ALLEGATO B

UNIVERSITÀ DEGLI STUDI DI MILANO
selezione pubblica per n._1__ posto/i di Ricercatore a tempo determinato ai sensi dell'art.24, comma 3, lettera a) della Legge 240/2010 *nell'ambito* del Piano Nazionale di Ripresa e Resilienza (PNRR), per il settore concorsuale O7G1 Scienze e Tecnologie Animali, settore scientifico-disciplinare AGR/18 presso il Dipartimento di Medicina Veterinaria e Scienze Animali
(avviso bando pubblicato sulla G.U. n. 96 del 06/12/22) Codice concorso 5147

[Tamil Selvi Sundaram]
CURRICULUM VITAE

(N.B. IL CURRICULUM NON DEVE ECCEDERE LE 30 PAGINE E DEVE CONTENERE GLI ELEMENTI CHE IL CANDIDATO RITIENE UTILI AI FINI DELLA VALUTAZIONE.
LE VOCI INSERITE NEL FACSIMILE SONO A TITOLO PURAMENTE ESEMPLIFICATIVO E POSSONO ESSERE SOSTITUITE, MODIFICATE O INTEGRATE)

INFORMAZIONI PERSONALI (NON INSERIRE INDIRIZZO PRIVATO E TELEFONO FISSO O CELLULARE)

COGNOME	**SUNDARAM**
NOME	**TAMIL SELVI**
DATA DI NASCITA	[20.07.1992]

TITOLI

TITOLO DI STUDIO
(indicare la Laurea conseguita inserendo titolo, Ateneo, data di conseguimento, ecc.)

Bachelor's degree:
Bachelor of Technology (B.Tech.) (Honors)- Biotechnology
Anna University, Chennai (India). **Date of Bachelor's degree achieved: 30th April 2014**

Master's degree:
MSc. Environmental Protection and Agricultural Food Production,
Universität Hohenheim, Stuttgart (Germany). **Date of Master's degree achieved: 20th August 2018**

TITOLO DI DOTTORE DI RICERCA O EQUIVALENTI, OVVERO, PER I SETTORI INTERESSATI, DEL DIPLOMA DI SPECIALIZZAZIONE MEDICA O EQUIVALENTE, CONSEGUITO IN ITALIA O ALL'ESTERO
(inserire titolo, ente, data di conseguimento, ecc.)

PhD degree:
PhD in Molecular Animal Nutrition (MANNA) on the thesis title, **"Establishing *in vitro* intestinal epithelial cell models in translational animal nutrition".**

(This is a European Joint Doctorate Degree Programme carried out jointly at the University of Milan (Italy) and the University of Veterinary Medicine and Pharmacy in Košice (Slovakia). This study was funded by the European Union's Horizon 2020 Programme under the Marie Sklodowsk-Curie grant agreement no. 765423).

Date of PhD degree achieved: 7.09.2022 (at University of Veterinary Medicine and Pharmacy in Košice); 12.09.2022 (University of Milan).

CONTRATTI DI RICERCA, ASSEGNI DI RICERCA O EQUIVALENTI
(per ciascun contratto stipulato, inserire università/ente, data di inizio e fine, ecc.)

Research grants during PhD study:

1) PhD degree (between October 2018 to September 2021): This is a European Joint Doctorate Degree Programme carried out between the **University of Milan (Italy)** in Department of Veterinary Medicine and Animal Sciences (DIVAS) and at the **University of Veterinary Medicine and Pharmacy in Košice (Slovakia)** in Department of Microbiology and Immunology. This study was funded by the European Union's Horizon 2020 Programme under the Marie Sklodowska-Curie grant agreement no. 765423.

2) Italian scholarship (L'area Scientifico-Disciplinare Delle Scienze Agrarie e Veterinarie, D.R. n. 1/2018 del 10/01/2018, Codice U-Gov H20MCITNIF18GSAVO) (between October 2021 to September 2022): Italian grant achieved for continuation of the PhD training of promising graduates for 1 year in the Department of Veterinary Medicine and Animal Sciences (DIVAS) at the University of Milan (Italy).

Research contracts during Master's and Bachelor's study:

3) Student Research Assistant (October 2015 to August 2016): Research work in Development of 3D cell culture scaffolds based on self-assembly of aromatic di-peptides in Microstructure group at the Leibniz Institute for Polymer Research, Max Bergmann Center of Biomaterials Dresden (Germany)**.**

4) Internship (January 2017 to September 2017): *In vitro* toxicity assessment of dendritic polymer-based nanoparticles in intestinal cell models for application in targeted drug system for colon cancer therapy. This research work was carried out at the Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf (HZDR) (Germany).

5) Master's thesis (January 2018 to August 2018): Thesis work on the topic, Influence of secondary bile acids (SBA) and SBA-producing probiotic bacteria against Clostridium difficile infection in mouse intestinal explant model, in the group of Microbial Immune Regulation at the Helmholtz - Zentrum für Infektionforschung (HZI) Braunschweig (Germany).

6) Bachelor's thesis (November 2013 to April 2014): Thesis work on the topic, Gold nanoparticles-mediated *in vivo* targeted-drug delivery system for cancer therapy in rat model at the Saveetha Dental College and Hospitals (India).

ATTIVITÀ DIDATTICA A LIVELLO UNIVERSITARIO IN ITALIA O ALL'ESTERO
(inserire anno accademico, ateneo, corso laurea, numero ore, ecc.)

1) Art.45 Tutorial activity aa 2021-2022 Biotecnologie per la nutrizione di alimenti di origine animale, UD Biotecnologia e Nutrizione animale, Corso di laurea Biotecnologia – University of Milan (20ore).

2) Given training to 3 bachelor and 2 master students for their thesis in animal cell culture techniques to carryout *in vitro* biochemical analysis (MTT, LDH, Apoptosis assays, Microscopy techniques) of bioactive feed supplements at the Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS), University of Milan, between the academic year 2018 - 2022 (3 months per student).

3) Seminars in "Nutrigenomics" course for bachelor and master students between the academic year 2018 – 2022 at DIVAS, University of Milan.

4) Seminars and practical course in "Animal cell culture techniques for *in vitro* assessment of bioactive feed supplements" for for bachelor and master students between the academic year 2018 – 2022 at DIVAS, University of Milan.

DOCUMENTATA ATTIVITÀ DI FORMAZIONE O DI RICERCA PRESSO QUALIFICATI ISTITUTI ITALIANI O STRANIERI;
(inserire anno accademico, ente, corso, periodo, ecc.)

Courses attended at the University of Milan during 2019-2020:

1) Bioinformatics tools to study OMICS data (ECTS: 2)
2) Next generation sequencing for cancer and beyond (ECTS: 4)
3) Flow cytometry in biomedical research (ECTS: 4)
4) Scientific writing (ECTS: 2)
5) How to write molecular and cell biology paper (ECTS: 2)
6) How to communicate your research (ECTS: 2)
7) Digital imaging and image integrity in scientific publication (ECTS: 2)
8) Transferable skill course: "Protecting and enhancing the Value of research results on the market" (Webinar)
9) Transferable skill course "Fake news, dissemination and scientific research" (Webinar)

Courses attended at the University of Veterinary Medicine and Pharmacy in Košice (Slovakia) during 2020:

10) Virology (ECTS: 8)
11) Immunology (ECTS: 5)
12) Usage of Statistical Methods (ECTS: 15)
13) *In vitro* cultivation of CNS cells and tissues (Practical training) (ECTS: 5)
14) Antigen detection methods (Practical training) (ECTS: 5)
15) English

Industrial trainings and workshops:

16) Common Core Training Course in Animal Science disciplines and introduction to OMICS, conducted by Molecular Animal Nutrition (MANNA) programme at the Scottish Center for Ecology and the Natural Environment, University of Glasgow, 1st – 10th October, 2018.

17) 1st Molecular Animal Nutrition (MANNA) Summer School in OMICS, at the Faculty of Veterinary Medicine in Lodi, University of Milan, 22nd – 26th July, 2019.

18) MANNA Training Course in Proteomics: From study design to scientific publication (Virtual course), University of Zagreb, 20th, 22nd, and 24th April, 2020.

19) 2nd Molecular Animal Nutrition (MANNA) Summer School on 'Essential Management Skills and Media Communication', (Virtual course), University of Zagreb, 21st – 25th September, 2020.

20) 3rd Molecular Animal Nutrition (MANNA) Summer School on 'From Research to Commercial Products and Applications: Perspectives and insights for the transition from PhD to job opportunities' (Virtual course), University of Bonn, 26th – 30th July, 2021.

21) Transcriptomics and Proteomics interactive workshop conducted jointly by the University of Veterinary Medicine and Pharmacy in Košice (Slovakia) and MetLabs (Bratislava) (Virtual course), 2nd – 4th March, 2021.

22) MANNA Proteomics workshop (Virtual course), University of Glasgow Polyomics, 22nd May 2021.

23) MANNA Metabolomics Refresher One-Day Course (Virtual), University of Glasgow Polyomics, 22nd July 2021.

24) MANNA Training course in Microbiota (Virtual), Porto Conte Recerche (Italy), 17th June, 2020.

25) Industrial training on the topic "Automation in Biotechnology at Matrix Arc Solutions (India), 17th - 22nd June, 2013.

26) Attended workshop on the topic "Regenerative Medicine: The Future Crux of the Health Sector" from 27 - 29th June, 2013.

27) Industrial training course in "Industrial Enzyme Technology" at Armats Biotek, Training and Research Institute Chennai (ABTRI), (India), 13th - 18th December, 2012.

DOCUMENTATA ATTIVITÀ IN CAMPO CLINICO
(indicare, data, durata, ruolo, ente presso il quale si è prestata attività assistenziale, ecc.)

REALIZZAZIONE DI ATTIVITÀ PROGETTUALE
(indicare, data, progetto, ecc.)

ORGANIZZAZIONE, DIREZIONE E COORDINAMENTO DI GRUPPI DI RICERCA NAZIONALI E INTERNAZIONALI, O PARTECIPAZIONE AGLI STESSI
(per ciascuna voce inserire anno, ruolo, gruppo di ricerca, ecc.)

(1) Worked as a **Student Research Assistant (October 2015 to August 2016)** in Development of 3D cell culture scaffolds based on self-assembly of aromatic di-peptides. This research work was carried out in "Microstructure group", Leibniz Institute for Polymer Research, Max Bergmann Center of Biomaterials Dresden (Germany)**.**

(2) Worked as a **student intern** in *in vitro* toxicity assessment of dendritic polymer-based nanoparticles in intestinal cell models for application in targeted drug system in treatment of colon cancer. This research work was carried out at "Nanoscalic Systems group", Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf (HZDR) (Germany).

(3) Participated in **research training and exchange activities** abroad during the PhD study programme for the period between January 2020 – September 2020 in the team of Laboratory of Biomedical Microbiology and Immunology (LBMI), Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice (Slovakia). The research activities involved both technical and practical trainings in Bioinformatics analysis applied.

(4) Participated in **industrial training** as a part of the PhD study programme (Academic year: 2021-2022) at Porto Conte Ricerche Srl (Strada Provinciale 55, 07041 Alghero SS, Italy). The research activities involved online trainings in application of proteomic analysis to animal cell cultures.

TITOLARITÀ DI BREVETTI
(per ciascun brevetto, inserire autori, titolo, tipologia, numero brevetto, ecc.)

ATTIVITÀ DI RELATORE A CONGRESSI E CONVEGNI NAZIONALI E INTERNAZIONALI
(inserire titolo congresso/convegno, data, ecc.)

Congress/Conference presentations:

(1) T.S. Sundaram, C. Giromini, R. Rebucci, A. Baldi. Establishment of inflammatory in vitro intestinal epithelial models for translational animal nutrition, In: ITALIAN JOURNAL OF ANIMAL SCIENCE. - ISSN 1828-051X. - 18:suppl. 1, pp. 159-159. (Intervento presentato al 23. convegno

ASPA tenutosi a Sorrento nel 2019. Animal Science and Production Association (ASPA) 23rd Congress, Sorreton, Italy. 11th -14th June 2019. (Poster)

Abstract:
Many naturally available compounds as n3-polyunsaturated fatty acids (EPA: Eicosapentaenoic acidand DHA: Docosahexaenoic acid), conjugated linoleic acid, milk exosomes and plant extract from Macleaya cordata exhibits anti-inflammatory effects. From previous studies, these bioactive compounds demonstrated a mul-titude of beneficiary effects in both human and animal health and are considered as potential therapeutic agents with pharmaceutical properties. Due to their health benefits, new ways to incorporate them in human diet through poultry and livestock nutrition is extensively studied and therefore, it is first important to determine its anti-inflammatory effects in cell-based inflammatory models. The gastrointestinal tract (GI) is the first site where food is broken down and nutrients are absorbed and therefore the GI cell models are widely preferred for food/feed analysis. In this respect, it becomes of paramount importance to establish inflammatory cell line models of intestinal epithelia. Therefore, in the present study, we demonstrated the inflammatory response of IPEC-J2 cell lines of neonate porcine intestinal epithelium challenged against dif-ferent stimuli as cell wall lipopolysaccharides (LPS) of Gram-negative bacteria as Escherichia coli and Salmonella, and chemical such as dextran sodium sulphate (DSS), analysed by MTT cell viability assay. The cells were treated with each stimulus in a dose-dependent manner (0.15–10% for DSS, 1.56–100 µg/mL for LPS) for 24 h and thereafter viability was measured and the con-centration at 50% inhibition (IC50) was calculated using regres-sion analysis. The IPEC-J2 cells exhibited an IC50 value of 2.89% for DSS challenge and 12.77 µg/mL for Salmonella LPS. The E. colidid not show any significant inflammatory response even with the challenge of highest dose as 100 µg/mL. These results suggest that the epithelial cells are specific for different biological challenge as bacterial LPS and the DSS chemical proves to be potent inflammatory agent even at small doses and can be effectively used to induce inflammatory response to study anti-inflammatory properties of food/feed additives.

(2) T. S. Sundaram, C. Giromini, R. Rebucci, A. Baldi, M. Bhide, J. Pistl . Transcriptomic profiling and functional assessment of omega-3 polyunsaturated fatty acids in porcine enterocyte model. Book of abstracts (DOI: 10.3920/978-90-8686-918-3). 72rd Annual Meeting of the European Federation of Animal Science (EAAP) in Davos, Switzerland, 30th August – 3rd September 2021 (Oral).

Abstract:
Marine and plant-based omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are widely incorporated in animal diet to improve growth and immunity. Especially, ω-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to exhibit strong immunomodulatory effects as anti-inflammation and anti-oxidation. However, its molecular activity on intestinal epithelium under inflammatory and oxidative stress is not fully understood. Presently, we evaluated the dose-response, anti-inflammatory and anti-oxidative effects of EPA and DHA against lipopolysaccharides (LPS) challenge in a non-transformed porcine enterocyte model, IPEC-J2. The results showed 24 h treatment with EPA or DHA exhibited proliferative effects in IPEC-J2 cells at concentrations of 6.25-50 µM ($P<0.05$). Further, 24 h pre-treatment of DHA (3.3 µM), EPA (6.7 µM) or DHA:EPA (1:2; 10 µM) increased the mitochondrial activity, decreased apoptotic caspase-3/7 release by two-fold post-LPS (24 h) challenge ($P<0.05$). For the first time, we demonstrated the proliferative and cytoprotective properties of EPA/DHA at low concentrations in IPEC-J2 cells. Increased intracellular mitochondrial activity by ω-3 PUFAs can play a crucial role in preventing enterocyte apoptosis during inflammatory and oxidative stress. Further, to identify the novel molecular pathway of ω-3 PUFAs activity, the gene expression was evaluated by high-throughput transcriptomics technique. cDNA library was constructed and sequenced by Illumina NextSeq. The sequences were further processed and aligned to reference pig genome. Around 293 common and 149 unique differentially expressed genes corresponding to LPS challenge with and without ω-3 PUFAs pre-treatment was identified. Thus, the present outcomes highlight ω-3 PUFA mediated cellular mechanisms underpinning their function in pig nutrition.

(3) Tamil Selvi Sundaram, Carlotta Giromini, Raffaella Rebucci, Salvatore Pisanu, Daniela Pagnozzi, Maria Filippa Addis, Mangesh Bhide, Juraj Pistl, Antonella Baldi. Transcriptomic and proteomic analysis of omega-3 fatty acids in the porcine enterocytes. Book of abstracts (DOI: 10.3920/978-90-8686-937-4). 73rd Annual Meeting of the European Federation of Animal Science (EAAP) in Porto, Portugal. 5th – 9th September 2022 (Poster).

Abstract:
Dietary omega-3 fatty acids (n-3 PUFA) are reported to improve the intestinal barrier integrity and maintain the gut homeostasis. Small intestinal enterocytes are the primary site of ω-3 PUFA uptake, but till date n-3 PUFAinduced global changes in the gene/protein expressions of these cells remains unexplored. Earlier, we demonstrated the anti-inflammatory, antioxidative and proliferative properties of the n-3 PUFA as Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) using a non-transformed porcine small intestinal enterocyte model, IPEC-J2. In the present study, n-3 PUFA-induced changes in the gene and protein expression profiles of the IPEC-J2 were explored using analysis was performed using the Illumina Nextseq technology, while proteomic analysis was performed using the filter-aided sample preparation with liquid chromatography bioinformatic analysis as transcriptomics and proteomics. To this, the IPEC-J2 cells were treated with EPA:DHA (1:2, 10 μM) for 24 h and subsequently samples were processed following the previously published methods for bioinformatic analysis. The cells without any treatment were included as the control. Transcriptomic (LC)-mass spectrometry (MS)/MS techniques. Presently, 51 differentially expressed genes (DEGs) and 40 differentially expressed proteins (DEPs) were identified when compared against the control. Further, Gene Ontology analysis revealed the participation of these DEGs/DEPs in several biological process including the lipid metabolic process, and cytokine signalling. The outcome of this study could contribute to comprehensive understanding on the role of n-3 PUFA in intestinal barrier for better planning of n-3 PUFA-based nutritional strategies in mammalian diets.

(4) R. Rebucci, C. Giromini, T.S. Sundaram, M. Comi, A. Baldi, L. Pinotti and F. Cheli. Effect of omega-3 fatty acids on porcine intestinal ex vivo model exposed to stress conditions. Università degli studi di Milano, Department of Health, Animal Science and Food Safety. Book of abstracts (DOI: 10.3920/978-90-8686-900-8). 71st Annual Meeting of the European Federation of Animal Science (EAAP) (Virtual meet, 2020), 1st - 4th December 2020.

Abstract:
Many naturally available compounds as n3-polyunsaturated fatty acids (Eicosapentaenoic acid, EPA and Docosahexaenoic acid, DHA) exhibit anti-inflammatory, anti-oxidative and other health beneficial properties in farm animals. The aim of the present study was to demonstrate the anti-inflammatory potential of EPA and DHA using ex vivo porcine duodenum tissue explants. Duodenal tissues were obtained from eight pigs at the slaughtering house. Each tissue was dissected in sections of about 1.0 cm2 and washed using phosphate buffer saline and randomly distributed into 24-well plates containing foam-pads to hold explant in place and 1.5 ml of pre-warmed control (only DMEM) and treatment medium. Explants were co-incubated with 10 μM of EPA:DHA (1:2) and/or lipopolysaccharide (LPS) at 10 μg/ml or Hydrogen peroxide (H2O2) at 2 mM (37 °C, 2 hours). Membrane damage was evaluated by measuring LDH release while the secretion of IL-8 was determined by enzyme linked immunosorbent assay (ELISA) in tissue culture supernatants. Data were analysed by one-way ANOVA using GraphPad, Prism. The treatment with H2O2 did not induce a significant release of LDH and did not alter IL-8 secretion. After LPS treatment, the LDH release increased up to 22% and the IL-8 secretion increased up to 27% in tissue culture supernatants, compared with control (no treatment). The co-incubation of LPS and EPA:DHA restored (P<0.05) the LDH release and also suppressed the secretion of IL-8 (P<0.05) in LPS challenged tissues compared to control (LPS alone). In conclusion, DHA:EPA consistently elicits strong anti-inflammatory activity. These results support the potential of n3-polyunsaturated fatty acids as anti-inflammatory agents in ex-vivo tissues of the gastrointestinal tract.

(5) C. Giromini, A. Baldi, R. Rebucci, T.S. Sundaram, S. Purup. Role of Short Chain Fatty Acids to counteract inflammatory stress in mucus secreting HT29-MTX cells, Book of abstracts (DOI: 10.3920/978-90-8686-918-3). 72rd Annual Meeting of the European Federation of Animal Science (EAAP) in Davos, Switzerland, 30th August – 3rd September 2021.

Abstract:
The integrity of the gastrointestinal barrier represents the first step in maintaining the gut health, which affects production animals' performance and welfare. Although SCFAs, especially butyrate, are known to promote intestinal health, their role in the protection of the intestinal barrier integrity and function is poorly characterized. The main aim of the study was to set-up an in vitro model of colonic epithelium to study the role of SCFAs as protective nutrient metabolites for the intestinal epithelial cells exposed to a stress condition. Intestinal goblet HT29-MTX-E12 (E12) cells were

differentiated and further stressed with Dextran Sodium Sulphate (DSS), to simulate intestinal inflammation. The effect of butyrate alone (BUT) and the SCFAs mixture (MIX) was tested on intestinal cell viability (LDH test), epithelial integrity (TEER) and permeability (FITC) of differentiated E12 cells exposed to inflammatory stress condition. MUC2 and MUC5AC gene expression modulation was also evaluated by RT-PCR. Results showed that the concentration of 10% DSS (24 hours) decreased the TEER about 50% compared to control (0% FCS). Treatment with a concentration of 10 mM of MIX for 1 and 24 hours significantly (P<0.05) counteracted the decrease of TEER. Treatment with a concentration of 10 mM of BUT for 24 hours significantly (P<0.05) counteracted the decrease of TEER induced with DSS. FITC data demonstrated that the treatment with concentrations of 0.1 mM and 10 mM of MIX for 1 hour significantly (P<0.05) reduced the epithelial permeability of HT29-MTX cells stressed with DSS. Finally, the concentration of 10% DSS for 24 hours significantly reduced MUC2 and MUC5AC gene expression, while treatment with 0.1 mM BUT and MIX for 24 hours significantly promoted MUC2 and MUC5AC gene expression (P<0.05). The present study demonstrates the suitability of E12 cells stressed with DSS as an inflammatory bowel diseases model to study the role of bioactive compounds in promoting intestinal health. SCFAs play an essential role in maintaining intestinal health by affecting epithelial integrity and mucus production.

(6) Carlotta Giromini, Raffaella Rebucci, Gabriella Tedeschi, Tamil Selvi Sundaram, Federica Cheli, Antonella Baldi. Angiotensin Converting Enzyme-1 inhibitory activity of milk proteins evaluated after *in vitro* digestion and peptidomic analysis. In: ITALIAN JOURNAL OF ANIMAL SCIENCE. - ISSN 1828-051X. - 18:suppl. 1, pp. 159-159. Animal Science and Production Association (ASPA) 23rd Congress, Sorreton, Italy. 11th -14th June 2019.

Abstract:
Milk proteins are relevant sources of bioactive peptides. Many hurdles still exist regarding the widespread utilisation of milk protein-derived bioactive peptides as they may be degraded during gastrointestinal digestion. A crucial issue in this field is the demonstration of a cause-effect relationship, from the ingested intact form to the bioactive form.The aim of this study was to evaluate in vitro digestion, digest-ibility (IVD, using two different hydrolysis methods) and Angiotensin Converting Enzyme-1 inhibitory activity (ACE-1i) of milk and plant proteins (used as control). Based on ACE-1i activ-ity, a peptidomic and proteomic profile analysis was performed on permeate and retentate samples.In particular, milk and plant protein samples were in vitrodigested, and the total digest was filtered using a 3 KDa mem-brane. A permeate fraction (<3 KDa) and retentate fraction (>3 KDa) were obtained. ACE-1i activity was measured as the ability of protein fractions (pre-digested, permeate and retentate) to decrease the hydrolysis of furanacroloyl-Phe-Glu-Glu (FAPGG) synthetic substrate for ACE enzyme. Furthermore, permeate was characterised by LC-nano ESI MS/MS using a shotgun-peptidomic approach, whereas retentate was further trypsin-digested prior the analysis with mass spectrometry using a shotgun-proteomic approach.We found a positive correlation among the IVD methods tested (p<.05; r = 0.85). Milk proteins exhibited higher values of IVD (>82.5%) with both methods used, compared with plant pro-teins. Milk proteins after in vitro digestion exhibited a signif-icant increase in ACE-1i (p<.05) (> 23.91 ± 0.64%) compared with plant protein tested (10.40 ± 1.07%). Based on proteomic and peptidomic analysis performed, specific peptides associ-ated with anti-hypertensive and ACE-1i effect have been iden-tified in permeate and retentate fractions of milk proteins. Our results demonstrated that milk and plant proteins are highly digestible and, in particular, milk proteins may represent valu-able sources of ACE-1i and anti-hypertensive peptides which may confer the ability to decrease blood pressure in vivo.

(7) Matteo Dell'Anno, Carlotta Giromini, Serena Reggi, Tamil Selvi Sundaram, Simona Coranelli, Ambra Spalletta, Luciana Rossi. Evaluation of *Lactobacillus plantarum* and *Lactobacillus reuteri* as feed additives for swine, Animal Science and Production Association (ASPA) 24th Congress (ASPA, Italy, 2021), Italian Journal of Animal Science 20(sup1):1-236, DOI:10.1080/1828051X.2021.1968170. 21st - 24th September 2021.

Abstract:
In swine farming, effective alternatives capable to reduce anti-biotic consumption are needed to cope with the increasing con-cern of antibiotic resistance. In this perspective, functional feed additives, such as probiotics, are able to sustain the health status and reduce the risk of diseases development, that have become a fundamental tool to prevent pathological conditions in livestock. The aim of this study was to evaluate Lactobacillus plantarumand Lactobacillus reuteri in vitro for their functional character-istics and in vivo for their effect on animal performance and health.

Firstly, L. plantarum 4.1 and L. reuteri 3X7, isolated from swine by Biotecnologie BT were genetically characterized by PCR reaction. Subsequently, their resistance to pH, temperature and digestive process were evaluated. Furthermore, the Lactobacilli mucosa adhesion ability was assessed on IPEC-J2 cell line. For the in vivo trial, 350 weaned piglets (28 ± 2d) were randomly divided into four experimental groups receiving basal diet respec-tively supplemented with: i) CTRL no supplementation; ii) PLA 2 × 108 CFU/g of L. plantarum; iii) REU 2 × 108 CFU/g of L. reuteri; iv) PROBIO 1 × 108 + 1 × 108 CFU/g of L. reuteri; iv) PROBIO 1 × 108 + 1 × 108 CFU/g for both bacterial strains. Growth performance and faecal consistency using a four-point scale (faecal score 0–3; considering diarrhoea ≥2) were recorded individually. Faecal samples were collected for the evaluation of main bacterial families, and blood serum aliquots were obtained for the assessment of metabolic parameters. In vitro characteri-zation revealed a great resistance to a wide range of pH (3,4,5,7) for both species. At pH 2 a statistically significant reduction of bacterial growth was observed ($p < .05$). Both species showed good tolerance to a wide temperature range, while at 60 and 70 °C a statistically significant reduction of bacterial growth was observed ($p < .05$). Both species survived well to all the steps of the digestion process. The LiCl treatment strongly inhibited the adhesion ability of L. reuteri ($p < .001$), while it showed no significant effects for L. plantarum strain. Piglets supplemented with Lactobacilli significantly decreased the faecal score ($p < .0001$) during the experimental period. L. plantarum and L. reuterirevealed interesting functional proprieties and health-improving effects as functional feed additives for weaned piglets.

(8) Tamil Selvi Sundaram, Hans Georg Braun. Liquid marbles based on self-assembly of oligopeptides as 3D scaffold for cell culture, HIPS symposium on pharmaceutical sciences in infection research organized by Helmholtz Institute for Pharmaceutical Research Saarland,Germany. 29th June 2017 (Poster)

Abstract:
Assemblies of oligopeptides based on diphenylalanine and its Fmoc protected derivate have been recognized as new supramolecular materials with a variety of both structural features (fibres, ribbons, 2,3 d networks by p-p stacking, colloids) as well as unusual materialsproperties (Piezoelectricity) and processibility (electrospinning). They offer a great potential for a variety of applications as biomaterials and nanotechnology. Interfacial controlled self-assembly of small oligopeptide units is presented as new versatile method to create ultrathin peptide mebreanes in which colloidal objects can become physically integrated. Using liquid marble technology the membranes are demonstrated to stablize macrocontainers in which cells can be cultivated and biological assays can be integrated.

(9) Ruchi Goswami, Tamil Selvi Sundaram, Hans Georg Braun. Magneto-responsive bioreactors (3D cell culture scaffolds) from self-assembled oligopeptides, Dresden Polymer Discussion on Polymer Materials in the Transition from Responsiveness to Interactivity and Adaptivity, organized by Leibniz Institute for Polymer Research. Meissen, Germany. 17th - 20th April 2016 (Poster)

Abstract:
Oligopeptide molecules like Fmoc-diphenylalanine dipeptide (FmocFF) is well known to self-assemble in three-dimensional hydrogel bulk phase. This self-assembly (gelation) process is triggered either by solvent mixtures (DMSO/Water) or pH. It has been demonstrated [1] that pH change at the liquid gas interface creates thin membrane. Using this approach, we demonstrate the integration of colloidal objects like silica beads (B) and magnetic beads (C) into this self-organised membrane. The integration of magnetic particles of different sizes into the membrane could open the direction of interaction of external magnetic fields with the magnetic particles integrated into membrane. Liquid phases can be encapsulated and stabilized by coating the liquid/gas interface with hydrophobic microparticles as for example PTFE powder. These so called "liquid marbles (LM)" [2] are prevented from wetting the underlying surface due to the rough interface originating from the hydrophobic powder particles. We demonstrate the pH triggered interfacial and bulk gelation of oligopeptide molecules (Fmoc-FF) to form stable mesoporous networks of ribbon like peptide assemblies [1]. Inclusion of magnetic particles into the membranes can offer an active mechanism to move the peptide containments in magnetic field or to change the shape of the oligopeptide containment. These high stable LMs can act as 3D scaffold for encapsulating cells, enzyme immobilization, gas sensors, etc. The shape, size, stability, limiting evaporation of material, controlling diffusion across the membrane and safe handling of the fragile LMs are some of the keys factors to be monitor in order to achieve high throughput micro-bioreactors.

CONSEGUIMENTO DI PREMI E RICONOSCIMENTI NAZIONALI E INTERNAZIONALI PER ATTIVITÀ DI RICERCA
(inserire premio, data, ente organizzatore, ecc.)

Italian scholarship (L'area Scientifico-Disciplinare Delle Scienze Agrarie e Veterinarie, D.R. n. 1/2018 del 10/01/2018, Codice U-Gov H20MCITNIF18GSAVO) (between October 2021 to September 2022): Italian scholarship achieved for continuation of the PhD training of promising graduates for 1 year to carryout research activities in *in vitro* assessment of bioactive feed supplements in the Department of Veterinary Medicine and Animal Sciences (DIVAS) at the University of Milan (Italy).

POSSESSO DEL DIPLOMA DI SPECIALIZZAZIONE EUROPEA RICONOSCIUTO DA BOARD INTERNAZIONALI (relativamente a quei settori concorsuali nei quali è prevista)
(indicare diploma, data di conseguimento, ecc.)

1) Master's degree:
Msc. Enviornmental Protection and Agricultural Food Production,
Universität Hohenheim, Stuttgart (Germany). **Date of Master's degree achieved: 20th August 2018**

2) PhD degree:
PhD in Molecular Animal Nutrition (MANNA) on the thesis title, **"Establishing *in vitro* intestinal epithelial cell models in translational animal nutrition".**

(This is a European Joint Doctorate Degree Programme carried out between the University of Milan (Italy) and the University of Veterinary Medicine and Pharmacy in Košice (Slovakia)). This study was funded by the European Union's Horizon 2020 Programme under the Marie Sklodowska-Curie grant agreement no. 765423).

Date of PhD degree achieved: 7.09.2022 (at University of Veterinary Medicine and Pharmacy in Košice); 12.09.2022 (University of Milan).

TITOLI DI CUI ALL'ARTICOLO 24 COMMA 3 LETTERA A) E B) DELLA LEGGE 30 DICEMBRE 2010, N. 240
(indicare se contratto di tipologia A o B, Ateneo, data di decorrenza e fine contratto, ecc.)

PRODUZIONE SCIENTIFICA

PUBBLICAZIONI SCIENTIFICHE
(per ciascuna pubblicazione indicare: nomi degli autori, titolo completo, casa editrice, data e luogo di pubblicazione, codice ISBN, ISSN, DOI o altro equivalente)

(1) Sundaram, T.S.; Giromini, C.; Rebucci, R.; Baldi, A. Omega-3 Polyunsaturated Fatty Acids Counteract Inflammatory and Oxidative Damage of Non-Transformed Porcine Enterocytes. *Animals (MDPI, Basel)*. 2020 June; 10(6): 956. Published online 2020 May 31. doi: 10.3390/ani10060956. (Open access)

Abstract:

Marine and plant-based omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are widely added to animal diets to promote growth and immunity. We tested the hypothesis that eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and their 1:2 combination could counteract acute or long-term damage of lipopolysaccharides (LPS), dextran sodium sulphate (DSS) and hydrogen peroxide (H2O2) in Intestinal Porcine Epithelial Cell line-J2 (IPEC-J2). The results showed that 24 h treatment with EPA or DHA exhibited proliferative effects in IPEC-J2 cells at low to moderate concentrations (6.25–50 μM) ($p < 0.05$). Further, 24 h pretreatment with individual DHA (3.3 μM), EPA (6.7 μM) or as DHA: EPA (1:2; 10 μM) combination increased the mitochondrial activity or cell membrane integrity post-LPS (24 h), DSS (24 h) and H2O2 (1 h) challenge ($p < 0.05$). Additionally, DHA: EPA (1:2, 10 μM) combination decreased the apoptotic caspase-3/7 activity around twofold after 24 h LPS and DSS challenge ($p < 0.05$). Our study confirms the proliferative and cytoprotective properties of EPA and DHA in IPEC-J2 cells. Increased intracellular mitochondrial activity and cell membrane integrity by ω-3 PUFAs can play a role in preventing enterocyte apoptosis during acute or chronic inflammatory and oxidative stress.

(2) Sundaram, T.S., Giromini, C., Rebucci, R. Pistl, J., Bhide, M., Baldi, A. Role of omega-3 polyunsaturated fatty acids, citrus pectin, and milk-derived exosomes on intestinal barrier integrity and immunity in animals. *Journal of Animal Science and Biotechnology* (BMC, Publisher: *Springer Nature)* 2022; 13:40. Published online 2022 April 11. doi: 10.1186/s40104-022-00690-7. (Open access)

Abstract:
The gastrointestinal tract of livestock and poultry is prone to challenge by feedborne antigens, pathogens, and other stress factors in the farm environment. Excessive physiological inflammation and oxidative stress that arises firstly disrupts the intestinal epithelial barrier followed by other components of the gastrointestinal tract. In the present review, the interrelationship between intestinal barrier inflammation and oxidative stress that contributes to the pathogenesis of inflammatory bowel disease was described. Further, the role of naturally existing immunomodulatory nutrients such as the omega-3 polyunsaturated fatty acids, citrus pectin, and milk-derived exosomes in preventing intestinal barrier inflammation was discussed. Based on the existing evidence, the possible molecular mechanism of these bioactive nutrients in the intestinal barrier was outlined for application in animal diets.

(3) Tamil Selvi Sundaram, Maria Filippa Addis, Carlotta Giromini, Raffaella Rebucci, Salvatore Pisanu3, Daniela Pagnozzi3, and Antonella Baldi1. Comprehensive proteomic analysis reveals omega-3 fatty acids to counteract endotoxin-stimulated metabolic dysregulation in porcine enterocytes. (Manuscript under revision in *Scientific Reports*, Publisher: Springer Nature, ISSN: 2045-2322). (Open access)

Abstract:
Omega-3 polyunsaturated fatty acids (n-3 PUFA), such as the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are reported to beneficially affect intestinal immunity. The biological pathways modulated by n-3 PUFA during infection at the level of intestinal epithelial barrier remain elusive. To address this gap, we investigated the proteomic changes induced by n-3 PUFA in porcine enterocyte cell line (IPEC-J2), in the presence and absence of lipopolysaccharide (LPS) stress conditions using shotgun proteomics analysis integrated with RNA-sequencing technology. A total of 33, 85, and 88 differentially abundant proteins (DAPs) were identified in cells exposed to n-3 PUFA (DHA:EPA), LPS, and n-3 PUFA treatment followed by LPS stimulation, respectively. Functional annotation and pathway analysis of DAPs revealed the modulation of central carbon metabolism, including glycolysis/gluconeogenesis, pentose phosphate pathway, and oxidative phosphorylation. Specifically, LPS caused metabolic dysregulation in enterocytes, which was abated upon prior treatment with n-3 PUFA. Besides, n-3 PUFA supplementation facilitated enterocyte development and lipid homeostasis. Altogether, this work for the first time comprehensively described the biological pathways regulated by n-3 PUFA in enterocytes, particularly during endotoxin-stimulated metabolic dysregulation. Additionally, this study may provide nutritional biomarkers in monitoring the intestinal health of human and animals on n-3 PUFA-based diets.

(4) PhD thesis in Molecular Animal Nutrition (MANNA), entitled "Establishing *in vitro* intestinal epithelial cell models in translational animal nutrition", Academic year 2018 – 2022 at University of Milan (Italy) and University of Veterinary Medicine and Pharmacy in Košice (Slovakia).

Abstract:
Immunomodulatory nutrients as the omega-3 polyunsaturated fatty acids (n-3 PUFA) and citrus pectins (CPn) are reported to beneficially affect the host intestinal immunity. However so far, the biological pathways modulated by these nutrients in intestinal inflammation at the level of intestinal epithelial layer (IEL) remains elusive. To bridge this knowledge gap, the aim of our present study was set in the direction to delineate the effects of n-3 PUFA in porcine IPEC-J2 cell line under (LPS) stress conditions underpinning pig nutrition, combining the state-of-the-art cell-based assays and bioinformatic analysis. The second part of our study was directed towards establishing a primary intestinal epithelial cell (IEC) culture from chicken embryos for the assessment of CPn against LPS stress, underpinning poultry nutrition. Utilizing different cell-based assays, we have successfully demonstrated the proliferative effects of n-3 PUFAs as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the IPEC-J2 cells. Besides, n-3 PUFA pre-treatment (DHA:EPA, 1:2, 10 μM, 24 h) was shown to counteract the cellular damage elicited by different stress factors as LPS, hydrogen-peroxide (H2O2) and dextran sulphate sodium (DSS). In addition, through system biology and multi-omics integration (transcriptomics and proteomics), we have demonstrated the cytoprotective properties of n-3 PUFA in IPEC-J2 cells against LPS-induced inflammatory and metabolic damage. Specifically, in these cells, we have identified that n-3 PUFA regulate the biological process as, (i) Axon guidance for developmental process; (ii) Defensin and interferon-mediated antimicrobial defense response for homeostasis; (iii) Cell junction assembly under stressrelated cell proliferation; (iv) Amelioration of TLR/MyD88 and cytokine signaling in innate immune response; (v) Fatty acid storage in lipid droplets for lipid homeostasis; (vi) Recovery of central carbon metabolic process from dysregulation under infection; (vii) Lipolysis of fatty acids stored in lipid droplets for prevention of cell starvation during infection. To the best of the knowledge, this is the first study to comprehensively map the bioactivity of n-3 PUFA in enterocytes using multi-omics approach. The outcome of the present study will enable us to better understand the role of n-3 PUFA in intestinal barrier for planning nutritional or therapeutic strategies. Further in the chicken in vitro study, we have preliminarily demonstrated a simplified method of cell isolation and establishment of primary IEC culture from chicken embryos using mechanical tissue disruptions method. We have also shown that the response of chicken cells is in accordance with the reference IPEC-J2 line using a dose-response study with CPn and LPS. This primary IEC culture model can further be utilized as a starting point for setting up poultry in vitro studies on intestinal barrier.

(5) Giromini C, Lovegrove JA, Givens DI, Rebucci R, Pinotti L, Maffioli E, Tedeschi G, Sundaram TS, Baldi A. In vitro-digested milk proteins: Evaluation of angiotensin-1-converting enzyme inhibitory and antioxidant activities, peptidomic profile, and mucin gene expression in HT29-MTX cells. *Journal of Dairy Science*, Publisher: Elsevier, 2019 December; 102(12):10760-10771. Published online: 11 September 2019. doi: 10.3168/jds.2019-16833. (Open access)

Abstract:
Over the past decades, several studies investigated the health-promoting functions of milk peptides. However, to date many hurdles still exist regarding the widespread use of milk-derived bioactive peptides, as they may be degraded during gastrointestinal digestion. Thus, the aim of our study was to in vitro digest intact whey protein isolate (WPI) and casein proteins (CNP), mimicking in vivo digestion, to investigate their bioactive effects and to identify the potential peptides involved. Whey protein isolate and CNP were digested using a pepsin-pancreatin protocol and ultra-filtered (3-kDa cutoff membrane). A permeate (<3 kDa) and a retentate (>3 kDa) were obtained. Soy protein was included as a control (CTR). Angiotensin-1-converting enzyme inhibitory (ACE1-I) and antioxidant activity (AOX) were assessed and compared with those observed in undigested proteins and CTR. Furthermore, the permeate was characterized by nano-liquid chromatography electrospray ionization tandem mass spectrometry (LC-nano ESI MS/MS) using a shotgun peptidomic approach, and retentate was further digested with trypsin and analyzed by MS using a shotgun proteomic approach to identify potentially bioactive peptides. Further, the effects of WPI, CNP, and CTR retentate on cell metabolic activity and on mucus production (MUC5AC and MUC2 gene expression) were assessed in intestinal goblet HT29-MTX-E12 cells. Results showed that WPI permeate induced a significant ACE1-I inhibitory effect [49.2 ± 0.64% (SEM)] compared with undigested WPI, CNP permeate, and retentate or CTR permeate (10.40 ± 1.07%). A significant increase in AOX (1.58 ± 0.04 and 1.61 ± 0.02 μmol of trolox AOX equivalents per mg of protein, respectively) upon digestion was found in WPI. Potentially bioactive peptides associated with ACE1-I and antihypertensive effects were identified in WPI

permeate and CNP retentate. At specific concentrations, WPI, CNP, and CTR retentate were able to stimulate metabolic activity in HT29-MTX-E12 cells. Expression of MUC5AC was increased by CNP retentate and unaltered by WPI retentate; MUC2 expression was significantly increased by 0.33 mg/g of CNP and reduced by 1.33 mg/g of CNP. Our results confirm that milk proteins may be rich sources of bioactive compounds, with the greatest beneficial potential of CNP at the intestinal goblet cell level.

(6) Dell'Anno M, Giromini C, Reggi S, Cavalleri M, Moscatelli A, Onelli E, Rebucci R, Sundaram TS, Coranelli S, Spalletta A, Baldi A, Rossi L. Evaluation of Adhesive Characteristics of *L. plantarum* and *L. reuteri* Isolated from Weaned Piglets. *Microorganisms* (Publisher: MDPI). 2021 July 26;9(8):1587. Published online: 26 July 2021. doi: 10.3390/microorganisms9081587. (Open access)

Abstract:
Limosilactobacillus reuteri and Lactiplantibacillus plantarum strains, previously isolated from weaned piglets, were considered for the evaluation of their adhesive characteristics. Lactobacilli were treated with LiCl in order to remove the surface protein layer, and probiotic activity was compared with those of untreated strains. The autoaggregation, co-aggregation to E. coli F18+, and adhesive abilities of LiCl-treated Limosilactobacillus reuteri and Lactiplantibacillus plantarum were significantly inhibited ($p < 0.05$) compared with the respective untreated strain. The hydrophobic and basic phenotypes were observed due to the strong affinity to chloroform and low adherence to ethyl acetate. In particular, L. plantarum showed higher hydrophobicity compared to L. reuteri, which may reflect their different colonizing ability. After treatment with LiCl to remove surface proteins, the adherence capabilities of L. reuteri and L. casei on IPEC-J2 cells decreased significantly ($p < 0.001$) and L. reuteri adhered more frequently. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that both L. reuteri and L. plantarum had several bands ranging from 20 to 100 kDa. Two-dimensional gel electrophoresis showed an acidic profile of the surface-layer polypeptides for both bacterial strains, and more studies are needed to characterize their profile and functions. The results confirm the pivotal role of surface proteins in the probiotic potential of L. reuteri and L. Plantarum.

(7) Carlotta Giromini, Antonella Baldi, Raffaella Rebucci, Davide Lanzoni, Martina Policardi, Tamil Selvi Sundaram, Stig Purup. Role of Short Chain Fatty Acids to Counteract Inflammatory Stress and Mucus Production in Human Intestinal HT29-MTX-E12 Cells. *Foods* (Publisher: MDPI). 2022 July 5;11(13):1983. Published online: 5 July 2022. doi: 10.3390/foods11131983. (Open access)

Abstract:
Short chain fatty acids (SCFAs), especially butyrate (BUT), are known to promote intestinal health, but their role in the protection of intestinal barrier integrity is poorly characterized. The aim of the study was to set up an in vitro model of human colon epithelium using HT29-MTX-E12 cells to delineate the potential role of SCFAs under stress conditions. Accordingly, the HT29-MTX-E12 cells were differentiated for 42 days and subsequently exposed to dextran sulphate sodium (DSS). Further, the effects of BUT or its mixture with acetate and propionate (SCFAs-MIX) were tested to study proliferation, epithelial integrity and mucus production. The results showed that the concentration of 10% DSS for 24 h decreased the TEER about 50% compared to the control in HT29-MTX-E12 cells. The pre-treatment on HT29-MTX-E12 cells with BUT or SCFAs-MIX at specific concentrations significantly (p < 0.05) reduced the DSS-induced damage on epithelial cell integrity and permeability. Further, the treatment with specific concentrations of BUT and SCFAs-MIX for 24 h significantly promoted ZO-1, MUC2 and MUC5AC mRNA expression (p < 0.005). The present study demonstrated the suitability of HT29-MTX-E12 cells treated with DSS as an in vitro stress model of inflammatory bowel disease, which enabled us to understand the effect of bioactive SCFAs on the intestinal barrier.

(8) T.S. Sundaram, C. Giromini, R. Rebucci, A. Baldi. Establishment of inflammatory in vitro intestinal epithelial models for translational animal nutrition, In: ITALIAN JOURNAL OF ANIMAL SCIENCE. - ISSN 1828-051X. - 18:suppl. 1, pp. 159-159. (Intervento presentato al 23. convegno ASPA tenutosi a Sorrento nel 2019. Animal Science and Production Association (ASPA) 23rd Congress, Sorreton, Italy. 11th -14th June 2019. (Poster)

Abstract:
Many naturally available compounds as n3-polyunsaturated fatty acids (EPA: Eicosapentaenoic acidand DHA: Docosahexaenoic acid), conjugated linoleic acid, milk exosomes and plant extract from Macleaya cordata exhibits anti-inflammatory effects. From previous studies, these bioactive compounds demonstrated a mul-titude of beneficiary effects in both human and animal health and are considered as potential therapeutic agents with pharmaceutical properties. Due to their health benefits, new ways to incorporate them in human diet through poultry and livestock nutrition is extensively studied and therefore, it is first important to determine its anti-inflammatory effects in cell-based inflammatory models. The gastrointestinal tract (GI) is the first site where food is broken down and nutrients are absorbed and therefore the GI cell models are widely preferred for food/feed analysis. In this respect, it becomes of paramount importance to establish inflammatory cell line models of intestinal epithelia. Therefore, in the present study, we demonstrated the inflammatory response of IPEC-J2 cell lines of neonate porcine intestinal epithelium challenged against dif-ferent stimuli as cell wall lipopolysaccharides (LPS) of Gram-negative bacteria as Escherichia coli and Salmonella, and chemical such as dextran sodium sulphate (DSS), analysed by MTT cell viability assay. The cells were treated with each stimulus in a dose-dependent manner (0.15–10% for DSS, 1.56–100 μg/mL for LPS) for 24 h and thereafter viability was measured and the con-centration at 50% inhibition (IC50) was calculated using regres-sion analysis. The IPEC-J2 cells exhibited an IC50 value of 2.89% for DSS challenge and 12.77 μg/mL for Salmonella LPS. The E. colidid not show any significant inflammatory response even with the challenge of highest dose as 100 μg/mL. These results suggest that the epithelial cells are specific for different biological challenge as bacterial LPS and the DSS chemical proves to be potent inflammatory agent even at small doses and can be effectively used to induce inflammatory response to study anti-inflammatory properties of food/feed additives.

(9) T. S. Sundaram, C. Giromini, R. Rebucci, A. Baldi, M. Bhide, J. Pistl . Transcriptomic profiling and functional assessment of omega-3 polyunsaturated fatty acids in porcine enterocyte model. Book of abstracts (DOI: 10.3920/978-90-8686-918-3). 72rd Annual Meeting of the European Federation of Animal Science (EAAP) in Davos, Switzerland, 30th August – 3rd September 2021 (Oral).

Abstract:
Marine and plant-based omega-3 polyunsaturated fatty acids (ω-3 PUFAs) are widely incorporated in animal diet to improve growth and immunity. Especially, ω-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to exhibit strong immunomodulatory effects as anti-inflammation and anti-oxidation. However, its molecular activity on intestinal epithelium under inflammatory and oxidative stress is not fully understood. Presently, we evaluated the dose-response, anti-inflammatory and anti-oxidative effects of EPA and DHA against lipopolysaccharides (LPS) challenge in a non-transformed porcine enterocyte model, IPEC-J2. The results showed 24 h treatment with EPA or DHA exhibited proliferative effects in IPEC-J2 cells at concentrations of 6.25-50 μM ($P<0.05$). Further, 24 h pre-treatment of DHA (3.3 μM), EPA (6.7 μM) or DHA:EPA (1:2; 10 μM) increased the mitochondrial activity, decreased apoptotic caspase-3/7 release by two-fold post-LPS (24 h) challenge ($P<0.05$). For the first time, we demonstrated the proliferative and cytoprotective properties of EPA/DHA at low concentrations in IPEC-J2 cells. Increased intracellular mitochondrial activity by ω-3 PUFAs can play a crucial role in preventing enterocyte apoptosis during inflammatory and oxidative stress. Further, to identify the novel molecular pathway of ω-3 PUFAs activity, the gene expression was evaluated by high-throughput transcriptomics technique. cDNA library was constructed and sequenced by Illumina NextSeq. The sequences were further processed and aligned to reference pig genome. Around 293 common and 149 unique differentially expressed genes corresponding to LPS challenge with and without ω-3 PUFAs pre-treatment was identified. Thus, the present outcomes highlight ω-3 PUFA mediated cellular mechanisms underpinning their function in pig nutrition.

(10) Tamil Selvi Sundaram, Carlotta Giromini, Raffaella Rebucci, Salvatore Pisanu, Daniela Pagnozzi, Maria Filippa Addis, Mangesh Bhide, Juraj Pistl, Antonella Baldi. Transcriptomic and proteomic analysis of omega-3 fatty acids in the porcine enterocytes. Book of abstracts (DOI: 10.3920/978-90-8686-937-4). 73rd Annual Meeting of the European Federation of Animal Science (EAAP) in Porto, Portugal. 5th – 9th September 2022 (Poster).

Abstract:

Dietary omega-3 fatty acids (n-3 PUFA) are reported to improve the intestinal barrier integrity and maintain the gut homeostasis. Small intestinal enterocytes are the primary site of ω-3 PUFA uptake, but till date n-3 PUFAinduced global changes in the gene/protein expressions of these cells remains unexplored. Earlier, we demonstrated the anti-inflammatory, antioxidative and proliferative properties of the n-3 PUFA as Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) using a non-transformed porcine small intestinal enterocyte model, IPEC-J2. In the present study, n-3 PUFA-induced changes in the gene and protein expression profiles of the IPEC-J2 were explored using analysis was performed using the Illumina Nextseq technology, while proteomic analysis was performed using the filter-aided sample preparation with liquid chromatography bioinformatic analysis as transcriptomics and proteomics. To this, the IPEC-J2 cells were treated with EPA:DHA (1:2, 10 μM) for 24 h and subsequently samples were processed following the previously published methods for bioinformatic analysis. The cells without any treatment were included as the control. Transcriptomic (LC)-mass spectrometry (MS)/MS techniques. Presently, 51 differentially expressed genes (DEGs) and 40 differentially expressed proteins (DEPs) were identified when compared against the control. Further, Gene Ontology analysis revealed the participation of these DEGs/DEPs in several biological process including the lipid metabolic process, and cytokine signalling. The outcome of this study could contribute to comprehensive understanding on the role of n-3 PUFA in intestinal barrier for better planning of n-3 PUFA-based nutritional strategies in mammalian diets.

(11) R. Rebucci, C. Giromini, T.S. Sundaram, M. Comi, A. Baldi, L. Pinotti and F. Cheli. Effect of omega-3 fatty acids on porcine intestinal ex vivo model exposed to stress conditions. Università degli studi di Milano, Department of Health, Animal Science and Food Safety. Book of abstracts (DOI: 10.3920/978-90-8686-900-8). 71st Annual Meeting of the European Federation of Animal Science (EAAP) (Virtual meet, 2020), 1st - 4th December 2020.

Abstract:
Many naturally available compounds as n3-polyunsaturated fatty acids (Eicosapentaenoic acid, EPA and Docosahexaenoic acid, DHA) exhibit anti-inflammatory, anti-oxidative and other health beneficial properties in farm animals. The aim of the present study was to demonstrate the anti-inflammatory potential of EPA and DHA using ex vivo porcine duodenum tissue explants. Duodenal tissues were obtained from eight pigs at the slaughtering house. Each tissue was dissected in sections of about 1.0 cm2 and washed using phosphate buffer saline and randomly distributed into 24-well plates containing foam-pads to hold explant in place and 1.5 ml of pre-warmed control (only DMEM) and treatment medium. Explants were co-incubated with 10 μM of EPA:DHA (1:2) and/or lipopolysaccharide (LPS) at 10 μg/ml or Hydrogen peroxide (H2O2) at 2 mM (37 °C, 2 hours). Membrane damage was evaluated by measuring LDH release while the secretion of IL-8 was determined by enzyme linked immunosorbent assay (ELISA) in tissue culture supernatants. Data were analysed by one-way ANOVA using GraphPad, Prism. The treatment with H2O2 did not induce a significant release of LDH and did not alter IL-8 secretion. After LPS treatment, the LDH release increased up to 22% and the IL-8 secretion increased up to 27% in tissue culture supernatants, compared with control (no treatment). The co-incubation of LPS and EPA:DHA restored (P<0.05) the LDH release and also suppressed the secretion of IL-8 (P<0.05) in LPS challenged tissues compared to control (LPS alone). In conclusion, DHA:EPA consistently elicits strong anti-inflammatory activity. These results support the potential of n3-polyunsaturated fatty acids as anti-inflammatory agents in ex-vivo tissues of the gastrointestinal tract.

(12) C. Giromini, A. Baldi, R. Rebucci, T.S. Sundaram, S. Purup. Role of Short Chain Fatty Acids to counteract inflammatory stress in mucus secreting HT29-MTX cells, Book of abstracts (DOI: 10.3920/978-90-8686-918-3). 72rd Annual Meeting of the European Federation of Animal Science (EAAP) in Davos, Switzerland, 30th August – 3rd September 2021.

Abstract:
The integrity of the gastrointestinal barrier represents the first step in maintaining the gut health, which affects production animals' performance and welfare. Although SCFAs, especially butyrate, are known to promote intestinal health, their role in the protection of the intestinal barrier integrity and function is poorly characterized. The main aim of the study was to set-up an in vitro model of colonic epithelium to study the role of SCFAs as protective nutrient metabolites for the intestinal epithelial cells exposed to a stress condition. Intestinal goblet HT29-MTX-E12 (E12) cells were differentiated and further stressed with Dextran Sodium Sulphate (DSS), to simulate intestinal inflammation. The effect of butyrate alone (BUT) and the SCFAs mixture (MIX) was tested on

intestinal cell viability (LDH test), epithelial integrity (TEER) and permeability (FITC) of differentiated E12 cells exposed to inflammatory stress condition. MUC2 and MUC5AC gene expression modulation was also evaluated by RT-PCR. Results showed that the concentration of 10% DSS (24 hours) decreased the TEER about 50% compared to control (0% FCS). Treatment with a concentration of 10 mM of MIX for 1 and 24 hours significantly (P<0.05) counteracted the decrease of TEER. Treatment with a concentration of 10 mM of BUT for 24 hours significantly (P<0.05) counteracted the decrease of TEER induced with DSS. FITC data demonstrated that the treatment with concentrations of 0.1 mM and 10 mM of MIX for 1 hour significantly (P<0.05) reduced the epithelial permeability of HT29-MTX cells stressed with DSS. Finally, the concentration of 10% DSS for 24 hours significantly reduced MUC2 and MUC5AC gene expression, while treatment with 0.1 mM BUT and MIX for 24 hours significantly promoted MUC2 and MUC5AC gene expression (P<0.05). The present study demonstrates the suitability of E12 cells stressed with DSS as an inflammatory bowel diseases model to study the role of bioactive compounds in promoting intestinal health. SCFAs play an essential role in maintaining intestinal health by affecting epithelial integrity and mucus production.

(13) Carlotta Giromini, Raffaella Rebucci, Gabriella Tedeschi, Tamil Selvi Sundaram, Federica Cheli, Antonella Baldi. Angiotensin Converting Enzyme-1 inhibitory activity of milk proteins evaluated after *in vitro* digestion and peptidomic analysis. In: ITALIAN JOURNAL OF ANIMAL SCIENCE. - ISSN 1828-051X. - 18:suppl. 1, pp. 159-159. Animal Science and Production Association (ASPA) 23rd Congress, Sorreton, Italy. 11th -14th June 2019.

Abstract:
Milk proteins are relevant sources of bioactive peptides. Many hurdles still exist regarding the widespread utilisation of milk protein-derived bioactive peptides as they may be degraded during gastrointestinal digestion. A crucial issue in this field is the demonstration of a cause-effect relationship, from the ingested intact form to the bioactive form.The aim of this study was to evaluate in vitro digestion, digest-ibility (IVD, using two different hydrolysis methods) and Angiotensin Converting Enzyme-1 inhibitory activity (ACE-1i) of milk and plant proteins (used as control). Based on ACE-1i activ-ity, a peptidomic and proteomic profile analysis was performed on permeate and retentate samples.In particular, milk and plant protein samples were in vitrodigested, and the total digest was filtered using a 3 KDa mem-brane. A permeate fraction (<3 KDa) and retentate fraction (>3 KDa) were obtained. ACE-1i activity was measured as the ability of protein fractions (pre-digested, permeate and retentate) to decrease the hydrolysis of furanacroloyl-Phe-Glu-Glu (FAPGG) synthetic substrate for ACE enzyme. Furthermore, permeate was characterised by LC-nano ESI MS/MS using a shotgun-peptidomic approach, whereas retentate was further trypsin-digested prior the analysis with mass spectrometry using a shotgun-proteomic approach.We found a positive correlation among the IVD methods tested (p<.05; r = 0.85). Milk proteins exhibited higher values of IVD (>82.5%) with both methods used, compared with plant pro-teins. Milk proteins after in vitro digestion exhibited a signif-icant increase in ACE-1i (p<.05) (> 23.91 ± 0.64%) compared with plant protein tested (10.40 ± 1.07%). Based on proteomic and peptidomic analysis performed, specific peptides associ-ated with anti-hypertensive and ACE-1i effect have been iden-tified in permeate and retentate fractions of milk proteins. Our results demonstrated that milk and plant proteins are highly digestible and, in particular, milk proteins may represent valu-able sources of ACE-1i and anti-hypertensive peptides which may confer the ability to decrease blood pressure in vivo.

(14) Matteo Dell'Anno, Carlotta Giromini, Serena Reggi, Tamil Selvi Sundaram, Simona Coranelli, Ambra Spalletta, Luciana Rossi. Evaluation of *Lactobacillus plantarum* and *Lactobacillus reuteri* as feed additives for swine, Animal Science and Production Association (ASPA) 24th Congress (ASPA, Italy, 2021), Italian Journal of Animal Science 20(sup1):1-236, DOI:10.1080/1828051X.2021.1968170. 21st – 24th September 2021.

Abstract:
In swine farming, effective alternatives capable to reduce anti-biotic consumption are needed to cope with the increasing con-cern of antibiotic resistance. In this perspective, functional feed additives, such as probiotics, are able to sustain the health status and reduce the risk of diseases development, that have become a fundamental tool to prevent pathological conditions in livestock. The aim of this study was to evaluate Lactobacillus plantarumand Lactobacillus reuteri in vitro for their functional character-istics and in vivo for their effect on animal performance and health. Firstly, L. plantarum 4.1 and L. reuteri 3X7, isolated from swine by Biotecnologie BT were genetically characterized by PCR reaction. Subsequently, their resistance to pH, temperature and digestive

process were evaluated. Furthermore, the Lactobacilli mucosa adhesion ability was assessed on IPEC-J2 cell line. For the in vivo trial, 350 weaned piglets (28 ± 2d) were randomly divided into four experimental groups receiving basal diet respec-tively supplemented with: i) CTRL no supplementation; ii) PLA 2 × 108 CFU/g of L. plantarum; iii) REU 2 × 108 CFU/g of L. reuteri; iv) PROBIO 1 × 108 + 1 × 108 CFU/g of L. reuteri; iv) PROBIO 1 × 108 + 1 × 108 CFU/g for both bacterial strains. Growth performance and faecal consistency using a four-point scale (faecal score 0–3; considering diarrhoea ≥2) were recorded individually. Faecal samples were collected for the evaluation of main bacterial families, and blood serum aliquots were obtained for the assessment of metabolic parameters. In vitro characteri-zation revealed a great resistance to a wide range of pH (3,4,5,7) for both species. At pH 2 a statistically significant reduction of bacterial growth was observed ($p < .05$). Both species showed good tolerance to a wide temperature range, while at 60 and 70 °C a statistically significant reduction of bacterial growth was observed ($p < .05$). Both species survived well to all the steps of the digestion process. The LiCl treatment strongly inhibited the adhesion ability of L. reuteri ($p < .001$), while it showed no significant effects for L. plantarum strain. Piglets supplemented with Lactobacilli significantly decreased the faecal score ($p < .0001$) during the experimental period. L. plantarum and L. reuterirevealed interesting functional proprieties and health-improving effects as functional feed additives for weaned piglets.

(15) Tamil Selvi Sundaram, Hans Georg Braun. Liquid marbles based on self-assembly of oligopeptides as 3D scaffold for cell culture, HIPS symposium on pharmaceutical sciences in infection research organized by Helmholtz Institute for Pharmaceutical Research Saarland,Germany. 29th June 2017 (Poster)

Abstract:
Assemblies of oligopeptides based on diphenylalanine and its Fmoc protected derivate have been recognized as new supramolecular materials with a variety of both structural features (fibres, ribbons, 2,3 d networks by p-p stacking, colloids) as well as unusual materialsproperties (Piezoelectricity) and processibility (electrospinning). They offer a great potential for a variety of applications as biomaterials and nanotechnology. Interfacial controlled self-assembly of small oligopeptide units is presented as new versatile method to create ultrathin peptide mebreanes in which colloidal objects can become physically integrated. Using liquid marble technology the membranes are demonstrated to stablize macrocontainers in which cells can be cultivated and biological assays can be integrated.

(16) Ruchi Goswami, Tamil Selvi Sundaram, Hans Georg Braun. Magneto-responsive bioreactors (3D cell culture scaffolds) from self-assembled oligopeptides, Dresden Polymer Discussion on Polymer Materials in the Transition from Responsiveness to Interactivity and Adaptivity, organized by Leibniz Institute for Polymer Research. Meissen, Germany. 17th - 20th April 2016 (Poster)

Abstract:
Oligopeptide molecules like Fmoc-diphenylalanine dipeptide (FmocFF) is well known to self-assemble in three-dimensional hydrogel bulk phase. This self-assembly (gelation) process is triggered either by solvent mixtures (DMSO/Water) or pH. It has been demonstrated [1] that pH change at the liquid gas interface creates thin membrane. Using this approach, we demonstrate the integration of colloidal objects like silica beads (B) and magnetic beads (C) into this self-organised membrane. The integration of magnetic particles of different sizes into the membrane could open the direction of interaction of external magnetic fields with the magnetic particles integrated into membrane. Liquid phases can be encapsulated and stabilized by coating the liquid/gas interface with hydrophobic microparticles as for example PTFE powder. These so called "liquid marbles (LM)" [2] are prevented from wetting the underlying surface due to the rough interface originating from the hydrophobic powder particles. We demonstrate the pH triggered interfacial and bulk gelation of oligopeptide molecules (Fmoc-FF) to form stable mesoporous networks of ribbon like peptide assemblies [1]. Inclusion of magnetic particles into the membranes can offer an active mechanism to move the peptide containments in magnetic field or to change the shape of the oligopeptide containment. These high stable LMs can act as 3D scaffold for encapsulating cells, enzyme immobilization, gas sensors, etc. The shape, size, stability, limiting evaporation of material, controlling diffusion across the membrane and safe handling of the fragile LMs are some of the keys factors to be monitor in order to achieve high throughput micro-bioreactors.

Data	15.12.2022	Luogo	Chennai (India)