

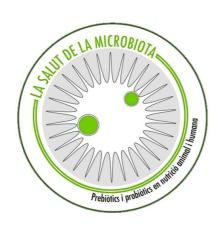








# III WORKSHOP ANUAL INSA-UB



# LA SALUT DE LA **MICROBIOTA**

Prebiòtics i probiòtics en nutrició animal i humana

# LLIBRE DE RESUMS

16 de novembre de 2017 Campus de l'Alimentació de Torribera Facultat de Farmàcia i Ciències de l'Alimentació Universitat de Barcelona

### Benvinguts al III workshop de l'INSA-UB

### LA SALUT DE LA MICROBIOTA

### Prebiòtics i probiótics en nutrició animal i humana

En nom de l'Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB) us donem la benvinguda al III Workshop Anual de l'INSA-UB. Aquesta jornada va néixer amb l'objectiu de convertir-se en un punt de trobada dels membres de l'Institut i d'aquests amb la comunitat científica al voltant d'un tema central abordat de forma transversal i multidisciplinària. La primera edició d'aquest Workshop es va dedicar a l'oli d'oliva ("L'univers de l'oli d'oliva") i la segona, al cacau i a la xocolata ("Cacau i xocolata. ciència i gastronomia"). Enguany, el tema proposat per al III Workshop és "La salut de la microbiota. Prebiòtics i probiòtics en nutrició animal i humana".

Per a aquesta edició, s'han convidat diferents ponents experts en aquest tema i investigadors de l'INSA-UB, amb l'objectiu que presentin els resultats obtinguts en el marc dels projectes FRI del mateix Institut. El Workshop està obert a investigadors tant de l'INSA-UB com d'altres institucions.

### Comité organitzador

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Dra. M. Teresa Brufau Bonet

Joan Campo Sabariz

### **Programa**

**Data**: Dijous 16 de novembre de 2017

Lloc: Edifici La Masia, Sala de les Voltes (Campus Torribera)

9:00 h Lliurament de la documentació

9:30 h Benvinguda institucional

Dra. Rosa M. Lamuela, Directora de l'Institut de Recerca en Nutrició i Seguretat Alimentària de la Universitat de Barcelona

Dra. Maria Izquierdo, Vicedegana de la Facultat de Farmàcia i Ciències de l'Alimentació en delegació del Degà de la Facultat de Farmàcia i Ciències de l'Alimentació de la Universitat de Barcelona

Sr. Luis Fuentes, regidor de Drets Socials, en delegació de l'alcaldessa de Santa Coloma de Gramenet

Dr. Josep Boatella, director del Campus de l'Alimentació de Torribera de la Universitat de Barcelona

### SESSIÓ 1: MICROBIOTA I SALUT

### Moderadora de la sessió: Dra. R. Martín

9:45 h	«Obesidad y microbiota en el entorno materno-infantil», Dra. M.C. Collado (Instituto
	de Agroquímica y Tecnología de Alimentos - CSIC València)
10:30 h	Presentació dels resultats del Programa FRI-INSA 2014: «Dieta rica en cacau i
	activitat inflamatòria en el teixit adipós de rata», Dra. S. Saldaña i Dra. M. Garcia-
	Aloy. Presentació: Dra. M. Camps-Bossacoma.
10:50 h	Presentació dels resultats del Programa FRI-INSA 2015: «Efecte immunomodulador

Presentació dels resultats del Programa FRI-INSA 2015: «Efecte immunomodulador i transmissió materna d'una soca probiòtica aïllada de llet humana», Dra. A. Tres i Dra. M.J. Rodríguez. Presentació: Dr. F.J. Pérez-Cano.

11:10 h Esmorzar + Sessió de pòsters 1: P1-P12

### SESSIÓ 2: PREBIÒTICS I PROBIÒTICS EN NUTRICIÓ ANIMAL

### Moderadora de la sessió: Dra. R. Ferrer

12:00 h	«Nova era de producció animal sense antibiòtics», Dr. J. Brufau (Institut de Recerca
	i Tecnologia Agroalimentàries, Generalitat de Catalunya)
12:30 h	«Efectes immunomoduladors de les alternatives a antibiòtics en dietes per a animals
	de granja», Dr. J. Tarradas (Institut de Recerca i Tecnologia Agroalimentàries,
	Generalitat de Catalunya)
13:00 h	Comunicacions orals: CO1-CO3
13:45 h	Dinar + Sessió de pòsters 2: P13-P24

### SESSIÓ 3: PREBIÒTICS I PROBIÒTICS EN NUTRICIÓ HUMANA

### Moderadora de la sessió: Dra. S. Guix

15:15 h	«Pre- i probiòtics en la prevenció i tractament de malalties intestinals», Dra. C.
	Herrera (Departament de Gastroenterologia, Vall d'Hebron Institut de Recerca)
15:45 h	«L'eix microbiota-cervell i els trastorns de l'ànim», Dra. I. Grande (Unitat de
	Trastorns Bipolars, Hospital Clínic, Institut de Neurociències, Universitat de
	Barcelona, IDIBAPS, CIBERSAM)
16:15 h	Comunicacions orals: CO4-CO6
17:00 h	«Pre- i probiòtics a la cuina», X. Torrado i M. Illán (Departament de Nutrició,
	Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia i Ciències de
	l'Alimentació, Universitat de Barcelona)

### CLOENDA I LLIURAMENT DE PREMIS

### Obesidad y microbiota en el entorno materno-infantil

Dra. M. Carmen Collado

Instituto de Agroquímica y Tecnología de Alimentos - CSIC València

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La obesidad y el sobrepeso representan en la actualidad un gran problema de salud pública debido al rápido aumento en su prevalencia, sobretodo en la población infantil, y también por su comorbilidad con diversas enfermedades, como el síndrome metabólico, la diabetes y enfermedades cardiovasculares. En este contexto, la identificación de nuevos factores y biomarcadores en obesidad son necesarios. Avances científicos recientes señalan un papel relevante de la microbiota intestinal en la salud humana. Alteraciones en la microbiota intestinal junto con la inflamación de bajo grado son factores que se han asociado al sobrepeso y la obesidad.

El desarrollo de la microbiota intestinal sucede de un modo paralelo con la maduración del sistema inmunitario y desempeña un importante papel en la fisiología intestinal, así como también en nuestro metabolismo. La microbiota materna resulta ser el primer inóculo y posteriormente y tras el nacimiento, ésta va aumentando gradualmente en diversidad filogenética para finalmente converger hacia una composición semejante a la adulta al final de los 3 años de vida. Factores perinatales tales como el tipo de parto, la dieta y la genética contribuyen en esta colonización microbiana. Una vez establecida, su composición es relativamente estable durante la vida adulta, pero puede ser alterado como resultado de infecciones bacterianas, el tratamiento con antibióticos, estilo de vida, intervenciones quirúrgicas y con la dieta. Dichas alteraciones en la microbiota están asociadas con un aumento en el riesgo de padecer diversas patologías. Por lo tanto, su adecuado establecimiento y su mantenimiento de la microbiota durante toda la vida podrían reducir el riesgo de la enfermedad tanto a corto como largo plazo.



Doctora por la Universidad Politécnica de Valencia (UPV) e investigadora del Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de Alimentos (IATA) del Consejo Superior de Investigaciones Científicas (CSIC) con sede en Valencia. Su trabajo de investigación es multidisciplinario y abarca las áreas de microbiología, ciencia de los alimentos y nutrición. Sus intereses se centran en los probióticos, la microbiota, la salud y la nutrición. Su trabajo actual incluye investigación básica y aplicada sobre análisis moleculares y evaluación de los efectos en la salud y en la enfermedad de las interacciones microbiota-huésped, así como también la influencia de la dieta (lactancia) y otros factores perinatales.

# Effect of cocoa's theobromine on metabolism and on plasma metabolomic profile in young rats

Camps-Bossacoma M<sup>1\*</sup>, Garcia-Aloy M<sup>2</sup>, Saldaña-Ruiz S<sup>1</sup>, Pérez-Cano FJ<sup>1</sup>, Andres-Lacueva C<sup>2</sup>, Castell M<sup>1</sup>

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\*Presenting author

### Background and objectives:

Cocoa intake influences carbohydrate and lipid metabolism. This study aimed to assess the relevance of theobromine in the cocoa effects on metabolism and inflammatory activity, together with its impact on plasma metabolic fingerprint.

### Methodology:

Young female Lewis rats were fed with either standard food, a diet containing 10% cocoa (CC group) or a diet containing the same amount of theobromine provided by 10% cocoa (TB group). After one week, an untargeted metabolomics analysis of plasma samples was carried out. Moreover, some metabolic variables, as well as the gene expression of hepatic and adipose tissue molecules associated with inflammation were quantified.

#### Results and conclusions:

The metabolomics study tentatively identified five endogenous and exogenous metabolites (i.e., theobromine) as discriminants of cocoa and/or theobromine consumption. The presence of plasma theobromine inversely correlated with the weight of retroperitoneal fat and spleen and the body weight. In addition, plasma theobromine positively correlated with higher plasma HDL-cholesterol. Both diets decreased hepatic triglycerides and fat faecal content. Furthermore, CC and TB diets decreased the mRNA levels of CD8, IL1- $\beta$  and IL-10 in liver whereas increased that related to macrophages in adipose tissue, although the effect on the gene expression of other molecules was not identical in both groups.

In conclusion, theobromine intake induces a particular metabolic fingerprint, similarly found in cocoa- and theobromine-fed animals. Theobromine seems to be the main responsible for the changes found in metabolic variables.

### Acknowledgements:

The authors thank INSA-UB for funding part of this study.

# Immunomodulatory effect and maternal transmission of a probiotic strain of Lactobacillus isolated from human milk

Tres  $A^{1,2}$ , Azagra-Boronat  $I^{1,3}$ , Massot-Cladera  $M^{1,3}$ , Franch  $\grave{A}^{1,3}$ , Castell  $M^{1,3}$ , Guardiola  $F^{1,2}$ , Pérez-Cano  $FJ^{1,3*}$ , Rodríguez-Lagunas  $MJ^{1,3}$ 

Little is known about the beneficial effects that probiotics exert in mothers during the pregnancy and breastfeeding periods. Among the probiotics of interest, those isolated from human milk, could be the best candidates for reinforcing immunity of both mothers and litters.

The aim of the present study was to elucidate whether a probiotic supplementation to rats during gestation and lactation periods may influence the immunity of mothers, breast milk composition and even the immune response of the offspring.

For that, Lewis rats were daily administered either with a *Lactobacillus* strain isolated from human milk or vehicle beginning the day of gestation and during the first two weeks of breastfeeding. At the end of the intervention, dam's immunity was evaluated by means of antibody levels as wells as lymphocyte phenotyping and phagocytosis function. Immune variables of the rats at day 14 of life were evaluated. Immune components of breast milk were also studied. The plasmatic lipid profile was evaluated in dams and neonatal rats and in breast milk of lactating mothers. The microbiota composition and the presence of the distribution of the probiotic were also studied.

The results demonstrate that the probiotic is able to modify breast milk composition and modulate the immunity and the intestinal microbiota in dams and offspring as well as their plasmatic lipid profile.

Thus, this strain of probiotic has an immunomodulatory potential in early life due to its ability to promote the immunity of dams and mothers and the quality of the mother's milk composition.

The authors thank INSA-UB for funding part of this study.

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<sup>\*</sup>Presenting author

## Eating behaviour dimensions and anthropometric indicators of adiposity and diet in Ecuadorian adults

Carpio-Arias TV<sup>1,2,3\*</sup>, Zerón-Rugerio MF<sup>1,3</sup>, Cando-Oñate F<sup>4</sup>, Izquierdo-Pulido M<sup>1,3,5</sup>

<sup>5</sup>CIBER Physiopathology of Obesity and Nutrition (CIBEROBN), Instituto de Salud Carlos III, Spain \*Presenting author

### Background and objectives:

Obesity is a growing and multifactorial pathology. There is a need to understand the cognitive-behavioral nature of food intake and its interaction with obesity. Among the cognitive-behavioral, two dimensions of eating behavior (EB) have been identified: uncontrolled and emotional, each with its own etiology. The aim of our work was to understand the link between the emotional and uncontrolled dimensions of the EB, adiposity indicators, and diet in a sample of overweight Ecuadorian adults.

### Methodology:

Two hundred adults ( $34.5 \pm 6.3$  years; 30% female) were recruited from the Nutrition Service from the general Hospital "Pedro Carbo" in Guayaquil-Ecuador. The following variables as parameters of adiposity were determined: BMI, waist circumference, visceral fat percentage and fat mass percentage and diet intake (3, 24-hour food recalls). EB were evaluated with the validated Three Factor Eating Questionnaire (TFEQ). The relationships between eating behaviors and obesity parameters were determined by ANCOVA adjusted by sex, age and physical activity level.

#### Results and conclusions:

Adults identified as high-emotional or high-uncontrolled eaters showed higher values of energy intake (p<0.001). On the other hand, the intake of all macronutrients was also higher in both high emotional or high-uncontrolled eaters (p<0.001). These individuals classified as high-emotional or high-uncontrolled eaters showed also higher values of BMI (p<0.05), higher waist circumference values (p<0.001) and higher values of fatty mass (p<0.001). Individual differences in EB may explain in part variations in diet, development of weight gain (adiposity), and subsequent obesity.

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The nutritional therapies should be redirected towards the predominance of the alimentary behavior of the patients.

### Acknowledgements:

Secretaria Nacional de Ciencia Tecnología e Innovación (SENESCYT-Ecuador), Consejo Nacional de Ciencia y Tecnología (CONACYT-México) Instituto de Salud Carlos III.

# Anthropometric indicators of adiposity and diet related with sleep quality in Ecuadorian adults

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### Background and objectives:

The implications on inadequate sleep in the development of overweight and obesity have been strongly evidenced. Previous studies indicate that sleep deprivation results in changes in the levels of some hormones including leptin, ghrelin, insulin, cortisol and growth hormone that contribute to energy imbalance, which in the long term is related to diseases such as hypertension, metabolic, type 2 diabetes and hypercholesterolemia. The aim of the present study was to examine the relationship between sleep quality and overweight-obesity and diet in a sample of Ecuadorian adults.

### Methodology:

Two hundred adults ( $34.5 \pm 6.2$  years; 30% female) were recruited from the Nutrition Service from the general Hospital "Pedro Carbo" in Guayaquil-Ecuador. Obesity parameters (BMI, waist circumference and fat mass percentage), diet (24-hour recall) and sleep quality (Pittsburgh Sleep Quality Questionnaire) were evaluated. Patients were categorized into two groups as a function of their sleep quality: Good (GSQ) and Poor sleep quality (PSQ). The potential relationships sleep quality, obesity, and diet were determined by ANCOVA adjusted by gender, age and physical activity level using STATA software.

### Results and conclusions:

Significant relationships between sleep quality, obesity measures and diet were found. Adults with **poor sleep quality** had higher body weight (p<0.001), higher BMI (p<0.001), higher waist circumference (p=0.023), higher fatty mass percentage (p<0.001), and they showed a higher intake of energy (p<0.001), carbohydrates (p=0.023) and fat (=0.021) than adults with a **good sleep** 

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**quality**. These results show that poor sleep quality could be an important risk for the development of overweight and obesity. This could be related to the fact that a higher intake of energy and carbohydrates and fat was observed in individuals who showed a poor sleep quality. Our results support the hypothesis of the metabolic implications of inadequate rest, so it is recommended to take this into account as part of an appropriate healthy style.

### Acknowledgements:

Secretaria Nacional de Ciencia Tecnología e Innovación (SENESCYT-Ecuador), Consejo Nacional de Ciencia y Tecnología (CONACYT-México) Instituto de Salud Carlos III.

# Making sense of metabolomic data: comprehensive analysis of altered metabolic pathways in diabetes and obesity

Palau-Rodriguez  $M^{1,2*}$ , Marco-Ramell  $A^{1,2*\dagger}$ , Alay  $A^3$ , Tulipani  $S^1$ , Sanchez-Pla  $A^{3,4}$ , Andres-Lacueva  $C^{1,2\dagger}$ 

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### Background and objectives:

Bioinformatic analysis and visualization techniques for 'omics' data are key tools for understanding complex biological systems. They reduce the complexity of data and allow generating hypotheses and searching for disease biomarkers. The aim of this work is to analyse the suitability of bioinformatic tools to interpret metabolomics datasets of a range of diseases including type 1 and 2 diabetes and obesity.

### Methodology:

We examined several disease datasets from metabolomics studies through different bioinformatic approaches: metabolic pathways, networks and disease-/functional-based analyses. Then we have analysed the accuracy of these tools to identify several traits of the datasets and their suitability to perform enrichment analyses

#### Results and conclusions:

The analysis of metabolic pathways, small-scale systems of biochemical reactions and events of regulation and signalling, proved to be the most appropriate approach to analyse metabolomics datasets. Tools based on KEGG metabolic pathways were the most suitable ones as they allowed us examining metabolic alterations in type 1 and 2 diabetes and obesity and formulating hypotheses about the physiopathology of these diseases. For instance, alterations in the metabolism of amino

acids, nitrogen, glutathione, sphingolipids and primary bile acids were revealed in diseased conditions.

The study of altered metabolic pathways allowed us interpreting data from metabolomics studies and extracting very valuable information from them that might help identifying disease biomarkers and possible metabolic alterations related to diseases. This information could be translated to the clinical practice to predict metabolic alterations before the onset of diseases.

### Acknowledgements:

This work was supported by Project PI13/01172 and CIBERfes co-funded by Fondo Europeo de Desarrollo Regional. 2014SGR1566 award from Generalitat de Catalunya's Agency AGAUR. M.P-R acknowlegde the APIF-INSA-UB fellowship (University of Barcelona), A.M-R and S.T. the Juan de la Cierva fellowship (MINECO), M.U-S the Ramon y Cajal fellowship (MINECO).

# Molecular metabolic response of diet-induced obese mice to a polyphenol mixture beverage based on Mediterranean Diet consumption

Sandoval  $V^{1,2}$ , Martínez  $U^{1,2*}$ , Sanz  $H^1$ , Marrero  $PF^{1,3}$ , Haro  $D^{1,3}$ , Relat  $J^{1,2*}$ 

### Background and objectives:

FGF21 is considered a therapeutic target for the treatment of metabolic diseases due to its beneficial effects on glucose/lipid homeostasis, body weight and energy expenditure. Polyphenols regular consumption has been associated with beneficial effects on obesity and insulin resistance and some of them are able to modulate the FGF21 expression and/or signalling. Based on the metabolic effects of FGF21 and the healthy benefits of polyphenols we hypothesised that **FGF21 could link dietary polyphenols and metabolism**. Concretely, our **objective** is **to describe the molecular mechanisms through which polyphenols impacts on metabolism looking for the role of FGF21.** 

#### Methodology:

We analysed the effects of a Polyphenol-supplemented beverage based on polyphenol consumption described on the PREDIMED study in diet-induced obese (DIO) mice. The nutritional intervention was introduced 4 weeks after the HFD, when the animals showed an impaired GTT and maintained for 12 weeks. We performed a molecular characterization of metabolism and FGF21 signalling in liver and subcutaneous white adipose (scWAT) tissue of the animals.

#### Results and conclusions:

Polyphenol-supplemented mice showed significant changes in the mRNA levels of Fatty acid synthase (FAS), SREBP1c and FGF21 receptor 1 (FGFR1) in liver and FGFR1 in scWAT. Obesity is described as an FGF21 resistant state and our results indicates that our polyphenols mixture can partially revert this phenotype by inducing the FGF21 receptors (FGFR1) and reducing the FGF21 expression in the liver but also have an effect on lipid metabolism by changing the lipogenesis capacity.

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### Acknowledgements:

Ministerio de Educación, Becas Conicyt- Chile. Consejo nacional de Ciencia y Tecnologia (Conacyt). México. Projecte 600004-Associació Catalana de la Diabetis (ACD).

# Effects of red wine and different doses of polyphenols from dealcoholised red wine on endothelial function in subjects with metabolic syndrome

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<sup>4</sup>Miguel Torres, Vilafranca del Penedés, Barcelona, Spain

### Background and objectives:

Cardiovascular disease (CVD) is the leading cause of death in the developed world. Several studies pointed out that mortality and risk from CVD are higher in subjects with Metabolic Syndrome (MetS), which is considered as a cluster of risk factors. Epidemiological studies and intervention clinical trials have shown that dealcoholised red wine (DRW) and moderate consumption of red wine (RW) are inversely associated with cardiovascular risk factors. Indicating a possible additional protective effect due to the non-alcoholic fraction of RW.

### Methodology:

A randomised, open, prospective and controlled clinical trial, running in parallel was performed in 72 subjects with metabolic syndrome; 130 or 260 mL/day of RW for women and men, respectively, 375 mL/day of DRW, dealcoholized red wine with grape extract (DRWEx) or water were administered during three months.

Peripheral blood mononuclear cells were isolated by Ficoll density-gradient centrifugation and, a FACS Calibur Flow Cytometer and CellQuest software were used to analyse levels of endothelial progenitor cells (EPC) and circulating endothelial cells (CEC).

#### Results and conclusions:

After the DRW interventions, the number of CEC significantly decreased, while after RW, it shows a noticeable decrease. There is a significant increase in the number of EPC after the RW intervention and, just a low increase in the DRWEx intervention.

<sup>\*</sup>Presenting author

The non-alcoholic fraction of wine, rich in polyphenols, may reduce CEC, known marker of severity of cardiovascular disease, and increase EPC, a marker of endothelial regeneration, in a population at high cardiovascular disease risk. These features might explain why DRW and moderate RW consumption suggest an improvement in the condition of the vascular endothelium and possibly contribute to delay the development of atherosclerotic plaques.

### Acknowledgements:

This work was supported by the INNPRONTA project, in part by CICYT (AGL2013-C3-1-R and AGL2016-79113-R), the Instituto de Salud Carlos III, ISCIII (CIBEROBN) from the Ministerio de Economía y Competitividad (MEC) (AEI/FEDER, UE) and the Generalitat de Catalunya (GC) 2014 SGR 773.

# Comparison of urinary excretion of total polyphenols after a high antioxidant diet and a low antioxidant diet

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### Background and objectives:

The hypothesis proposed was that the bioavailability of bioactive compounds, such as polyphenols, may be higher when the body is under a stressful situation (such a low antioxidant diet). The aim of this work was to compare the urinary excretion of total polyphenols before and after the intake of a single dose of *sofrito* after following either a high or a low antioxidant diet.

#### Methodology:

A crossover, randomized and controlled interventional study was carried out. Twenty-two healthy volunteers between 18 and 32 years performed two interventions, a high antioxidant diet and a low antioxidant diet for two weeks. Then, they consumed a single dose of *sofrito*. Polyphenols in urine were assessed by the Folin-Ciocalteu method before and after *sofrito* intake (from 0h to 24h).

#### Results and conclusions:

The concentration of polyphenols in urine was significantly higher after having the high antioxidant diet. However, the urinary concentration of polyphenols between 0 a 3 hours after the *sofrito* intake was higher among participants who followed the low polyphenolic diet. This response may be due to a higher absorption of polyphenols after following a low antioxidant diet.

### Acknowledgements:

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### Different polyphenol excretion between two populations following a 40th parallel diet

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### Background and objectives:

Polyphenols are non-nutrients of the human diet, secondary metabolites produced by plants in response to stress. Their consumption has recently been related with the lowering of risk factors of different conditions such as cancer or cardiovascular disease. Polyphenol excretion in urine is a measure of the polyphenol intake, which is very dependent on diet, among other factors. In the present study this variable was compared between two populations: California (USA) and Barcelona (Spain).

### Methodology:

In order to obtain the total polyphenol content in urine, the Folin-Ciocalteau method was used after a purification by solid phase extraction. The method is based on the reduction of fosfomolibdate and fosfotungstate originated by the antioxidant capacity of the polyphenols, which can then be determined by a colorimetric measurement. Besides, creatinine in urine was also measured to get a corrected and more accurate polyphenol concentration.

Data were compared using a t-test (P<0,05) of IBM SPSS Statistics software.

### Results and conclusions:

The comparison showed that California subjects had a significantly higher total polyphenol excretion related to that of the population from Barcelona (429,33 µg/ml vs. 274,33 µg/ml).

These results could derive from a higher polyphenol intake of the population from California than that of Barcelona, but dietary data is still being analyzed.

<sup>\*</sup>Presenting author

### Acknowledgements:

We would like to thank the Walnuts and Healthy Aging Study (WAHA) for providing the samples that were used in this study.

# Changes in intestinal immune system after training and exhausting physical exercise in rats

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# Background and objectives:

An exhausting physical exercise induces adverse effects on the immune system including the gut-associated lymphoid tissue. In this situation, nutrition may play a role by reducing the exercise-induced immunodepression. This study aimed to develop a suitable rat model for training and exhausting exercise in which biomarkers of intestinal immune system could be defined.

### Methodology:

Three-week-old female and male Wistar rats were distributed into runner and sedentary groups. Runner rats were progressively trained in a treadmill for 4 weeks. In the last week, intestinal permeability was measured thought the oral administration of  $\beta$ -lactoglobulin, and its further quantification in serum samples collected every 30 min. At the end, an exhausting test was carried out and samples were immediately collected. Immunoglobulin A (IgA) concentration was quantified in serum and caecal content by ELISA. The proportion of caecal IgA-coated bacteria was determined with fluorescent-labelled anti-rat IgA followed by flow cytometry analysis.

#### Results and conclusions:

Although rats' training and exhausting exercise did not affect serum IgA concentration, it induced changes at intestinal level. In particular, runner rats had lower intestinal permeability than the sedentary group. In addition, caecal IgA content was lower in runners than the sedentary group. Nevertheless, the proportion of caecal IgA-coated bacteria increased in male runners.

In conclusion, these results suggest the influence of physical exercise on the rat intestinal immunity and represent a first approach to establish biomarkers of intestinal changes.

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# Lactobacillus paracasei supplementation throughout the rat suckling and early post-weaning periods as strain-specific modulators of body growth and antibody response

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### Background and objectives:

Probiotics are functional foods that can play a beneficial role in the immune system. Early infancy is a critical period for immune development, and therefore it could be an interesting model to apply probiotics. The aim of this study was to ascertain whether the supplementation of two strains of *Lactobacillus paracasei* in early life influence morphometric and immune variables.

### Methodology:

For this purpose, Wistar rats were daily supplemented by oral gavage with two strains of *L.paracasei* from day 3 to day 28 of life. Body weight (BW) was monitored throughout the study. At days 21 (suckling ending) and 28 (one week post-weaning), spleen, thymus and small intestine were weighted. Plasma and intestinal immunoglobulin concentrations were determined at day 28.

#### Results and conclusions:

A strain of *L.paracasei* increased BW in the last week of suckling and also the relative splenic weight at day 21. However, the supplementation with the other strain did not affect BW but increased the relative weight of the small intestine at day 28. Both probiotics demonstrated a differential strain-specific effect in the immunoglobulins' concentrations: a strain was able to enhance both intestinal and plasma IgA levels whereas the other strain reduced the intestinal IgM content.

These results demonstrate that the supplementation with *Lactobacillus paracasei* throughout rat suckling and early post-weaning periods can promote the growth and development of some

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lymphoid tissues as well as modulate antibody response. However, it must be taken into account that these effects depend on the particular *L.paracasei* strain.

### Acknowledgements:

This study was supported by a grant from Centro para el Desarrollo Tecnológico Industrial CDTI (SMARTFOODS PROJECT, IDI-20141206).

# Leptin and adiponectin variations in rat's milk and plasma throughout the lactation period

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### Background and objectives:

Breast milk provides to the newborn a large range of biofactors that promotes the immune system development. Among them, there are metabolic hormones such as leptin and adiponectin, also named adipokines. Although their composition has been widely established in human breast milk, studies in rat milk are practically non-existent. The aim of this study was to establish milk leptin and adiponectin concentrations throughout the lactation in rat dams and to assess their relationship with their corresponding plasma levels.

### Methodology:

For this purpose, milk and blood samples were collected on days 3, 7, 10, 14, 17, 21 of lactation from rat dams. Leptin and adiponectin concentrations where determined in milk whey and plasma samples by ELISA.

#### Results and conclusions:

Regarding milk samples, the leptin concentration was 1000-2000 times lower than adiponectin levels. During the lactation period, leptin concentration rose in the first week and its levels kept stable until the end of the study. No significant changes in milk adiponectin levels were found throughout this period. However, a progressive decrease in the content of both adipokines in plasma samples was observed. There was a positive correlation between milk adiponectin and its plasma levels, which was not found for leptin contents.

This study shed light to the knowledge of the leptin and adiponectin concentrations in rat milk during lactation, and their relationship with plasma levels. It could be useful for understanding their biological effects in the dam and the offspring during the lactation period.

<sup>\*</sup>Presenting author

# Acknowledgements:

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### Lessons learnt from a norovirus outbreak caused by bottled mineral water

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### Background and objectives:

A norovirus gastroenteritis outbreak affecting 4,136 individuals was reported in Catalonia (Spain) in April 2016. The Catalonian Public Health Agency pointed towards drinking spring water bottled in Andorra as the source of infection. The company producing the bottled water recalled as a precautionary measure more than 6,150 water coolers. The water complied with all requirements of the European Commission directive on the exploitation and marketing of natural mineral waters. Our objective was to estimate the risk of infection in conditions of natural exposure.

### Methodology:

A questionnaire on water consumption and occurrence of symptoms was performed on 26 exposed individuals. Saliva samples were collected to determine norovirus susceptibility (secretor status). Water analysis was performed RTqPCR following ISO/TS 15216-1:2013, and treatment with a viability dye prior to RTqPCR was included to provide a better estimation of the infectious viral titer.

### Results and conclusions:

GII infections were only detected in secretor individuals, while GI infections were detected in both secretors (73%) and non-secretors (27%).

High levels of total genome copies (gc) per liter of both norovirus genogroup I (1.1x103) and II (5.8x103) were detected in the water samples. Infectivity of GI viruses was higher than for GII. ID50 causing illness may be figured for an ingested dose of ~400 gc/day for GI, and of ~3000 gc/day for GII. The use of PMA indicated that only 0.3-5.6% of genomes detected by regular RTqPCR contained undamaged capsids, rendering much lower illness doses.

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Management of microbial risks of commercially produced mineral waters could benefit from additional analysis for relevant viral pathogens such as norovirus.

### Acknowledgements:

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### Emotional eating is associated to sleep quality in Spanish young adults

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### Background and objectives:

Poor sleep quality (SQ) independently influences dietary intake (food and nutrients) and weight outcomes. However, few studies have studied the potential relationship between SQ with eating behaviors (EB) (the cognitive-behavioral nature of food intake) and its three dimensions: emotional (EE), uncontrolled (UE) and restraint (RE) eating. Our aim was to study potential associations between SQ and EB in young adults.

### Methodology:

One hundred forty-nine students (20–32 years; 78.5% females) from the University of Barcelona (UB) were included in a cross-sectional study. SQ was assessed with the Pittsburg Sleep Quality Index (PSQI) and EB with the Three Factor Eating Questionnaire, which allows to evaluate the three dimensions of eating: EE, UE, and RE. Lowest scores for PSQI indicate good SQ, whereas highest scores of each factor indicate higher EB. All the data were collected using the free software Open Data Kit. Scores were treated as continuous variables, and the significance level was considered at p<0.05. Linear regression was performed and EE questions were categorized by the median and divided into two groups: high emotional and low emotional eating. ANCOVAs were also carried out to compare PSQI scores within EE groups and adjusted by confounding factors.

#### Results and conclusions:

Higher PSQI scores predicted higher EE ( $\beta$  =0.298, p<0.029, r<sup>2</sup>=0.056), but not UE and RE. We also found that high emotional eaters with poor SQ tend to overeat when anxious (p=0.030), sad (p=0.039) or depressed (p=0.022). We conclude that poor SQ is associated with EE, specifically people tend to overeat when sad, depressed or anxious. These findings suggest that improving SQ in young population may reduce individual susceptibility to overeat when coping with emotionally negative situations, which could help to attenuate weight gain during adulthood.

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# Nova era de producció animal sense antibiòtics

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La producció animal, amb alt nivell de desenvolupament, ha pres la determinació de reduir l'ús d'antibiòtics com a promotors de creixement i com a preventius (pinsos medicats), i ha introduït el concepte d'ús prudent. Aquesta decisió dóna resposta a un compromís amb l'Organització Mundial de la Salut (WHO-2014). Per tant, entrem en un període sense ús d'antibiòtics en la producció. Segons la publicació de T. Vam Boeckel *et al.*, 2015, a l'any 2030 es consumiran 105 000 Tm d'antibiòtics i serà superior al consum de l'any 2010, amb un increment del 63%. Però, per què s'incrementa el consum antibiòtics a nivell global? La resposta, segons la publicació esmentada anteriorment, ve donat per l'important creixement de consum de productes d'origen animal en zones en fase inicial de desenvolupament. La reducció d'ús d'antibiòtics i la prudent aplicació terapèutica a Europa i altres zones econòmicament sostenibles, ha estat possible mitjançant una millora productiva, una millora en el maneig, en el benestar animal, en l'alimentació, en la nutrició, així com a un millor coneixement de tot el que fa referència a la relació entre el síndrome general d'adaptació i la salut del tracte gastrointestinal.



Doctor en Veterinària i director del centre de Mas de Bover de l'IRTA. Membre de l'SCAN (Scientific Committee of Animal Nutrition of UE Commission, 1994-2002) i del comitè FEEDAP de l'EFSA (European Food Safety Authority, fins al 2009). Ha estat involucrat des de 1986 en estudis sobre la digestibilitat, l'eficàcia i la seguretat de diferents additius, fonamentalment d'enzims en pollastres i porcs. També ha participat en estudis sobre els efectes de la composició de la dieta i d'additius alimentaris sobre la salut intestinal.

# Efectes immunomoduladors de les alternatives a antibiòtics en dietes per a animals de granja

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Les resistències antimicrobianes (AMR) generades per l'ús massiu dels antibiòtics en producció animal és una amenaça real per la salut humana. Les AMR provoquen la mort de 700.000 persones anualment i la tendència indica que al 2050 aquesta xifra augmentarà fins als 10 milions. S'estima que l'augment de la demanda de carn, sobretot d'au, incrementarà l'ús d'antibiòtics un 67% al 2030 agreujant aquesta situació. La prohibició europea de l'ús d'antibiòtics en pinso com a factors de creixement (AGP), les AMR, la seguretat alimentària i la consciència dels consumidors sobre el benestar animal fa indispensable trobar alternatives als antibiòtics.

Per tal de que aquestes alternatives siguin viables, cal que tinguin uns efectes similars als AGP (modulació del sistema immunitari intestinal, modificació de la microbiota i afectació directa a patògens). Actualment, s'està estudiant intensament el mode d'acció de diferents agents i substàncies que, afegits a la dieta, poden esdevenir alternatives reals als AGPs. Tot i que s'han obtingut resultats prometedors, sobretot amb l'ús de probiòtics, prebiòtics, àcids orgànics i pèptids antimicrobians, actualment no es disposa de cap estratègia que sigui capaç d'aportar individualment els beneficis dels antibiòtics en dosi sub-terapèutica.

Tot i així, l'estudi dels modes d'acció d'aquests productes sobre les rutes metabòliques (via NF- $\kappa\beta$ , reclutament de cèl·lules immunitàries, expressió de TLRs, etc.) i l'afectació directa sobre patògens intestinals permetrà dissenyar estratègies simbiòtiques capaces d'induir efectes beneficiosos similars als AGPs en un futur proper.



Llicenciat en Biotecnologia i Doctorat en Medicina i Sanitat Animals per la UAB. Actualment és investigador del programa de Nutrició de Monogàstrics de l'IRTA (Mas Bover – Constantí). L'any 2006 inicia la seva carrera científica al Departament de Millora Genètica Animal (UAB). L'any 2015 s'incorpora a l'IRTA on desenvolupa un projecte licitat per la European Food Safety Authority (EFSA) titulat "Revisió de substàncies / agents immunoestimuladors susceptibles de ser usats como a additius en pinso: mode d'acció i identificació dels end-points per l'avaluació de la seva eficàcia". Actualment, està iniciant una nova línia d'investigació en pollastres. L'objectiu d'aquesta línia és la inducció d'un estat homeostàtic a l'intestí mitjançant productes alternatius als antibiòtics que evitin la inflamació intestinal i incrementin la salut intestinal sense reduir la capacitat defensiva de l'animal.

SESSIÓ 2 Comunicació Oral 1

# Effect of Salmosan, a β-galactomannan-rich product on intestinal barrier function

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#### Background and objectives:

The use of antibiotic growth promoters (AGP) in animal feed is prohibited in the European Union (regulation [EC] no. 1831/2003) due to the risk of developing resistance to antibiotics in human consumers. However, natural feed additives such as mannan oligosaccharides (MOS) are promising alternatives to AGP. MOS, natural substances obtained from cell walls of yeast and plants, have health-promoting effects as they have potential prebiotic properties. Salmosan® (S- $\beta$ GM) is a hydrolyzed and highly  $\beta$ -galactomannan-rich MOS product developed from the carob bean of the *Ceratonia siliqua* tree and guar bean of *Cyamopsis tetragonoloba*.

#### Methodology:

We have studied the effect of S- $\beta$ GM in chicken intestine and in an *in vitro* model of intestinal Caco-2 cells in culture.

### Results and conclusions:

We have found that dietary supplementation with S- $\beta$ GM in chickens infected with *Salmonella* Enteritidis increases mucus production and prevents ZO-1 delocalization. These effects are associated with improved intestinal barrier function. Moreover, in Caco-2 cell cultures, we have confirmed the capacity of S- $\beta$ GM to agglutinate *Salmonella* Enteritidis and to prevent epithelial barrier function disruption induced by the bacterium colonization. Furthermore, we have demonstrated that in combination with *Lactobacillus plantarum*, a recognized probiotic accepted to be used as an additive in animal nutrition (EFSA regulation [EC] no. 1831/2003), restores intestinal barrier function disrupted by TNF- $\alpha$  and LPS in co-cultures of Caco-2 cells and macrophages. This

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protective effect could be related to the modulation of cytokine production and to the fact that S- $\beta$ GM increases the growth of *Lactobacillus plantarum*.

# Acknowledgements:

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SESSIÓ 2 Comunicació Oral 2

# Early Life Prebiotic effect on the intestinal microbiota composition and functionality

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# Background and objectives:

Breast milk is an important source of a broad variety of bioactive factors that influence the intestinal immune system and microbiota development. The objective of this work was to study the effect of an Early Life Prebiotic (ELP) supplementation on the development of the intestinal microbial composition and its functionality in suckling rats.

#### Methodology:

Neonatal Lewis rats (n=16 per group) received either ELP or PBS (control) daily from the 2nd to the 16th day of life. Changes in microbiota composition and functionality were evaluated on the 8th day of life by means of metagenomic and metabolomic analyses. In addition, immunological changes in intestinal structure of histological samples was also studied. Moreover, at the 16th day of life the concentrations of caecal short chain fatty acids (SCFA) as an indicator of microbiota functionality were quantified.

#### Results and conclusions:

ELP supplementation promoted intestinal development by increasing villus length and mucosal thickness. Profound changes were detected in specific microbial groups, such as an increasing proportion and diversity of Lactobacillus species. Regarding SCFA, although its total production was reduced, the relative proportion of butyric acid was increased in the ELP-supplemented animals as compared to control.

In conclusion, the ELP influences the development of the intestinal tissue and microbiota, at both composition and functionality levels, suggesting its key role in early life.

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SESSIÓ 2 Comunicació Oral 3

# Morningness and sleep quality improve adherence to Mediterranean Diet

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# Background and objectives:

Chronotype (morning or evening) and sleep quality (SQ) have been linked to obesity as well as to dietary intake (food and nutrients). Morning oriented people tend to have a more balanced diet and a healthier lifestyle compared to evening types; similar relationships have been found with SQ. However, no study has examined the relationship between chronotype, SQ, and adherence to the Mediterranean Diet (AtMD), which is a healthy dietary pattern that provides the right proportion of most nutrients. Our aim was to examine the potential relationship between chronotype and SQ with AtMD.

#### Methodology:

One hundred forty-nine students (20–32 years; 78.5% females) from the University of Barcelona (UB) were included in a cross-sectional study. Chronotype was assessed using the Spanish version of Horne and Östgeberg's morningness–eveningness questionnaire (MEQ), SQ with the Pittsburg Sleep Quality Index (PSQI) and AtMD with the KIDMED questionnaire. Higher scores of MEQ and KIDMED indicate morningness and higher AtMD respectively, whilst lower scores of the PSQI indicate good SQ. All the data were collected using the free software Open Data Kit. Scores were treated as continuous variables, significance level was considered when p<0.05 and, after testing interactions, a linear regression model was performed and adjusted by confounding factors.

#### Results and conclusions:

Higher MEQ scores ( $\beta$  =0.047, p=0.006, r<sup>2</sup>=0.058) and lower PSQI scores ( $\beta$  = -0.163, p<0.001, r<sup>2</sup>=0.058) predicted higher AtMD. Our results suggested that morningness and good SQ improve AtMD, these findings could contribute to provide holistic guidelines in order to reduce the prevalence of obesity during adulthood.

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# Synergism effect of home cooking and ingredients enhance tomato polyphenols in Mediterranean *sofrito*

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# Background and objectives:

There has been increasing the interest in the food matrix study since the bioavailability of bioactive compounds, like polyphenols, are affected by intrinsic food proprieties, processing and interaction of these factors. *Sofrito*, a typical home-made Mediterranean tomato based sauce, present a complex matrix by the addition of ingredients like olive oil, onion and garlic that can influence the content, type and bioavailability of polyphenols. The aim of this study was to evaluate whether home cooking and ingredients addition in Mediterranean *sofrito* sauce may interact and improve tomato's polyphenols.

# Methodology:

A full factorial design 2<sup>4</sup> was applied to clarify the contribution of the ingredients: extra virgin olive oil (5-10%), onion (20-40%) and garlic (2-4%), and cooking duration (30-60 min) on the polyphenols composition of *sofrito*. The identification of tomato polyphenols was performed by UPLC-ESI-QqQ-MS/MS and the quantification using external calibration curves with standards.

#### Results and conclusions:

Short cooking time was able to increase the content of chlorogenic acid, ferulic acid hexoside and naringenin. The presence of olive oil enhances the extractability of some polyphenols from tomato improving the bioaccessibility. Onion shows to be capable to protect some phenolic compounds from oxidation during cooking process. The use of olive oil and onion with adequate cooking time may improve tomato's polyphenols stability.

# Acknowledgements:

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# Stability of carotenoids in commercial sofrito

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#### Background and objectives:

Tomato products are an important source of carotenoids, such as *sofrito*, present a high level of them especially lycopene. However, the interaction between ingredients and lycopene can change the amount and isomers production during storage. The production of Z-lycopene isomers is interesting, because is more bioavailability and has more antioxidant capacity than the -E forms. The aim of the study was identified, quantify the carotenoids behavior during an accelerate stability test in how many different commercial *sofrito*.

#### Methodology:

The effect of ingredients on carotenoids change kinetics of nine different commercial *sofrito* was investigated during storage at 40°C for 0, 4,8,16 and 32 weeks. The identification of the carotenoids was based on retention time; standards; UV/VIS absorption spectrum:  $\lambda_{max}$ , %III/II and %Ab/II. Quantification was performed by HPLC-DAD, using external calibration curves with standards.

#### Results and conclusions:

The commercial *sofritos* A, B, D and G showed an increase in the content of *cis*-lycopene isomers during the stability assay, which could be correlated with a high content of onion and olive/sunflower oil in the nutritional label. The samples C, E and F that increased the content of trans-lycopene presented more oil than onion in their composition. The sample H was stable during the storage and correlated with low content of oil and onion. The presence of onion and oil must be encouraged in the *sofrito* formulation to improve carotenoid isomers content.

#### Acknowledgements:

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# The fruit of Olea europaea L. as a dietary source of bioactive compounds

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# Background and objectives:

The fruit of *Olea europaea* L. is a typical food of Mediterranean diet with a high content of monosaturated fatty acid, followed by minor components such as tocopherols, sterols, triterpens and phenolic compounds. Among them, polyphenols stand out due to their antioxidant, anti-inflammatory, cardioprotective and cancer-preventive activities. Due to the mentioned properties, table olives could be considered to be a functional food and for this reason, the concentration of polyphenols and its bioavailability have been analyzed by HPLC-MS/MS, and the effect on blood pressure was evaluated.

#### Methodology:

A homogenous suspension, prepared from destoned Arbequina table olives at a dose equivalent to ingestion of 30 olives taken by a 70 kg-human, was analyzed and quantified using HPLC-MS/MS previous extraction with methanol-ethanol (1:1,v/v). The prepared olive suspension was orally administered to overnight fasted male Sprague-Dawley rats. Blood was taken from saphenous vein after 30 min of administration and subsequently analyzed. The olive suspension was also given to spontaneously hypertensive and Wistar Kyoto rats and the acute blood pressure was measured by tail-cuff method.

#### Results and conclusions:

The analysis of table olives indicated that hydroxytyrosol, tyrosol and luteolin are the most abundant phenolic compounds. After administration, the hydroxytyrosol was the polyphenol found in the highest concentration in the plasma. The ingestion of olives suspension did not affect the blood pressure in hypertensive and normotensive rats. The study extends the knowledge that the olives are an important source of bioactive polyphenolic compounds and could become a potential functional food.

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# Volatile fingerprinting for the verification of virgin olive oil sensory quality

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#### Background and objectives:

Sensory quality, assessed by trained panels following a standardized method<sup>1</sup>, is one of the parameters defining the commercial category of virgin olive oil (VOO). Considering the need to reduce the number of samples to be assessed by sensory panels, as well as decrease the number of cases related to an uncertain or debated classification; the setting up of instrumental methods can be a solution to achieve a preliminary rapid screening and supporting the sensory panels in the discrimination of boundary samples. In this context, volatile profile analysis can be an excellent fit-for-purpose tool as the volatile fraction is responsible for both VOO's positive and negative sensory attributes<sup>2</sup>.

Finding specific patterns in highly dimensional analytical data, known as fingerprints, is a state-of-the-art strategy in food analysis. Thus, a preliminary fingerprinting approach on the volatile profile was applied to verify the quality grade of VOOs. The capability of this approach to grade VOOs' sensory attributes was also evaluated.

#### Methodology:

The volatile profile of 94 VOOs graded as Extra Virgin (EVOO), Virgin (VOO) or Lampante (LOO) by six EU sensory panels, was analyzed by HS-SPME-GC-MS<sup>3</sup>.

Data analysis was performed after the alignment of GC-MS chromatogram by applying shrinkage statistical methods for data exploration and development of classification models.

#### Results and conclusions:

PCA showed that samples tended to group according to VOO's commercial categories. PLS-DA classification models were developed following two strategies: i) a model with three categories

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(EVOO, VOO & LOO) and ii) two sequential models with two categories: a first one in which the samples were classified as EVOO or Non-EVOO and a second one, where all Non-EVOO samples were classified as VOO or LOO.

Prediction results were satisfactory for both strategies despite the percentage of correct classification for sequential model being slightly high. Still, further optimization of chromatogram alignment and external validation are necessary.

#### Acknowledgements:

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<sup>3</sup>Vichi et al., 2003 J Chromatogr A, 983: 19-33.

# Grape cane extracts: an opportunity for the development of novel additives for food Chilean industry

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Grape canes, obtained after annual pruning of wines, is a waste estimated in more than 120.000 tons/year in Chile<sup>1</sup>. The grape cane can be storage at controlled temperature and humidity. The system enable increase the polyphenolic compounds in the grape cane biomass<sup>2</sup>. The general aim was: "To enhance the use of waste from the Chile wine industry to obtain extracts rich in stilbenes and procyanidins for future applications as functional compounds in food industries"

In this work an extract of Pinot Noir grape canes was produced at industrial scale in a reactor of 750 L. The extract was encapsulated with  $\beta$  cyclodextrins, and then dried by spray drying. Profiles and content of stilbenoids and procyanidins were determined by HPLC-DAD-ESI-MS/MS. Other chemical constituents as carbohydrates, organic acids, metals and lignin were also detected and quantified. Antioxidant capacity of the whole extract, using cells and cell-free assays was also evaluated. The antioxidant capacity of the Pilot scale extract was compared with a commercial product Resveravid®.

The dry extract obtained under optimal conditions (T:80 °C, t:100 min, ratio S/L:1:10) showed, a yield of 2.4 g stilbenoids/kg of dry grape cane and total stilbenoids concentration was 5.45% w/w. The antioxidant capacity (ORAC) was high 14760.66 µmol trolox equivalent/g of extract. On the other hand, the antioxidant activity of the extract is higher than a commercial product of resveratrol (Resveravid®).

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# Reference:

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# Resum no disponible

# Resum no disponible

# Effect of cocoa, cocoa polyphenols, theobromine and hesperidin on the gene expression of tight junction proteins in Caco-2 cells

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# Background and objectives:

Cocoa powder contains over 500 different compounds with polyphenols and methylxanthines as the most recognized active ones. The human adenocarcinoma cell line (Caco-2) has been widely used as model of the intestinal epithelial layer for barrier investigations. The aim of this study was to establish the effect of cocoa powder, a cocoa polyphenol extract (CPE), theobromine and hesperidin on the gene expression of tight junction proteins in the Caco-2 cell model.

# Methodology:

The Caco-2 clone TC7 was grown in DMEM medium supplemented with non-essential amino acids, HEPES, heat inactivated foetal calf serum, glutamine and penicillin-streptomycin. After 21 days, Caco-2 cells were incubated either with cocoa powder (10 μg/mL), CPE (10 μg/mL), theobromine (10 μM- 100 μM) or hesperidin (10 μM- 100 μM) for 30, 60 and 120 min. Total RNA was isolated using Trizol and the RNeasy RNA isolation kit including a DNase digestion. Later, cDNA was obtained by the High Capacity cDNA Reverse Transcription Kit and a PCR quantitative assay was performed to determined gene expression of claudin-1, claudin-4, occludin, zonula occludens (ZO)-1 and ZO-2.

#### Results and conclusions:

The incubation of cocoa powder for 120 min increased claudin-1 gene expression of Caco-2 cells. No differences were obtained for claudin-4, occludin, ZO-1 and Z0-2 in the three studied time points and for all tested compounds.

In conclusion, the present data suggest that cocoa polyphenols, as well as the polyphenol hesperidin and the alkaloid theobromine do not compromise the intestinal epithelial barrier *in vitro*. Further studies are needed to in depth evaluate the effect of these food compounds in the intestinal layer.

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# Model of Se deprivation in Caco-2 cells and macrophages in culture

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# Background and objectives:

Modern poultry industry involves high rates of animal growth, which leads to a high sensitivity to stress situations. Selenium (Se) could contribute to an optimal state of health by the formation of selenium-proteins that maintain the oxidation/reduction state. The hypothesis is that dietary supplementation with Se can protect chickens from stress. Our objective was to establish a model of Se deprivation in cultures of intestinal Caco-2 cells and macrophages to further investigate the effects of supplementation with different Se sources. The indicators considered to validate the model are glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) activity and selenoprotein P (SEPP1) protein and gene expression.

# Methodology:

Given that fetal bovine serum (FBS) is the main source of Se in cultures, FBS deprivation would induce Se deficiency. Caco-2 cells and macrophages were incubated in absence of FBS during 6 days and 24 h, respectively. A positive control of cells maintained with FBS was also included.

#### Results and conclusions:

In Caco-2 cells, GPx activity and SEPP1 protein and gene expression in the absence of FBS show statistically lower values than the positive control, whereas no differences were detected for TrxR activity. In macrophages, GPx and TrxR activity as well as SEPP1 protein and gene expression in the absence of FBS show lower values than the positive control, although only statistical differences were detected for GPx activity and SEPP1 protein expression. The results obtained allow us to conclude that the model of deprivation for both cell cultures is established.

#### Acknowledge:

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# Comparison of metagenomic profile of patients, meeting criteria for non-celiac gluten sensitivity, with and without response to restrictive diets

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# Background and objectives:

Non-celiac gluten sensitivity is a disorder with similar symptoms to celiac disease, but with negative biomarkers, that is linked to the consumption of wheat and other foods containing gluten.

Despite being a fairly common disorder, there is no consensus among the medical community and studies to determine its etiology and diagnosis, to address the right treatment.

The hypothesis proposed was that dysbiosis might favor the development of gluten intolerance, since by causing an alteration of intestinal permeability and the potential trespass of the gut barrier by proteins that could be detected as antigens, triggering an immune and inflammatory response. Thus, the use of probiotics might be of help to their treatment.

#### Methodology:

Randomized pilot study (n=50) with parallel groups (25 each) design.

Patients meeting inclusion criteria were randomized to one of the two treatment groups: restrictive diet or diet plus treatment with probiotics.

### Results and conclusions:

Although the study has not been completed, it has observed that approximately 8-10% of patients, meeting the inclusion criteria, and have been discarded celiac disease or any other pathology that could justify these symptoms, did not improve their symptoms, neither with the restrictive diet, nor with the restrictive diet plus probiotics.

It would be interesting to design a new project, including a metagenomic analysis of the intestinal flora to compare both, patients who do not respond to the treatment and patients that treatment

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works. Differences could help to elucidate the problem of these patients and the right approach of their pathology.

# How to calculate number of samples in the design of pre/pro-biotics studies (metagenomic studies)

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#### Background and objectives:

Nowadays a high amount of pre/probiotic studies use metagenomics to control the effectiveness of the use of pre/probiotic. Many bacterial species are non-cultivable out of their natural environment and, therefore, some sets of species can only be studied all together within their environment (e.g. human gut) using metagenomics, sampling and sequencing DNA from all species. Sample size in metagenomics is an unresolved problem in which statistics and computational methods have much to say, especially from the point of view of Bayesian approach. One possibility is the use of a bietapic method: conduct a pilot study, prior to the final study.

#### Methodology:

We propose the use of a Bayesian method based on Markov Chain Monte Carlo simulations (MCMC) to calculate the richness, supposing a Dirichlet-multinomial probability distribution for the matrix of richness-abundance. The sample size on the base of sampling effort (90, 95 and 99%) is then calculated assuming saturation or not using parametric and non-parametric nonlinear models.

#### Results and conclusions:

These calculations have been implemented in the new library 'BDSbiost3' for R, which has been used in different simulation scenarios of the common practice in metagenomics (low abundance, high richness, oversampling, under sampling) and the results (Sp) are presented with the effort curve and a value proposed of the correct number of samples to reach the extrapolated richness of the 90, 95 and 99%. This function can help researchers in pre/probiotics to better design studies and save on number of samples.

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# Lignans and chronic diseases: results from the PREDIMED trial

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# Background and objectives:

Lignans are phenolic compounds found in olive oil, whole grains and seeds, among others. A healthy lifestyle and a healthy diet can reduce the risk of chronic diseases, which are a worldwide burden.

The objective was to prospectively evaluate the association between lignan intake and the incidence of chronic diseases within the PREDIMED study (ISRCTN 35739639), which includes 7,447 participants at high cardiovascular risk that were followed a median of 4,8 years.

#### Methodology:

Dietary intake of lignans was obtained from yearly food frequency questionnaires using the Phenolexplorer database. Time-dependent Cox proportional hazard models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for chronic diseases and mortality according to lignan intake. Statistical analyses were conducted by using SAS software, version 9.3.

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#### Results and conclusions:

After multivariate adjustment and comparing the highest versus the lowest categories, lignan intake was inversely associated with cardiovascular events (HR=0.51, 95% CI 0.30-0.86, P-lineal=0.007), incidence of type 2 diabetes (HR=0.71, 95% CI 0.51-0.98, P-lineal=0.05), and all cause mortality (HR=0.60, 95% CI 0.37-0.97, P-lineal=0.03). In this population, olive oil was the main source of lignans (72%).

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# Resum no disponible

#### L'eix microbiota-cervell i els trastorns de l'ànim

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En ple segle XXI, ens trobem per una banda, que dietes poc saludables han estat descrites com a factor de risc per a la depressió i que la dieta mediterrània s'ha identificat com un possible factor protector. D'altra banda, s'ha identificat un increment de la prevalença dels trastorns afectius en la població mundial. Una incipient línia de recerca ha sorgit al voltant del possible paper que pot jugar la dieta i l'eix intestí- cerebral en les malalties cerebrals així com la probable funció de la microbiota en aquest eix. Existeix evidència pre-clínica convincent que mostra que la microbiota intestinal pot induir conductes ansiògenes i que la manipulació de la microbiota intestinal amb probiòtics específics o antibiòtics pot induir conductes depressògenes. A més, estudis recents han confirmat que fenotips psiquiàtrics poden ser transferits a través de la microbiota intestinal. Al llarg d'aquesta presentació mostrarem quina transferència clínica poden tenir aquests resultats en el nostre dia a dia.



Psiquiatra especialista en la Unitat de Trastorns Bipolars a l'Hospital Clínic, investigadora de l'Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) i del Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), així com professora clínica de Psiquiatria a la Universitat de Barcelona. Participa en diversos projectes d'investigació sobre microbiota i l'eix intestí-cervell i sobre estratègies farmacològiques en el trastorn bipolar. A més a més, investiga sobre possibles biomarcadors perifèrics en el trastorn bipolar i en la conceptualització d'aquesta malaltia en el model d'estadificació.

SESSIÓ 3 Comunicació oral 4

# Dietary supplementation with spray-dried animal plasma proteins modifies the profile of the fecal microbiota in young mice

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## Background and objectives:

Oral administration of spray-dried porcine plasma (SDP) reduces the magnitude of the gut-associated lymphoid tissue (GALT) response in mice challenged with the Staphylococcus aureus enterotoxin B1. We have studied if feeding mice with 8% SDP, starting at weaning and for 14 days, can modify the profile of fecal microbial communities.

#### Methodology:

DNA was extracted from fecal samples using phenol-chloroform method. The genomic study was done with the MiSeq Illumina, analyzing DNA from amplicon sequencing of prokaryotic 16S small subunit rRNA genes. For the statistical analysis the False Discovery Rate test was applied. Control and SDP-supplemented diets were supplied by APC Europe (Granollers, Barcelona, Spain).

#### Results and conclusions:

At Phylum level, SDP reduced the relative weight of Actinobacteria (from 10.3% to 2.6%) and Verrucobacteria (from 15.2% to 11.3%) while increasing the Firmicutes (from 25.6% to 36.3%) population. At Family level, SDP strongly increased *Lactobacillaceae*, with well-known probiotic anti-inflammatory effects; *Porphyromonadaceae*, with species that are negatively correlated with the expression of pro-inflammatory cytokines; and *Lachnospiraceae*, involved in the regulation of regulatory T-lymphocytes homeostasis and the restoration of mucosal permeability. In conclusion, the SDP-induced changes in the microbiota profile are compatible with the well-known role of SDP on GALT modulation reducing intestinal inflammation.

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SESSIÓ 3 Comunicació oral 5

# Resum no disponible

SESSIÓ 3 Comunicació oral 6

# Microbial metabolites are associated with a high adherence to a Mediterranean dietary pattern using a 1H-NMR-based untargeted metabolomics approach

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## Background and objectives:

The study of biomarkers of dietary patterns including the Mediterranean diet (MedDiet) is scarce and could improve the assessment of these patterns. We aimed to determine a robust and accurate biomarker associated with a high adherence to a MedDiet pattern that included dietary assessment and its biological effect.

#### Methodology:

In this cross-sectional study we included 56 and 63 individuals with high (H-MDA) and low (L-MDA) MedDiet adherence categories, respectively, all from the Prevención con Dieta Mediterránea trial. A <sup>1</sup>H-NMR-based untargeted metabolomics approach was applied to urine samples. Multivariate statistical analyses were conducted to determine the metabolite differences between groups, as well as to build and evaluate the prediction model for H-MDA.

#### Results and conclusions:

Thirty-four metabolites were identified as discriminant between H-MDA and L-MDA. The H-MDA included higher excretion of food metabolome metabolites, and decreased amounts of metabolites related to glucose metabolism. The microbial metabolites: phenylacetylglutamine, p-cresol and 4-hydroxyphenylacetate were included in the prediction model of H-MDA, thus being the biomarker

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that defined high adherence to the MedDiet. The overall metabolite profiling identified reflects the metabolic modulation produced by H-MDA. The proposed biomarker may be a better tool for assessing and aiding nutritional epidemiology in future associations between H-MDA and the prevention or amelioration of chronic diseases.

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SESSIÓ 3 Taller

## Pre- i probiòtics a la cuina

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Caipirinha de kombucha. Còctel sense alcohol elaborat amb probiòtic (kombucha)

Kombucha de te verd Concentrat de caipirinha sense alcohol Llima fresca Poma àcida Sucre integral

**Tostones** de plàtan verd. Aperitiu que combina aliments que contenen midó resistent i inulina (prebiòtics)

Plàtan verd fregit Carxofa confitada Ceba caramel·litzada Radicchio

**Bombes de** *pickles*. Boles de puré de patata farcides i arrebossades elaborades amb ingredients probiòtics i prebiòtics (xucrut, *pickles* i patata cuita i refredada)

Puré de patata Xucrut/pickles

Tostones dolços amb garrofí. Dolços elaborats amb ingredients prebiòtics (plàtan verd i garrofí)

Plàtan verd fregit Xocolata de garrofí Sucre i canyella en pols

Lassi de papaia i pinya. Beguda dolça de quefir (probiòtic) amb fruites

Quefir Papaia Pinya Xarop d'atzavara Gel o aigua gelada

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# Amb la col·laboració de:













