety, as combined exposure to multiple mycotoxins can lead to synergistic toxic effects, increasing human and animal health risks.

Key words: wheat, barley, emerging, LC-MS/MS, multimycotoxin, co-occurrence

IDENTIFICATION OF PROCESSED ANIMAL PROTEINS (PAPS) FROM INSECTS IN FEED BY HIGH RESOLUTION LC-MS/MS

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Due to the risk associated with Transmissible Spongiform Encephalopathy (TSE), the European Parliament established a ban concerning the use of processed animal proteins (PAP) from mammals in feed ((EC) No. 999/2001). Since 2007, this feed ban has been gradually relaxed and EU regulations (EC) NO. 893/2017 and 1925/2021 authorized eight specific insects for the use in aquaculture as well as in pig and poultry feed: black soldier fly (*Hermetia illucens*), house fly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domestica*), banded cricket (*Gryllobates sigillatus*), field cricket (*Gryllus assimilis*) and silk moth (*Bombyx mori*). The official control method as laid down in regulation (EC) No. 152/2009 annex VI, is a combination of light microscopy (LM) and polymerase chain reaction (PCR). However, LM cannot distinguish between particles derived from authorized and non-authorized insects and a validated PCR method for the control of insects in feed is not implemented so far. This analytical gap can be closed by a targeted LC-MS/MS approach.

The aim of this project is to develop a qualitative targeted LC-MS/MS method to differentiate between authorized and non-authorized insects in feed. For this purpose, target proteins were extracted, digested by trypsin, and the resulting peptides were purified. Specific marker peptides for all eight authorized insects were identified based on untargeted discovery proteomics by data dependent (DDA) and data independent analysis (DIA) approaches using a high-resolution UPLC-QToF-MS system. To detect also non-authorized insects including contaminations, peptides of fruit fly (*Drosophila melanogaster*) which occur also in many other insects were identified. With the 27 selected specific marker peptides a targeted parallel reaction monitoring (PRM) method was established and optimized. The specificity of all peptides was proofed *in silico* by BLAST-analysis using the SwissProt database and experimentally using different sample materials which were commercially available. The results demonstrated excellent selectivity of all tested peptides, confirming that the method can identify both authorized and non-authorized insects in feed. The method can be applied in routine feed control to verify label declarations, as well as to distinguish potential contaminations from the authorized insects.

Keywords: processed animal proteins, insects, high resolution LC-MS/MS, proteomics, feed

Acknowledgements: Thanks to the Austrian Research Promotion Agency (FFG) for funding.

WIDE SCOPE SCREENING AND TARGET QUANTIFICATION OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN FEED AND FEED INGREDIENTS TO FARMED SALMONIDS AND THEIR FEED-TO-FILLET TRANSFER

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Recently, the EU has set maximum levels (MLs) for per- and polyfluoroalkyl substances (PFAS) in food products after an EFSA risk assessment these compounds. Following, the EU aims to set MLs for PFAS in animal feed and feed ingredients in order to protect the safety of food products from farmed animals. The present paper extends on earlier studies reporting on the presence of target PFAS in fish meal, which is considered the main source for PFAS in animal feeds. We provided wide- scope occurrence data of PFAS in commercial feed ingredients and feed for farmed Atlantic salmon, and assessed the relative feed-to-salmon transfer of the most predominant PFAS in salmon feed including the four PFAS that currently are under EU legislation.

For occurrence data, we employed a comprehensive analytical strategy to identify and quantify PFAS in Norwegian salmon feeds, and relevant commercial plant- and fish-based protein and oil ingredients. The samples include 4 feed, 4 fish meal (FM), 3 fish oils (FO), 3 plant oils (PO), and 2 plant meal (PM) samples. Targeted analyses included a list of 28 PFAS, including novel precursors, analysed using ultra-HPLC coupled with triple quadrupole mass spectrometry (QqQ/MS). Additionally, suspect and non-target wide-scope screening is performed utilizing a suspect list of approximately 49,000 PFAS with UHPLC-HRMS. The study also examines the role of unidentified precursors in the formation of perfluoroalkyl acids (PFAA), utilizing Total Oxidizable Precursor (TOP) assay.

To assess feed-to- salmon fillet transfer, Atlantic salmon were fed with PFAS vacuum-top coated spiked feed for 3 months followed by a 3-month depuration period in triplicate tanks. Time-course samples were taken, in the exposure period three fish per tank were sampled at day 0, 7, 14, 30, 56, 70 and during the depuration period fish were sampled at day 80, 85, 95, 112 and 126. This provides PFAS congener uptake and elimination kinetics that can be used in physiological based toxicokinetic (PBK) feed-to-transfer models.

All feed and feed ingredients (100% of analysed samples) had detectable PFAS levels. Of all main feed ingredients, fishmeal (FM) had highest PFAS loads with 12 detected PFAS of which PFUnDA and PFOS as the dominant compounds (mean 0.34 and 0.32 $\mu\text{g kg}^{-1}$ wet weight, respectively). Other feed ingredients, including fish oil (FO), plant oil (PO), and plant meal (PM), generally contain lower PFAS levels. However, PFOS was detected in all FO and PO samples, and as salmon feed contains mainly (>70%) plant ingredients, they do significantly contribute to overall PFAS load in feed. More importantly, salmon feed contained high levels of PFAA precursors, at concentrations by far (up to two-fold) exceeding the legacy PFAA. As these precursors can be transferred into legacy PFAA, as confirmed by a total oxidizable precursor (TOP) assay. Based on the uptake and elimination kinetics of a congener specific feed-to-fillet transfer potential was seen (here expressed as steady state kinetic based biomagnification factor; $\text{BMF}_{\text{ss}} \times 10^{-2}$) with relative transfer rates in the following order PFOS (1.2) >> PFHxS (0.7) > PFBS~PFDA~PFNA (0.2-0.3) > PFOA (0.1). The difference on transfer potential was mostly explained by congener specific half-lives ($T_{1/2}$ days); PFOS (97)>> PFHxS (54)> PFBS~PFDA~PFNA (28-17)> PFOA (12).

The present study shows that not only FM but also other feed contaminants need to be surveyed to assess the source of PFAS in commercial salmon feed, as both fish oil and plant oil can contribute significantly to PFOS loads. Congener specific kinetic data is provided to assess the relative transfer from feed to fillet, with PFOS having by far the highest transfer potential. However, the presence of the precursors can “mask” additional

PFAS sources and loads in salmon feed and can contribute to “unseen” PFAA transfer in farmed salmon.

Keywords: PFAS, wide scope screening, feed, transfer.

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INSECT PROCESSED ANIMAL PROTEINS DETECTION AND IDENTIFICATION BY DEEP LEARNING MICROSCOPIC IMAGE RECOGNITION

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The introduction of terrestrial invertebrate processed animal proteins (PAPs) from insects in animal feed has introduced challenges for official controls in the European Union. Currently, as imposed by regulation EC/152/2009, their detection relies only on light microscopy and therefore is entirely dependent on the expertise of trained operators. Recent collaborative studies have highlighted two primary problems regarding insect PAP detection: (1) specificity issues due to the misidentification of non-insect structures as insect derived ones, and (2) the difficulty in categorizing a wide morphological diversity of particles resulting from the grinding of whole insect larvae. Furthermore, insect species identification is nearly impossible without rigorous entomological expertise. To address these primary limitations, a proof of concept was developed involving automated microscopic image analysis and classification by deep learning. A classification pipeline integrating a YOLO object detection model with an improved ConvNeXt architecture coupled with transformer blocks was proposed and tested. After supervised learning, training and validation of the model, obtained results allowed (a) to successfully distinguish insect particles from other non-insect materials, and (b) to further discriminate PAPs particles derived from two insect species (*Tenebrio molitor* and *Hermetia illucens*) achieving Matthews Correlation Coefficient (MCC) values above 0.90 and accuracies exceeding 95% for both tasks. The robust performance and the rapid processing speed of the model enabled to couple it to a digital microscope for real-time automated particle classification, including confidence scores for individual particle predictions. This artificial intelligence model for microscopic identification of complex structures, such as cuticular fragments from ground insect larvae, opens new perspectives for official controls achieving performance scores never reached before. It provides to microscopists an optimisation of image analysis and a valuable decision-support tool in complex feed matrices where human expertise is frequently challenged.

Keywords: deep learning, microscopy, insect meal, feed

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POSTER PRESENTATIONS

LOOKING FOR THE MOST PROMISING REGIONAL ALTERNATIVES TO FISHMEAL IN AUSTRIA – FIRST STEPS

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Fish is becoming increasingly popular as a food in Austria, while consumers are placing growing importance on local production. At the same time, Austrian aquaculture companies face significant challenges, such as rising prices for fish feed—an important factor in operating costs, particularly in trout farming. Beside general inflation, price increases are driven by limited availability of raw materials (for fishmeal and fish oil), strong competition (e.g. from the pet food industry) and global uncertainties affecting supply chains (e.g. due to COVID-19, the war in Ukraine, extreme weather etc.).

As part of the project, a comprehensive analysis is being conducted to explore options for producing and marketing a regional, sustainable, and affordable fish feed for predatory fish species in Austria, using locally raw materials.

Many studies highlight the substitution potential for marine fishmeal and fishoil, but long-term analyses and economically viable implementation strategies are still lacking. Research and regulatory gaps also exist regarding fish by-products. Fish silage is seen as a simple and cost-effective solution, though its quality depends on the raw material, storage and fermentation.

The following main categories of alternative protein sources have been identified:

- Animal proteins (PAPs): Poultry meal shows promising results and, combined with fish silage, can fully replace fishmeal. However, nutrient losses during processing may pose limitations.
- Plant proteins: Fermented soy (up to 50% fishmeal replacement), corn gluten (high protein content but lacking essential amino acids) and brewery by-products (limited use). Challenges include the presence of mycotoxins and competition with human food production.
- Insects: The black soldier fly shows great potential, though it has low omega-3 content and high production costs.
- Single-cell organisms and algae: Yeasts, bacteria, and microalgae are considered promising alternatives.

For the next phase of the project, four key areas with the highest potential were identified and discussed with stakeholders:

- Replacement of fishmeal with regional poultry PAPs, plant proteins and insects (highest stakeholder interest)
- Transport system for single-variety processing of fish by-products
- On-farm processing (fishmeal or silage)
- Delivery of fish carcasses to the pet food industry

Stakeholders recommended visualizing on-farm fish processing steps (to optimize circular economy interfaces) and supported route modelling for relevant raw materials.

Regarding the availability of key raw materials, the sourcing area was defined and research initiated. So far, 103 fish processing companies, 133 poultry processing companies, and 21 insect producers have been identified. Initial route modelling shows plausible results but high complexity. Research and modelling are ongoing.

A legal analysis identified the intra-species ban as a major challenge. The processing of fish by-products requires veterinarian approval, HACCP concepts, and strict species separation.

Keywords: fish feed, regional raw material, aquaculture, sustainability

Acknowledgements: This project is funded by the Federal Ministry of Agriculture and Forestry, Climate and Environmental Protection, Regions and Water Management, Austria

EFFECTS OF PARTIALLY REPLACING SOYBEAN MEAL WITH THERMALLY PROCESSED LUPIN ON THE MILK FATTY ACID PROFILE AND MILK QUALITY OF DAIRY COWS

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Lupinus albus cultivation offers a sustainable alternative to imported soybean meal, supporting local protein self-sufficiency. This study tested the hypothesis that partial replacement of soybean meal with thermally processed lupin seeds improves milk quality without compromising oxidative stability.

The trial was conducted in the Zagliveri region of Greece over 90 days, involving 60 Holstein cows randomly assigned to control and treatment groups. The treatment group received a modified concentrate in which 7% of soybean meal was replaced with lupin seeds thermally processed in ovens at 130°C. Milk yield and quality were monitored throughout the trial.

Gas chromatography (GC-FID) revealed an improved fatty acid profile in milk from the treatment group, with a significant increase in polyunsaturated fatty acids (PUFAs) and a more favorable omega-6/omega-3 ratio compared to the control. Near-infrared spectroscopy (NIR) was used to evaluate feed and milk composition. Lipid oxidation remained stable, with no increase in malondialdehyde (MDA) levels in milk.

Feed analysis showed a higher total phenolic content (TPC, mg GAE/g) and slightly lower MDA concentration (ng/g) in the lupin-based diet compared to the basal diet, indicating improved antioxidant potential. These results align with enhanced oxidative stability and improved milk quality in the treatment group.

In conclusion, partial replacement of soybean meal with thermally processed lupins at a 7% inclusion rate improved the nutritional profile of milk without compromising oxidative stability, while increasing the total phenolic content of feed. Further research will focus on optimizing lupin genotypes to meet the nutritional needs of livestock and market demands.

Keywords: lupin seeds, dairy cows, milk fatty acid profile, lipid oxidation, total phenolic content

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ENRICHMENT OF BOVINE MILK FATTY ACID PROFILE VIA DIETARY PHYTOBIOTICS AND OMEGA-3 SUPPLEMENTATION

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Plant-based phytobiotics and omega-3 polyunsaturated fatty acids have been explored as dietary strategies to enhance the lipid profile and oxidative stability of milk. In this study, we evaluated the effects of a supplement blend, comprising oregano essential oil, dried oregano, dried sage, and flaxseeds, on the milk fatty acid composition and lipid peroxidation in lactating dairy cows. 100 Holstein cows in Valtino, a small village near Trikala, Greece, received a concentrate enriched with this mixture, and milk samples were collected both prior to and during the supplementation period. Gas chromatography analysis revealed that supplemented cows produced milk with reduced saturated fatty acids, from 79.39 % to 75.60. In addition, monounsaturated fatty acids increased from 18.74 % to 22.01 %, and polyunsaturated fatty acids rose from 1.87 % to 2.35 %. Specifically, omega-3 fatty acids increased from 0.21 % to 0.41 %, omega-6 fatty acids from 1.62 % to 1.86 %, α -linolenic acid from 0.18 % to 0.32 %, and EPA + DHA from 0.03 % to 0.04 %. Finally, the milk exhibited greater resistance to oxidation. These findings suggest that combining phytobiotics with omega-3 PUFAs in the diet can effectively remodel milk lipid composition and enhance its oxidative stability, supporting the development of value-added dairy products.

Keywords: Omega-3, aromatic plants, dairy cows, fatty acids

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MICROBIOLOGY IN THE FEED CHAIN: FOUR-YEAR OVERVIEW OF OFFICIAL CONTROL RESULTS

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The Agri-food Laboratory, situated in Cabrils, Barcelona, Spain, is a public institution responsible for analyzing food and feed samples as part of official control programs within the food chain. The laboratory participates in the official control of agri-food products safety and quality, processing samples from inspections, legal proceedings, and those intended for export. Additionally, the laboratory holds accreditation in compliance with the UNE-EN ISO/IEC 17025 standard.

This study evaluates, the results obtained over the past four years for *Salmonella* spp. and *Enterobacteriaceae*, from the official feed safety control program. The methods applied were according to ISO 6579-1:2017 for the detection of *Salmonella* spp. and ISO 21528-2:2017 for the detection and enumeration of *Enterobacteriaceae*.

Between 2021 and 2024, a total of 536 and 560 samples were analyzed for *Salmonella* spp. and *Enterobacteriaceae*, respectively. These samples consisted of animal-derived feed materials, compound feed, dog chews and raw pet food. They were primarily collected by inspectors from the autonomous government of Catalonia, as well as from Border Inspection Posts under the authority of the Spanish central government, during the official inspections conducted at the production sites and feed establishments. During this period, 25 samples (5%) tested positive for *Salmonella* spp. (16 feed materials, 4 dog chews, 4 raw pet food and 1 compound feed), and 32 samples (6%) for *Enterobacteriaceae* (12 feed materials, 9 dog chews, 7 compound feed and 4 raw pet food).

By types of feed, non-compliances were predominantly detected in samples consisting of raw pet food for both parameters (4 non-compliances out of 4 samples, 100%). Animal-derived feed materials ranked second (16 out of 176, 9%) in the case of *Salmonella* spp., while dog chews ranked second (9 out of 68, 13%) for *Enterobacteriaceae*. In terms of origin, samples collected by the regional competent authority exhibited a higher contamination rate compared to imported samples (88% regional and 12% imported for *Salmonella* spp., 69% regional and 31% imported for *Enterobacteriaceae*).

In conclusion, compound feed presents a low microbiological risk, whereas other feed types exhibit significant non-compliance rates. Based on analytical results, sampling efforts by the competent authorities responsible for official control should prioritize raw pet food, followed by dog chews and animal-derived feed materials.

Keywords: feed chain microbiology, *Salmonella* spp., *Enterobacteriaceae*, safety in the feed chain, official controls on feed.

Acknowledgements: We acknowledge the personnel responsible for microbiological analyses at the Agri-food Laboratory.

EFFECT OF DIFFERENT AMOUNTS OF LIVE YEAST CELLS (*Saccharomyces cerevisiae*) IN FEED ON THE ECONOMIC EFFICIENCY OF BROILER FATTENING

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This study was conducted to assess the impact of supplementing broiler feed with two different concentrations (0.25 g/kg and 0.65 g/kg) of live yeast (*Saccharomyces cerevisiae*) over a 42-day fattening period on the economic efficiency of broiler production. The data collected for body weight (BW), survival rate (SR), average daily gain (ADG), and feed conversion ratio (FCR) were utilized to calculate the European Production Efficiency Factor (EPEF) and the European Broiler Index (EBI) for the entire rearing period. Broilers in the group receiving 0.25 g/kg of yeast (E-I group) exhibited statistically significantly higher ($p<0.05$) EPEF and EBI values compared to both the control group (C) and the group receiving 0.65 g/kg of yeast (E-II group), primarily due to improved production performance. Specifically, in the E-I group, the average values for BW, ADG and FCR were 2.611 ± 0.247 kg, 61.14 ± 5.00 g/day and 1.71 ± 0.017 , respectively. In the E-II group, these parameters were 2.548 ± 0.270 kg, 59.64 ± 4.80 g/day and 1.73 ± 0.012 , whereas the control group showed values of 2.499 ± 0.386 kg, 58.50 ± 4.14 g/day and 1.76 ± 0.013 . The survival rate (SR) was 100% across all groups. The calculated EPEF for the E-I group (363.41 ± 25.45) was significantly higher ($p<0.05$) than that of both the E-II group (350.67 ± 27.24) and the control group (338.07 ± 26.58). Moreover, the EPEF of the E-II group was also significantly higher ($p<0.05$) compared to the control. A similar trend was observed in the EBI values: the E-I group achieved the highest value (357.54 ± 25.25), followed by the E-II group (344.91 ± 26.01) and the control group (332.39 ± 25.91), with all differences being statistically significant ($p<0.05$). These findings suggest a dose-dependent effect of yeast supplementation, whereby the lower dose (0.25 g/kg) yields the most beneficial outcomes, likely through improved gut health and enhanced nutrient absorption. Conversely, the higher dose (0.65 g/kg) does not confer additional benefits and may even be counterproductive.

Keywords: production results, ROSS 308, EBI, EPEF, yeasts

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ADVANCING FISH HEALTH: ORAL ADMINISTRATION OF AUTOGENOUS VACCINES VIA FUNCTIONAL FEED

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Vaccination is a key strategy for preventing bacterial diseases in aquaculture and reducing antibiotic use. While commercial vaccines are widely used, farm-specific bacterial pathogens often require tailored approaches. Autogenous vaccines, prepared from pathogens isolated on-site, offer a targeted and sustainable solution. Traditionally administered by injection or immersion, these vaccines can now be delivered through functional feed, enabling non-invasive oral immunization. This study aimed to develop and evaluate a prototype of functional feed for oral booster delivery of autogenous inactivated vaccines. The research, supported by the Innovation Fund of the Republic of Serbia, targeted key pathogens affecting trout and carp: *Aeromonas salmonicida* subsp. *salmonicida*, *Lactococcus garvieae*, *Yersinia ruckeri*, and *Aeromonas hydrophila*. Vaccine formulations were incorporated into feed under conditions ensuring antigen stability and palatability. Field trials on commercial farms demonstrated successful immune stimulation in vaccinated fish, with no adverse effects on feed intake or growth performance. Booster administration via feed prolonged the protective effect of the initial vaccination, reduced stress from handling, and improved overall fish welfare. These results confirm that oral delivery of autogenous vaccines via functional feed represents a promising innovation in fish health management. The approach allows adaptation to specific farm conditions, enhances disease control, and supports sustainable aquaculture by reducing reliance on antimicrobials.

Keywords: autogenous vaccines, functional feed, aquaculture, oral booster, fish welfare

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FISH-BASED DIET IN CHANGING LIPID STATUS AND OXIDATIVE PARAMETERS IN POLICE DOGS

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Fatty acids (FA) as bioactive lipid components of plasma and erythrocytes phospholipids as structural lipids have biological roles in integrity of cell membranes. FA, depending on the chain length and the degree of saturation and its synthesis pathway, can alleviate inflammation and oxidative stress caused by excessive exercise. Concerning that changing in food intake or diet can influence fatty acids phospholipids metabolism, our study aimed to determine effects of fish-based diets in working (police) dogs undergoing intensive training concerning bioactive lipids such as FA phospholipids of plasma and erythrocytes.

Methods: 15 male working dogs (3-7years, Belgian Shepard Malinoa) were fed with fish based food for 12 weeks, water ad libitum. Fatty acid esters composition of plasma and erythrocytes phospholipids as a bioactive lipids, in addition to markers of oxidative stress and metabolic parameters, were analysed by GC chromatography. Biochemical analysis were done spectrophotometrically (Cobas 111, Roche, Swicerland). Level of lipid peroxidation were determined by usual TBARS measuring method.

Results. After the treatment with fish-based food, blood glucose, total, and LDL cholesterol levels were significantly reduced, indicating a positive biochemical profiles of dogs. Correlations of FA phospholipids composition between plasma and erythrocytes have shown that both plasma and erythrocytes could represent markers of omega-3 eicosapentaenoic and docosahexaenoic acid intake levels in dogs as well as caused a significant reduction in lipid peroxidation.

Conclusion: Enrichment of dogs' diets with marine fish could improve oxidative status and improve roles and status of bioactive lipids such as membrane phospholipids and FA as its components in plasma and erythrocytes in police dogs exposed to intensive exercise.

Key words: bioactive lipids, fatty acids, phospholipids, oxidative stress, working dogs

Acknowledgement: This study was conducted with the permission of Ministry of Police, Republic of Serbia.

DIETARY EFFECTS OF RAW vs. FERMENTED RAPESEED CAKES ON ANTIOXIDANT PROFILE OF BROILER'S LIVER

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The dietary effects of fermented vs. unfermented rapeseed cakes on antioxidant profile of broiler's liver were evaluated in 300-day-old ROSS 308 broilers over 35 experimental days.

The chicks were divided into 3 groups, 4 replicates/group, 25 chicks/replicate raised on permanent wood shaves litter and fed different diets: control group (CON) based on corn-soybean meal, raw rapeseed cakes (RRC, 20%) and fermented rapeseed cakes (FRC, 20%) during growing and finisher phases. During the trial, the performances were monitored and efficiency production indices were calculated. Blood samples were collected at the end of the trial from 8 chicks/group, afterwards chicks were slaughtered to assess: total antioxidant capacity (DPPH%), lipid peroxidation (TBARS), enzymatic activity (CAT and GPx, UI/mg protein) and organoleptic study during 3 shelf-life periods evaluation (0, 7, 14 days) from liver samples. Initially, the FRC group significantly increased in TAC (79.28%), CAT and GPx (158.81 and 0.508 UI/mg protein, respectively) which was maintained throughout the storage period (7 and 14 days, at 4 °C) compared to CON group. Also, the TBARS values were significantly lower ($p \leq 0.0001$) intragroup and throughout the storage period in the FRC compared to the CON group.

No significant differences ($p > 0.05$) were observed regarding the productive performances, but blood parameters were improved.

In conclusion, the FRC group improved significantly the antioxidant profile during different period of shelf-life which can be considered an alternative source to the conventional dietary soybean meal for broilers.

Keywords: broiler, fermented rapeseed cake, meat quality, antioxidant capacity, lipid peroxidation

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DIFFERENTIATING THE PROCESSING DEGREE OF ANIMAL-DERIVED FEED INGREDIENTS BY MASS SPECTROMETRY-BASED PROTEOMICS

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In order to protect livestock from transmissible spongiform encephalitis (TSEs), the European Union implemented detailed regulations regarding the use and processing requirements for animal material in feed. For example, typically raw or spray-dried blood products from pig and poultry may be fed to all farmed species except ruminants, whereas pig- and poultry-derived ‘processed animal proteins’ such as meals made of e.g. meat, bone and/or blood shall undergo harsher processing and may not be fed to farmed insects and pig or poultry, respectively.

To provide an - as yet non-available – analytical method able to distinguish the differently processed feed ingredients, we investigated whether processing-induced covalent changes on proteins originating by Maillard reaction and (lipid) oxidation may provide suitable processing markers. Thorough high resolution mass spectrometry-based proteomic analyses of in total 28 porcine, 17 ruminant, and 14 poultry samples identified a total of 19,512, 21,747, and 11,851 peptides, more than half of which were post-translationally modified. A script normalizing peptide intensities to those of their assigned proteins (accounting for their concentration differences across all samples) was developed and implemented into the automated data analysis workflow. Filtering according to separation power between differently processed materials as well as parameters scoring peptide identification quality revealed 18 porcine, 59 ruminant, and 20 poultry marker candidates.

Analytics was transferred to a set-up available to official control by developing targeted methods on a low resolution mass spectrometer for both marker candidates and reference peptides representing their corresponding proteins. Candidate utility was verified with extended sample sets (81 porcine, 65 ruminant, 58 poultry materials) and all peptides’ identities were proven by synthetic analogues (except three markers non-accessible to standard synthesis).

Transferring the remaining one to four markers per species (group) to a fully quantitative and validated method will enable authorities to distinguish differently processed and regulated feed ingredients.

Keywords: Maillard reaction, oxidation, protein backbone cleavage, post-translational modification, blood

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SAFFLOWER OIL QUALITY IN RELATION TO GENOTYPE AND ENVIRONMENT: IMPLICATIONS FOR ANIMAL NUTRITION

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Safflower (*Carthamus tinctorius* L.) is an oilseed crop valued for its high oil content (35–48%) and favorable fatty acid composition. Safflower oil is rich in unsaturated fatty acids (UFA), including both monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), and low in saturated fatty acids (SFA). Its incorporation into animal feed has been shown to improve immune response, reproductive performance, and growth of broilers, cattle, and sheep, while enhancing the composition of unsaturated fatty acids in animal products such as milk and meat, offering potential benefits for both animal and human nutrition. The objective of this research was to analyze the fatty acid composition of oil obtained from two safflower genotypes (SO₁ and SO₂) cultivated in three regions of Serbia (Pančevo, Rimski šančevi and Sombor). Oil was extracted from seeds using a hydraulic press, and the fatty acid composition was analyzed by gas chromatography (Konik HRGC 4000) equipped with a flame ionization detector. The analysis focused on quantifying the levels of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids to assess the nutritional quality and potential health benefits of safflower oil. PUFA content ranged from 73.65% (SO₂-Sombor) to 77.10% (SO₂-Pančevo), MUFA from 11.34% (SO₂-Pančevo) to 16.13% (SO₂-Sombor), and SFA from 10.03% (SO₁-Pančevo) to 11.92% (SO₂-Rimski šančevi). Linoleic acid was the predominant PUFA (74.10%–76.90%), while oleic (11.18%–15.94%) and palmitic acids (6.15%–7.56%) were the major MUFA and SFA, respectively. Analysis of variance showed that safflower oil fatty acid composition was significantly affected by genotype, locality, and their interaction, with variations depending on the fatty acid group. MUFAs were influenced by all three factors ($p < 0.01$), PUFAs mainly by locality and its interaction with genotype ($p < 0.01$), while genotype alone had no effect ($p = 0.902$). Total UFAs and SFAs were significantly affected by genotype and genotype $\times$ locality interaction ($p < 0.001$), with no significant effect of locality alone ($p > 0.3$). These findings emphasize the predominant role of genotype and genotype–environment interaction in determining oil quality, particularly for UFA and SFA, while highlighting the influence of environmental conditions on PUFA. This study underscores the importance of selecting genotypes tailored to specific growing environments to optimize the nutritional composition of safflower oil.

Keywords: safflower oil, feed, MUFA, PUFA, health benefits

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COMPARISON OF FLUORINE DETECTION IN ANIMAL FEED BY ISE, CIC AND ICP-MS/MS IN RELATION TO FEED SAFETY

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Fluorine contamination in animal feed poses safety concerns, especially natural feeds in regions with high geogenic fluorine or industrial related fluorine emissions. Fluorine is included in the EU feed legislation (Directive 2002/32, and later amendments), with maximum levels established for fluorine in various feeds and feed materials. Accurate monitoring requires reliable analytical methods capable of quantifying fluorine across diverse feed matrices. This study compares three analytical techniques, ion-selective electrode (ISE), combustion ion chromatography (CIC), and inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) for their performance in detecting fluorine in a wide range of natural or commercially sourced animal feeds.

Preliminary data suggest that each of the three techniques; ICP-MS/MS using BaF⁺ as a proxy ion, CIC and ISE offer advantages depending on the analytical context. Fluorine quantification by ISE has an established CEN method (EN 16279:2012) and is the most commonly used method currently. ISE also presents a more straightforward and accessible option (lower instrumentation costs) that might be suitable for simpler matrices or routine applications. CIC appears to demonstrate promising sensitivity and high matrix tolerance, particularly in complex feed samples. Additionally, CIC calls for minimal sample preparation of simply weighing homogenized sample into quartz combustion vessels prior to analysis. ICP-MS/MS offers higher sample throughput at the cost of sensitivity, and lower sample matrix tolerance.

Despite differing instrumentation, all three methods provide comparable quantitative results at higher fluorine concentrations. These findings support the potential of a multi-method approach to fluorine monitoring in feed. The study also highlights the importance of selecting appropriate analytical techniques based on analytical goals (cost, throughput, or sensitivity), sample type, and regulatory context.

Keywords: fluorine, animal feed, CIC, ISE, ICP-MS/MS

INVERTED SUGAR SYRUP FOR BEES: HOW HEATING AND ACIDITY AFFECT ITS SAFETY

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Honey is a natural substance containing essential nutrients and bioactive compounds such as glucose, fructose, proteins, minerals, vitamins, enzymes, and phenolic antioxidants, which contribute to its nutritional and medicinal benefits. Bees primarily rely on carbohydrates for energy, making sucrose a common substitute for nectar in supplemental feeding due to its rapid breakdown and absorption. During winter, when bees have higher energy needs, inverted sugar syrup is preferred because it contains glucose and fructose, which are more easily digestible and require less metabolic effort than sucrose. Inverted sugar syrup is typically produced by heating a mixture of commercial sugar and water in an acidic medium, a process known as sugar inversion. However, these conditions can lead to the formation of 5-(hydroxymethyl)-2-furfural (HMF), a toxic compound harmful to bee health. Therefore, it is critical to control processing parameters to minimize HMF formation. The recommended maximum concentration of HMF in inverted syrup without adverse effects on bees is 20 mg/kg. This study aims to investigate how variations in the acidity of the medium and the duration of heating affect the efficiency of sucrose inversion and the formation of HMF in inverted sugar syrup intended for bee nutrition. Results showed that increasing both the heating time and the proportion of the acidic component accelerates sucrose inversion into glucose and fructose but also raises HMF levels. Consequently, optimizing these parameters is vital to maximize energy availability for bees while reducing the risk of HMF toxicity. Careful control of the inversion process is essential to produce safe and effective supplemental feeding syrup that supports bee health and colony productivity.

Keywords: bee, feeding, sugar

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LABEL-FREE QUANTIFICATION OF MAJOR PROTEINS AND TRYPSIN INHIBITORS IN SOYBEANS BY HIGH RESOLUTION LC-MS/MS

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Soybeans are a very important source for animal feed due to their high protein content (up to 40 %). As soybeans contain anti-nutritional factors (ANF) such as trypsin inhibitors a thermal treatment (roasting) is required. To ensure consistently high quality and digestibility, processing must be carried out in a controlled manner to maximise the reduction of ANF and preserve valuable components. Soybean treatment is currently controlled in the final product, by analysing the trypsin inhibitor activity (TIA), which is time-consuming and costly. This often leads to overprocessing, undesirable protein damage and loss of valuable amino acids. The use of high-resolution proteomics approaches for characterisation of protein composition is an innovative solution to get an in-depth knowledge of soybean proteins. Differences in protein composition between different cultivars and growing locations, as well as the composition of the different trypsin inhibitors, should enable the prediction of the most suitable process parameters thus guaranteeing a proper treatment resulting in high quality soybean products for feed and food production. The aim is the development of a targeted LC-MS/MS method for relative quantification of the six trypsin inhibitor types Kunitz-type trypsin inhibitor KTI 1 – 3 and Bowman-Birk type proteinase inhibitor I, C-II and D-II as well as the development of an untargeted LC-MS/MS method for relative quantification of the major proteins.

For this purpose, the protein profiles of untreated and treated soybeans (different periods of time and temperatures) were analysed. For each protein specific marker peptides were identified, and specificity was proofed *in silico* by BLAST-analysis using the SwissProt database. There are clear differences in the proteome profile depending on the variety and differences in the heat stability of the individual proteins. The relative contents of the types of trypsin inhibitors match the results of values for determination of trypsin inhibitor activity.

Keywords: soybean, trypsin inhibitors, high resolution LC-MS/MS, proteomics

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THEOBROMINE AND CAFFEINE IN FORMER FOOD PRODUCTS INTENDED FOR ANIMAL NUTRITION

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The integration of Former Food Products (FFPs) into animal feed represents a promising circular economy strategy to reduce food waste while maintaining feed safety and animal productivity. FFPs are foodstuffs originally intended for human consumption that are excluded from the retail chain due to non-safety-related issues such as packaging defects, aesthetic imperfections or logistical oversupply. Despite their removal from the food market, FFPs retain high nutritional value. However, potential safety concerns may arise from naturally occurring compounds like methylxanthines, including theobromine and caffeine, especially when FFPs contain chocolate or cocoa-based ingredients. This study aimed to quantify theobromine and caffeine in FFPs containing various food industry products such as baked goods, pasta, confectionery, and cereals by-products. Compliance was evaluated against EU Directive 2002/32/EC, which sets maximum levels for theobromine. Caffeine is not regulated with a maximum limit in feed. However, caffeine can still have physiological effects on animals, so its presence is often monitored. An initial screening for theobromine and caffeine was performed by High-Performance Thin Layer Chromatography (HPTLC); following positive results, quantitative analysis was carried out using High-Performance Liquid Chromatography with UV detection (HPLC-UV). Results indicated that all FFPs were generally compliant with the EU maximum levels (ML) for theobromine in complete feed for ruminants (300 ppm) and pigs (200 ppm), assuming a worst-case inclusion rate of 30% of total dry matter intake. FFPs primarily composed of savoury products exhibited lower theobromine and caffeine concentrations compared to those with a higher inclusion of bakery products. These findings emphasize the need for species-specific evaluations, careful consideration of inclusion rates, and consistent compositional monitoring. Overall, this study supports the safe incorporation of FFPs into livestock diets within regulatory boundaries, highlighting their potential to improve feed sustainability and reduce environmental burdens associated with food waste.

Keywords: former food, ex-food, theobromine, alkaloids, circular economy

A PROPOSAL FOR THE DEVELOPMENT OF A FEED CONSUMPTION DATABASE USING A STANDARDISED FEED CLASSIFICATION SYSTEM: A PROOF OF CONCEPT FOR GMO

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There is currently no comprehensive feed consumption database, leading to animal dietary exposure assessments that rely on default values for body weights, daily intakes and diet composition. The use of these default values, selected from heterogeneous sources (e.g. OECD guidances, EFSA guidances, scientific publications), can lead to inconsistent estimates of feed consumption and may result in over and/or underestimations. Such variations may hinder adequate risk characterisation. In this study, the development of a feed consumption database was proposed. First, a thorough mapping of existing systems and methodologies for feed classification and feed was conducted. Then, a model database and data structure were built, utilising SSD2 controlled terminologies. To validate our model database, two case studies were originally conducted: the first on genetically modified (GM) feed and the second on contaminants. Focusing on genetically modified feed, three different GM maize were selected. The animal dietary exposure to GM proteins was I) retrieved from the correspondent EFSA Scientific Opinions; II) calculated using default values present in the Excel calculator proposed and published by the EFSA GMO Panel in 2023; III) calculated using data retrieved from our model database. Overall, we observed that using the input data extracted from the model database resulted in general lower estimates of animal dietary exposure for the selected case studies. The proof of concept demonstrated that our model database has the potential to harmonise animal dietary exposure assessments and provide values that may better reflect real-world conditions. However, it also highlighted the existing gaps in consumption data and underscored the need for further development. From these results, we recommend establishing an EU classification system where the main features and items reported in the European Catalogue of Feed Materials are harmonised with FoodEx2. Following this harmonisation, we suggest collecting consumption data to populate the database from the feed industry, animal nutritionists, breeders, and farmers, in collaboration with relevant stakeholders.

Keywords: feed consumption database, animal dietary exposure, GMO, feed consumption data

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REPLACING FISH MEAL WITH UNTREATED AND ENZYMATICALLY TREATED TORULA YEAST (*Cyberlindnera jadinii*) AFFECTING PELLETING DIE FLOW RESISTANCE AND PHYSICAL PROPERTIES OF THE FEED PELLETS

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Yeast is gaining importance as a novel feed ingredient. Although fishmeal generally provides better feed conversion in farmed aquatic animals, recent studies suggest that the torula yeast *Cyberlindnera jadinii* is a promising alternative. This study examines the effects of replacing fishmeal with torula yeast, both untreated and treated with protease and endo-exo 1,3-beta-glucanase, on pellet production and quality characteristics. The first experiment evaluated changes in flow resistance and pellet quality when fishmeal was replaced with yeast. The second experiment focused on how enzyme-treated yeast influenced these factors. Diets containing 20% yeast, whether treated or not, showed increased flow resistance and higher pellet strength. Pellets with 10%, 20%, and 100% yeast exhibited water-repellent properties, which improve feed intake, reduce waste, and support sustainable production. However, pellets with 10% and 20% enzyme-treated yeast showed fat-repellent behavior, which may hinder post-production processes. Enzymatic treatment reduced underwater swelling in these pellets, and limited degree of hydrolysis is recommended to prevent pellet disintegration. Enzyme treatment reduced surface roughness, especially in pellets containing 20% treated yeast, which exhibited the smoothest texture.

Keywords: pellets, enzyme, physical characterization, single die pelleting

SINKING SPEED AS A NOVEL QUALITY INDICATOR IN FEED AND FOOD PRODUCTION

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Sinking speed is a sensitive and underexploited parameter for assessing physical product quality in feed and food production. A fully developed and industrially implemented measurement system, called the *sidometer*, now enables precise, automated quantification of this property. The technology provides a statistically robust dataset that offers valuable insight into product behaviour, consistency, and performance across a range of applications.

The most common current method for evaluating aquatic feed performance is the binary sink/float classification, which oversimplifies particle behaviour and conceals variation within a batch. While bulk density is sometimes used as an indirect indicator, it provides only an average and cannot reliably predict individual particle properties. This can lead to quality issues going undetected – especially when slow-sinking particles later float under different environmental conditions.

The *sidometer* overcomes these limitations by capturing the true sinking speed of individual particles as they fall through a water column. This allows for real-time monitoring, earlier detection of process deviations, and faster corrective actions – minimizing waste, reducing environmental impact, and improving end-user satisfaction.

Moreover, even in products not designed with specific sinking characteristics in mind, variation in sinking behaviour may reflect deeper inconsistencies in structure, composition, or process stability. In this way, sinking speed becomes a valuable proxy for quality and process control across both aquatic and terrestrial production environments.

The system also supports simultaneous measurement of particle size and shape, enabling a more comprehensive understanding of how physical attributes influence performance. By making such parameters measurable and monitorable at industrial scale, the *sidometer* represents a significant advancement in the physical characterization of feed and food products.

Keywords: sinking speed, sink/float test, size and shape analysis, feed quality, process monitoring

IMPROVING SAFETY IN ORGANIC FERTILIZATION: TRACKING GLYCEROL TRIHEPTANOATE (GTH) FROM ANIMAL BY-PRODUCTS TO SOIL

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Sustainable agriculture helps protect soil, human health, and the environment by promoting the reuse of by-products and recyclable materials to produce organic fertilizers.

The Fertilizing Products Regulation (FPR) defines safety and quality standards for fertilizers used in the EU. It allows certain processed materials that no longer pose a risk to be freely employed as fertilizers. These organic materials provide essential nutrients and could improve soil health.

In the EU, animal by-products are strictly regulated to protect humans, animals, and the food chain. These by-products are classified into three risk categories, and their handling depends on their associated risk level.

High-risk materials (Categories 1 and 2) must be rigorously separated from low-risk materials (Category 3). High-risk materials must be marked with a synthetic fat called glycerol triheptanoate (GTH) and they are not allowed to enter the food chain.

Delegated Regulation (EU) 2023/1605 defines the end points in the production process of organic fertilisers made in the EU, beyond which they are no longer subject to animal by-product regulations—provided they are used in EU-approved fertilizing products.

A method using GC-MS/MS was validated to measure GTH in fertilizers derived from cat 3 materials and cow manure. The study evaluated the method's performance in terms of fat and GTH recovery, matrix effects, accuracy, and repeatability.

Fat recovery was higher in samples with greater organic matter and lower manure content (15%). GTH recovery ranged from 80% to 120%. Matrix effect acceptability depended on the manure content. The coefficient of variation was below 16%, and the repeatability limit was 51.6 at a GTH concentration of 250 mg/kg. This research supports the monitoring of GTH levels in fertilizers to ensure compliance with EU regulations.

Keywords: organic fertilizer, animal by-product, glyceroltriheptanoate (GTH), sustainable agriculture, GC-MS/MS

Acknowledgements: This research was funded by the Italian Health Ministry Research Grants (Project n. IZS PLV 01/23 RC).

AN ION-SELECTIVE ELECTRODE (ISE) METHOD FOR IODINE DETECTION IN FOOD AND FEED

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Iodine is naturally present in certain foods and is essential for health at all life stages. However, the introduction of novel foods/feeds such as algae may pose a risk of iodine overexposure for both humans and animals. While iodine is necessary for growth and development, excessive intake can lead to adverse health effects, potentially causing hyperthyroidism or hypothyroidism.

A simple ion-selective electrode (ISE) method was developed to determine iodine exposure, particularly in foods naturally high in iodine. Sample preparation involved extraction with 3% (v/v) acetic acid, followed by the addition of an acetate buffer. The extract was then diluted with ultrapure water and filtered. For quantitative iodine determination, an ion meter (Metrohm) equipped with an iodide ion-selective electrode, a pH electrode, a reference electrode, and a magnetic stirrer was used.

The method showed linearity in the range of 5-500 mg/kg ($R^2 > 0.99$) with a quantification limit of 5 mg/kg. EURL-MN PT-2019-01 seaweed meal was used for recovery assessment (assigned value 393±10 mg/kg).

Foods of terrestrial and marine origin, petfood, infant food, supplements, and animal feeds, were analysed. Iodine levels ranged from 5 to 69 mg/kg, with higher concentrations found in marine products and seaweed-based supplements.

Keywords: iodine, feed, ISE

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AUTHENTICATION OF INSECT PRODUCTS IN FEED USING DNA-BASED METHODS

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Insect proteins are gaining global attention as sustainable alternatives to conventional protein sources. Most of the time, insect products are processed into powders with issues mainly concerning species identification, product authenticity, food safety and potential fraud. Increasingly used as ingredients in food and animal feed, it will become mandatory to control that insect-based products are compliant with the strict European legislation especially regarding the insect species and the rearing substrates.

A total of 21 single-species insect meals for feed and 43 multi-ingredient feed products containing insects were collected from various origins (Austria, Belgium, Denmark, France, Germany, Lithuania, Portugal but also New Zealand, UK and USA) and analysed using species-specific real-time PCR assays targeting *Tenebrio molitor*, *Hermetia illucens*, *Alphitobius diaperinus*, *Acheta domesticus*, *Bombyx mori*, and *Gryllodes sigillatus* as well as high-throughput sequencing (HTS) using metabarcoding to check whether the insects claimed on the packaging were actually present. Official real-time PCR assays for ruminant, porcine, and poultry DNA were also performed on single-species insect meals to detect potential remaining animal-derived DNA from unauthorized substrates.

The 21 single-species insect meals for feed all contained the declared species but at least one additional species was detected in 10 samples by real-time PCR and in 6 samples by HTS. Among the 43 multi-ingredient feed products containing insects and predominantly destined to pets, the rate of discordance between the label and the composition is higher.

If most samples contained the declared species, cases of mislabeling, substitution and cross-contamination were identified.

Real-time PCR and high-throughput sequencing (metabarcoding) proved to be highly complementary approaches for species identification. Real-time PCR enables rapid and sensitive detection of specific, targeted species, while HTS offers a broader overview of the taxonomic composition, including the ability to detect non-target or unexpected species (storage pests). Animal DNA was also detected in a limited number of insect meals indicating a potential use of prohibited substrates. These results highlight the urgent need for robust DNA-based authentication methods to support official controls of the European regulatory framework, particularly regarding insect species composition, rearing practices, and accurate labelling, ultimately contributing to the safety and transparency of insect-based products.

Keywords: PCR, HTS, metabarcoding, insects, feed, authentication

Acknowledgements: This research was funded by the European Commission in the frame of Horizon 2020 Public-Private Partnership Bio-Based Industries Joint Undertaking (topic BBI.2018.F2—proposal number 837750 —Large-scale production of proteins for food and feed applications from alternative, sustainable sources) through the FARMYNG project.

DIETARY INCLUSION OF PEA PROTEIN CONCENTRATE: IMPACT ON PRODUCTION TRAITS AND BLOOD CHEMISTRY OF AFRICAN CATFISH FINGERLINGS

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Soybean products are commonly used ingredients in aquafeeds in addition to components of animal origin. Pea protein is a locally produced plant protein source, which is promising candidate for substituting imported soy. Fish feeding research mainly focuses on reducing the fishmeal content of the diets, and there are few results available on whether pea protein/pea protein concentrate can be used to replace soy protein. Aim of our study was to evaluate the suitability of pea protein concentrate (PPC) in the diet of hybrid African catfish (*Clarias gariepinus* x *Heterobranchus longifilis*) fingerlings; furthermore, to determine whether this ingredient can replace the soy content of the feed. Soybean content of a commercial catfish feed was substituted with pea protein concentrate in 25, 50 and 75 %. Thus, the experimental diets contained 5.5%, 11% and 16.5% of PPC. The same diet without PPC inclusion was used as control. After feeding fish for 6 weeks with the experimental diets there were no significant differences in terms of certain production traits (weight gain, specific growth rate, etc.). However higher inclusion levels of PPC showed positive effect on feed conversion ratio and protein efficiency ratio. No significant differences were found in blood chemistry parameters between the treatments, which indicating no signs of metabolic diseases. It can be concluded that PPC was well utilized even in the highest dose (16.5 %) without compromising the zootechnological parameters. These results suggest that pea protein concentrate is a promising alternative to soybean meal as a dietary protein source in the nutrition of African catfish fingerlings.

Keywords: African catfish, alternative protein, pea protein concentrate, production traits, blood biochemistry

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ANIMAL FEED PRODUCTION AND SUSTAINABILITY? THE ROLE OF INSECTS IN AGRICULTURE

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Providing the world's population with adequate quality and quantity of food is an urgent problem due to the limited resources available. In order to ensure high levels of sustainable agricultural production, the importance of insects needs to be reconsidered in this context, highlighting the growing demand for animal proteins, the efficient use of land and water, and the limitations of non-renewable energy sources. Insects are the largest and most diverse group of animals. They have been part of human nutrition since ancient times, and their consumption is still part of traditions in many regions today. A decade ago, FAO advocated for the increased utilization of insects in the production of animal feed, with the dual objectives of environmental preservation and resource conservation. The market for edible insects is constantly growing due to their high nutritional value, the growing demand for insect proteins in animal feeds, and the low risk of zoonoses. The production costs of conventional protein sources (soybean, pea, fishmeal) are exorbitant, and their availability is already constrained. The utilization of insect meal as a supplement to primary protein sources is a viable option, as it provides valuable protein, energy and fat to the animal diet. Furthermore, the palatability of alternative diets is satisfactory, with 25-100% of soy or fishmeal being replaceable, depending on the species. However, due to the low production volumes and high production costs, insect proteins are currently not competitive with fishmeal or soymeal. Nevertheless, efforts to increase production will increase both the availability and quality of the products, thereby reducing costs. In summary, the literature review indicates that expanding the output and use of insects for food and feed can greatly contribute to sustainable agricultural production. However, this requires continued research in this emerging field to expand the knowledge already available, facilitating more efficient production and use.

Keywords: insects, sustainability, insect products, farm animals, feed production

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CIRCULAR ECONOMY: VALORIZATION OF SOLANACEE PLANTS CULTIVATION WASTE PRODUCING INSECT MEAL FROM BSF LARVAE (*Hermetia illucens*) REARING

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The Solanaceae plant family includes several agronomically and industrially significant crops, such as potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum*) and eggplant (*Solanum melongena*). Although their edible parts have a well-established economic value, the voluminous biomass of their aerial residues constitute a serious environmental burden because their rich content in toxic alkaloids prohibits their valorization.

Study herein concerns the utilization of Solanaceae potential of the aerial plant residues as feeding substrates for Black Soldier Fly (BSF, *Hermetia illucens*) larvae rearing. Final goal is their bioconversion to high protein content insect meal for use as livestock feed.

For this purpose the residues of potato, tomato and eggplant cultivations were incorporated into BSF rearing experiments as replacement (by 0%, 10%, 20%, 40%, 60%, 80% and 100%) of the standard poultry feed used for their nutritional experiments. Each experiment included 200 larvae and performed in five replicates, under the following conditions: 30 °C, absence of light and 70% humidity. After their harvesting, larvae were dried, homogenized and their phytochemical content was determined using UHPLC-HRMS Spectrometry. Their nutritional aspects were evaluated through the determination of their nitrogen, protein, fiber, cellulose, lignin, lipids, ash and carbohydrate content, while the absence of harmful alkaloids was verified by performing the *Daphnia magna* bioassay.

Respective the inclusion of 80% Solanaceae aerial biomass in BSF feeding rations. Results herein demonstrate the usefulness of agricultural by-products upcycling as a promising circular bioeconomy strategy.

Keywords: Solanaceae, black soldier fly, insect meal, bioconversion, high protein feed, circular bioeconomy

EXPLOITATION OF MEDITERRANEAN BIODIVERSITY FOR THE DEVELOPMENT OF POTENT ANTHELMINTIC ACTIVITY AGAINST GASTROINTESTINAL NEMATODES IN SHEEP

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Gastrointestinal nematodes (GINs) constitute a major health issue for grazing ruminants worldwide, causing health disorders such as nutrient malabsorption, various clinical symptoms (e.g., anaemia, diarrhoea), reduced productivity, and even death. Currently, their control relies almost exclusively on synthetic anthelmintic (AH) drugs, causing resistance problems and emerging the need to develop their eco-friendly, sustainable alternatives. In this context, the exploitation of tannin and polyphenol rich plant extracts constitutes an intriguing case.

Study herein aimed the evaluation of phytochemical content and *in-vitro* anthelmintic activity of 31 endemic in Mediterranean tannin-rich plant species against third-stage larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis*, using the Larval Exsheathment Inhibition Assay at four concentration levels (1200, 600, 300, and 150 µg/mL in PBS).

Results revealed a strong, dose-dependent AH activity of *Arbutus adrachnae*, *Onobrychis peloponnesiaca*, *Punica granatum*, *Pyrus spinosa*, and *Potentilla hyrardiana* against both parasites. *Quercus coccifera*, *Coronilla varia*, *Olea europaea pulp* and *Aremonia agrimonoides* were effective only at highest concentrations. Species such as *Robinia pseudoacacia*, *Onobrychis alba-laconica*, *Amorpha fruticosa*, *Arbutus unedo* and *Erica arborea* were more effective against *H. contortus*, while *Erica manipuliflora* showed activity primarily against *T. colubriformis*. Finally, *Rosa canina*, *Prunus webii* and *Fragaria vesca* were effective only against *T. colubriformis*, while *Alchemilla bulgarica* only against *H. contortus*.

The determination of plant's phenolic and tannin content highlighted as richest the extracts of *A. andrachne*, *E. manipuliflora*, *Am. fruticosa*, *R. pseudoacacia* and *A. unedo*. Notably, the most effective extract (*On. peloponnesiaca*) contained high levels of both tannins (7.17%) and phenols (7.70%).

These findings support the valorisation potentials of tannin-rich plants as sustainable alternatives of synthetic AHs. Results variability highlights the necessity for further investigation with *in-vivo* experiments to validate their efficacy and safety to support their utilization into integrated parasite management strategies.

Keywords: bioactive plants, anthelmintic activity, tannins, polyphenols

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UTILIZATION OF FRACTIONATED CAMELINA CAKE IN ANIMAL NUTRITION

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The aim of this study was to investigate the effect of fractionation of cold-pressed camelina cake by sieving on the nutritional and functional properties of different fractions. Two *Camelina sativa* varieties, NS Zlatka and NS Slatka, developed by the Institute of Field and Vegetable Crops, Novi Sad, Serbia, were cultivated in 2024 in the South Bačka District, located in the northern part of Serbia. Cleaned and dried camelina seeds were mechanically pressed using a screw press. The obtained camelina cake was then ground into flour in a laboratory mill equipped with a water-cooling system to prevent additional heating during milling. The ground material was subsequently fractionated into three particle-size fractions: >250 µm, >180 µm, and <180 µm, using a laboratory dry sieve system. The analyses included proximate composition, amino acid and fatty acid profiles, antioxidant potential, and water and oil absorption capacities. The finest fraction (<180 µm) contained the highest levels of protein (39.4% in NS Zlatka and 40.3% in NS Slatka), fat (19.4% and 17.7%), and essential amino acids, as well as the highest concentrations of glucosinolates (24.3 and 27.4 µmol/g) and phytic acid (41.1 and 38.4 g/kg). In contrast, the coarsest fraction (>250 µm) was richer in crude fiber (13.0% and 15.3%), exhibited higher antioxidant potential, and showed the greatest water absorption capacity (9.55 g/g for NS Zlatka and 9.61 g/g for NS Slatka), while containing the lowest levels of glucosinolates (19.8 and 21.1 µmol/g) and phytic acid (17.6 and 20.6 g/kg). The obtained results indicate considerable potential for the application of camelina cake and its fractions in animal nutrition, with the possibility of targeted utilization of specific fractions depending on their composition and functional properties.

Keywords: camelina cake, particle-size fractions, antinutritional factors, animal nutrition, functional properties

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EFFECT OF TOTAL REPLACEMENT OF CORN AND SOYBEAN MEAL WITH WHEAT SILAGE AND HEMPSEED CAKE IN LACTATING DAIRY COWS

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The study assessed the impact of substituting corn silage with wheat silage and soybean meal with pelleted hempseed cake on performance, milk composition, and health in lactating dairy cows.

For this purpose, a crossover trial was conducted at the farm of the University of Padua (Italy). Four primiparous Italian Simmental cows (148±85 days in milk, 24±5 kg/d of milk yield) were divided in 2 homogeneous groups and housed in 4 individual pens. The trial comprised 2 periods of 4 weeks each (1 week for adaptation and 3 for experimental), during which the control (CTR), and the wheat silage and hempseed (WSHS) diets were alternately offered to each group. Diets were balanced for crude protein (15.05±0.51%), lipids (2.55±0.12%), NDF (35.86±1.74%), starch (23.81±1.08%) and net energy (1.54 Mcal/kg), all expressed on DM basis.

The DM intake of cows feed with WSHS diet was tendentially higher than CTR diet (21.66 vs 19.30 kg/d, P=0.05). No differences were observed for milk yield (on average 25.46 kg/d) and feed efficiency (on average 1.26 kg of milk/kg of DM intake). On the other hand, compared to CTR diet, milk from the WSHS diet showed higher PUFA content (5.49 vs. 4.19% on the total of fatty acids; P<0.05) especially for n-3 (0.69 vs. 0.50% on the total of fatty acids; P<0.001), enhancing the nutritional profile of milk. No adverse effects were observed on animal health, as confirmed by similar blood glucose, urea, BHB and NEFA. Similar values in both groups within optimal range were observed for pH and rumination time (on average 6.82 and 518 min/d resp.).

These findings suggest that wheat silage and pelleted hempseed cake can effectively replace the conventional feedstuffs in dairy diets, enhancing milk fatty acid profiles without compromising performance or health.

Keywords: alternative feedstuffs, wheat silage, hempseed cake, dairy cows, nutritional strategies

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NUTRITIONAL CHARACTERIZATION AND REGIONAL VARIABILITY OF CORN GRAINS FOR MONOGASTRIC ANIMAL FEEDING IN WESTERN ROMANIA

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The objective of this study was to evaluate the nutritional quality and variability of corn grains used in the diet of monogastric animals, with a focus on samples collected from three counties in western Romania: Timiș, Arad, and Caraș-Severin. The analyzed corn kernels originated from the local hybrid Fundulea 350 were collected from regional grain collection centers after harvest and analyzed to determine their chemical composition, amino acid profiles, and energetic parameters relevant to swine and poultry nutrition. Near-infrared reflectance spectroscopy (NIR) was employed to assess dry matter, crude protein, ether extract, crude fiber, ash, starch, and the concentrations of essential and non-essential amino acids.

The results revealed significant interregional differences. Dry matter content ranged from 85.40% (Caraș-Severin) to 90.50% (Timiș), indicating generally good preservation conditions. Crude protein concentrations exhibited marked variability between regions, with highest values recorded in Timiș (up to 10.72%) and lowest in Caraș-Severin (minimum 6.67%). Lysine levels varied between 0.213% and 0.312%, while methionine ranged from 0.167% to 0.219%. Total starch content was consistently high (61.9–66.6%), supporting substantial energy contributions. Metabolizable energy for growing pigs (ME_{GP}) ranged from 13.61 MJ/kg in Caraș-Severin samples to 14.62 MJ/kg in Timiș samples. All samples contained a high proportion of phytic phosphorus (74–76%), underscoring the need for phytase supplementation to improve phosphorus bioavailability. The variability observed among regions emphasizes the importance of locally specific nutrient analysis to inform precise diet formulation and optimize performance in monogastric animal production.

These findings highlight the critical importance of regional nutrient profiling to support precision diet formulation in swine and poultry production. Targeted selection and strategic blending of corn batches can improve protein supply, amino acid adequacy, and feed energy density, ultimately enhancing production efficiency and sustainability in monogastric systems.

Keywords: corn, nutrition, swine, poultry, NIR

INSECT REARING ON LIVESTOCK RESIDUES: ANALYTICAL CHALLENGES LINKED TO DIET COMPOSITION

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As part of the EU Green Deal and its Circular Economy Action Plan, one objective is to reduce reliance on critical feed materials like soy and minimize food waste. In this context, recycling industrial and agricultural residues for insect rearing offers promise, but also potential sanitary risks. Although currently prohibited, the key question is whether this practice can be effectively controlled.

This study investigated the rearing of Black Soldier Flies (BSF) on waste-based substrates: hatchery by-products, poultry manure, poultry sludge, and fish offal. After the rearing period, larvae were collected, rinsed, oven-dried, and ground into insect meal.

To assess the feasibility of detecting dietary components in larvae, a UHPLC-MS/MS-based proteomics approach was employed. The analysis successfully detected poultry collagen in larvae reared on hatchery by-products. However, matrix-matched evaluation revealed strong matrix effects for other substrates.

Matrix effects in mass spectrometry refer to the influence of complex biological matrices on ionization efficiency and analyte detection. Understanding and mitigating these effects is crucial for reliable and accurate MS-based analysis, especially at trace levels.

This study aimed to identify the sources of matrix effects and explore mitigation strategies. BSF meals were analyzed using both data-dependent acquisition (DDA) and data-independent acquisition (DIA) mass spectrometry. Analyses were conducted on a timsTOF HT (Bruker) coupled to a nanoElute 2 system. DDA data were processed using Mascot and Scaffold 5; DIA data were analyzed using DIA-NN.

The protein database included SwissProt entries for *Sus*, *Bos*, *Gallus*, *Salmo*, and *Hermetia illucens*. In the DDA workflow, various parameters were tested (e.g., specific/semi-specific tryptic digestions and non-specific cleavages). The DIA approach increased sensitivity for low-abundance proteins, crucial for detecting potential dietary contaminants.

Keywords: insect, safety, proteomics, mass spectrometry, circular economy

SAFETY AND NUTRITIONAL EVALUATION OF DUCKWEED (*LEMNA* L.) FOR USE IN ANIMAL FEED

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The growing demand for sustainable alternatives to conventional animal feed ingredients has renewed interest in duckweed as a potential protein source capable of substituting unsustainable ones. This study evaluated the safety parameters and nutritional value of the dried duckweed (*Lemna* L.) powder as a raw material for animal feed formulation. Safety assessment involved the determination of heavy metals and pesticide residues, while nutritional evaluation included proximate composition (moisture, protein, fat, ash, fibre, and carbohydrates), as well as macro- and microelement contents analysed by atomic absorption spectrometry. The fatty acid profile of dried duckweed powder was determined using GC-FID, and amino acid composition was assessed with an amino acid analyser. All analyses were conducted in duplicate.

Results showed that levels of arsenic, cadmium, and lead were below detection limits, while mercury remained below the maximum permissible level for animal feed, reflecting the controlled cultivation of duckweed in clean water. Pesticide residues, including organochlorines, organophosphates, and triazines, were also below detection thresholds, confirming the safety of duckweed for feed use. Nutritional analysis revealed that duckweed has high protein (33.0%) and carbohydrate (50.0%) contents, with relatively low levels of fat (5.3%), ash (5.6%) and moisture (6.0%). The total amino acid content was 33.56 g/100 g, of which 16.97 g/100 g were essential amino acids (EAAs). Leucine, lysine, valine, and arginine were the most abundant EAAs, while glutamic acid was the predominant non-essential amino acid, indicating the nutritional relevance of duckweed protein, particularly its contribution to essential amino acid requirements in animal feed formulations. Duckweed was also rich in iron and manganese. Its fatty acid profile was dominated by polyunsaturated fatty acids (PUFAs), primarily α -linolenic and linoleic acids, with an omega-6 to omega-3 ratio of 0.68, falling within the recommended optimal range for supporting animal health. Overall, duckweed represents a safe, sustainable, and nutritionally valuable feed ingredient for livestock and aquaculture, owing to its favourable safety profile, balanced amino acid composition, mineral richness and beneficial fatty acid profile.

Keywords: feed ingredient, protein source, amino acids, feed safety

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MICROPLASTIC PRESENCE IN ANIMAL FEED OF DIFFERENT ORIGINS

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Microplastic contamination in animal feed is an emerging concern with implications for animal health, food safety, and environmental sustainability. While growing evidence confirms the presence of microplastics in various feed types, it remains unclear to what extent different animal species are exposed through their diets. This abstract presents a critical overview of the current understanding of microplastic contamination in feed, with a focus on how feed composition and ingredient origin contribute to differences in contamination levels across production systems.

Feeds formulated for different species—such as fish, poultry, or ruminants—often vary significantly in their ingredients and sourcing. Marine-derived components like fish meal, commonly used in aquaculture and monogastric livestock diets, are especially vulnerable to contamination due to the high levels of plastic pollution in marine ecosystems. In contrast, plant-based feeds may accumulate microplastics through pathways such as soil contamination, irrigation with polluted water, or airborne deposition. These environmental and compositional factors result in notable differences in both the quantity and type of microplastic particles present in feeds.

This variation has important implications for assessing species-specific exposure risks and for understanding how microplastics may be transferred through the food chain. Furthermore, the lack of standardized sampling and analytical protocols complicates direct comparison between studies and species. Addressing these gaps requires coordinated efforts to monitor microplastic levels across a wide range of feed types and production environments.

This highlights the need for further investigation into the origins and variability of microplastic contamination in animal feed. By improving our understanding of these pathways, we can better evaluate potential impacts on animal health and develop more effective strategies to mitigate microplastic exposure in agricultural systems.

Keywords: microplastics, animal feed, contamination sources, species-specific exposure, food safety.

Acknowledgements: This work was supported by the ECO(RE)ACT project, co-funded by the Interreg IPA Cross-border Cooperation Programme Croatia–Serbia.

DETECTION OF LIVESTOCK RESIDUES USED TO REAR BLACK SOLDIER FLY LARVAE BY NEAR INFRARED MICROSCOPY

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For many years, insects have been seen as one of the solutions for ensuring food security, particularly in view of the increasing demand for alternative protein. Insects offer a wide range of environmental advantages, like the ability to degrade and recycle waste. In Europe, legislation prohibits the use of substrates derived from livestock products and food waste to rear insects¹. Authorizing of these substrates could encourage a circular economy, but this must be supported by appropriate analytical methods to ensure safety. In this context, the Near Infrared Microscopy (NIRM) technique was evaluated on *Hermetia illucens* larvae reared on several fermented substrates containing lactose, based on hatchery residues, slaughterhouse sludge and aquaculture residues. After 10 days of development, the larvae were separated from the substrates, rinsed, dried and finally ground for analysis. A sample of each substrate was collected to establish a reference spectral bank to detect any residues of these substrates in the larvae.

For each substrate type, a PLS-DA model was built. All three PLS-DA models performed very well, with sensitivity and specificity values above 90%. Based on these models, some spectra from analyses carried out on larvae reared on hatchery residues and on slaughterhouse sludge were predicted as being substrate. Besides, no spectra were predicted as substrates for analyses carried out on larvae reared on aquaculture residues. These initial analyses suggest that it is possible to detect the presence of substrate in larvae using NIR spectroscopy methods. However, genomic and proteomic analyses are still required to support these results. Combining these different analytical techniques with improved insect-based products processing could encourage the approval of new substrates without affecting food safety and thus promote a circular economy.

Keywords: insects, livestock waste, feed safety, vibrational spectroscopy

¹European Commission. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). Official Journal of the European Union. 2009; L300: 1–33.

THE INFLUENCE OF FEED COMPOSITION IN CHICKEN FATTENING ON FATTY ACID PROFILE AND HEALTH LIPID INDICES IN THIGH MEAT

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Chicken meat is an important source of nutrients for a balanced diet. This study aimed to determine the impact of n-6 and n-3 PUFA dietary supplementation, from different feed sources, on the fatty acid profile and lipid quality of chicken thigh meat. The study used 700 Ross 308 chickens, divided into two groups: control and experimental (C and E). Fattening lasted 42 days. In the first three weeks, all chickens received a starter mixture, and in the last three weeks a finisher mixture. The finisher mixtures differed in the oil source. The control group received a mixture with 5% sunflower oil, while the experimental group was fed a mixture containing 1.0% fish + 3.0% linseed + 1.0% rapeseed oil. The composition of the mixtures affected the fatty acid profile in thigh meat. A significantly higher content of Σ MUFA and Σ n-3 PUFA was found in the E group compared to the C group ($P < 0.05$). The content of Σ n-6 PUFA was significantly higher in the C group than in the E group ($P < 0.001$). The more favourable Σ n-3 PUFA content in the thigh meat of the E group compared to the C group influenced the Σ n-6/ Σ n-3 PUFA ratio, which was better in the E group ($P < 0.001$). The Σ PUFA/ Σ SFA and LA/ALA indices were significantly higher in the C group than in the E group ($P < 0.05$). The EPA+DHA content was significantly higher in the E group than in the C group ($P < 0.05$). The atherogenic and hyper/hypocholesterolemic indices were similar in both groups, while the thrombogenic index was lower in the E group compared to the C group ($P < 0.001$). The results indicate that the use of designed feed mixtures in chicken fattening can increase the omega-3 fatty acid content in thigh meat, supporting its recommendation for human consumption due to its potential to reduce the risk of thrombosis.

Keywords: chicken, thigh meat, fatty acids, qualitative indices, nutritional indices

Acknowledgements: The data for the paper are part of the research project "Profile of fatty acids in oils and animal samples", which is carried out as part of the IT QualSec activity.

DETERMINATION AND REGULATION OF INORGANIC ARSENIC IN FEED – ARE WE READY?

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The content of arsenic in feed and feed ingredients are regulated through maximum levels (MLs) set in European Union (EU) Directive 2002/32 and amendments. The MLs are for total arsenic, not inorganic arsenic, which is considered as the most toxic form of arsenic. But for some of the MLs a footnote applies; asking “the responsible operator” to demonstrate that the content of inorganic arsenic is lower than 2 mg/kg. MLs for inorganic arsenic are currently discussed by the European Commission (EC), but so far not established. In addition, the EC has issued a recommendation of the monitoring of inorganic arsenic in feed (2022/C 202/01). A question is, are the laboratories in Europe ready for future regulation of inorganic arsenic in feed?

The European Committee for Standardization (CEN) has published a method on the determination of inorganic arsenic in animal feed by HPLC-ICP-MS (EN 17374:2020). The method determines inorganic arsenic (as sum of arsenite and arsenate) using HPLC-ICP-MS following water-bath extraction with dilute nitric acid. The method applies to feed and feed ingredients of marine, animal and plant origin, but not to mineral feeds.

The CEN method is used by several national reference laboratories (NRLs) within the EURL-MN/NRL network, and the EURL-MN has in recent years organised hands-on training on the analysis of inorganic arsenic in feed and proficiency tests to improve the analytical capabilities of the network.

We will provide an overview on the performance on the determination of inorganic arsenic in feed by the EURL-MN/NRL network laboratories and discuss whether the laboratories are ready for an introduction of MLs on inorganic arsenic in feed matrices and future compliance testing.

Keywords: Inorganic As, total As, HPLC, ICP-MS, feed legislation

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COMPARATIVE PERFORMANCE OF MELTING CURVE QPCR FOR SALMONELLA DETECTION IN CULTURES, INOCULATED SAMPLES, AND FEED

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Real-time PCR (qPCR) is a rapid method for detecting pathogens, enabling results in under one day. Feed matrices are challenging for pathogen detection using PCR due to rich endogenous microflora and PCR-inhibitory substances. In this study, we evaluate the specificity of SYBR Green-based qPCR with melting curve analysis as a rapid, cost-effective alternative for *Salmonella* detection in feed. SYBR Green was chosen over probe-based systems due to its lower cost, absence of probe design/validation steps, and demonstrated success in inhibitory matrices.

To our knowledge, this is the first application of fimA-targeted SYBR Green qPCR for *Salmonella* identification in naturally contaminated feed matrices. The fimA gene was selected for its conservation among environmental *Salmonella* strains frequently found in feed.

Four sample types were analyzed: (1) pure *Salmonella* cultures isolated from animal feed, (2) artificially inoculated feed samples (spiked with *Salmonella enteritidis* and *S. agona*), (3) feed experimentally contaminated with a mixed enterobacterial suspension (*S. enteritidis*, *S. agona*, *Escherichia coli*, and *Citrobacter freundii*), and (4) naturally contaminated commercial feed mixtures. DNA extraction was performed using the boiling method for pure cultures and commercial kits for complex matrices. Melting temperature (T_m) profiles were analyzed for all samples.

Results showed consistent T_m values in pure cultures (mean = 85.65 °C, SD = 0.29 °C), confirming specificity. Artificially spiked feed showed slight T_m elevation (mean ≈ 86.21 °C, SD = 0.62 °C), while mixed bacterial contamination resulted in further T_m shifts (up to 87.24 °C, SD = 1.28 °C) and reduced specificity. Naturally contaminated samples exhibited broad T_m variability (positives: mean = 83.78 °C, SD = 5.38 °C; negatives: mean = 86.08 °C, SD = 5.45 °C), with overlap between groups.

In conclusion, while effective in pure cultures, SYBR Green-based qPCR performance decreases in complex feed due to inhibitors and non-specific amplification.

Keywords: *Salmonella*, feed matrices, PCR inhibition, diagnostic reliability, SYBR Green

EVALUATION OF THE POTENTIAL OF PLANT EXTRACTS TO REDUCE SURFACE TENSION AND CORROSION OF ANTIMICROBIAL FEED ADDITIVES

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Traditional antimicrobial feed additives, based on organic acids, salts and surfactants are widely used to control microbial contamination in feed and feed materials. Due to growing awareness of health and environmental sustainability, the demand for natural feed additives has increased. The organic market is expanding rapidly (5-6% CAGR 2030). Synthetic surfactants and corrosion inhibitors are not allowed in organic feed. This study aimed to identify natural alternatives. Phytochemicals like saponins, exhibit surface activity and can reduce metal corrosion. However in plant extracts, saponins are highly variable in composition and effectiveness.

A screening of 37 plant extracts was carried out. Surface tension was measured using a Drop Shape Analyzer system (DSA100; KRÜSS GmbH). Corrosion inhibition was evaluated in an accelerated test measuring weight losses of mild steel (S235jr) after 11 days incubation at 55°C. An antimicrobial feed additive product (AMFA) was formulated based on the most potent plant extracts. Physical stability of the formulation was visually evaluated at different temperatures. The efficacy of 3 kg/T AMFA against *Enterobacteriaceae* was evaluated in an artificially contaminated broiler feed. *Enterobacteriaceae* enumeration was performed two days after treatment.

An optimised blend of plant extracts could significantly lower both surface tension and corrosivity (ANOVA, $p < 0.05$). In AMFA, the surface tension was lowered from 50.54 mN/m to 38.90 mN/m when the blend was added. The blend of plant extracts significantly reduced corrosivity of AMFA, with mild steel plate weight loss dropping from 1.22% to 0.98%. AMFA caused a reduction in *Enterobacteriaceae* of 0.79 log cfu/g compared to the untreated control.

The study demonstrated that a carefully selected blend of plant-based extracts can effectively replace synthetic additives. The new AMFA formulation offers numerous benefits including antimicrobial activity, corrosion inhibition and surface tension reduction, making it suitable for applications in organic feed.

Keywords: antimicrobial feed additives, plant extracts, organic feed market

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KINETIC STUDY OF TERPENE TRANSFER FROM DIET TO MILK AND CHEESE IN DAIRY COWS

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Dairy cow diets are often rich in terpenes, which can influence the composition, properties, and aroma of milk and derived products such as cheese. This study investigated the kinetics of terpene transfer from feed to milk and cheese, using two aromatic plants, hemp (*Cannabis sativa* L.) and savory (*Satureja hortensis* L.), as terpene sources. Six dairy cows were randomly assigned to three groups: a control group fed a total mixed ration (TMR group) only, and two treatment groups receiving TMR supplemented with either fresh hemp (CS group) or savory leaves (SH group). Over six days, treatment groups were fed increasing doses of the respective fresh leaves (0.1% to 2% of TMR dry matter). From day 7 to 14, all cows received only the standard TMR. Milk was sampled daily (from day 1 to 14), and milk collected on day 7 was used to produce raw milk cheese, aged for three months. Terpene concentrations in feed, milk, and cheese were analyzed via HS-SPME/GC-MS. A total of 24 terpenes were identified across feed and milk samples. Hemp had the highest total terpene content (85,962 µg/kg), followed by savory (11,313 µg/kg) and TMR (228 µg/kg). Milk terpene levels peaked on day 7 and declined rapidly after supplementation ceased, with compound-specific half-lives ranging from 0.67 to 1.99 days. Transfer efficiency of terpenes was highest for TMR cows (15.4%), followed by CS (8.7%) and SH (6.2%), likely due to differences in molecular structure, ruminal degradation, and metabolic fate. Key terpenes such as β-caryophyllene, α-farnesene, and p-cymene showed effective dose- and time-dependent transfer from feed to milk. Although no terpenes were detectable in the aged cheeses, sensory and structural properties were notably influenced by dietary supplementation. These results highlight that while terpenes are not retained in the final product, their transient presence can still significantly alter the sensory profile of dairy products.

Keywords: terpene transfer, dairy cows, officinal plants, milk and cheese flavor, volatile compounds.

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THE EFFECTS OF DIETARY MILK LIPIDS ON DIABETES-RELATED METABOLISM: A BIBLIOGRAPHIC STUDY

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The paper explores the complex relationship between milk lipids and human health, with a particular focus on their implications on diabetes-related metabolic pathways. Lipids are not only energy-rich nutrients but also essential bioactive compounds that influence cardiovascular, metabolic and immune functions. thus, the most relevant milk lipids are presented along with their potential effects on consumers health.

Saturated fatty acids, such as palmitic and myristic acid, are linked to elevated LDL cholesterol, inflammation, insulin resistance, and hepatic lipid accumulation. In contrast, monounsaturated (oleic, palmitoleic) and polyunsaturated fatty acids (linoleic, alpha-linolenic) are linked to reduced inflammation, enhanced insulin sensitivity, and protection against cardiovascular disease. Lipids from other classes such as sphingolipids and phospholipids support intestinal, neural and immune health, while also showing potential anticancer and anti-inflammatory effects.

The paper presents details on how excessive intake of saturated and trans fats contributes to obesity, cardiovascular diseases, type 2 diabetes, and chronic inflammation, highlighting the importance of balanced lipid intake. It also discusses the interdependence of lipid and glucose metabolism, emphasizing the Randle effect and the role of insulin in energy homeostasis.

Future research directions are discussed, including optimizing the fatty acid profile of animal-derived foods, understanding lipid-mediated cellular mechanisms and exploring their role in preventing metabolic disorders for improved consumer health.

Keywords: milk, lipids, health, diabetes

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HISTAMINE IN PET FOOD

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Histamine is a biogenic amine formed by microbial decarboxylation of histidine and serves as a significant indicator of protein degradation in animal-based raw materials. Although traditionally associated with fish and fishery products, recent studies have confirmed its presence in pet foods containing fish and meat meals. Factors such as improper storage, elevated temperatures, and prolonged pre-processing intervals significantly contribute to the accumulation of histamine. Regulatory limits for histamine exist in foods intended for human consumption; however, comparable legal thresholds for pet food are currently lacking. Nevertheless, elevated histamine levels may negatively impact palatability, nutrient absorption, and animal health, particularly in sensitive populations such as juveniles and immunocompromised pets. This study examines histamine levels in various animal-derived raw materials and commercial pet foods (both dry and canned), with a particular focus on chicken, game, and fish meals. An HPLC-UV method was used to analyze 13 protein meal samples and 14 pet food samples. Histamine was not detected in shrimp, lamb, quail or pork protein meals. In chicken and fish meals, histamine content ranged from 23.86±2.60 to 256.98±14.69 mg/kg. In 8 out of 10 dry pet food samples, histamine levels ranged from 9.18±0.56 to 54.24±4.12 mg/kg. Canned pet food samples were free of detectable histamine.

Keywords: histamine, meat meal, fish meal, pet food

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NATURAL ALTERNATIVES TO REDUCE THE IMPACT OF CLIMATE CHANGE ON COCCIDIOSIS

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Climate change is reshaping the epidemiology of coccidiosis in poultry by influencing key environmental factors on farms. Warmer temperatures accelerate sporulation and increase oocyst viability, which enhances infection potential. Rising heat and frequent heatwaves place poultry under thermal stress, leading to immunosuppression and worsening the severity of coccidiosis. At the same time, shifts in precipitation patterns create humid conditions that favor oocyst survival and persistence in litter, raising the risk of environmental contamination and transmission within flocks. Changing climate conditions may also expand the geographic distribution of *Eimeria* species into regions that were previously unaffected. This not only increases the incidence of the disease but also creates new challenges for areas lacking established control strategies, often resulting in significant economic losses.

Another pressing issue is the growing reliance on anticoccidial drugs in response to increased disease pressure. Such dependence can accelerate the development of resistant strains of *Eimeria*, complicating management and contributing to treatment failures. Moreover, many chemical coccidiostats are less suitable under high-temperature conditions, as they can alter water intake and disturb electrolyte balance, further undermining flock health.

These limitations highlight the importance of natural alternatives. Phytogenic compounds offer a sustainable solution by combining anticoccidial efficacy with broader health benefits. In one of our recent studies, 1,150 one-day-old ROSS 308 broilers were allocated into seven groups with nine replicates each to evaluate phytomolecule-based products. Results confirmed that natural alternatives can be successfully integrated into coccidiosis control programs. Beyond reducing infection, these compounds provided strong anti-inflammatory and antioxidant effects, supported gut health, and reduced the need for conventional coccidiostats, thereby helping mitigate antimicrobial resistance. Based on the anticoccidial index, in-feed prophylaxis with P1 and P2 was partially effective in controlling experimental coccidiosis, while in-feed prophylaxis with P3 was moderately effective, comparable to standard coccidiostats.

Keywords: coccidiosis, antimicrobial resistance, natural additives

EFFECTS OF DEOXYNIVALENOL AND FUMONISIN B1 ON GROWING-FINISHING BROILERS AND THEIR ATTENUATION WITH IN-FEED INTERVENTION

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Mycotoxins are secondary metabolites of fungi, commonly found in cereals, and proven to exert toxicity in production animals. In-feed interventions that reduce mycotoxin exposure and effects can be used to mitigate their impact on poultry. Broilers in growing and finishing phases are considered less sensitive to mycotoxins than young broilers. However the consequent higher exposure can hinder their health and performance. Fumonisin B1 (FB1) and deoxynivalenol (DON) were tested at levels representative of naturally occurring contamination in commercial feeds to evaluate their effects in growing-finishing broilers with and without an in-feed intervention (IFI). Four-hundred and eighty day-old broiler chickens were divided in four groups with 10 replicates of 12 birds. All groups received the same diet from day 1 to 10. From day 11 to 42, the broilers were fed either the control diet (CON – no challenge) or the challenge diet (CHL –ca. 5000 µg/kg FB1 and 2000 µg/kg DON), each supplemented (IFI, 2g/kg feed) or not with the test product. The CHL birds showed lower weight and growth rate between day 11 and 24 ($p<0.05$), and a higher feed conversion ratio (FCR) from day 24 to 42 ($p<0.05$). In both periods, birds in the CHL+IFI group had similar performance to the CON group. On day 30, the intestinal mucosa of CHL broilers showed higher inflammation (increased AGP and iALP, $p<0.01$), and permeability (elevated serum *Escherichia coli* LPS, $p=0.001$); effects also mitigated by the IFI. Intestinal lesion scoring and histomorphometry, as well as hepatic histopathology revealed a negative impact of the mycotoxin challenge ($p<0.05$), avoided by the IFI. In summary, the study confirms that a co-exposure to DON and FB1, at levels lower than the EU guidance, affects the metabolism and performance of growing-finishing broilers. Moreover, the tested in-feed supplementation effectively prevented the mycotoxin-induced changes, making it an interesting strategy to manage mycotoxin risk in broiler production.

Keywords: mycotoxin, broilers, gut health, deoxynivalenol, fumonisin

IN VITRO CHARACTERIZATION OF OLIVE POMACE AS A FUNCTIONAL INGREDIENT FOR SUSTAINABLE FEED SYSTEMS

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The growing need to enhance sustainability and ensure food security in agri-food and livestock systems has driven the valorization of agro-industrial co-products. In the present olive pomace (OP), a co-product of oil production, was analyzed *in vitro* for its nutritional and functional properties in feed and cellular food production. An *in vitro* digestion model was used to evaluate the bioaccessibility of phenolic compounds and the antioxidant capacity of OP. Olive pomace showed an interesting nutritional profile, in particular for lipid content (18.10 ± 0.07 g/100 g), although characterized by a low dry matter digestibility (31.49 ± 1.57 %). In parallel, OP showed a relevant antioxidant capacity over the digestive process. The inclusion of OP in piglet diets at 2.5% and 5% improved functional activity without significantly compromising digestibility values, compared to the control diet. Finally, OP as a functional ingredient in C2C12 muscle cell culture medium, for cellular agriculture application, sustained cell viability at concentrations of 0.015 mg/mL (105.76 ± 5.91 % viability), 0.03 mg/mL (101.27 ± 5.28 %) and 0.07 mg/mL (102.51 ± 5.95 %), although statistically ($p < 0.05$) lower than 10% FBS. The results obtained suggest the potential of OP as a sustainable functional ingredient, yet to be optimised for cellular agriculture.

Keywords: Co-product; functional activity; in vitro digestion; olive; pomace.

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OPTIMIZING PROTEIN EXTRACTION FROM EDIBLE INSECTS: THE ROLE OF PRETREATMENT IN *TENEBRIO MOLITOR* PROCESSING

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The growing demand for sustainable protein sources has positioned edible insects as promising alternatives, making optimization of protein extraction critical for advancing their application in food and feed industries. This study investigated the effects of pretreatment methods, extraction conditions, and processing parameters on protein extraction efficiency from *Tenebrio molitor* larvae. Three pretreatment methods were evaluated: freeze-dried full-fat larvae, defatted freeze-dried larvae, and blanched-and-dried larvae. Alkaline extraction was performed at pH 10, 11, and 12, followed by isoelectric precipitation at pH 4.0, 4.5, and 5.0, using dilution ratios of 1:4 and 1:6 (w/v). Protein content was determined using the Kjeldahl method, with protein recovery calculated as percentage of the initial protein content.

The pretreatment methods significantly affected extraction efficiency. The highest protein content (83.5%) and recovery (17.5%) under optimal conditions (pH 11 extraction, pH 4.5 precipitation, 1:6 dilution) were obtained from freeze-dried defatted larvae. The defatted samples consistently produced protein concentrates exceeding 80% purity with recovery rates greater than 14%, while the full-fat larvae achieved 55-75% protein content and 11-15% recovery. The blanched-and-dried samples showed inferior performance with protein content ≤50% and recovery ≤10%.

Extraction pH optimization revealed that increasing pH from 10 to 11 improved both protein purity (2-5% increase) and recovery (~8% increase) across all pretreatment methods. Further pH increase to 12 improved recovery in defatted samples without additional purity gains. Precipitation at pH 4.5 produced maximum protein purity, while 1:6 dilution ratio improved both purity and recovery compared to 1:4 ratio.

The findings indicate that defatting before alkaline extraction, combined with pH 11-12 extraction, pH 4.5 precipitation, and 1:6 dilution ratio, optimizes protein extraction from *T. molitor*, achieving protein concentrates with >80% purity and recovery rates approaching 18%. These results provide crucial insights for formulating industrial processes designed to optimize both yield and quality of insect protein.

Keywords: *Tenebrio molitor*, protein extraction, alkaline solubilization, protein recovery, edible insects

CHARACTERIZATION OF PACKAGING REMNANTS AND CHEMICAL CONTAMINANTS IN FORMER FOODSTUFF PRODUCTS FOR ANIMAL NUTRITION

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Former foodstuff products (FFPs) are increasingly seen as promising circular feed ingredients that can enhance sustainability in livestock production by reducing food waste, improving resource efficiency, and promoting feed self-sufficiency. However, their safe use requires monitoring for physical and chemical contaminants, particularly from packaging residues and legacy pollutants.

This study aimed to (1) quantify packaging remnants and regulated contaminants in FFPs, and (2) investigate correlations between packaging materials and contaminant levels. Fifteen FFP samples were analyzed for packaging fragments using μ -FTIR spectroscopy, identifying plastics, aluminium, and polysaccharides (e.g., paperboard). Metallic trace elements (MTEs: Al, As, Cd, Pb) were measured by ICP-MS, while persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), dioxins, and furans (PCDD/Fs) were assessed by HRGC-MS/MS.

Packaging remnants were frequently detected, particularly polysaccharides (16.9 ± 11.7) and plastics (6.5 ± 6.6 pieces per 60 g sample). Although contaminant concentrations varied across samples, all remained below EU regulatory thresholds. Arsenic ranged from 0.01–0.12 mg/kg, Pb from 0.03–0.19 mg/kg, and Cd was detected in only five samples (0.05–0.06 mg/kg). The sum of indicator PCBs ranged from 0.14–0.63 μ g/kg, and total TEQ values for PCDD/Fs and dioxin-like PCBs ranged from 0.03–0.11 ng TEQ/kg.

Spearman correlation analysis revealed associations ($P \leq 0.05$) between aluminium residues and hepta/octa-chlorinated PCDD/Fs ($r = +0.56$ to $+0.71$), polysaccharides with PCDD/F TEQ ($r = +0.77$) and Pb ($r = +0.59$), and plastics with Pb ($r = +0.61$). However, these results should be interpreted with caution due to unknown background contamination, variable amount and type of packaging materials identified, and small number of samples analyzed.

Although current contamination levels pose no immediate risk, the frequent presence of packaging fragments highlights the need for improved processing strategies and continued monitoring to ensure the safe integration of FFPs in sustainable feed systems.

Keywords: Former foodstuff products, feed safety, packaging residues, persistent organic pollutants, sustainable livestock

SLAB51 PROBIOTIC FORMULATION IN RABBIT DIET: EFFECTS ON GROWTH PERFORMANCE, ANTIOXIDANT STATUS, CAECAL ENVIRONMENT, AND INTESTINAL HISTOMORPHOLOGY

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This study investigated the impact of dietary supplementation with the probiotic formulation SLAB51 on growth performance, blood biochemical parameters, antioxidant enzyme activity, caecal short-chain fatty acid (SCFA) profile, intestinal morphology, and caecal microbiota composition in growing rabbits. Eighty Italian White rabbits, six weeks of age, were randomly allocated to two dietary groups. The control group received a standard diet without additives, while the experimental group was fed the same diet supplemented with SLAB51 (SivoMixx®) at a concentration of 250 mg/kg. The trial lasted until 12 weeks of age. Rabbits receiving the probiotic-supplemented diet exhibited significantly greater daily weight gain, final body weight, and feed conversion efficiency compared to the control group. Additionally, improvements in slaughter weight and carcass dressing percentage were observed. No significant differences were found in meat protein, lipid, or ash content between groups. Probiotic supplementation significantly reduced serum levels of total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides. Antioxidant enzyme activities, including glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD), were significantly increased, while levels of thiobarbituric acid-reactive substances (TBARS) were significantly decreased in the probiotic group. Although caecal pH, ammonia-N, and total volatile fatty acid concentrations were not significantly affected by diet, the molar proportions of individual VFAs were altered, with significantly higher concentrations of acetic and butyric acids observed in the probiotic group. Furthermore, SLAB51 supplementation promoted beneficial microbial populations (*Lactobacillus* spp., *Bacteroides* spp.) while suppressing potentially pathogenic bacteria (*Escherichia coli*, *Bacillus* spp., *Clostridium* spp.). Histomorphological analyses revealed enhanced gut structure in probiotic-fed rabbits, evidenced by increased villus height, villus width, and crypt depth. Overall, dietary inclusion of SLAB51 improved growth performance, modulated serum biochemical and antioxidant profiles, supported intestinal health, and promoted a beneficial microbial balance in the caecum of growing rabbits.

Keywords: rabbit, probiotic, health; gut features; microbiota.

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VALORIZATION OF *HERMETIA ILLUCENS* LARVAL FRASS: COMPOSITION AND AGRICULTURAL POTENTIAL

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The by-product of *Hermetia illucens* (black soldier fly) larval rearing-larval frass-represents a value-added and sustainable material with significant potential for circular economy applications in agriculture. This study evaluated the physico-chemical composition and agronomy potential of frass derived from *H. illucens* larvae reared on agricultural by-products, primarily grains and bran, processed to an optimal consistency for larval digestion. The frass was naturally dried without additional heating to preserve its inherent properties.

The analyses revealed a high content of stable organic matter, with organic carbon at 28.7% and total organic matter at 87.3%. The C/N ratio of 11.56 indicates a well-balanced and mature product. Total nitrogen was measured at 2.49%, reflecting a moderate nutrient level, while total phosphorus (P₂O₅) at 1.34% and total potassium (K₂O) at 5.16% demonstrate adequate phosphorus and high potassium content, respectively. Additionally, micronutrient analysis showed calcium (Ca) at 0.89%, magnesium (Mg) at 0.27%, manganese (Mn) at 29.8 mg/kg, iron (Fe) at 327.01 mg/kg, zinc (Zn) at 53.44 mg/kg, and copper (Cu) at 18.5 mg/kg, all contributing essential elements for plant growth and metabolic functions.

These characteristics position *H. illucens* frass as an effective organic fertilizer that enhances soil health, structure, and microbial activity. Its elevated potassium levels make it particularly suitable for fruiting vegetables, root crops, and orchards. Furthermore, its micronutrient profile supports essential plant metabolic functions, making it suitable for both, conventional and organic farming systems. Additionally it could be also used in precision agriculture system. Overall, *H. illucens* larval frass offers a sustainable and circular economy solution for agricultural waste valorization, promoting eco-friendly practices and improved soil fertility.

Keywords: larval frass, *Hermetia illucens*, organic fertilizer, circular economy, sustainable agriculture

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INFLUENCE OF GRINDING PARAMETERS ON DRY FRACTIONATION EFFICIENCY IN SUNFLOWER MEAL PROCESSING

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Sunflower meal is a relatively cost-effective protein source for animal feed. However, its high cellulose content limits its broader use in diets for monogastric animals. Fractionation methods can reduce the cellulose content while simultaneously improving the protein content of sunflower meal. This study aimed to optimize a dry fractionation process for sunflower meal in order to reduce cellulose content and enhance protein concentration, thereby improving its suitability for monogastric animal diets.

Applied dry fractionation process included two-step grinding (hammer and roll milling) followed by sieving. The sunflower meal was first pre-ground using a hammer mill equipped with sieves of different opening diameters (SOD – 2, 4, and 6 mm). The pre-ground meal was subsequently milled on a laboratory roll stand, where all operating parameters, except for the differential ratio ($d=2$), were varied according to a central composite design at three levels: roll gap (0.15; 0.20; 0.25 mm), feed rate (0.100; 0.175; 0.250 kg/cm·min), and fast roll speed (400; 500; 600 rpm). Following grinding, the sunflower meal was fractionated using a sieve with an aperture size of 500 μm . Grinding parameters were evaluated in relation to cellulose content, low-cellulose fraction yield, and grinding energy consumption.

The developed models for all the three responses demonstrated good predictive performance, with coefficients of determination (R^2) of at least 0.82. Additionally, the lack-of-fit tests confirmed the adequacy of the models, as indicated by statistically insignificant p-values ($p > 0.05$). Only the input parameters with statistically significant effects ($p < 0.05$) were considered when evaluating their influence on individual response variables. Results showed that SOD had a strongly dominant influence (74.6%) on cellulose content, with its increase leading to a reduction in this response. The greatest impact on the yield of the low-cellulose fraction was observed for the roll gap (41%), followed by SOD (25.6%) and feed rate (21.4%), where an increase in each of these parameters resulted in a decrease in fraction yield. The most significant effect on the grinding energy consumption was attributed to the roll gap (63.2%), followed by the feed rate (19.4%). Wider roll gap led to a decrease in grinding energy consumption, whereas an increase in feed rate caused grinding energy consumption to rise. These results, which highlight the dominant influence of roll gap and feed rate (parameters related to the roll mill) on the studied responses, are particularly important since they represent technological parameters that can be easily adjusted during operation, thereby directly influencing the outcomes of fractionation. In addition, the proposed dry fractionation process is simple, economical, and easily scalable, requiring no large investments for industrial implementation, while offering flexibility in producing fractions with different protein contents and yields.

Keywords: Dry fractionation process, sunflower meal, hammer mill, roll mill, cellulose content

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ACHETA DOMESTICUS PROTEIN ISOLATE: A PROMISING INGREDIENT FOR THE FEED INDUSTRY

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Due to population growth, economic development, rising energy consumption, and changing consumer habits, the global demand for food continues to increase. In line with sustainability and circular economy principles, edible insects have gained attention as a valuable protein source for both food and feed. *Acheta domesticus* (house cricket), originally found in Southwest Asia, is now one of the most widely farmed insect species worldwide. While consumed in some cultures, it is also widely used as feed for exotic pets.

This study aimed to optimize protein isolation from *Acheta domesticus* and evaluate the techno-functional properties of the extracted proteins. Protein isolation was performed using an alkaline extraction method, modified with pH shifting and double extraction to improve yield and purity. Additionally, various biological and techno-functional characteristics of the obtained protein isolate were assessed.

The isolated protein content reached 68.88%, with a favourable amino acid profile; glutamic acid was most abundant, while methionine was the limiting amino acid. Electrophoresis revealed five prominent protein bands, including low molecular weight proteins (<14 kDa) known for their biological functions. Antioxidant activity was confirmed through ABTS assays, demonstrating the antioxidative potential of the cricket protein isolate. Additionally, enzymatic hydrolysis of the protein isolates yielded hydrolysates with antimicrobial activity. The oil and water binding capacities were 0.51 and 1.51, respectively, higher than those of some leguminous plants.

These findings suggest that protein isolates from *Acheta domesticus* could be valuable in the feed industry to improve the biological and nutritional quality of animal feed.

Keywords: crickets, proteins, nutritional value, biological properties

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QUALITY AND SAFETY ASSESSMENT OF COMPLETE FEED MIXTURES FOR MONOGASTRICS IN COMMERCIAL PRODUCTION: A TWO-YEAR MONITORING STUDY IN SERBIA

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This two-year study aimed to systematically evaluate the quality and safety of complete feed mixtures formulated for monogastric animals by analyzing samples collected from a commercial feed production facility. The research focused on a comprehensive assessment of microbiological contaminants, chemical hazards-including mycotoxins, organochlorine pesticides, and heavy metals-and core nutritional parameters, in order to detect potential deviations from legally prescribed safety and quality standards prior to feed market placement.

Among all feed safety parameters, aflatoxin contamination has become the most critical concern, primarily driven by adverse climatic conditions and climate change-an issue of increasing importance both in Serbia and worldwide. Throughout the years 2023 and 2024, a total of 60 feed samples were systematically collected and analyzed as part of the official regulatory controls to ensure compliance with safety and quality standards. Aflatoxin B1 was detected in 25% of the samples, with 8.3% exceeding the maximum allowable limit of 5 ppb or 20 ppb, depending on the specific category of animals, while 16.7% contained levels within the acceptable legal range. In the remaining 75% of samples, aflatoxin B1 levels were below detectable limits (<1 ppb). Nutritional analysis revealed that the feed mixtures were rich in essential nutrients, with crude protein content ranging from 13.0% to 18.8%, and fat content reaching up to 4.3%, supporting their classification as valuable dietary components for monogastrics.

Although the majority of samples met current regulatory criteria, the presence of non-compliant results-particularly in relation to mycotoxin contamination-highlights the importance of continuous quality control and monitoring throughout the feed production process. These findings contribute to the body of evidence supporting proactive risk management in feed manufacturing and offer a scientific basis for the refinement of safety protocols aimed at protecting animal health and promoting sustainable livestock production systems.

Keywords: complete feed, monogastric animals, feed safety, mycotoxins, nutritional quality

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SCALE-UP EXTRACTION OF PROTEIN FROM BROCCOLI LEAF WASTE WITH POTENTIAL FOR FOOD AND FEED APPLICATIONS

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In line with the principles of circular economy and sustainable resource use, this study explores the scale-up extraction of protein from broccoli leaf waste—an underutilized by-product of vegetable production with notable nutritional and functional potential.

A scale-up process was implemented using a 45 L temperature-controlled extractor with mechanical stirring, enabling efficient handling of viscous suspensions. Dried broccoli leaves (1.5 kg) were suspended in 25 L of water (16.6% w/v), and proteins were extracted under alkaline conditions (pH 10) at 35 °C for 30 minutes. After separation and isoelectric precipitation (pH 4), the obtained protein isolate was centrifuged, dried, and milled. The final product contained 45% protein and demonstrated excellent techno-functional properties, particularly in terms of solubility, suggesting applicability within food and feed systems.

Amino acid analysis confirmed a high content of essential amino acids (>40%), with an EAA/NEAA ratio of 0.77, indicating a favourable nutritional profile for use as an additive in animal feed, especially in premium pet food and aquaculture. Solubility of the broccoli leaf protein was highest under alkaline conditions (36.4 mg/mL at pH 10), which may positively influence digestibility and functional behaviour in food and feed formulations.

Importantly, the fibrous residue remaining after protein isolation retained a high content of fiber and structural polysaccharides, indicating potential use as valuable component in ruminant feed. This enables complete valorisation of the biomass.

This scalable approach demonstrates the feasibility of converting agricultural side-streams into value-added feed and food ingredients, contributing to agri-food waste reduction and sustainable protein sourcing. The process supports global sustainability goals through improved resource efficiency and development of novel plant based source, particularly in the context of functional feed formulations.

Keywords: broccoli leaf waste, plant protein, scale-up extraction, feed additive, fiber-rich residue

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SAME INGREDIENTS, DIFFERENT OUTCOMES: A BUOYANCY-BASED EXTRUSION STUDY IN EUROPEAN AND AFRICAN CATFISH FEED

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While having the similar nutritional requirements European catfish (EC) and African catfish (AC) require the feed of different buoyancy. While the EC is bottom dweller and needs sinking feed, AC as surface feeder, requires floating pellets that can be consumed near the water surface.

The aim of the study was to develop the extruded feed for two separate feeding trials for EC and AC using pilot scale twin-screw extruder. The same diet for both species was formulated, containing approximately 450 g/kg of protein and 110 g/kg of fat, where 50 g/kg of black soldier fly oil was added directly in the mixer and the flexibility of extrusion technology was used to develop both sinking and floating pellets for EC and AC, respectively.

The required pellet size was 3.0 – 3.5 mm, thus die with one round opening of 3 mm was used for both feeds. The moisture content of mash was targeted in preconditioning step at 250 g/kg prior extrusion. The promotion of product expansion was needed to produce floating pellets, thus temperature profile of the barrel was set to ensure temperature above 100 °C at the die together with higher pressure build up in the barrel for AC feed. For this reason, screw configuration for AC feed ensured more reversed screw elements to promote cooking of starch and build up more pressure in the extruder barrel. Also, increase in feed rate (35 vs. 40 kg/h for EC and AC, respectively) and screw speed (450 vs. 600 rpm for EC and AC, respectively) had to be executed for getting the product with floating ability. The higher moisture content of the material in the barrel was needed for floating feed extrusion (270 vs. 300 g/kg for EC and AC, respectively), for promoting product expansion, which resulted in almost the same energy consumption of the extrusion process (approximately 65 Wh/kg). Listed alterations of the input extrusion parameters resulted in two feeds with required physical characteristics and the same nutritional trait for both catfish species, while no differences in extrusion energy consumption was recorded.

Keywords: catfish, aquafeed, extrusion, pellet buoyancy

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BIOFILM FORMATION OF *Listeria monocytogenes* STRAINS ISOLATED FROM MEAT UNDER DIFFERENT GROWTH CONDITIONS

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Listeria monocytogenes is an important gram-positive foodborne pathogen that causes the serious illness known as listeriosis in both humans and animals. Due to its ubiquitous nature, *L. monocytogenes* can tolerate harsh conditions such as grow at refrigeration temperatures, and many outbreaks are associated with the consumption of contaminated food, particularly ready-to-eat products like deli meats, unpasteurized dairy, and fresh produce, as the main route of transmission to humans. The persistence of *L. monocytogenes* in food-related environments may be due to incomplete removal of bacterial contamination in the food supply chain caused by biofilms. With this in mind, the present study aimed to evaluate the ability of the reference strain *L. monocytogenes* ATCC 19111 and eight *L. monocytogenes* strains isolated from the meat industry to form biofilm on polystyrene surfaces. Bacterial biofilms were formed in two different media such as Tryptone soya yeast extract broth (TSYEB) and Brain-heart infusion broth (BHI) at different temperatures 7 °C, 25 °C, 37 °C, 42 °C for 5 days. The method used to assess biofilm formation was crystal violet staining. Results showed that all strains were able to form biofilm, highlighting that biofilm formation capacity varies among strains and was influenced by growth conditions. The most effective medium in promoting biofilm production by *L. monocytogenes* isolates from meat was BHI medium while for reference strain *L. monocytogenes* ATCC 19111 it was TSYEB. On the other hand, data indicated that temperature is a very important factor that considerably affects the biofilms of tested strains, with the lowest biofilm formation observed at 7 °C. Overall, this study provides valuable information for better understanding of *L. monocytogenes* biofilm formation on polystyrene surface, and gives awareness to food supply chain and environment for contamination with microorganisms capable to adhere.

Keywords: biofilm formation, *Listeria monocytogenes*, growth medium, temperature

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CAMELINA, HEMP AND FLAX SEEDS AS VALUABLE PLANT-BASED SOURCES OF PROTEIN AND AMINO ACIDS IN ANIMAL FEED

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The increasing global demand for livestock products creates a need for more sustainable and efficient animal feed, particularly in protein-rich sources. Camelina, hemp and flax seeds offer promising, plant-based alternatives to traditional protein sources like soybean meal, addressing challenges related to feed production and sustainability. The aim of this study was to evaluate the potential of camelina, hemp and flax seeds as alternative protein sources for animal feed, focusing on their amino acid profile. Chemical composition analyses of the seeds were determined by AOAC methods, while determination of amino acid composition were performed by ion exchange chromatography using a Biochrom 30+ automatic amino acid analyzer. The results showed a high protein content of camelina seeds reaching 30.61% and a more favorable amino acid profile, especially with regard to lysine, the limiting amino acid in many seed-based proteins. Camelina, hemp and flax seeds had different levels of total essential amino acids (TEAA), with the highest TEAA content of 10.67 g/100 g in camelina seeds, followed by hemp seeds with 8.84 g/100 g, and flax seeds with 6.87 g/100 g. These essential amino acids, which include threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, and lysine, accounted between 30.60% and 36.77% of the total amino acids. Among the non-essential amino acids in these seeds, aspartic and glutamic acids together accounted for the largest portion, specifically between 24.12% and 29.81%, of their non-essential amino acid content. These cultures, rich in protein and essential amino acids, can improve the nutritional profile of animal products and reduce reliance on conventional protein sources.

Keywords: protein, amino acid composition, camelina seed, hemp seed, flax seed

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ANTIMICROBIAL AND ANTI-ADHESION POTENTIAL OF ESSENTIAL OILS AGAINST *Salmonella* Typhimurium AND *Staphylococcus aureus*

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The growing resistance of microorganisms to existing antimicrobial agents has significantly intensified the research into new, naturally derived and more potent antimicrobial drugs with broad-spectrum activity. The antimicrobial properties of natural substances such as essential oils (EOs) and their bioactive compounds are becoming increasingly important for food preservation and the control of microbial diseases in humans. The aim of this research was to examine the antibacterial and anti-adhesion activity of fifteen EOs and their compounds against gram-negative *Salmonella* Typhimurium ATCC 14028 and gram-positive *Staphylococcus aureus* ATCC 25923 bacteria. Antibacterial activity was determined by testing the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of EOs and their compounds. The method used to assess adhesion was crystal violet staining. The results showed that the EOs of *Cinnamomum zeylanicum* and *Eugenia caryophyllus* were the most effective with the MIC values ranging from 0.078 to 1.25 mg/mL, and from 0.039 to 1.25 mg/mL, respectively. On the other hand, EOs of *Eucalypti aetheroleum* and *Salvia officinalis* had significantly lower antimicrobial potential than the others. Further, MICs were used to assess the inhibition of adhesion of both bacteria in a microtiter plate. Based on the percentage of adhesion inhibition, *E. caryophyllus* EO showed the strongest effect with a percentage of inhibition of 53.5 and 73% on *S. Typhimurium* ATCC 14208 and *S. aureus* ATCC 25923, respectively. EOs of *C. zeylanicum*, *Origanum vulgare* and *Thymus vulgaris* also showed a strong effect on the adhesion of the tested bacteria. The most active compounds were carvacrol and thymol, which showed the remarkable anti-adhesion effect against bacterial strains with a percentage of inhibition up to 66%. A deeper understanding of the antimicrobial activity of EOs and their mechanism of action will greatly contribute to the development of novel strategies for treating infectious diseases and food preservation in the future.

Keywords: essential oils, antibacterial activity, anti-adhesion activity, *Salmonella* Typhimurium, *Staphylococcus aureus*

Acknowledgements: This work was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia [Grant Number 451-03-136/2025-03/200222].

PROTECTIVE EFFECTS OF *ENTEROCOCCUS FAECIUM* AGAINST T-2 MYCOTOXIN-INDUCED TOXICITY IN BROILER CHICKS

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Mycotoxins, secondary metabolites produced by filamentous fungi, pose a significant threat to poultry production due to their detrimental effects on health, growth, and product safety. T-2 toxin, a trichothecene mycotoxin, is particularly hazardous, inducing cytotoxicity, immunosuppression, oxidative stress, and histopathological lesions in liver and intestinal tissues. Chronic exposure impairs nutrient absorption, disrupts biochemical homeostasis, and compromises flock productivity, highlighting the need for effective mitigation strategies.

Probiotics, including *Enterococcus* strains, have emerged as promising interventions to counteract mycotoxin toxicity. They exert protective effects by modulating gut microbiota, enhancing intestinal barrier integrity, stimulating immune responses, and reducing oxidative damage. Probiotic supplementation can partially restore biochemical balance, preserve tissue architecture, and improve growth performance in toxin-challenged poultry.

This study evaluated the protective effects of *Enterococcus faecium* against T-2 toxin-induced toxicity in broilers under controlled conditions. Eighty one-day-old chicks were randomly assigned to six groups: control, two T-2 toxin doses (0.125 and 0.250 mg/chick), probiotic only, and probiotic combined with each toxin dose. T-2 toxin was administered orally in three doses, while the probiotic was supplied via drinking water (0.2 g/L) from day one. Blood and tissue samples were collected on days 6 and 8 for biochemical and histopathological analyses.

T-2 toxin alone significantly disrupted serum biochemical parameters, increased oxidative stress, reduced body weight, and caused liver and intestinal lesions ($p < 0.05$). Co-administration with *E. faecium* attenuated these effects, improving biochemical profiles, reducing tissue damage, and enhancing resilience to toxin-induced stress.

These findings indicate that *E. faecium* effectively mitigates T-2 toxin toxicity in broilers, supporting its inclusion in poultry diets to improve health, growth performance, and sustainability in modern poultry production systems.

Keywords: t-2 mycotoxin, broiler chickens, *Enterococcus faecium*, probiotics, oxidative stress

YEAST-BASED PROBIOTICS AS SUSTAINABLE FEED ADDITIVES IN POULTRY PRODUCTION

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The expansion of the global poultry production has created an urgent demand for sustainable feeding strategies that ensure animal health, optimize productivity, and reduce environmental impact. Yeast-based probiotics, particularly *Saccharomyces boulardii*, have emerged as promising alternatives to antibiotic growth promoters, aligning with the current shift toward natural and eco-friendly solutions in animal nutrition. These yeasts provide multifaceted benefits in poultry production through modulation of the gut microbiota, enhancement of digestive efficiency, and stimulation of immune responses. By improving nutrient absorption and supporting intestinal health, *Saccharomyces boulardii* can lower feed conversion ratios and enhance growth performance, thereby reducing overall feed costs and resource use. Furthermore, their antagonistic effects against pathogenic bacteria and mycotoxins lower disease prevalence, minimizing the need for antibiotics and mitigating the risk of antimicrobial resistance, a critical issue in both veterinary and human medicine.

Beyond production efficiency, studies have shown that yeast supplementation enhances poultry welfare by reducing oxidative stress and improving resilience under environmental or dietary stressors. Their role in stabilizing gut health also translates into better carcass quality and safer poultry products for consumers. From an ecological perspective, improvements in feed utilization and pathogen control reduce the environmental footprint of poultry farming, thereby making yeast supplements key contributors to a more sustainable and resilient food system.

To further substantiate this challenge, the Faculty of Veterinary Medicine Skopje will undertake a controlled study to evaluate the effects of *Saccharomyces boulardii* supplementation in poultry production. The investigation focuses on gut microbiota composition and microbiome diversity, growth efficiency, feed conversion ratio, physiological and biochemical health parameters, as well as immune response modulation. By integrating these multidimensional outcomes, the study seeks to generate robust empirical evidence to guide the development of sustainable feeding strategies that enhance both production efficiency and animal welfare in modern poultry production systems.

Keywords: *Saccharomyces boulardii*, probiotics, poultry production, feed efficiency sustainability

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ANTIMICROBIAL CONTAMINATION IN FEED ADITIVES: EMERGING RISKS, REGULATORY CHALLENGES, AND SUSTAINABLE MITIGATION STRATEGIES

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The global burden of antimicrobial resistance (AMR) represents a critical public health threat, with an estimated 4.95 million deaths associated with bacterial AMR in 2019, including 1.27 million directly attributable. Although the use of antibiotics as growth promoters in animal feed has been banned in the European Union since 2006, the risk of antimicrobial contamination in feed and feed additives persists. Contamination may occur at various stages of production, including sourcing of raw materials, manufacturing, storage, and transportation, as well as through cross-contamination in feed mills. Exposure of animals to antimicrobials can generate multiple adverse effects. Beyond the selection and spread of AMR, it may lead to disruptions of the intestinal microbiome, alterations in gut morphology and function, impaired immunological status, and the presence of antibiotic residues in edible animal products. These risks highlight the need to balance sustainability efforts with stringent feed safety measures. This paper examines the regulatory frameworks governing feed safety in the European Union (EU) and Serbia, with emphasis on the requirement that feed additives be free from harmful antimicrobial residues. It highlights risk assessment as a key element of governance, involving hazard identification, exposure assessment, and evaluation of AMR development potential. Case examples from EU monitoring programs illustrate the persistence of sub-inhibitory antibiotic concentrations that may disrupt gut microbiota and select for resistant bacterial populations. Sustainable mitigation strategies are proposed, including strengthening good manufacturing practice (GMP) and hazard analysis and critical control point (HACCP) systems, enhancing surveillance at national and EU levels, implementing rapid analytical screening methods, and adopting “green” feed additives such as probiotics and phytogenics. Integrating One Health principles into feed production safety assessments is essential to safeguard animal health, ensure food chain integrity, and prevent AMR dissemination while advancing environmental sustainability goals.

Keywords: antimicrobial contamination, antimicrobial resistance, feed additives, One Health, risk assessment

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SOYBEAN MOLASSES VS. SUGAR BEET MOLASSES: EFFECTS ON PELLET PRESS ENERGY CONSUMPTION AND PELLET QUALITY IN DAIRY COW FEED

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Soybean molasses, a by-product of soybean protein concentrate production, and sugar beet molasses, a by-product of the sugar industry, are both characterized by high carbohydrate content. While sugar beet molasses is widely used in animal feed production, soybean molasses used less despite its low price. This study evaluated the effects of these two molasses types on pellet press energy consumption and the physical quality of dairy cow pellets.

The primary objective of this research was to compare the effect of soybean molasses and sugar beet molasses on pellet press specific energy consumption (SEC), quantity of fines (QoF), pellet hardness (PH), pellet durability index (PDI) and bulk density of pellets (BD). Both soybean molasses and sugar beet molasses were included at 9% in the feed, with an additional control group without molasses. Moisture content of all smashes before pelleting was 16%, and the sieve opening diameter of the hammer mill was 6 mm.

Results showed that soybean molasses (21.97 kWh/t) significantly reduced the SEC compared with the control sample (45.56 kWh/t) and sugar beet molasses (26.85 kWh/t). Sugar beet molasses also significantly reduced SEC compared with the control sample. The control sample (620.50 g/l) had higher BD than both samples with molasses, while the sample with soybean molasses (582.16 g/l) showed higher BD than the sample with sugar beet molasses (561.81 g/l). PH did not differ between soybean molasses (11.90 Kahl unit kg) and sugar beet molasses (11.80 Kahl unit kg), but both were lower than the control sample (17.90 Kahl unit kg). No significant differences were observed in QoF and PDI.

Keywords: soybean molasses, sugar beet molasses, pelleting, dairy cow pellets, pellet press

Acknowledgements: This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (451-03-136/2025-03/200222).

CAROTENOID STABILITY IN COMPLETE FEEDS DIFFERING IN MAIZE HYBRID, VITAMIN A SUPPLEMENTATION LEVEL AND TRACE MINERAL FORM

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Maize grain is an important source of carotenoids for yolk pigmentation. However, carotenoids are prone to degradation during storage of complete feeds, and some ingredients may increase the extent of degradation. The aim of this study was to investigate the effect of vitamin A supplementation level (5,000, 10,000 and 20,000 IU/kg) and trace minerals (Zn, Mn, Cu, Fe and Se) in inorganic and organic form on the stability of carotenoids during three months of storage in complete feeds for laying hens differing in 2 commercial maize hybrids (soft- and hard-type). The complete feeds were stored at an average daily temperature of 20±2 °C and a relative humidity of 50-60% and subjected to carotenoid analysis [lutein (LUT), zeaxanthin (ZEA), α- and β-cryptoxanthin (αCX, βCX), α- and β-carotene (αC, βC) and total carotenoids (TC)] after 0, 14, 28, 56 and 84 days of storage. After 84 days, degradation averaged 30.7% for LUT, 25.2% for ZEA, 52.12% for αCX, 24.13% for βCX, 39.09% for αC, 39.59% for βC and 29.03% for TC. Compared to the hard-type, the soft-type hybrid showed higher stability of most individual and total carotenoids (3.99 vs. 4.80 ×10³ day⁻¹). Trace mineral form had a minor effect, with organic minerals reducing LUT degradation (4.88 vs. 4.52 ×10³ day⁻¹) and inorganic minerals reducing αC degradation (7.05 vs. 5.57 ×10³ day⁻¹). The level of vitamin A supplementation affected the stability of LUT, βCX, βC and TC, with the rate of degradation increasing with increasing supplementation level. Overall, changes in the composition of the complete feed could affect the stability of maize carotenoids as the only pigment source for yolk pigmentation.

Keywords: carotenoids, complete feed, degradation rate, storage

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UTILIZATION OF INVASIVE CRAYFISH AND SILVER CARP IN SUSTAINABLE WET CAT FOOD FORMULATION

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This study presents the development of a complete wet food formulation for adult cats, enriched with whole spiny-cheek crayfish (*Faxonius limosus*) and bone-in meat of silver carp (*Hypophthalmichthys molitrix*), two invasive and underutilized freshwater species. The main objective was to assess the nutritional adequacy, technological feasibility, and broader sustainability potential of incorporating these alternative protein sources into pet food production. The formulated product contained 10.2% protein, 11.6% fat, 2.29% ash, 0.81% crude fiber, and 71.5% moisture, with a calculated metabolizable energy of 6.04 MJ/kg (approximately 1443 kcal/kg). These parameters comply with the current nutritional guidelines for adult cats set by both the FEDIAF (European) and AAFCO (American) pet food standards. Technologically, both *F. limosus* and *H. molitrix* were shown to be compatible with homogenized wet food systems without the need for added stabilizers. From an ecological perspective, the use of *F. limosus*, a highly invasive species in European freshwater systems, contributes to biodiversity protection by mitigating its impact on native crayfish populations (*Pontastacus leptodactylus*) and juvenile fish stocks, which are under pressure from its aggressive expansion. Similarly, *H. molitrix* has shown ecological disruption in some regions and its controlled exploitation is considered beneficial. Economically, these raw materials are locally available, cost-effective, and currently underexploited, offering an opportunity to lower production costs while promoting circular and ecologically responsible resource use in the pet food industry. The results demonstrate that alternative aquatic ingredients such as invasive crayfish and silver carp can provide nutritionally adequate, environmentally beneficial, and economically viable options in the development of next-generation wet pet food products.

Keywords: invasive species, *faxonius limosus*, wet cat food, sustainable pet nutrition, silver carp

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ULTRA-PROCESSED CARBOHYDRATE-BASED DIETS IN PETS: TRENDS AND HEALTH IMPLICATIONS

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Ultra-processed foods (UPFs) are a hallmark of modern human food systems and have been associated with adverse health effects in humans; similarly, they are gaining attention in veterinary nutrition, where they are commonly referred to as ultra-processed carbohydrate-based diets (UPCDs). This study aims to review the UPCDs consumption trends and effects on health in companion animals. UPCDs include dry, canned and any other pets' food that are manufactured using more than one thermal or pressure processing step to produce the final product. Typical UPCDs include extruded kibble, baked treats, and canned or wet industrially cooked foods, which dominate the commercial pet food market and pet diets, being considered traditional pet food. UPCDs are considered a source of advanced glycation end products (AGEs) produced in Maillard reaction during the processing and have been reported to result in higher dietary AGEs loads and elevated plasma and urinary AGEs concentrations, particularly in wet food diets. AGEs intake is associated with reduced bioavailability of essential amino acids, oxidative stress due to their pro-oxidant properties, and a slow rate of tissue turnover. These effects have been linked to atherosclerosis, nephropathy, retinopathy, osteoarthritis, and neurodegenerative diseases, and in dogs, elevated tissue AGEs levels have been associated with multiple pathological conditions. Processing can also generate acrylamide and other Maillard reaction products, which may contribute to oxidative stress and long-term metabolic disturbances. It has been suggested that early life feeding patterns have long-term effects, UPCDs significantly increased the risk of chronic enteropathy, whereas non-processed and human food leftovers were associated with a lower incidence later in life. In response, there is growing interest in pets' diet, as it plays a significant role in health and longevity, leading to a growing trend of feeding pets homemade and raw animal-based diets.

Keywords: Pet nutrition, feeding practices, ultra-processed carbohydrate-based diets, advanced glycation end products, pet health

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COMPARATIVE DIGESTIBILITY OF FUNGAL BIOMASS FROM SUBMERGED AND SOLID-STATE FERMENTATION: A NILE TILAPIA *IN VITRO* MODEL

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Sustainable aquaculture requires novel protein sources to reduce reliance on conventional ingredients with high environmental impacts. Hence, accelerating the assessment of alternative protein is essential. Although *in vivo* trials are resource-intensive, simulated fish digestion systems offer a practical and efficient alternative for evaluating novel sustainable protein sources. This study assessed the *in vitro* digestibility of fungal-derived protein using gastrointestinal enzymes extracted from Nile tilapia (*Oreochromis niloticus*). Chamber bioreactors with semi-permeable membranes operated at 25 °C for 360 minutes, simulating stomach (pH 4.5) and intestinal (pH 7.5) phases. We evaluated four ingredients obtained from two fermentation processes with the edible filamentous fungi *Neurospora intermedia* and *Aspergillus oryzae*. Submerged fermentation (SmF) produced pure fungal biomass, while solid-state fermentation (SSF) used bread waste as the substrate. Statistical comparisons among treatments were performed using analysis of variance (ANOVA) followed by post-hoc tests to determine significant differences ($p < 0.05$). SmF biomass achieved significantly higher ($p < 0.05$) amino acid hydrolysis, with 213 ± 6 mg for *N. intermedia* and 204 ± 5 mg for *A. oryzae*. These values were substantially greater than SSF products (88 ± 2 mg and 79.2 ± 10.6 mg, respectively), which were significantly higher ($p < 0.05$) than fishmeal (109.7 ± 1.4 mg) and unfermented bread (54.8 ± 1.9 mg). Acid protease activity in SmF biomass was 0.50 ± 0.15 U g⁻¹ for *N. intermedia* and 0.14 ± 0.06 U g⁻¹ for *A. oryzae*, while SSF products recorded 0.13 ± 0.01 U g⁻¹ and 0.53 ± 0.01 U g⁻¹, respectively. Endogenous proteases activity contributed significantly ($p < 0.05$) to protein hydrolysis in fungal derived ingredients. Overall, these results position fungal protein as a promising and sustainable addition to Nile tilapia diets. The notable enzyme activity observed could be a key driver in improving protein digestibility and supporting better growth performance.

Keywords: submerged fermentation, solid-state fermentation, filamentous fungi, *In vitro*, Nile tilapia

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MYCOTOXINS IN MAIZE: CLIMATE-DRIVEN RISKS TO FOOD AND FEED SAFETY IN SERBIA

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Over the past two decades, Serbia has experienced significant climate change, leading to an escalation of food and feed safety risks. Among these, the prevalence of mycotoxins - key indicators of food and feed contamination - has become a growing and persistent concern. The combination of rising temperatures, prolonged droughts, and shifting precipitation patterns has considerably increased the frequency and severity of mycotoxins occurrence, particularly in maize, one of the country's most important arable crops.

Since 2012, frequent hot and dry weather conditions in Serbia have led to the detection of aflatoxins in 24% to 85% of maize samples, in almost every year or at least every other year. In addition to aflatoxins, fumonisins are also frequently detected in Serbian maize, with their concentrations largely dependent on weather conditions. Moreover, numerous other mycotoxins and fungal metabolites have been reported in maize over the past decades. Given that maize is a staple food and feed ingredient, its contamination poses a significant risk for further transmission throughout the production chain. One of the most concerning consequences is the recurrent contamination of milk and dairy products, which has been observed across Serbia in multiple years.

The long-standing occurrence of mycotoxins in maize used for both human and animal consumption raises significant concerns due to the multiple potential health risks. Beyond the national level, maize contamination in Serbia represents a broader issue, as the country is among the leading maize producers and exporters in Europe and globally. This issue has a substantial economic impact across various sectors, including agriculture, food and feed production, trade, and public health.

To effectively address these climate-driven mycotoxin risks, it is crucial to implement comprehensive monitoring programs, develop predictive models, promote resistant maize varieties, and strengthen regulatory frameworks to safeguard food and feed safety throughout Serbia's agricultural production and supply chains.

Keywords: mycotoxins, maize, Serbia, climate change

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THE EFFECTS OF PROTEIN AND ENERGY LEVELS IN THE NUTRITION OF *Tenebrio molitor* ON THE CHEMICAL COMPOSITION OF MEALWORMS

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The energy and protein requirements of domestic animals are well understood in nutrition. Properly balancing these two factors can lead to lower production costs and a more favourable chemical composition of the final product. However, knowledge about the nutritional requirements of *Tenebrio molitor* is limited due to it being a relatively new research field. The concept of utilizing mealworms as a protein source has gained popularity in recent years.

In this trial, three energy levels (low, medium, and high) and three protein levels (low, medium, and high) were used, resulting in a total of nine different diets. Wheat bran served as the base, with additions of different percentages of soybean meal and corn to obtain the protein and energy levels for various energy and protein levels in the diet. The trial was conducted with four replicates per diet, each replicate consisting of a tray containing 2 grams of the insect *Tenebrio molitor*, which were as uniform as possible in age (28 days old) and weight. The experiment lasted until the first several pupae appeared in any of the nine diets, which occurred 32 days after the start. Statistically significant differences were observed in the protein, fat, ash, and ADF contents of the mealworms. The protein content of dry larvae ranged from 44.8% to 51.9% and it was influenced by the level of the energy and protein and their interaction. In contrast, the fat content was affected just by the energy levels and ranged from 25.2 to 40.4%. The ADF content, which derived from the chitin of insect cuticles, ranged between 5.51% and 6.51% and it was effected by both energy and protein level.

Keywords: *Tenebrio molitor*, protein and energy levels, chemical composition

Acknowledgements: This research was supported by the Science Fund of the Republic of Serbia, 7284, Advancing Sustainable Solutions by Developing Insect based Protein as New Feed Options PRO-SUSTAIN.

FROM STRESS TO SUCCESS: FEEDING AND DENSITY CHOICES FOR COMMON CARP (CYPRINUS CARPIO) IN SCARCE WATER

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Freshwater pond aquaculture faces mounting challenges due to increasing water scarcity, driven by climate change, population growth, and competing demands for freshwater resources. Traditional semi-intensive systems, though productive, risk unsustainable nutrient loading and water quality deterioration under such constraints. This study investigated how water supply level, stocking density, and feeding regime interactively affect growth, water quality, and stress physiology in common carp (*Cyprinus carpio*) juveniles. A full factorial design (2³) was applied in twelve experimental ponds (~300 m² each) over a three-month growing period. Treatments were tested against optimal and limited water supply, low and high stocking density, and traditional (plankton enhancement + cereals) versus commercial (formulated, high protein) feeding. Growth performance (weight gain, SGR, FCR, and survival), water quality (DO, TAN, and chlorophyll a), and stress responses (plasma cortisol) were evaluated.

Results revealed strong main and interactive effects. Water limitation consistently reduced growth and survival, with the most pronounced impacts in high-density ponds using traditional feed, where critical DO drops and elevated TAN were observed. These conditions triggered heightened stress markers and poor fish welfare. Conversely, commercial feed markedly mitigated these effects under the same high-density, water-limited conditions, supporting higher growth rates, improved FCR, and more stable water quality profiles. Under optimal water supply, differences between feeding regimes were less pronounced, indicating that the benefits of formulated feeds are greatest under environmental stress.

Although high stocking densities are often perceived as ecologically risky, this study shows they can be sustainable when paired with high-quality feeds and proactive water quality management. Traditional feeding, while economically attractive, increases the risk of eutrophication and stress in drought-prone systems. Adaptive strategies integrating efficient feed use, density management, and water optimization are essential for resilient aquaculture. These findings inform future practices to sustain carp productivity while optimising environmental risk under climate uncertainty.

Keywords: carp, intensive, feeding, water-lack, fingerling

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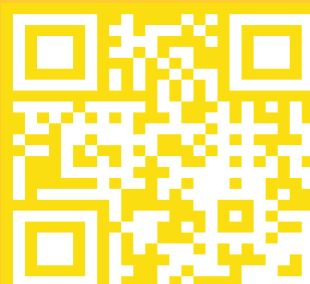
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IKA Mills IKA MultiDrive control

IKA MultiDrive Control – Sve u jednom uređaju za mlevenje i mešanje

Univerzalni laboratorijski uređaj za drobljenje, mlevenje i disperziju – pogodan za tvrde, meke i vlaknaste uzorke.

Snaga: 1000 W, 3000–20.000 o/min

Funkcije: drobljenje, mlevenje, mešanje i disperzija

Ugrađena vaga – merenje i mlevenje u istoj posudi

Intervalni rad za zahtevne uzorke

Sistem hlađenja za zaštitu osetljivih materijala

TFT ekran i USB priključak za jednostavno upravljanje i dokumentaciju

Različite posude za suvo i mokro mlevenje, kao i



IKA

BINDER ECO serija – održivost i energetska efikasnost bez kompromisa

BINDER

Best conditions for your success



U sve većoj potražnji za održivim rešenjima, BINDER stavlja akcenat na efikasnost i ekološku odgovornost. Zahvaljujući optimizovanoj Peltier tehnologiji i patentiranom odvođenju toplote, BINDER inkubatori i klimatske komore spadaju među najefikasnije uređaje za simulaciju na tržištu.

ECO porodica uključuje:

- KBF-S ECO – komore za testiranje pod homogenim klimatskim uslovima
- KB ECO – rashladni inkubatori za bezbednu i reproduktivnu inkubaciju



LignoBond® DD

SUSTAINABLE BINDING POWER FOR ANIMAL FEEDS

LignoBond® DD je visoko efikasno vezivno sredstvo za pelete napravljeno od 100% kalcijum lignosulfonata. Stvoreno je da poboljša trajnost peleta, tvrdoću i apsorpciju pare, uz istovremeno smanjenje sitnih čestica i sprečavanje raspadanja.

Zašto izabrati LignoBond DD?

- Pruža veliku trajnost peleta
- Smanjuje sitne čestice i sprečava raspadanje peleta
- Održivo rešenje dobijeno iz drveta
- Pogodno za sve životinjske vrste
- Efikasno već pri dodavanju od 0,5 do 0,8%



Read more: LignoBond.com
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Efikasna kontrola mikotoksina u hrani za životinje

Neogen® Reveal® Q+ MAX testovi nude jednostavan imunohromatografski format koji se lako koristi, dajući kvantitativne rezultate za nekoliko minuta. Dostupni su testovi za detekciju

Aflatoksina, **Deoksinivalenola (DON)**, **Ohratoksina**, **T-2/HT-2**, **Zearalenona**



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