NATIONAL AGRICULTURAL AND FOOD CENTRE

NanoFEED

NANOSTRUCTURED CARRIERS FOR IMPROVED CATTLE FEED

NATIONAL AGRICULTURAL AND FOOD CENTRE
2023

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NANOSTRUCTURED CARRIERS FOR IMPROVED CATTLE FEED

H2020-EU.1.3.3. – Stimulating innovation by means of cross-fertilisation of knowledge
MSCA-RISE – Marie Skłodowska-Curie Research and Innovation Staff Exchange (RISE)

National Agricultural and Food Centre Lužianky
2023

Title: **Nanostructured carriers for improved cattle feed**
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Content

About the NANOFEED project

EUROPEAN RESEARCH EXECUTIVE AGENCY (REA)

REA.A – Marie Skłodowska-Curie Actions & Support to Experts

A.3 – MSCA Staff Exchanges

General information

Project title: Nanostructured carriers for improved cattle feed

Project Acronym: NanoFEED

Grant Agreement Number: 778098

Call (part) identifier: H2020-MSCA-RISE-2017

Topic MSCA-RISE-2017: Research and Innovation Staff Exchange

Coordinator: National Agricultural and Food Centre, Hlohovecká 2, 951 41 Lužianky, Slovak republic, http://www.nppc.sk

Consortium of 8 partners – 5 academic and 3 industrial institutions:

National Agricultural and Food Centre, Slovak republic

Agricultural University of Athens, Greece

Selçuk University, Turkey

University of Veterinary Medicine Vienna, Austria

German Institute of Food Technologies, Germany

InoCure s. r. o., Czech republic

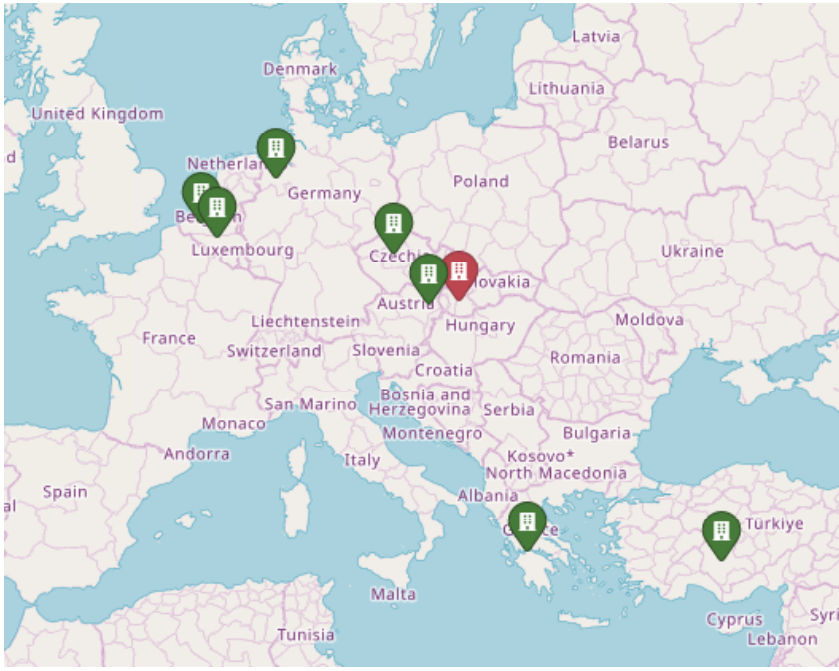
Nutrition Sciences N. V., Belgium

Bio Base Europe Pilot Plant, Belgium

Starting date: 01/02/2018, duration 48 months (prolongation to 63 months, during the coronavirus pandemic)

Budget: 733 500 Euro

Project website: https://cordis.europa.eu/project/id/778098



The consortium of the project NanoFEED – partners.

Foreword

It is always a great honour for a R&D organisation, particularly one under the auspices of the Ministry of Agriculture and Rural Development, *i.e.*, partly governmental institution, to be given the opportunity to participate in an international science and innovation project. Moreover, it is a specific honour, which is also binding, if you manage such a consortium of partners in the position of leader. The National Agricultural and Food Centre undertook this task with the awareness of maximum responsibility both towards partners as well as the European Commission, not only to sustain the fulfilment of all declared goals and project indicators.

From many aspects, the Nanofeed project is rather unique – it connects different branches of multidisciplinary research with practice and at the same time, creates space for sharing the knowledge and experience through the international exchange of scientific expertise. At the same time, it offers space for the development of knowledge and innovations in its research part.

Not only NPPC as the leader, but all the involved partners – foremost scientific institutions across the Europe approached the project mainly with the expectation of intensifying cooperation with other institutions. However, none of us expected that the global pandemic of Covid 19 would affect its implementation in a significant way, when instead of exchanging knowledge through personal meetings and truthful discussions, we would meet for almost half of its duration via the computer screens from our workplaces or the living rooms of our apartments. Changes and transfers of partners were also dynamic and affected the project very intensively.

However, it must be said that the research life and the activities of the Nanofeed project did not stop, and despite the above-mentioned limitations and problems, mostly thanks to mutual understanding and support of all the partners involved as well as the managing authorities from the European Commission, unique knowledge and results were achieved. Several of them are already or will soon be the subject of patent protection or publications that were created during the project.

In the same way, the goal of establishing and developing contacts across the Europe in the area of the food & agriculture sector was fulfilled to the maximum possible extent. We only have to believe that these contacts will stay alive and that the relationships we have established within this project will soon be transformed into other projects of international scientific cooperation. We have all the prerequisites for that.

Briefly, what was the Nanofeed project about? The topics that were investigated are highly current and relatively complex. We can mention few of them:

- Problem of animal productivity and quality as a basic prerequisite for sustainable and self-sufficient production of a wide range of products (not only) in the food industry

- Increasing the efficiency of livestock breeding as one of the key strategies to increase the competitiveness of agri-food sector
- Innovations in the field of effective livestock nutrition and thereby reduction of the prevalence of several serious animal diseases with an impact on increasing productivity

NanoFEED responded to these factors by nano/microencapsulation approach to advanced core/shell particles with pH sensitive properties. The proposed technology of encapsulation of diverse active molecules brought the simplification of the protection against the degradation in rumen for wide range of active molecules. Thanks to encapsulation and improved delivery to small intestine, novel active molecules may be used to treat nutritional deficiencies. At the same time, the process of encapsulation is based on green and biobased methods with minimal carbon footprint helping in development of sustainable bioeconomy.

In addition, the project fulfils the aim of RISE project and delivers significant social impact, increases knowledge-based economy and creates long lasting cooperation strengthening the European research area.

The presented brochure summarizes the research orientation, cooperation possibilities, main tasks, expectations, results and contributions of all partners in a limited area. Through the rich photo documentation, it also maps the development of the project and cooperation between the partners during the entire period of its implementation. Moreover, several ambitious visions and perspectives for the direction of future cooperation are presented.

We believe that its content will fulfil the expected purpose, both in relation to the European Commission as the funder of project activities, but also in relation to the wider professional and public audience, with the aim of demonstrating that if cooperation is desired, a pandemic or other barriers are not a significant obstacle and science and research bring real results.

One project ends today, but we believe that the partnership and cooperation will continue.

On behalf of the project partners

Martin Polovka

Benefits of the NANOFEED project

Project goals

- Intersectoral cooperation to deliver novel fodder based on nano- and microencapsulation techniques for the controlled release of nutrients solving nutritional deficiencies of cattle, while preventing and reducing diseases and boosting their immunity.
- The innovation is based on encapsulation of feed supplements into polymeric coaxial nano and microparticles with core/shell structure, which allows the retention of biological activity of transferred substances and their release in the small intestine.

Kick off meeting of NanoFEED project, Prague, Czech republic, 18 – 19. 4. 2018.

Work packages (WP)

- WP1 Networking activities.
- WP2 Research and training.
- WP3 Workshop activities and guidance for onsite experiments.
- WP4 Innovation activities and Industrial Experiments.
- WP5 Dissemination and Outreach activities.
- WP6 Management activities.
- WP7 Ethics requirements.

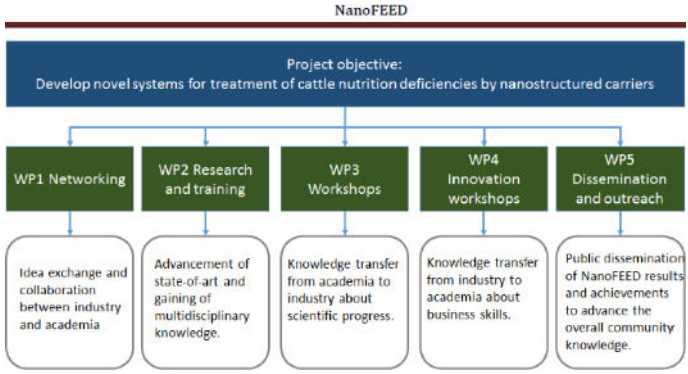
NanoFEED advantages.

Excellence

- Intersectorial cooperation to deliver novel fodder based on nano- and microencapsulation techniques.
- Controlled release of nutrients solving nutritional deficiencies of cattle, while preventing and reducing diseases and boosting their immunity.
- The goals have been achieved through the accomplishment of the following specific sub-objectives through the proposed activities:
  - Ensure a high degree of collaboration between academic and industrial organizations: Networking Activities.
  - Go beyond the current state of art: Researcher & Training Activities.
  - Increase intersectoral transfer of knowledge: Workshop Activities and on-site training.
  - Ensure high level of innovation capacity
  - Industrial Scale Experiments.
  - Ensure communication to the scientific community and to the public.
  - Dissemination and Outreach Activities.

Viability

- Actual problem of productivity and quality of livestock production.
- Nutritional deficiencies are key factors reducing the efficiency of stock raising, due to high prevalence of serious diseases.
- Boosting the immune system.
- The consortium have expertise in veterinary medicine, tissue engineering, chemistry, nanomaterials, toxicology, pharmacology, physiology, molecular biology, nutrition and feed production, business, operational management, marketing product implementation.
- The drug delivery system is prepared from ecological and fully degradable biopolymers.
- The innovation is based on encapsulation of feed supplements into polymeric nano- and microparticles with core-shell structure, which allow the retention of biological activity of transferred substances and their release in the small intestine where they are better utilized for the animal's needs and therefore for economy.
- Knowledge transfer between universities and companies and increase the prestige and quality of agricultural research in the EU.
- Global demand due to population growth and increasing global living standards is growing – according to OECD predictions. Increased efficiency in production can help solving food deficiency.



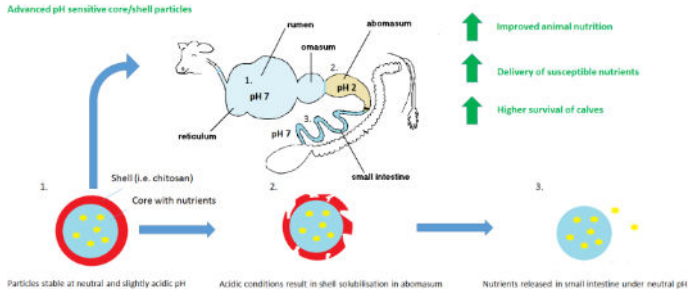
NanoFEED aim and methodology.

- The approach involves utilization of green polymers, biopolymers and manufacturing technologies eliminating the carbon footprint of nanoparticle and micro particle production.
- Production of fresh and local food has been emphasised which is the main benefit for the end user. This has a positive effect on customer health quality in both developed world and third-world countries.

- Nevertheless, an indirect effect is the reduced number of animals required to fill nutritional needs leading to more ethical breeding conditions; and also reduced energy requirements of breeding (water, electricity) and decreased emissions of greenhouse gases produced by ruminants. This generates significant social impact of the project outcomes.

Socio-economic impact

- Improved health of cattle and decreased mortality.
- Improved quality of products (*i.e.,* meet, milk).
- More energy efficient, ethical and green livestock industry.
- Increased awareness on nutritional deficiency problems of cattle.



NanoFEED approach.

Publicity and outputs of the project NanoFEED

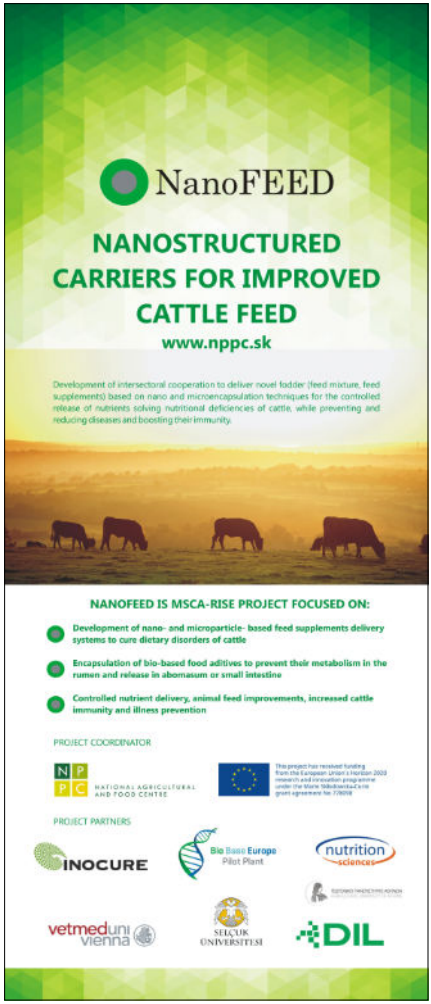
- Publicity of the project was carried out during the entire duration of the project, while it was implemented in several ways.
- The publicity activities contributed to the spread of positive information about the H2020-EU Program. 1.3.3. – Stimulating innovation by means of cross-fertilisation of knowledge - MSCA-RISE – Marie Skłodowska-Curie Research and Innovation Staff Exchange – RISE.
- Organization of events, where there was an important exchange of information and knowledge transfer - Workshops, International film festival, Agricultural exhibitions, Field Days and others.
- Implemented events supported the intersectoral transfer of knowledge between academic and non-academic institutions and public.
- Important publicity outputs were articles in scientific and professional journals as well as the publication of the presented brochure.
- Networking activities in the NanoFEED were meant to encourage and facilitate long-term collaboration between the non-commercial and commercial sectors.

International agricultural and food exhibition Agrokomplex, NPPC exhibition, 18. – 21. 8. 2022, Nitra, Slovakia. Presentation of information about the H2020-EU Program and the NanoFEED.

Workshop "Nanostructured carriers for improved cattle feed, NPPC, 13 – 14 September 2022, Bratislava, Modra, Lužianky – Slovakia" Publicity and outputs of the NanoFEED and Future perspectives (3 figures).

- Final feed/developing of optimized feed – the project focused on encapsulation of nutrients to prevent their degradation in rumen and support release in abomasum or small intestine.
- Drug delivery systems for improvement of cattle breeding strategies – to cure dietary disorders of cattle.
- The innovation is based on encapsulation of nutrients into polymeric nano- and microparticles with core-shell structure, which allow the retention of biological activity of transferred substances and their release in abomasum or small intestine, where these nutrients are better used for the animal's needs.

Project outcomes – encapsulated final feed. Feed production is based on the use of nano- and microencapsulation techniques with controlled release of nutrients in the rumen, which help eliminate nutritional and health problems in cattle. Feed was produced by a Belgian partner Nutrition Sciences.



From the publicity of the project NanoFEED – roll-up.

Project consortium – partners and their roles in the project

National Agricultural and Food Centre (NPPC) Slovak Republic

Project coordinator

National Agricultural and Food Centre.

Mission

Comprehensive research and gathering of knowledge in the sustainable use and protection of natural resources, especially soil and water for crop production and animal husbandry, quality and safety, innovation and competitiveness of food and non-food products of agricultural origin, productive and non-productive impact of agriculture on the environment and rural development and the transfer of knowledge from agricultural and food research to end users. NPPC is research organization of the Ministry of Agriculture and Rural Development of Slovak republic.

Focal Issues

- Sustainable agriculture and food production.
- Regional development.
- Competitiveness.
- Quality of life.
- Environment.
- Bioeconomy.

Strong partner for complex research and services

- We offer various models for research, innovation and collaboration to meet the needs of all stakeholders.
- We provide to our customers research, services and analyses, consulting based on our outstanding scientific and technological expertise.
- We use high-quality research environments and experimental facilities and long term extensive scientific.
- Data resources.
- H2020 projects: BIOSKOH, ECOBREED, RUSTWATCH, NANOFEED, AGENT, EJP SOIL.
- Strategic thinking in bioeconomy - participation in the BIOEAST knowledge hub.
- Knowledge Transfer and Dissemination.

Key mission in NanoFEED project

- Project coordinator.
- Expert: in vivo and in vitro testing, clinical biochemistry, clinical haematology and clinical rumenology.
- Expert: isolation of active molecules, spray drying and bioprocessing.
- Conduct a workshop: Microparticle development and drug delivery.
- Leader, WP2, WP6, leader research Task 5. of WP2 – In vivo testing.
- Leader, WP3, research Sub-Task 2.3. of WP2 – Spray drying for production of microparticles.

Outcomes / benefits of the project implementation

Two research institutes of NPPC were involved in the research part of the NanoFEED project:

1. NPPC – Food Research Institute Bratislava, Department of Technology, Innovation and Cooperation with Practise (Biocentre Modra) focused on:

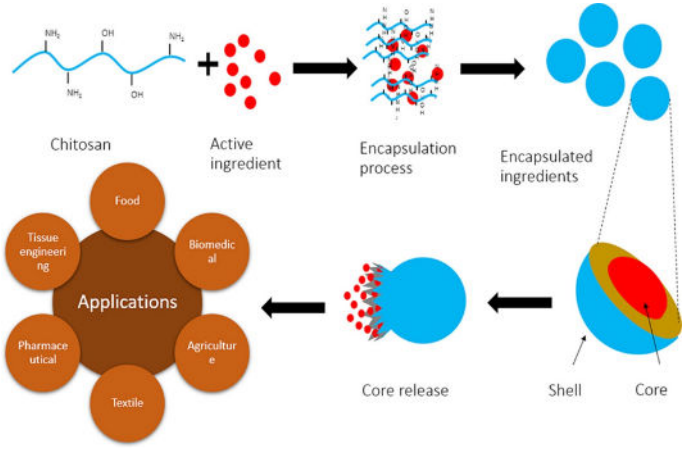
Spray dryer.

APV homogenizer.

Spray drying process.

Dried powder.

- Technology of additive and food/feed preparation with the use of separation and concentration techniques.
- Development of new kind of additives and food of the health products type.
- Collection and isolation of natural microbial food cultures, optimalization of their production conditions and their use in fermented foods vegetable and animal origin.
- Patent registered methods and developed products.

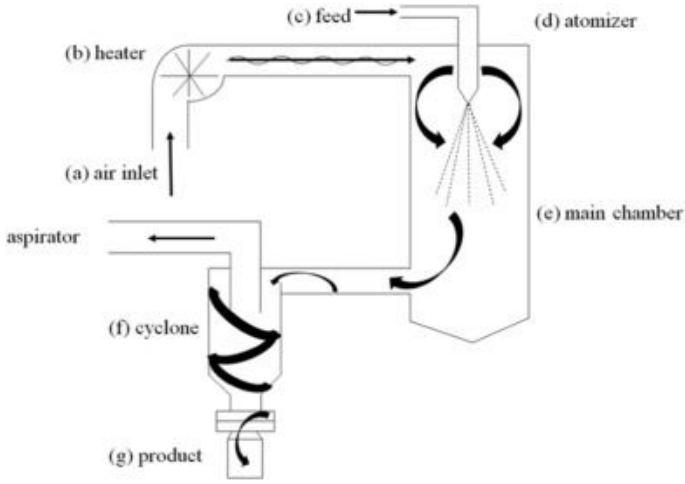


Chitosan encapsulation active ingredients.

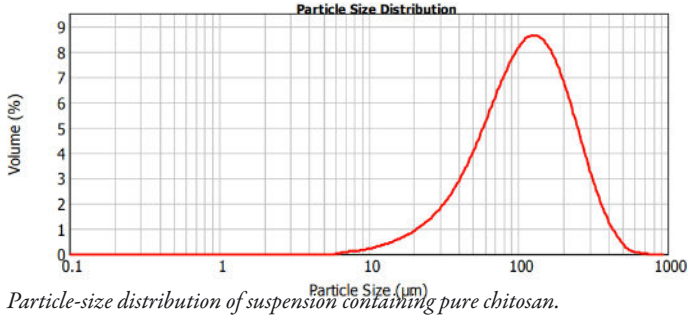
Encapsulation involves the development of capsules containing the active ingredient as a core, which is surrounded by, for example, chitosan, gum arabic and others as a shell. The shell itself plays an important role in slowly releasing the core material. Microencapsulation using chitosan is used due to its non-toxicity, biodegradable and biocompatible properties.

We prepared encapsulated samples where we encapsulated lysine and essential oil. We used chitosan and gum Arabic as packing material. Chitosan itself dissolves in an acidic environment and we prepared 10 and 15% suspensions. Subsequently, the suspension was homogenized on an APV homogenizer. A stable emulsion was created, because chitosan is soluble only in acid solvent, but lysine is not soluble in this system.

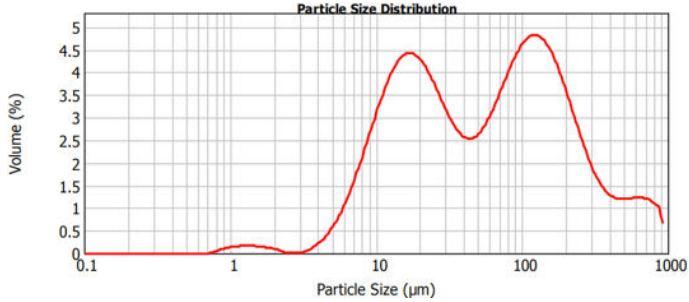
Wall material or carrier is the layer that embraces the core material (bioactive and valuable compounds) with the aim of protection – encapsulation. Since wall material has a direct influence on the storage stability, protection, and controlled release of the core material, and ultimately the encapsulation efficiency of the process, it is necessary to select the right carriers.



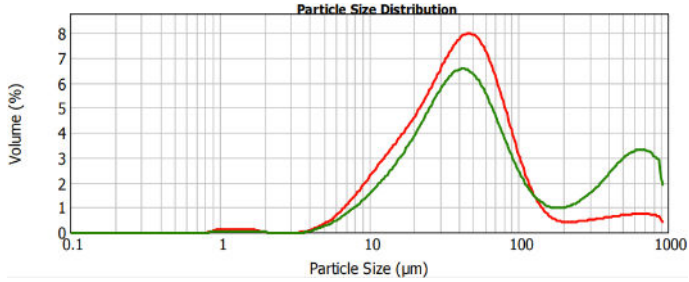
Schematic representation of spray drying without recirculation loop, (a) air is sucked by aspirator; (b) air is heated up to defined inlet temperature and flows into drying chamber; (c, d) feeding liquid is pumped into atomizer with predefined flow rate; (e) heated air comes in contact with atomized feed and drying takes place in the main chamber; (f) dried particles are separated from the air stream in the cyclone due to the pressure drop; (g) the final particles are collect.



Particle-size distribution of suspension containing pure chitosan.



Particle-size distribution of suspension containing Chitosan + lysine + APV homogenizer.



Particle-size distribution of suspension containing gum Arabic (20%, Red line) and mixture of essential oil and gum Arabic (20 %, green line).

The ability to encompass large amounts of essential fatty acids, protecting them against chemical degradation (like oxidation or hydrolysis) during storage, to have a release at a specific site and controlled rate, compatibility with the targeted food matric without any adverse changes on the techno-functional and sensory levels are some of the important properties the composition of wall material should have.

We prepared 10, 15, and 20 % suspensions with gum Arabic as packing material. The

suspensions were homogenized on an APV homogenizer. Prepare "bigger" particles – by increasing the concentration of matric in solution. The image shows a comparison between gum Arabic and essential oil (juniperus essence) encapsulated in gum Arabic.

2. NPPC – Research Institute for Animal Production Nitra, Department of Nutrition focused on problems of precise nutrition:

- Physiology of digestion in animals (ruminants, pigs, rabbits, cloven hoofed game, rats).
- Nutritional requirements of animals.
- Methods in vitro, in vivo, in sacco and mobile bag are used to study nutritional value of feeds, properties, digestion, utilisation and transformation of nutrients in individual parts of the digestive tract.
- Influence of feed treatment on utilisation of nutrients.
- Optimisation of composition of feed rations and mixtures to consider real requirements of animals; minimisation of content of undesirable and anti-nutritive matters; utilisation of probiotic, stimulating and biologically effective matters.
- Effective utilisation of traditional and alternative feed resources, inclusive of waste and by-products from industry.
- Control of feed quality.
- Study of problems in production, storage, conservation and treatment of roughage using effective conservation preparations.

One of the key tasks of the Department of Nutrition in NanoFEED was the participation in the development of new feed and its testing on cattle.

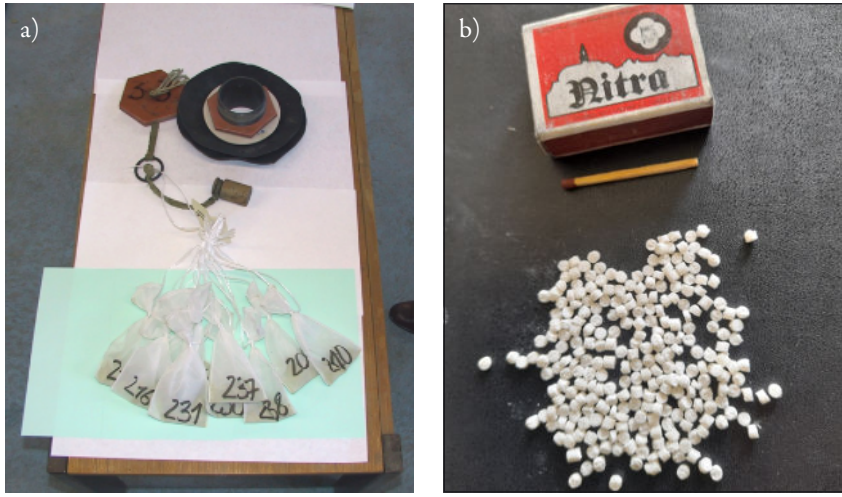
- Rumen degradability and intestinal digestibility of undegraded feed protein is an important parameter in modern protein evaluation systems for ruminants.
- For better protein utilization it is possible to use protection technologies to reduce the negative enzymatic effect of rumen microorganisms to nutrients and thus increase their bypass into the small intestine.
- Protecting feed proteins increasing their intestinal digestibility and thereby protecting the environment, by reducing the excretion of excess ammonia, by artificially influencing the solubility, degradability.
- The implementation of protected nutrients in order to decrease fast degradation through controlled release was the goal of the NanoFEED.

Laboratory of Physiology of Ruminant Nutrition of the Department of Nutrition, NPPC – VÚŽV Nitra. Testing of encapsulated final feed by in sacco and mobile bag method.

- Precise nutrition increase feed utilization, reduce nutritional disorders and reduce ammonia emissions.
- Livestock sector produces emissions such as ammonia (NH_3), carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O).
- Determination of changes in the rumen degradability of nutrients after feed treatment (by in *sacco* and mobile bag method) – as a prerequisite for reducing the release of ammoniac nitrogene.
- Results on the degradability and intestinal digestibility of individual feeds help to set the rations of cattle within the framework of precise nutrition, so that there is no unnecessary excretion of nutrients into the environment; this is the reason why it is important to know the nutritional characteristics of individual feeds and to continuously monitor and test them.
- The Final feed, as one of outcomes of NanoFEED project, was tested in canulated cows using in sacco and mobile bag method – at the Department of Nutrition - Laboratory of Physiology of Ruminant Nutrition we determined the degradability of crude protein in several feeds, both unmodified and modified (treated).
- The results revealed, that *e.g.,* the effective degradability of proteins varies from 29.7% with encapsulated soybean meal to 87.4% with untreated peas.
- Results of encapsulation efficiency tests clearly demonstrated that the crude protein degradability of untreated soybean meal is 72.2%, toasted soybean meal 40.2 and encapsulated soy bean meal 29.7 %.

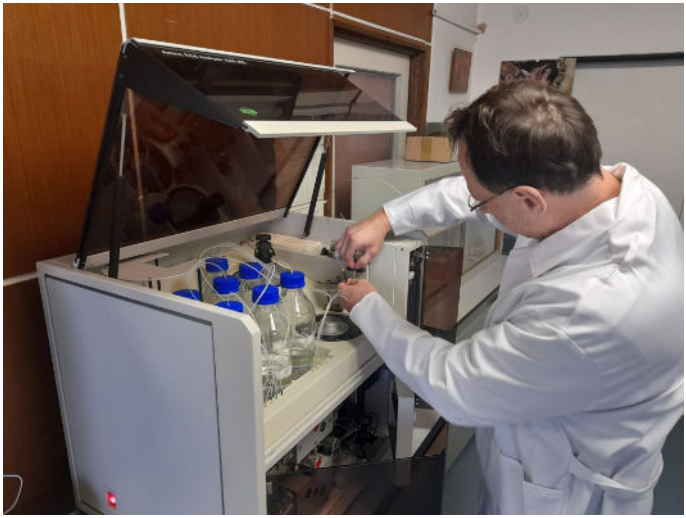
Degradability of crude protein. Laboratory of Physiology of Ruminant Nutrition of the Department of Nutrition, NPPC – VÚŽV Nitra.

Feed	Degradation of crude protein in the rumen (%)
Soy bean untreated	59.3
Soy bean hydrothermally treated	43.4
Extracted soybean meal untreated	72.3
Extracted soybean meal toasted	40.2
Extracted soybean meal encapsulated	29.7
Extracted rape seed meal untreated	76.2
Extracted rape seed meal (expansion)	36.1
Peas untreated	87.4
Peas extruded	68.3



a) The in sacco method is based on the incubation of feed samples in nylon bags at appropriate time intervals in the rumen. This method ensures direct contact of rumen microorganisms (active enzymatic activity) with the tested feed through the wall of the nylon bag. In this way, it is possible to accurately quantify the course of the degradability process of a cannulated animal.
b) Encapsulated final feed. Feed production is based on the use of nano- and microencapsulation techniques with controlled release of nutrients in the rumen.

Secondee from Nutrition Sciences Belgium at the NPPC – Research Institute for Animal production Nitra.



Determination of rumen undegegrad lysin content in encapsulated feed. Amino Acid Analyzer AAA 400. Laboratory of feed analytics at the Department of Nutrition, NPPC – VÚŽV Nitra.

Agricultural university of Athens (AUA): Twenty-eight building complexes, six Schools with thirteen Departments.

Vision

- High-level undergraduate and postgraduate Education
- Research in Agricultural Science, and its vision is to achieve Educational and Research Excellence so as to occupy a dynamic position in the international academic environment.
- True to its traditional role of responding to the productive and developmental needs of the Greek economy and society, the AUA intervenes and develops in line with the development and orientation of modern education and science.
- Continuous contact with society to make proposals and provide solutions to problems that arise within the agri-nutrition sector.

Research and Technology Transfer

Key focus

Research in AUA focuses at creating new and using existing knowledge in all sectors of agronutrition, environmental ecology and agricultural economics, with the view of contributing to sustainable development and meeting modern challenges.

Areas of interest

Soil, atmosphere, water recourses, microorganisms, plant and animal organisms, feed and food are among the areas of interest of the AUA scientific community, aiming at improvement of production processes, safety and quality of products and processes, development, innovation and sustainability.

Means for realisation

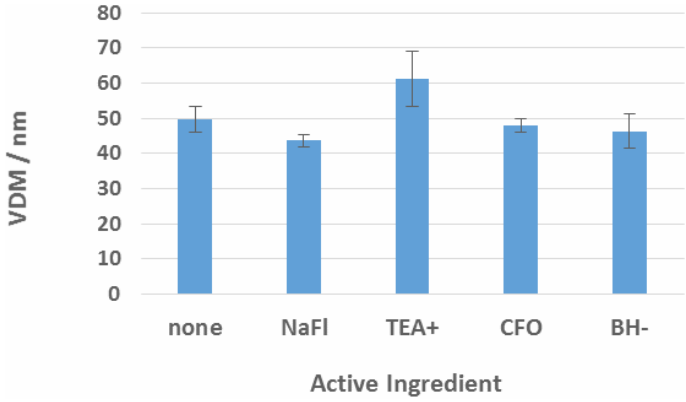
Research aims are achieved through a combination of sciences, such as genetics, biotechnology, plant protection, hygiene, physiology, nutrition, bioinformatics, technologies of process and bioprocess, and agricultural economics, always implementing rules of sound management and practices. Interdisciplinarity, cooperation and innovation are the keywords in AUA research projects. The wide range of research areas actually contributes to the international recognition of the basic and applied research conducted by the AUA academic and scientific community members. Research is funded by National and European scientific/research programmes, public or private organizations or endowments, whereas the financial management is carried out by the AUA Special Research Funds Account (E.L.K.E.).

Key mission in NanoFEED project

- Expert, in vitro testing, pharmacokinetics, nutrient effect evaluation
- Leader, research Sub-Task 3.3. of WP2 – In vitro testing of active molecules

Outcomes / benefits of the project implementation

The Laboratory of Cell Technology (LCT) belongs to the School of Applied Biology and Biotechnology, Department of Biotechnology of AUA. LCT has a long track



Results after DLS at 250kcps, illustrating the mean values of the particle size distribution (± SD) for different active ingredients.

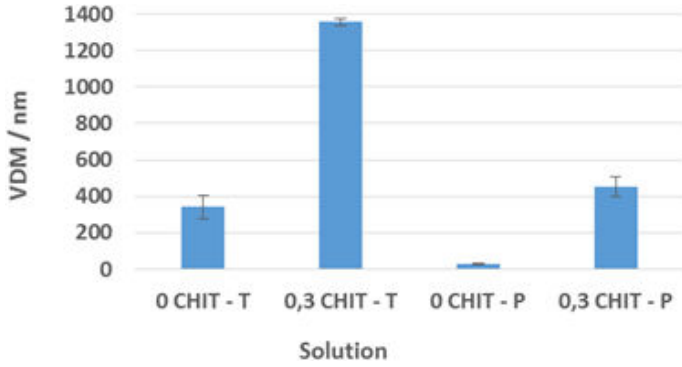
record in toxicology research in vitro, in particular the assessment of toxicity of drugs, pesticides, mycotoxins, xenobiotics, as well as research oriented towards the identification of natural bioactive compounds with pharmacological potential, in particular dopamine agonists/antagonists and anticancer drugs.

During the project researchers form AUA mainly worked on the influence of the charge of the active molecules on particle stability, to form the solid particles with positively, negatively and neutral active ingredients. More specifically, the ability to form solid lipid particles with positively, negatively and neutral active ingredients was evaluated.

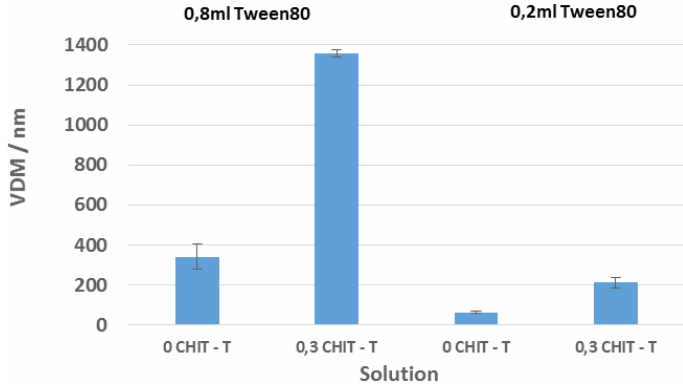
Solid lipids were prepared with sodium fluorescein (NaFl), β-glycerol phosphate (BH-) (negatively charged), triethylamine (TEA+) (positively charged), and caffeine (CF0) (neutral). These experiments are part of the efforts to develop a universal core for delivering any active ingredients. The result of the DLS showed that except for TEA+, all solutions seem to have the same influence.

Moreover, the influence of the chitosan content on the particle size and the influence of the amount of the Tween in different concentrations of chitosan have also been investigated.

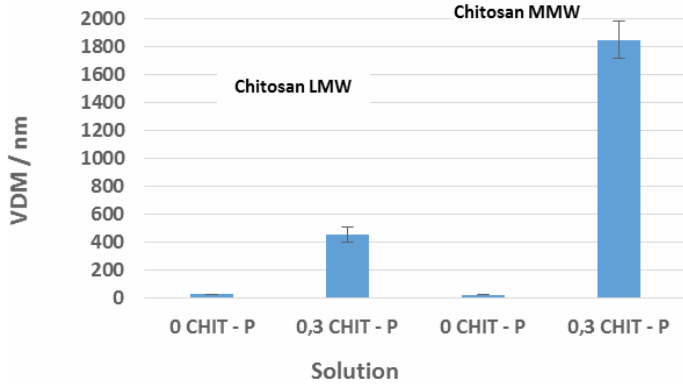
The experiments aimed to develop nanocarriers for cattle feed and thus, particles need to go through the ruminant stomachs, which means that particles have to remain stable while in pH7 and pH2 and start to degrade in subsequent pH7. In this study, the results showed that particles are much smaller when PVP10 is used as surfactant instead of Tween80.



Different surfactants (Tween80 and PVP10) with two chitosan concentrations.



Results after DLS at 250kcps, illustrating the mean values of the particle size distribution (± SD) for different Tween80 concentrations using lmw chitosan.

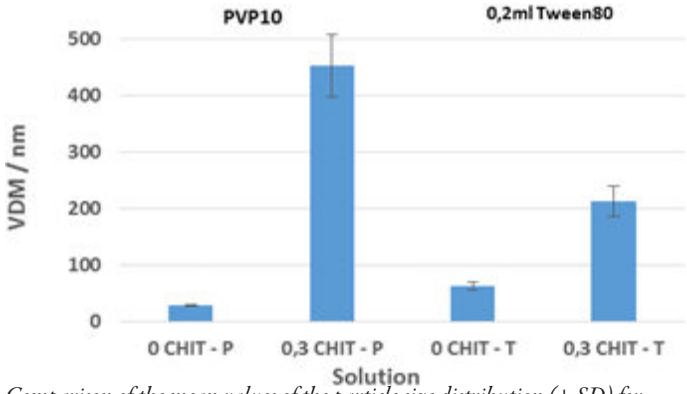


Results after DLS at 250kcps, illustrating the mean values of the particle size distribution (± SD) for different chitosan sizes in two different concentrations, using PVP10.

Due to fact that in the initial study the particle size remains the same in acid and becomes higher with the addition of chitosan, much smaller amounts of Tween80 were tested in order to evaluate the influence of Tween80 on particle size using the same lmw chitosan. In another study, PVP10 and mmw chitosan were used.

In case of the different chitosan sizes, with the use of PVP10 as a surfactant, the DLS analysis presented smaller particle size for lmw chitosan in both concentrations, compared to the respective mmw chitosan.

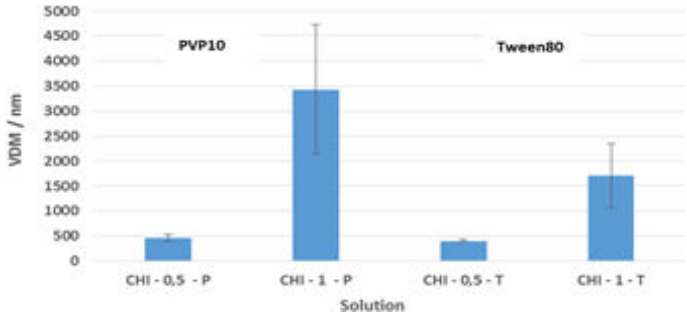
As regards the effect of surfactant, two surfactants with the best responses were tested for lmw chitosan. The application of Tween80 combined with chitosan gives smaller particle size compared to the respective with PVP10.



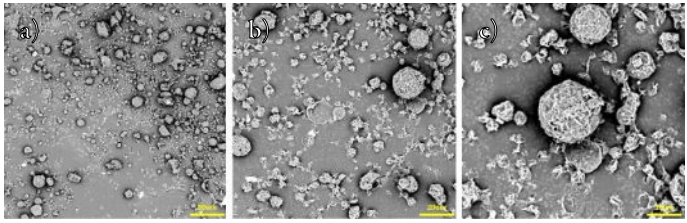
Comparison of the mean values of the particle size distribution (± SD) for PVP10 with Tween80, using the same chitosan size (LMW).

Additionally, coating SLPs with methacrylates studies were performed. More specifically, the feasibility of the coating SLPs with water insoluble polymers was evaluated. Initial experiments were implemented with 0,3wt% chitosan coating concentration and showed bigger particle size for

PVP10 (450 nm) and smaller particle size for Tween80 (200 nm). In this case, larger chitosan concentrations (0.5wt%, 1wt% and 2wt%) were tested taking into account the size and the stability in salts. From the following pictures, it can be easily seen that for solutions using PVP10 and Tween80 as well, apart from the 0,5wt% chitosan concentration, the rest of the samples appear to have big particles. 0.5wt% was chosen to be sprayed as it is the sample with the best particle size showed from DLS analysis.



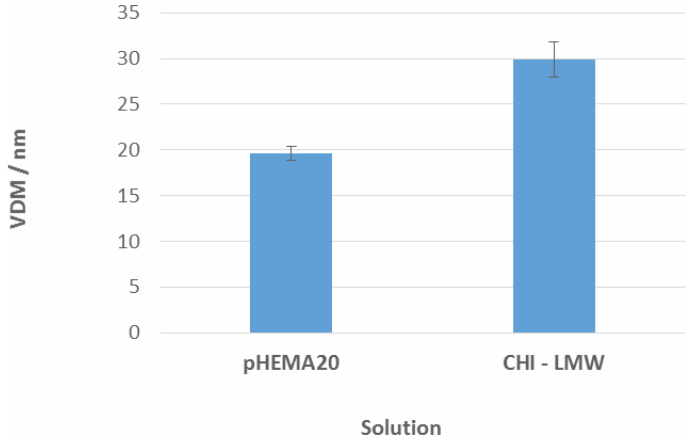
Results after DLS at 250kcps, illustrating the mean values of the particle size distribution (± SD) for different chitosan concentrations using PVP10 and Tween80 as surfactants.



Results after SEM, illustrating the nanoparticles coated with 0.5wt% chitosan after spray drying of Pluronic F 127 in three different scales: 80um (a), 20um (b), and 10um (c).

Images from the sample chosen to be spray-dried were produced using a scanning electron microscope (SEM), provided from INOCURE. The results showed nanoparticles coated with 0.5wt% chitosan.

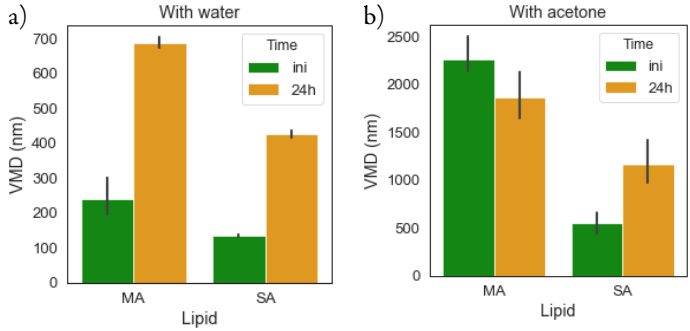
Except from the feasibility of the coating SLPs with water insoluble polymers, the influence of the pHEMA coating to the particle size and stability was also evaluated. For the NanoFEED project, the active ingredient should be encapsulated so that it is not destroyed in the cattle rumen.



Results after DLS at 250kcps, illustrating the mean values of the particle size distribution (± SD) for pHEMA and lmw chitosan.

The encapsulation must be stable at pH7, then be activated in pH2, and only released at the following pH7. In this experiment, the SLP particles were coated with 3 polymers: PMMA with different Mw and pHEMA. For comparison purposes, chitosan was also tested. From the following pictures, it can be easily seen that both solutions with PMMA are not compatible with acetone, as acetone 'destroys' the particles, opposed to the solutions with pHEMA (dissolved in water) and chitosan (dissolved in acetic acid) which both allow the creation of nanoparticles. The results

of the DLS analysis showed that PHEMA gives smaller particles than chitosan in acetic acid.



Results after DLS at 250kcps, illustrating the mean values of the particle size distribution (± SD) for MA and SA dissolved in water (a) and dissolved in acetone (b).

In addition, the spray–drying technology was used on pHEMA coated particles for the evaluation of the spray – dried particles for the stability at different pH.

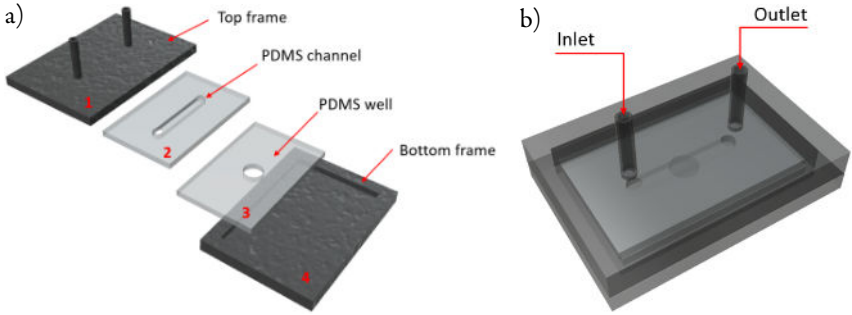
Previous experiments showed that particles could not be coated with PMMA as lipid particles dissolved as soon as added to PMMA solution in acetone. Moreover, particles could be mixed with pHEMA but the solution was not stable (particles sediment after 24h). In this experiment, the fabrication procedures for coating with pHEMA were evaluated, as well as the stearic acid (SA) versus myristyl alcohol (MA) cores. The specific experiment was completed by testing the pH behaviour. The results showed that after the DLS analysis particles prepared with acetone are much larger but may be more stable.

In order to evaluate the stability of the spray-dried particles, a specific procedure was followed. The pHEMA coated lipid particles were tested and adapted on pH7. Side group hydrolysis occurs when they change their pH7 to pH2 with little change in their size or no change at all. When they are brought back to pH7, size group ionization takes place, as well as swelling of the shell, which increases the size of the particles. Finally, on the last stage at pH7, there is dissolution of the shell, which allows the decrease of the particles' size.

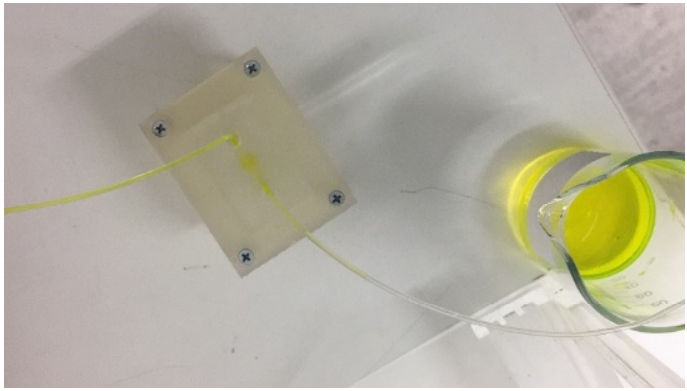
Following this hypothesis, pH was changed by adding HCl and NaOH to the particle solutions. Three samples were chosen for the testing in pH levels: PMMA, pHEMA, and PHEMA-coated SA. Each sample was tested for four different times: initial, 2h, 18h and 24h. After the DLS analysis, in the case of PMMA, although the particle size is too large for any conclusions, there is some decrease in particle size that could be observed.

For pHEMA particles, some pH-triggered change in size may occur, as particles might aggregate or de-aggregate from pH2 to pH7. In the last sample for PHEMA-coated SA particles, some low pH triggered change in particle size may occur, again as particles might aggregate or de-aggregate from pH2 to pH7. A second task was the implementation of 3D designs for the fabrication of microfluidic systems, special for drug delivery. The first sketch of the microfluidic systems was designed. More specifically, this system will be suitable to support cell cultures and measure the properties of the cells. For the construction of the well and the channel as well, polydimethylsiloxane (PDMS) was used as it is reported from many researchers PDMS membranes are biocompatible for studies based on embryonic and stem cell differentiation. Figure presents the sketch of the microfluidic system.

Fused filament fabrication and stereolithography constitute two methods of 3D printing process. The best printing technique was chosen, taking into account the retention of the biological activity of the transferred substances. For this model, stereolithography was used for the printing of each layer (top, bottom and mask for PDMS fabrication). The microfluidic system was successfully tested for leaks and mixing rate.



3D of the microfluidic systems with PDMS channel and well: analytical view of the layers (a) and input and output of the flow (b).



Testing of the microfluidic system for leaks and mixing rate.

Researcher from Agricultural University of Athens performed secondment at NSNV Belgium. Picture from NSNV factory visit.

Selçuk University, Turkey

Mission

Selçuk University's mission is to contribute to the betterment of the nation, humankind, and the environment through the implementation of progressive, excellence-oriented, and consciousness-raising educational and research activities that align with fundamental social and universal principles.

Vision

Selçuk University's vision is to be a world-renowned research university that continuously adds value to society and humanity.

Main Values

- The universality of science and academic freedom.
- Institutional autonomy.
- Transparent, accountable, participatory, and stakeholder-based governance.
- Sustainable quality management.
- Student-centered approach.
- Sensitivity towards the environment.
- Awareness-raising sense of social responsibility.
- Commitment to ethical values.

Department of Chemistry has been focused on material science and technologies, smart surfaces, interface chemistry, BCP technology, energy, sensors development, graphene chemistry & applications, magnetic NPs including synthesis, patterning, functionalization and, directed self-assembly of nanostructures for CMOS technologies.

A major goal is to expedite the development of novel technologies and advanced products to address the needs of local and global technology leaders and take an active part in tackling societal challenges. Researcher has been focused to carry out research in the areas of nanotechnology, advanced materials, and biotechnology.

They can be classified as:

- Nanotechnology (synthesis, patterning, functionalization, surface treatment).
- Synthesis of block copolymers, nanoparticles and quantum dots for different applications.
- Electrochemistry at interfaces.
- Graphene chemistry applications (materials, optoelectronics, sensors, flexible electronics, energy, nanocomposites).
- Membrane technology (bulk, supported, activated and composite membranes, preparation and applications, UF/NF/RO processes and applications).

- Film preparation and composite processing (CVD, PVD, LB, spin coating, layer-by-layer assembly, electrodeposition).
- Synthesis of organic semiconductors, organic electronics, dye sensitized solar cells, organic and hybrid light emittion diodes, metal based quantum dots and their modifications.
- Directed self assembly of nanostructures for CMOS Technologies.
- Standardisation in research and innovation.

Nanotechnology & Surface Engineering Laboratory

- Nanoparticles & magnetic NPs (synthesis, patterning, functionalization, surface treatment).
- CVD systems to form advanced organic and inorganic materials with desired properties.
- Characterization and application of the deposited materials, also the heterogeneous reaction kinetics and mechanism of the deposition processes.
- Processing techniques (in the films, fibers, fillers, coatings, etc).
- Advanced studies on smart surfaces (hydrophobic, hydrophilic, oleophobic, oleophilic, antibacterial, conductive, responsive (thermo, photo, pH etc.), SWCNTs, MWCNTs.
- Graphene chemistry & applications (materials, optoelectronics, sensors, flexible electronics, energy and nanocomposites).

Key mission in the project

- Conduct a workshop: Material and particle characterization
- Leader research Sub-Task 3.1. Nano- and microparticles characterization

Sub-Task 3.1. Nano- and microparticles characterization

Each polymeric systems were optimized to enable maximal loading of active molecule not disrupting the structure of polymeric particles. In order to modify the release time, the particles that crosslinked by standard methods (i.e. glyoxal crosslinking of gelatine, cellulose, polyvinyl alcohol, chitosan and starch, calcium crosslinking of alginate). Particle morphology and material properties were analysed by Selçuk . Morphology of particles was evaluated by SEM and HRTEM.

Outcomes / benefits of the project implementation

Selçuk offered an integrated infrastructure and advanced instrumentation equipment. Facilities such as the thin film, plasma systems, laser laboratories, advanced microscopy laboratory, cleanroom, biotechnology laboratories are managed by technical specialists and can be accessed by researchers from a wide variety of academic backgrounds, as well as a broad range of industry sectors. The key infrastructure includes: advanced microscopy Laboratory (UHR-TEM, SEM), electron Spin Resonance (ESR), nuclear Magnetic Resonance (NMR), inductively

Coupled Plasma Mass Spectrometer (ICP-MS), atomic Force Microscopy (AFM) – Scanning Nearfield Optical Microscopy (SNOM), spectroscopy Laboratory (UV-Vis, FTIR, Flourescent, NIR), chromatography laboratory (HPLC; GC-MS, GPC), thermal Analysis (TA, DSC, TGA) and dynamic Light Scattering (DLS), BET.

The infrastructure supports the role in nanomaterial characterization which was characterised by Selçuk :

The knowledge about strategies and methods for nanoparticle characterization was delivered by SU reseachers for the preparation of advanced drug delivery systems. The ESRs from SU were trained on nanoparticle morphology and stability characterization by INCU and *the knowledge about nanoBioEngineering of bioinspired biopolymers and industrial production of feed supplements and bioprocessing and biopolymer production and characterisations methods by NSNV.*

The workshop activities performed by Selçuk Researchers.

Summary of the workshop on "Micro Encapsulation Techniques and Applications" delivered by ERs: Microencapsulation is the process which is applied for isolation purposes by surrounding or enveloping one substance within another substance on different scales and produced capsules ranging from less than one micron to several hundred microns in size.

Microencapsulation is the application of a very thin coating to a small particle of a solid or a droplet of a liquid & dispersion. The composition of the core material can be varied as the liquid core can include dispersed and/or dissolved material. The selec-

tion of coating material decides the physical and chemical properties of the resultant microcapsules/microspheres. While selecting a polymer, the product requirements that should be taken into consideration are: stabilization, reduced volatility, release characteristics, environmental conditions, inert toward active ingredients, controlled release, pliable, tasteless, stable and non-hygroscopic, low viscosity, soluble in an aqueous media or solvent, should be flexible, brittle, hard, thin.

Researchers from Selçuk performed the research activities related to electrospinning and electrospraying of nanoparticles -microparticles and nanofibrous matrices at InoCure. Active molecule encapsulation strategies, encapsulated nanomaterials were characterised.

Chitin $(C_8H_{13}O_5N)n$ is a natural polysaccharide and is the second most abundant biopolymer after cellulose. The following studies were performed:

- Differences in chitin structures isolated from the female and male of the four orthopteran species.
- Description of the gamma chitin in detail.
- Isolated chitin from ladybug.
- Chitin hoops obtained from *Ommatoiulus sabulosus* (*Diplopoda, Julidae*).
- Natural chitin film production from grasshoppers abdominal skeleton
- Hexagonal chitin isolation from insect eyes.
- Isolation of chitinious microcages from resting eggs.
- Effect of glutaraldehyde cross-linking degree of chitosan/sporopollenin microcapsules for various applications.
- Chitosan/sporopollenin microcapsules: preparation, characterization and applications.

- Preparation and characterization of biodegradable pollen-chitosan microcapsules and applications.
- Microfungal spores immobilised chitosan microcapsules and applications.

Microencapsulation technology was utilized to create a unique delivery system that can provide targeted delivery of nutrients and other bioactive compounds. One of the most promising applications of NanoFEED project is in animal nutrition. By encapsulating nutrients such as vitamins, minerals, and probiotics in a nanostructured matrix, these compounds can be protected from degradation and delivered directly to the animal's digestive system, resulting in increased absorption and utilization of these important nutrients.

University of Veterinary Medicine Vienna, Austria

vetmeduni
University of Veterinary Medicine, Vienna

University of Veterinary Medicine Vienna.

Institute of Animal Nutrition and Functional Plant Compounds

- The institute contributes significantly to the field of animal nutrition, nutritional physiology, feed hygiene, and feed processing through research, service, and practice-oriented teaching
- Following the reorganization in 2012, the Institute of Animal Nutrition and Functional Plant Compounds was formed and has functioned under the leadership of Qendrim Zebeli. Currently, the Institute consists of three working groups including Animal Nutrition, Functional Plant Compounds, and Nutrigenomics
- The Animal Nutrition working group focuses on nutritional and clinical aspects of pet food and animal feed, and also on the significance of the diet fed to livestock in terms of its impact on the production of foods of animal origin. Research activities at the Institute encompass basic as well as applied research in the field of animal nutrition. These efforts are carried out in cooperation with institutions within the university and externally and also with international partners

Key mission in the project

Our key mission is to expand our research connections with academic and industrial partners. The institute joins forces with the industrial partner Nutrition Sciences, Belgium to perform research activities dealing with the efficient use of raw materials for cattle feeding. Various activities were performed including:

- Meta-analysis to investigate the influence of supplementation of rumen-protected choline on the health and performance of dairy cattle

- First secondment in 2019 to find simple yet reliable methods to differentiate the apparent availability of Mg in supplemental sources
- Second secondment in 2022 to validate the chosen method
- Webinar in 2020 "Innovations in the nutrition for improving health of dairy cattle"

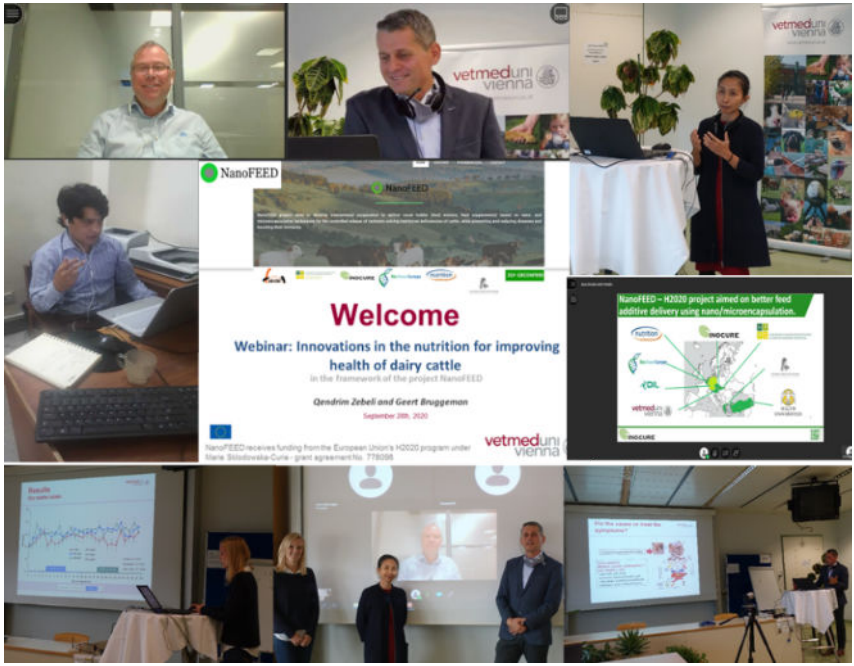
Photos from the secondments.

Outcome of the project implementation and future perspective

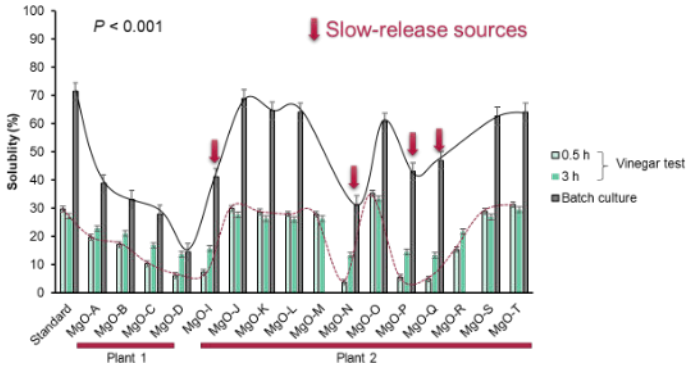
We proved the concept of using simple chemical tests to differentiate potentially available Mg contents of the supplemental sources based on their solubility and alkaline properties. The findings and recommendations can be easily implemented in feed plants to assist in selecting better supplemental sources of magnesium for feed formulation.

The vinegar test (i.e., incubation of magnesium sources with a 5% acetic acid solution at 0.5 or 3.0 h) is the most promising method due to its simplicity, reproducibility, and robustness. We further showed that the content of solubilized magnesium can be estimated from pH readings after incubation when a feed plant does not have access to an expensive ICP instrument for mineral analysis.

However, the current protocol of the vinegar test has some limitations for its application in magnesium sources with very high alkaline properties and it could mislead the



Webinar presentations by Nutrition Sciences, Belgium, Vetmeduni Vienna, Austria, UVAS, Pakistan and InoCure, Czech Republic.



Solubility of Mg oxide sources subjected to 0.5 and 3 h of incubation with an acetic acid solution (vinegar test) or a 24-h in vitro rumen fermentation (batch culture).

result of relatively inert sources. Moreover, factors other than the solubility affect the ruminal absorption of magnesium and thus the true availability of the magnesium for the maintenance and performance of animals.

Fine-tuning the protocol of the vinegar test (and possibly other chemical tests) and importantly, its incorporation in related research in vivo in the future will be necessary to overcome these challenges with the prospect of using a chemical test for estimating bioavailability of the magnesium supplemental sources. This will bring the reality for the routine application of a chemical test in feed plants to improve feed formulation, which is key to reach the ultimate goal of an efficient use of raw materials in dairy production.

Secondee from VUW Austria visiting NSNV Belgium: The main scope of experimental work was related to the efficient use of raw materials in ruminant feeding. Work aimed at finding simple yet optimal laboratory methods for differentiating the quality of supplemental Mg sources.

German Institute of Food Technologies (DIL)

DIL Institute site – 9.000 square metres working space.

Introduction

The DIL, which is the German Institute of Food Technologies operates as a research institute working in the areas of food structure and processing, food safety and authenticity, and sustainability and food data. The institute's competencies and technical capabilities span the full range of food technologies.

Our strengths

- Research and development expertise from product and process development till market introduction.
- Eminently well-equipped technical schools and laboratories with cutting-edge equipment.
- Test production, manufacturing and sales of selected products keep us close to the market.
- Multidisciplinary know-how.
- International cooperation with partners from industry and academia.
- Fostering entrepreneurship and start-ups (DIL Innovation Hub).
- Decades of experience in recipe and process optimisation focusing on:
  - Processing technologies.
  - Food & feed technology.
  - Laboratory analytics.

Key mission in the project

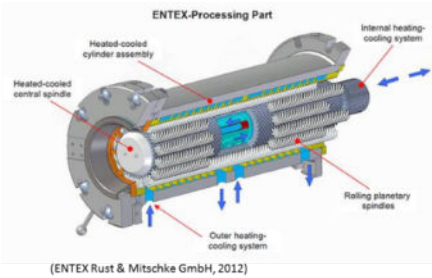
- Expert: biopolymers, microencapsulation, cold extrusion, preparation of feed.
- Leader, WP5.
- Leader, research Sub-Task 3.2. of WP2 – Release of active molecules and particle stability Bio Base Europe Pilot Plant (BBEU).

- Expert, biopolymers, chitosan and bioprocessing (lab-to-industrial systems).
- Leader, research Sub-Task 2.1. of WP2 – Polymers for drug delivery systems.

Outcomes/benefits of the project implementation





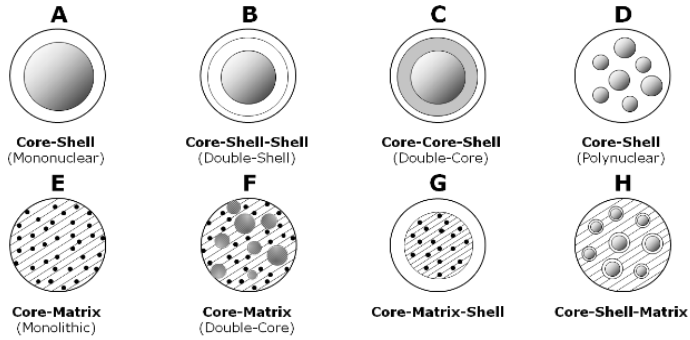


(ENTEX Rust & Mitschke GmbH, 2012)


DIL's Process Engineering group focuses on development and implementation of innovative technologies for the sustainable production of high-quality foods and feeds. To reach this goal, we investigate novel mechanical and thermal processes and evaluate their impact on resulting product characteristics and functionality. We work on industry-ready solutions in the fields of **mixing, foaming, extrusion, and comminution** including design and scale-up of related processes.

Micro-encapsulation

Encapsulation is the packaging of material in a coating or matrix to protect it from the environment. Micro-encapsulation of active components by extrusion processing can



be regarded as variant of the cold extrusion process. In the feed zone, a wall material (flour at low pressure) is generated to homogenize the material. In the following zones an active core material is added and homogenized and compressed.

In the final part of the barrel, a constant screw design helps to maintain a continuous high pressure to ensure a uniform delivery rate of molten material out of the extruder. The barrel is also divided into sections to allow for section-controlled variation in temperature. Addition of the active ingredient might be in the mixing/dispersing zone of the extruder at about halfway to minimizes the residence time of the active ingredients. At the end of the barrel, a "pre die" and "die head" determine the shape of the final product (*e.g.*, sheets, ropes or threads). It can be equipped with a chopper/cutter to obtain granular extrudates.

1. Research Department – Advanced Technologies

Advanced Technology is working on development of new technologies and their customised applications in research and production processes. Our research focus lies in increasing existing process efficiency, product quality and safety. Technological focus is on:

- Technologies based on electromagnetic phenomenon
  - Pulsed Electric Fields (PEF).
  - Ohmic Heating (OH).
- Technologies based on application of pressure:
  - High Hydrostatic Pressure (HHP).
  - High Dynamic Pressure (ultra high-pressure homogenisation – UHPH).
  - Application of supercritical fluids (water and CO_2).
- Technologies based on pressure waves
  - Shockwave (SW).
  - Ultrasound (US).
- Radiation based technologies
  - UV light, pulsed light (PL) and infra-red (IR).
  - Electron beam treatments (E-Beam).

2. Research Department – Product Innovation

The Product innovation at DIL is equipped with all the necessary processes for food production: deep-frying, pasteurizing, homogenizing, freezing, molding, shaping, drying, mixing, etc. We also have an industrial kitchen where we can develop all kinds of food and feed products from scratch.

On the research side, the product innovation focuses mainly on protein science and quality within meat technology, alternative proteins such as insects, algae and side stream proteins, *e.g.*, pumpkin, sunflower, hemp and rapeseed with a focus on quality, efficiency and sustainability.

3. Research Department – Chemical Analytics

The quality of the work at the Chemical Analytics is ensured by the comprehensive expertise of our employees, including certified food chemists, chemists, food technologists and nutrition scientists.

The analyses are performed with state-of-the-art technical equipment:

- Microbiological & chemical test labs with broad analysis spectrum (qualitative/ quantitative) analytical chemistry methods.
- Quick review inspections due to extensive QM expertise (UV / Vis Scanner, HPTLC-MS, HPTLC-Biolunminizer).
- Analysis of allergens relevant to food.
- Reliable and up-to-date analysis for herbicides & residues of veterinary medicine.

Analysis of processes and products

- Sensory, chemical, microbiological & molecular biological tests.
- Continue water analyzes and immunological tests.
- Quality management: "SusFood"- system solution to support small and medium-sized companies and optimize resources.
- Hygiene checks, microbiological or biochemical tests such as PCR or ELISA e.g. allergens analysis.

Specialty analytics

- Development, establishment & validation of new methods.
- Influence of ingredients on the technological properties.
- Close cooperation with other research departments.
- Food physics, structure and functionality, process engineering.
- Food Authent (NMR lab since 2018).

4. Research Department – Petfood and Feed Technology

There are in fact 3 main areas of focus, all driven by the protein challenge of our days: Petfood technology, Feed technology and Meat Technology.

The activities of the Feed Technology division are divided into an **analytical division**

related to food safety and a division in which **process engineering** solutions are developed. In addition to the grinding, mixing and dosing of the various components, **structure-giving processes** serve as an important tool for optimally adapting the use of energy, protein and fat to meet the animal's needs.

Innovation drivers of the Institute

DIL Innovation Hub

DIL Innovation Hub is one of the leading national stakeholders in the startup ecosystem for Food-Tech startups, providing a platform to promote innovations in the food industry and to network actors from this area. For startups from the agri-food sector, we provide a wide range of offers for every development phase. DIL Innovation Hub is one of the five EIT Food Hubs in Europe implementing the Seedbed Pre-Accelerator programme and is connecting startups to other national Agri- and Food-Tech programmes and to DIL's extensive partner network.

INOCURE s. r. o., Czech Republic

Introduction

InoCure is a technological company focused on development of modular electrospinning devices. InoCure currently markets InoSPIN (lab-to-pilot electrospinning unit) and performed several custom production line developments including industrial roll-to-roll system for wound dressing applications. The core of the devices is a patent pending high-throughput electrospinning electrode used for processing nanofibers at a higher speed compared to competitors. The strategy of the company is to expand utilization of technology to novel segments, such as food, medicine, energy and other industries.

Key mission in NanoFEED project

- Expert, nanoencapsulation, drug delivery, electrospraying, electrospinning, nanoparticle unit engineering, pharmacokinetics, in vivo testing, production.
- Leader, WP1, WP4, WP5.
- Leader research Sub-Task 2.2. of WP2 – Electrospraying for production of nanoparticles.

Outcomes/benefits of the project implementacion

Functional nanofibers

Personalized medicine has been characterized in recent decades by a massive transfer of nanotechnologies. Small diameter favors nanofibers for preparation of highly effective filters and/or biosensors. Clearly, randomly deposited nanofibers of small diameter led to formation of nets with small pores resulting in a highly effective filtration process. Nanofibers are versatile, flexible, nano-biomimetic and, namely, they are suitable for functionalization both on their surface and in their core. In addition, they can be produced easily and, with a novel way of their preparation by AC technology, they can be also prepared in a bulk quantity and, thus, cost-effectively.

Electrospinning is a fiber-forming nanotechnology that enables the production of nanofibers and submicrometer fibers drawn from polymer solutions and melts. Modulation of polymer solution properties (concentration, viscosity, and polymer molecular weight), electrospinning setup (spinning electrode, collector, flow rate, collector distance, and applied voltage), and environmental properties (humidity and temperature) reflects in production of fibers with diameters ranging from micrometers to tens of nanometers. Thus, the electrospinning process and its controlling allows us fabrication of structures with nanoscale topography, tunable porosity, and large surface-area-to-volume ratios. The problem hindering extensive application of these technologies is a low production rate.

Bare nanofibers alone are already an interesting material, but their main potential in medicine seems to be hidden in their subsequent functionalization, *i.e.* preparation of so called smart nanofibers. This means specific modification of nanofiber surface and/or core to induce their new specific properties. There are several ways for modification of nanofiber surfaces, mainly based, first, on physical modification by cold plasma followed by, second, a specific chemical intervention resulting induction of specific chemical groups and linkers necessary for subsequent binding and, third, attachment of specific molecule (*e.g.* miRNA). Internally functionalized nanofibers are commonly prepared by "coaxial electrospinning" and there are characterized with a strictly organized core-shell structure. Both nanofiber surface and core can be employed for the immobilization of diverse bioactive substances such as RNA, proteins, antibiotics, or other drugs. Preparation of nanofiber-based detectors functionalized on nanofiber surface with relevant antibodies belong among the key steps towards highly sensitive and specific bionanosensors.

Thus, among important goals of NanoFEED project will be identification of up-regulated or down-regulated markers at early stages of serious diseases for clinical applications. Recent progress in the preparation of protein-compatible nanofibers has widely open the door for Additive Manufacturing (AM) techniques that are considered as a novel tool for fabrication of customized microarchitectures both for specific bionanosensors, theragnostic systems and for smart scaffolds. AM technology could bring a large profit also for the concept of the interactome, namely for detection of extremely low concentrated targets (proteins). Clearly, extremely large ratio between surface and volume of nanofibers and small pores in nanofiber net favors nanofiber nets as a highly effective filtration unit.

What is our expertise?

Nanofibers are excellent filter due to their small pores and easy surface modification.

Nanofeed project: Smart nanofibers in medicine seem to have an extremely high potential due to their unique biomimetic properties. Nanofibers can be functionalized both on their surface and in their core. The surface modification can be highly selective, which opens the door for controlled and targeted drug delivery. The greatest advantages of the use of nanofibers are their topographical properties as good absorption capacity, controllable degradation, adhesive layers, and the ability to produce multilayered materials.

Many methods have already been developed to fabricate nanofiber carriers. The size of individual nanofibers depends on the manufacturing method by which the fiber is created. The DC electrospinning method has achieved the greatest use. The applications of electrospinning in drug delivery are almost limitless. Electrospun nanofibers are promising drug delivery systems as their huge surface can serve for physical absorption or chemical conjugation. Several bioactive molecules, such as ascorbic acid and toco-

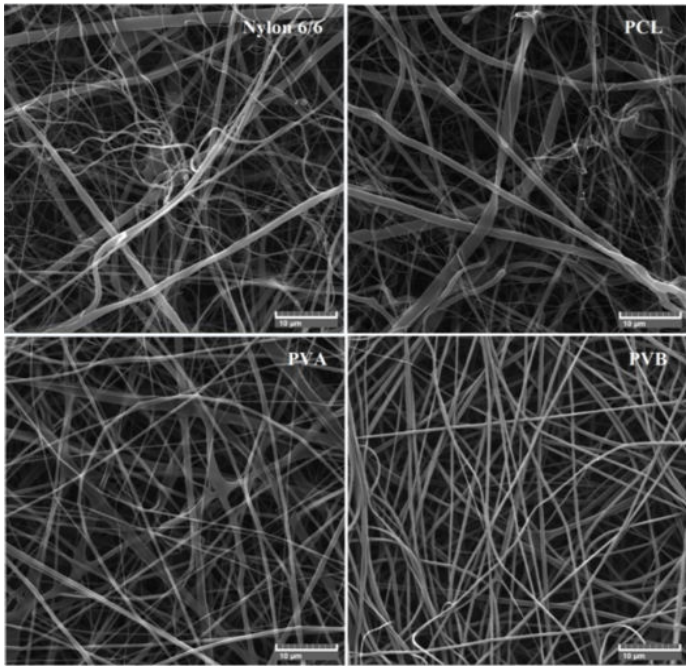
pherol, can be encapsulated into conventional liposomes and, subsequently, encored on the nanofiber surface or be encapsulated into nanofiber core. Conventional liposomes cover artificial vesicles where a hydrophobic membrane can encapsulate lipid-soluble substances while an aqueous interior hydrophilic molecule. Due to their desirable properties such as low toxicity, good biocompatibility, targeted delivery of bioactive compounds to the required site of action, incorporation of hydrophobic/hydrophilic drugs, *etc.* (Mickova *et al.* 2012; Akbarzadeh *et al.* 2013; Vocetkova *et al.* 2014).

How does it look mcroscopically?

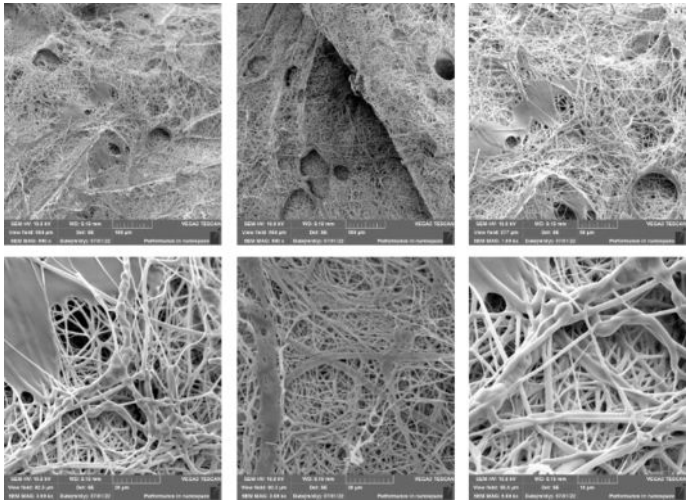
The Nanofeed project has significantly contributed to the development of this encapsulation technology with the goal to bring new, cheaper, and more efficient controlled drug delivery nanosystems. The proposed technology enables encapsulation of diverse active molecules and simplify the protection against degradation in rumen for wide range of active molecules. Nevertheless, due to encapsulation and improved delivery to small intestine, novel active molecules may be used to treat nutritional deficiencies.

In order to achieve project goals in the framework of the Project, the secondees received by InoCure, were trained on the development and optimization of liposomes preparation protocol for the encapsulation of bioactive compounds, before starting the experiments they received appropriate training concerning laboratory safety issues and techniques. Liposome preparation using film hydration method incubated with PBS at 32°C for 24 h while shaking at 150 rpm. They were also trained in liposome visualization using fluorescent dye. The secondees were also trained in nanofiber preparation using electrospinning technology. The nanofibers with embedded liposome were analyzed by scanning electron microscopy (SEM), the most common method used for characterization of nanofibers, which allows the analysis of the nanofiber diameter / size, the pore distribution (porosity of nanofibers), and also the nanofiber distribution. During the experimental work, different polymers coated liposomes using PVA and PVP as polymers were prepared.

In addition, cell viability assays have been used to assess the effects of the encapsulated bioactive compounds in the liposome preparations. The assays involved also cell-based biosensors according to the Bioelectric Recognition Assay (Moschopoulou *et al.* 2006) principle in order to identify biomarkers such as oxidative stress molecules.



How does it look microscopically before use?



Fabrication with NanoSpider Phosphatidylcholine liposomes encapsulated with a-Tocopherol and coated with PVA polymer.

One of the elderly secondee from NPPC visiting InoCure Prague.

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Nutrition Sciences N.V., Belgium

General information

Belgian is a private R&D company well-known for its R&D activities in animal production and pays much attention to the introduction of new technologies in animal livestock production for its customers.

It provides:

- Research of functional feed ingredients and further successful development towards the market.
- Several patents and different successful innovations.
- Significant contributor (including coordinator activities) in a number of Belgian and European research and innovation projects.
- International bilateral collaborations with universities, research institutes and companies and is an active partner in the European framework program as well as internationally recognized organizations.

Competences

- Nutrition Sciences N.V. actively participates in projects by performing screening for functionalities and by validating those functionalities in *in vitro* and *in vivo* trials for the project outcomes.
- In that context, Nutrition Sciences N.V. runs its own innovation laboratory, in order to perform bioactivity tests and specific analyses on the functional ingredients. Following analyses can be performed: humidity, ash content, protein and fat content, minerals, metabolizable energy, buffering capacity, sugars. In addition, different bioassays and gastrointestinal simulations are performed in order to reveal functionalities such as antimicrobial and prebiotic activity.
- In addition, Nutrition Sciences N.V. goes on the development of innovative functional ingredients with special focus on nutritional and physiological functionality, improving animal health and wellbeing and resulting in improved zootechnical performances (daily growth, daily feed intake and feed conversion ratio). In that respect, Nutrition Sciences N.V. owns it animal trial facilities and supporting diagnostics.

Key mission in NanoFEED project

- Expert, active molecule isolation, feed development, bioprocessing, animal and human nutritional additives, production, commercialization.
- Leader, research Task 1. of WP2 - Compound feed formulation and Task 4. of WP2 – Final feed formulation manufacture for in vivo testing.
- Expert, material characterization, physical chemistry, nanoparticle morphology, chemical analysis.

- Conduct a workshop: Material and particle characterization.
- Leader, research Sub-Task 3.1. Nano- and microparticles characterization.

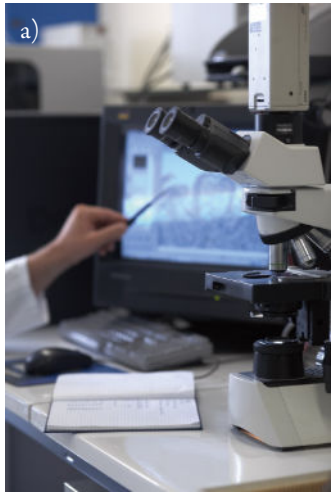
Innovation laboratory at Nutrition Sciences N.V.
Secondee from NPPC visiting Nutrition Sciences N.V.

Outcomes/benefits of the project implementation

- Functional ingredients as sound basis for safe and healthy feed and food.
- Nutrition Sciences N.V. strongly focuses on gastrointestinal health (and overall health) of farm animals. In this context some novel natural and sustainable feeding concepts are developed within projects.
- One of the most recent innovations is the launch of health supporting portfolio for livestock, based on 2 pillars: optimal nutrition and health. The NanoFeed project fits into this strategy as well, since it empowers a more nutritive pilar of this strategy, at the level of encapsulation.

For Nutrition Sciences N.V., NanoFeed is an interesting project in order to share competences that are lacking within the organization. By participation in secondments, researcher of Nutrition Sciences N.V. learned new technologies for testing additional functionalities in vitro and in vivo. In that respect secondments to the Greek and Slovakian partners were performed. In order to work on additional formulation technologies, a secondment to the German partner was performed. In addition, Nutrition Sciences also invited secondees from different partners, for example partner NPPC.

Participation and co-organization in (innovation) workshops within NanoFeed helped sharing experiences on technologies, intellectual properties and markets.



a) Functionality testing in animal tissue (gastrointestinal sampling); b) Secondee from NPPC visiting Nutrition Sciences N.V.

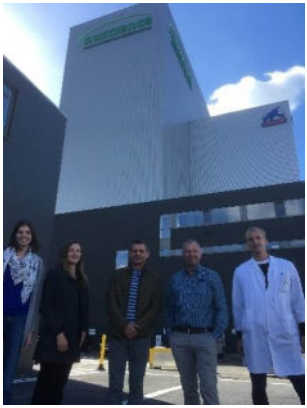
Secondees from NPPC visiting Nutrition Sciences N.V.

NanoFeed fits in the main interest for Nutrition Sciences N.V. for co-developing innovative functional feed ingredients with special focus on nutritional and physiological functionality, improving animal health and wellbeing and resulting in improved zootechnical performances (daily growth, daily feed intake and feed conversion ratio).

Additionally, Nutrition Sciences developed via NanoFeed sustainable nutritional con-

cepts able to reduce the use as antibiotics in livestock production. These concepts are mainly based on natural compounds empowered by nanotechnology.

This way, NanoFeed contributed to a multidimensional solution raising interest among various stakeholder groups, including consumers and policy-makers, highlighting the need for an integrated approach to protect consumers from antimicrobial resistance related risks in the food chain by establishing appropriate preventive and control measures.



Nutrition Sciences N.V. hosts a networking activity with partner VUW and DIL in Belgium.

Production protocol – NanoFeed ingredients for animal feeds

Based on lab trials at partner Innocure and pilot trials at partner DIL, protein source (fine soy, 30%) and carrier (fat, 70%) are coated to (nano) aggregates at high pressure and high temperature. This process is a semi-continues process with following parameters: aggregation flow 20 kg/h at 65°C followed by cooling (5°C) screw speed 100 l/h. This resulting (nano) aggregate concept is further integrated as a functional ingredient in a conventional concentrate or premix at partner Nutrition Sciences, for fortifying the final animal feed application.

NanoFeed concept for inclusion in final feed.

Bio Base Europe Pilot Plant (BBEPP)

BBEPP in the nanofeed project: bridging the science and industrial production

The BBEPP team combines over 50 years of experience in fermentation and scale-up, and also has extensive expertise in biocatalysis, green chemistry, and the development of industrial recovery and purification techniques. Furthermore, it has built up significant expertise with biopolymer production and purification in particular, including chitooligosacharides (COS).

56 Pilot scale state-of-the-art 1.5 m^3 and 15 m^3 fermentation equipment for industrial bioprocessing at BBEPP.

Key mission in NanoFEED and Outcomes/Benefits of the project implementation

- Expert, biopolymers, chitosan and bioprocessing (lab-to-industrial systems)
- Leader, research Sub-Task 2.1. of WP2 – Polymers for drug delivery systems

To meet the increasing global food demands from a steadily growing population with high living standards, efficient and high-quality production of livestock is imperative and has been identified as a priority area by the European Commission.

Yet, to enhance the efficiency of stock raising and its competitiveness in the food industry, nutritional deficiencies present a key challenge which needs to be overcome. In this light, the NanoFEED project aimed to contribute to the development of novel feed solutions to treat cattle nutrition deficiencies, based on the use of nanostructured carriers from sustainable biopolymers such as chitosan and cellulose. Here, insight into the techno-economic feasibility as well as the environmental performance of the novel biobased production processes is crucial, whereas the transfer of a process from a lab environment to industrial systems

often forms a principal barrier to wide industrial implementation of such innovations.

As a flexible state-of-the-art pilot facility for the bioeconomy and a partner with the NanoFEED project, Bio Base Europe Pilot Plant (BBEPP) aims to close this gap in the innovation chain, developing and scaling bioprocesses from a laboratory level to an industrial multi-ton scale and hence bridging science and industrial production. It is operating according to the open innovation service model, where companies and research institutes throughout the world that are active in the bio-based economy can make use of this test facility for their technological developments.

By increasing knowledge transfer and providing expert input in the NanoFEED project through active participation in project consortium meetings and workshops, BBEPP has contributed to the enhanced insight into applied research and development processes and to connecting research and industry, hence stimulating innovations and new value chains in the bioeconomy.

Future perspectives

Where should the started cooperation and research go further?

What is the NanoFEED project impact on preparation and implementation of other related projects?

- **All partners: The topic of using nanotechnology in cattle nutrition was a challenge.** Despite the obstacles during the implementation of the project (corona pandemic, personnel changes and changes in partner organizations), thanks to the unique composition of the Project Consortium - organizations that are leaders in the field of their professions, the goals of the project were achieved.
- **Selcuk University, Turkey:** NanoFEED project has shown to revolutionize the field of encapsulation by providing a highly efficient and effective way to protect and deliver bioactive compounds. Overall, the future perspective of NanoFEED project looks promising, and continued research and development in the encapsulation which will likely lead to even more innovative and effective delivery systems for a wide range of bioactive compounds.
- **Nutrition Sciences, Belgium:** Further research will translate the Nanofeed outcomes into functional ingredients for reducing the use of antibiotics in livestock production. This way, NanoFeed contributes to safe animal nutrition for healthy human food.

  The NanoFeed project brought promising insights into the use of (nano)coating technologies in protection of functional feed ingredients, such as amino acids. This increases bioavailability of nutrients and can be of interest in lowering feed losses and nitrogen emissions affecting climate change.

  This topic becomes more urgent in today's animal husbandry, and for that reason the partners are looking for further exploitation of the NanoFeed project outcomes for other nutrients, such as new emerging amino acids like valine and histidine. Opportunities for further collaboration into that field and to substantiate the European farmer as part of the solution for coping with climate change - Nutrition Sciences is happy to help validating and bringing the concepts towards the EU market for introducing sustainable farming practices.
- **National Agricultural and Food Centre, Slovak Republic:** European cooperation on next project proposals in wide field of NanoFEED topic and its impact on agriculture and food production – developing, producing, testing of new feed / food additives with high value in smart farming, precision nutrition, circular economy and emission reduction.

  The implementation of the NanoFEED project provided in addition to its main project outputs, also support for the creation of proposal of new project in frame of general call 2022 of the Slovak Research and Development Agency.

This demonstrates the benefits and impact of the NanoFEED project. Proposal of the new project was prepared by NPPC workplaces involved in NanoFEED project: Department of Nutrition at the Research Institute for Animal Production in Nitra (in sacco and mobile bag testing methods using cannulated cows in precision nutrition research and applications) and Department of Technology, Innovation and Cooperation with Practise (Biocentre Modra) at the Food Research Institute Bratislava (feed and food production technology).

NATIONAL AGRICULTURAL AND FOOD CENTRE

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 778098

NANOFEED IS MSCA – RISE PROJECT FOCUSED ON:

- **Development of nano- and microparticle – based feed supplements delivery systems to cure dietary disorders of cattle**
- **Encapsulation of bio-based food aditives to prevent their metabolism in the rumen and release in abomasum or small intestine**
- **Controlled nutrient delivery, animal feed improvements, increased cattle immunity and illness prevention**

PROJECT COORDINATOR

NPC NATIONAL AGRICULTURAL AND FOOD CENTRE

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PROJECT PARTNERS

INOCURE

DIL

nutrition sciences

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Bio Base Europe Pilot Plant

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