Sociedad de Biología de Cuyo

Abstracts of the XL Annual Meeting

Mendoza - Argentina December 6th and 7th, 2022

In memoriam

Dra. Ana María Valentina Teresa Stefanini de Guzmán

(1935–2021)

Professor Emeritus

Microbiology and Immunology – Faculty of Chemistry, Biochemistry and Pharmacy

Universidad Nacional de San Luis

She was born in San Luis and graduated National Pharmacist, Licentiate and Dr in Biochemistry at the National University of San Luis (UNSL), where she worked her whole life with great vocation as teacher and researcher in the Microbiology Area. She formed uncountable students, directed scholarships and postgraduate thesis at UNSL and CONICET on themes related to *Yersinia enterocolitica* and anaerobics, in which she was a region pioneer. She developed several extension activities, services and government functions, being director of the School of Biochemistry in the 1987-1989 period. She was recognized for her huge scientific production; in 1999 she got Category I in the Research Incentive and in 2001 she received the professor emeritus recognition. Founder of the Microbiology Laboratory of the Faculty of Chemistry, Biochemistry and Pharmacology at UNSL together with Dr. Olga P. de Centorbi and prestigious colleges, she reached her dream of seeing it in the new building with modern equipment to continue the research in molecular aspects of Microbiology, with applications on the human and animal health.

Her example is always with us, thank you so much for your legacy dear Dr. Guzmán!

DIRECTIVE BOARD

President

Dra. Nidia Noemí GOMEZ

Vicepresident

Dra. Claudia M. CASTRO

Secretary

Dr. Juan Gabriel CHEDIACK

Treasurer

Dra. Silvina Mónica ALVAREZ

Members

Dr. Esteban LOZANO

Dra. Gabriela FERESIN

Dra. Verónica P. FILIPPA

Dra. Marcela Alejandra MICHAUT

Lic. Glenda MARTIN MOLINERO

Dra. Lorena Celina LUNA

Accounts' Reviewers

Dra. María Verónica PEREZ CHACA

Dr. Walter MANUCHA

ORGANIZING COMMITTEE

Dr Walter Manucha
Dra Marcela Michaut
Dr Esteban Lozano
Lic Omar Klinsky
Dra Isabel Quesada
Dra Claudia Castro
Dra Silvina Alvarez
Dra María Eugenia Ciminari
Dra Nidia Gómez
Dr Juan Gabriel Chediack
Lic. Silvana Piguillem
Gino Verteramo
Sol Giménez Santobuono

SCIENTIFIC COMMITTEE

Dr. Walter Manucha
Dra. Marcela Michaut
Dr. Esteban Lozano
Dr. Carlos Gamarra Luquez
Dr. Diego Grilli
Dr. Fernando Saraví
Dr. Martin Pavarotti
Dra. María Esther Escudero
Dra. Liliana Villegas
Magister Mónica L. Gatica
Dra. Claudia Gatica Sosa
Dra. Edith Pérez Romera
Dra. Silvina Álvarez
Dra. María Verónica Pérez Chaca
Dr. Luis S. Mayorga
Dra. Gabriela Feresin
Dr. Juan Chediack
Dra. Hilda Pedranzani
Dra. Graciela Wendel
Dra. Lucia Fuentes
Dra. Alejandra Rotelli
Dra. Marta Moglia
Dra. Jesica D. Paredes
Dra. Ana C. Anzulovich
Dra. Laura Sosa
Dra. Cristina Barcia
Dra. Alba Vega
Dra. Andrea González Reyes
Dra. Susana Ferrari
Dra. María Eugenia Ciminari

OPENING LECTURE:

A1
HISTORY OF THE CUYO BIOLOGY SOCIETY

Piezzi R, Castro-Vazquez A
Universidad Nacional de Cuyo

History of the last 40 years of the scientific meetings of the Cuyo Biology Society, from the refoundation of the society in 1973 to the present day.

SYMPOSIUM I: APLICATIONS OF NATURAL PRODUCTS CHEMISTRY ON HEALTH AND ENVIRONMENT

A2
MICROPROPAGATION AS A STRATEGY FOR THE SUSTAINABLE PRODUCTION OF THERAPEUTIC ACTIVE PRINCIPLES: THE AMARYLLIDACEAE ALKALOIDS AS AN EXAMPLE

Bastida J
Grup de Productes Naturals. Departament de Biologia, Sanitat i Medi Ambient. Facultat de Farmàcia i Ciències de l'Alimentació, Av. Joan XXIII #27-31, 08028-Barcelon, España. E-mail: jaumebastida@ub.edu

The conservation of biodiversity currently rests on two well-established pillars: the United Nations Convention on Biological Diversity (1993), an outcome of the Earth Summit in Rio de Janeiro (1992), and the subsequent Nagoya Protocol (2010), which was ratified by the signatory states in 2020. The study of plant diversity, an inexhaustible source of bioactive molecules, has had to find ways of adapting to these agreements to ensure the available therapeutic arsenal can continue to grow. Plant chemists have modified their methodologies and reduced plant batch size by an order of magnitude. Playing a key role in this process of adaptation is plant micropropagation, which allows the generation of sufficient biomass for research from very small quantities of starting material. We discuss the strategies employed in the study of a monocotyledonous family of bulbous plants, the Amaryllidaceae, producers of an exclusive group of alkaloids, most notably galanthamine, which is used for the palliative treatment of Alzheimer's disease. We describe the GC-MS (compound library) and LC-SPE-NMR techniques for the separation and identification of compounds and the use of molecular docking to simulate compound inhibitory activity against various enzymatic targets. Special focus is given to the micropropagation of species of high therapeutic interest, an approach that avoids the depletion of plant biodiversity and can provide enough plant material for the complete characterization of bioactive components and *in vitro* assessment of their neuroprotective activity. This study is part of a thematic network subsidized by CYTED (Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo), in the area of Sustainable Development.

A3
CHEMISTRY AND BIOLOGY: NATURAL PRODUCTS INVOLVED IN CHEMICAL ECOLOGY AND ITS APPLICATIONS

Borges WS
Universidade Federal do Espírito Santo, Brasil. E-mail: warley.borges@ufes.br

According to data published in the Journal of the natural products more than half of all drugs approved by the FDA in the last 40 years are natural products or natural product derivatives. To exemplify the importance of these compounds to current society, in 2015, the Nobel Prize in Medicine was ceded to researchers for their discoveries concerning novel therapy based on natural products against infections caused by roundworm parasites and the discoveries concerning novel therapy against Malaria. Recent studies have demonstrated that chemical compounds involved in ecological relationships have the capacity to present different biological activities, and some examples of these types of studies will be presented.

A4

INNOVATION AND DEVELOPMENT TABLE
HOW TO INCREASE THE IMPACT OF SCIENCE IN SOCIETY: THE UNIVERSITY THIRD MISSION

Tarolli P, Silva F, Estrella Orrego J
University of Padova, Dept. of Land Environment Agriculture and Forestry, E-mail: paolo.tarolli@unipd.it; Universidad Nacional de Cuyo y Conicet, E-mail: msilva@fca.uncu.edu.ar; Universidad Nacional de Cuyo, Facultad de Ciencias Agrarias, E-mail: jestrella@fca.uncu.edu.ar

As key actors in the social scenario, universities fulfill their mission by offering academic courses, undertaking research, and developing outreach activities. This last role requires understanding universities as transforming institutions with the knowledge to generate impact on different stakeholders. Intense debate arose after the pandemic on how these outreach activities must occur. While the pandemic has increased science's popularity, it has generated intense debate about how universities perform their role and interact with different stakeholders. There are countless ways to promote this interaction. Entrepreneurship is one of the most developed ones, looking to generate companies based on students and researchers with distinctive skills matched with market opportunities. These so-called spin-off companies offer the community intense knowledge of products or services and help universities finance activities. The way to generate companies requires training programs for researchers and students on entrepreneurship skills, generating contests, enabling direct dialogue with successful entrepreneurs, and helping them get financial aid. Intellectual property issues play a central role in this and must be assessed strategically. Technical services are another way to do outreach and offer external organizations knowledge and equipment on a certain subject. Public governments widely use these technical services when certain issues need to be assessed, and an impartial view is required. These services were traditionally focused on one specific subject, but they have changed to be more interdisciplinary for a while. Researchers normally generate a group of students to carry out these services, enabling them to get real contact with public agencies or companies, increasing their skills and their employability rate. A step forward in carrying out outreach is through an innovation strategy. To develop this, universities must put social and productive needs on a central role, understanding and defining the challenges organizations face and conveying researchers and students to propose solutions. These solutions must help organizations create value and not only solve a technical issue. The substantial difference with previous approaches is the centrality of society. Superior communication skills are required on both sides to fulfill a successful innovation strategy and therefore achieve social-economic and environmental impact. Lastly, one key aspect of outreach is creating special public events, workshops, and scientific café for citizens. In such circumstances, adapting communication skills is necessary to better disseminate the results. This is the key point to raise awareness among citizens and stimulates policymakers to better adopt solutions according to scientific results. Adequate use of social media is necessary to maximize the impact of science.

SYMPOSIUM II: MICROBIOME IN HEALTH AND DISEASE

A5
MODULATION OF THE INTESTINAL MICROBIOTA THROUGH FERMENTED FOODS, PROBIOTICS AND PREBIOTICS

Vinderola G
Instituto de Lactología Industrial (INLAIN, UNL-CONICET), Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe. E-mail gvinde@fiq.unl.edu.ar

The human intestinal microbiota, the set of microorganisms that colonize the intestinal tract, has become relevant because of its fundamental role as an interface between our diet and the immune system, but also as a modulator of the gut-brain or gut-skin axis. The COVID-19 pandemic further directed our attention to the gut microbiota as a key factor in the susceptibility to infection and its resolution: association studies showed that countries with higher per capita consumption rates of fermented plant foods had lower rates of coronavirus infection and lower severity of infection. The combination of factors such as cesarean section, limited breastfeeding, and consumption of antibiotics and antimicrobial compounds in the diet that, in many cases, is also deficient in fibers, has progressively impoverished the taxonomic composition of our intestinal ecosystem decreasing its diversity and abundance and leading to a progressive increase in chronic diseases, developmental and functional gastrointestinal disorders. In a context of a "microbiota in extinction", fermented foods, probiotics, and prebiotics have gained attention as nutritional strategies to address this issue. Fermented foods are a very heterogeneous family of foods, with asymmetric scientific evidence on their effects on microbiota and health. Some fermented foods are a source of live microorganisms (yogurt, cheeses, kefir, kombucha, sauerkraut, kimchi), while in other cases the microorganisms are inactivated by cooking processes (sourdough bread) or removed in the final processing of the food (beer, wine). Some fermented foods may also contain varying amounts of alcohol (kefir,

kombucha). Probiotics, on the other hand, are live microorganisms, with microbiological identity at the strain level and beneficial effects demonstrated in scientific studies. Modification of the structure and/or function of the microbiota is not a condition for the efficacy of fermented foods or probiotics, but it is for prebiotics (Inulin, FOS, GOS), for which evidence shows that they are able to selectively stimulate the development of the indigenous bifidobacteria of the intestine. Recent intervention studies show that the progressive incorporation of fermented foods in the diet is able to decrease several biological parameters related to chronic low-grade inflammation. The knowledge of the microbiological particularities of fermented foods and probiotics, and of the presence of prebiotics in foods or in supplements, allows a rational approach for their use, with the aim of providing viable microorganisms, or not, and their metabolites, to temporarily colonize the intestine, to contribute to the diversification and abundance of microorganisms in this ecosystem, for better functioning of the digestive and immune system, for the management of chronic conditions and for the prevention of intestinal or respiratory tract infections. Fermented foods, probiotics, and prebiotics are gaining popular interest, but also, they attract the interest of the medical community and the industry, although much more slowly by the regulatory agencies. A proper regulatory framework is needed for rational and scientifically based management. The incorporation of fermented foods, probiotics, and prebiotics in the dietary guidelines of the population is the next challenge.

A6
NEW MECHANISMS IN THE ETIOPATHOGENESIS OF ARTERIAL HYPERTENSION: ROLE OF THE INTESTINAL MICROBIOME

Choi MR
Cátedra de Anatomía e Histología, Facultad de Farmacia y Bioquímica, UBA. Instituto Alberto C. Taquini de Investigaciones en Medicina Traslacional, UBA-CONICET. E-mail: marcelinkchoi@yahoo.com.ar

The gut microbiota comprises approximately one trillion microorganisms with 150-fold more genes than the human genome. Considered an essential part of the human body, a healthy gut microbiota that generates bioactive metabolites can benefit the host in multiple organs and systems. The term dysbiosis involves an imbalance in gut microbiota composition and its metabolic capacity and is associated with the development of several chronic diseases, including hypertension. In addition to the harmful impact caused by hypertension on different target organs, gut dysbiosis is capable of causing direct damage to critical organs such as the brain, heart, blood vessels, and kidneys. The role of gut microbiota and its metabolites in cardiovascular disease has become a topic of great interest in recent years. Several metabolic pathways involving a wide range of metabolites from the gut microbiota may be implicated in the link between gut dysbiosis and critical cardiovascular disease risk factors. Therefore, the comprehension of the complex interactions between the gut microbiota and the cardiovascular system results in great importance to develop improved pharmacological therapies for hypertension prevention and treatment.

A7
OBESITY AND GUT MICROBIOTA: FROM RESEARCH TO ACTION

Quesada IM
Instituto de Bioquímica y Biotecnología, Facultad de Ciencias Médicas UNCUYO e IMBECU-CONICET. E-mail: iquesada@mendoza-conicet.gob.ar

Obesity is a complex metabolic disorder caused by a variety of genetic and non-genetic factors (such as environmental factors), its incidence is increasing every year and it is considered a public health problem. Despite the fact that the COVID-19 pandemic occupied priority places in the guidelines during 2020–2021 in the national public health research agenda (ANISP) of the Argentine Ministry of Health, non-communicable chronic diseases such as obesity and its related diseases continue to occupy the first places of priority. Obesity not only manifests as changes in appearance, but is also associated with lipid and glucose metabolism disorders, chronic inflammation, oxidative stress, and increased risk of cardiovascular disease, diabetes, and cancer. The gut microbiota may be a relevant environmental factor in obesity and may be positively or negatively modulated by different lifestyle and dietary factors. In addition, microbial metabolites can induce epigenetic modifications, which would imply susceptibility to obesity. Given the importance of nutrition in modulating the intestinal environment and its relationship with obesity, the objective of this conference is to show how the management of intestinal microbiota can be used as a prevention or treatment method for obesity in the context of our country. At this conference, the results of our research group will be presented, which is focused on the study of the relationship between intestinal dysfunction, dysbiosis, and obesity, both in *in vitro*, *ex vivo*, and *in vivo* models using animal models of obesity/metabolic syndrome and with patients with overweight/obesity and type 1 diabetes. It is important to direct scientific research efforts in order to respond to priority public health problems such as obesity and its related diseases.

LECTURE:

A8
BIOREMEDIATION OF ENVIRONMENTS WITH MIXED CONTAMINATION: CHALLENGES AND ADVANCES

Polti MA[1,2], Aparicio JD[1,3], Saez JM[1,2], Benimeli CS[1,4]
[1]Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), S. M. de Tucumán, Argentina.
[2]Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán (UNT), Argentina.
[3]Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán (UNT), S. M. de Tucumán, Argentina. [4]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca (UNCA), Argentina.
E-mail:marta.polti@conicet.gov.ar

In the last decades, the growing industrial activities, rapid urbanization, highest consumption rates, and non-safe human practices have greatly increased soil pollution with different contaminants, being pesticides, heavy metals, hydrocarbons, chlorophenols, and polychlorinated biphenyls the most frequently found. Therefore, it is important to devise eco-friendly remediation technologies to restore the ecosystems affected by inappropriate anthropogenic action. Gentle Remediation Options such as bioaugmentation, phytoremediation, vermiremediation, and biostimulation have received considerable attention in recent years as effective risk-management strategies to reduce the transfer of contaminants to local receptors, through *in-situ* stabilization or extraction of pollutants. These treatments can provide a cost-effective, environmentally friendly solution to soil co-pollution and are increasingly employed in place of traditional remediation technologies. Each biological technology for soil remediation has certain limitations, and the simultaneous presence of inorganic and organic pollutants poses its own particular problems. These restrictions could be counteracted by a combination of technologies to remediate soil pollution, together with the recovery of soil health. Moreover, the selection of the appropriate remediation technique/s to effectively reduce contaminant concentrations to acceptable levels will depend on the costs, type and concentration of pollutants, edapho-climatic characteristics, and requirements of the soil. Principles, advantages, disadvantages, and applications of the main bioremediation technology employed for polluted soil will be discussed. Later, case studies will be presented, to evaluate the efficiency and safety of the bioremediation process of soil polluted with Cr(VI) and lindane. In this sense, it is essential to have tools of ecological relevance to assess the biological impact of pollutants on the environment. Bioassays to evaluate the effectiveness of a bioremediation process of co-contaminated soils were applied, using five model species: four plant species (*Lactuca sativa*, *Raphanus sativus*, *Lycopersicon esculentum*, and *Zea mays*) and one animal species (*Eisenia fetida*). The biomarkers showed different sensitivity levels. However, two key species, *L. esculentum* and *E. fetida*, were the most sensitive to evaluating the toxic impact of Cr(VI) and lindane. On the other hand, single and combined bioremediation strategies were evaluated: phytoremediation (*Brassica napus*), microbial remediation (actinobacteria consortium), phytoremediation (*E. fetida*), biostimulation. The combination of all strategies was the most successful treatment and would be a suitable strategy to reduce contamination and improve the health of soils co-polluted with hexavalent chromium and lindane.

A9
PRENATAL STRESS AND NEURODEVELOPMENT: SEARCHING FOR NOVEL MEDIATORS AND BIOMARKERS

Sánchez M, Nardocci G, Corvalán D, Méndez-Ruette M, Oyarce S, Monteiro L, Wyneken U, Tenorio M, Arango P, Godoy MI, Illanes S, Bátiz LF
Center for Biomedical Research and Innovation (CiiB), Universidad de los Andes, Santiago, Chile.
E-mail: lbatiz@uandes.cl

Maternal psychological distress during pregnancy, also known as prenatal stress (PS) increases the risk of poor neurodevelopmental outcomes in the offspring. However, the mechanisms and mediators by which maternal stress is communicated to the fetus remain poorly understood. In addition, there are no early biomarkers to predict neurodevelopmental outcomes in the context of PS. Our group is investigating a novel mother-to-fetus (placenta) communication pathway mediated by small extracellular vesicles (sEVs) that might regulate fetal neurodevelopment under PS conditions. sEVs are a heterogeneous population of membrane-bound vesicles of varying biogenesis, size, content, and bioactivity. sEVS are specifically packaged with a complex cargo of molecules of different types, comprising lipids, proteins, and RNAs; they are actively released into biofluid compartments, such as the bloodstream, and can regulate the physiology of distal target cells. Thus, sEVs represent a complex integral signaling pathway mediating intercellular communication. Moreover, most of the cargo of EVs is composed of products from the donor cell and is cell-type and cell-status specific. Thus, the content of EVs is considered a “fingerprint” of the releasing cell that reflects its physiological or pathophysiological status and, as such, sEVs have been proposed as useful biomarkers for different conditions and pathologies. We have developed a rat model of PS by repetitive movement restraint, and we are studying

the consequences on the fetal neurogenic process. In addition, we are investigating whether maternal sEVS can mediate these changes. These studies are being complemented by in vitro assays using primary cultures of neural stem/progenitor cells. On the other hand, we have studied the consequences of exposure to a high-magnitude earthquake and the COVID-19 pandemic on maternal psychological distress (depressive symptoms and perceived stress) during pregnancy and changes in neurodevelopmental outcomes in the offspring at different postnatal stages. Our results strongly suggest that sEVs can mediate stress-related signals and have the potential to be used as biomarkers of PS-induced neurodevelopmental changes. *[Acknowledges: Fondecyt 1211384 Grant (ANID, Chile)]*

A10
CARDIOVASCULAR RISK ASSESSMENT: ATHEROGENIC AND CARDIOPROTECTIVE LIPOPROTEINS

Brites FD
Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, UBA. CONICET.
E-mail: fdbrites@hotmail.com

Atherosclerotic cardiovascular disease (ACVD) is one of the leading causes of death worldwide, and the main strategy for primary prevention is the identification of asymptomatic individuals at high risk of developing this pathology. This is how risk assessment tables have been prepared, built using regression equations in population samples, which give value to the various known risk factors. Currently, we have the guidelines published by different American (2018 AHA/ACC Guideline on the Management of Blood Cholesterol) and European (2019 ESC/EAS Guidelines for the management of dyslipidemias: Lipid modification to reduce cardiovascular risk) organizations, as well as with the recommendations of the Lipid National Association (2021). In turn, there are specific recommendations regarding the parameters that must be evaluated to validate a cardiac marker. Among the criteria to be evaluated, it is expected that they have clinical utility, that they discriminate between people who have the disease of interest and those who do not, and that they help to reclassify subjects, especially those at intermediate risk. The results, for their part, must be easy to interpret and use in a primary prevention care system, must be internationally standardized, and involve reasonable costs. In the literature, mention is made of a wide variety of ASCVD risk factors and biomarkers, for many of which there are commercial kits. These include the lipid and lipoprotein basic profile, with LDL-C being the primary target for treatment, apo A-I and B, Lp(a), lipoprotein remnants, high-sensitivity C-reactive protein, interleukins 1ß and 6, lipoprotein-associated phospholipase A_2, metalloproteases, endothelial adhesion molecules, etc. However, few of them meet the aforementioned requirements, which highlights the need for a more in-depth analysis regarding the specific clinical utility of each one and the limitations of their measurement methods. In particular, the determination or estimation of LDL-C deserves particular attention due to its clinical relevance and the limitations of both the chemical methods used for its measurement, as well as the equations proposed for its calculation (Friedewald, Martin-Hopkins, and Sampson formulas). On the other hand, the aforementioned parameters, to a greater or lesser extent, have pro-atherogenic and/or proinflammatory properties, being in opposition to HDL, the only lipoproteins with antiatherogenic functions. In recent years, some controversy has arisen regarding its cardioprotective role, based, among other reasons, on evidence showing that the relationship between HDL-C levels and ECVA follows a U-shape, thus showing that not only low values but also very high ones are associated with a high rate of cardiovascular events. However, the aforementioned controversy could be related to the parameter used to evaluate HDL (HDL-C) and with alterations in the ability of HDL to uptake free cholesterol from lipolysis of triglyceride-rich lipoproteins, a recently developed hypothesis. Finally, a crucial aspect deals with the conditions that patients must present to undergo a lipid study, among which fasting must be considered. These conditions are decisive for the reliability and clinical value of the risk factors and biomarkers used in the estimation of the risk of developing ACVD.

SYMPOSIUM III: INVITED BIOLOGY SOCIETES FROM ARGENTINA. ONE HEALTH: A BETTER HEALTH FOR PEOPLE, ANIMALS AND OUR PLANET

A11
AIR POLLUTION AND UNDERNUTRITION: TWO SYNERGISTIC RISK FACTORS ON CARDIOVASCULAR AND PULMONARY HEALTH.

Kurtz ML
Laboratorio de Bio-Toxicología Ambiental, Instituto de Tecnologías Emergentes y Ciencias Aplicadas, ECyT, UNSAM – CONICET. E-mail: mkurtz@unsam.edu.ar

Children encompass a highly susceptible subpopulation to the adverse effect of environmental pollutants and malnutrition. Air pollution (gases and particulate matter-PM) has a negative impact on the lung, inducing oxidative stress and pro-inflammatory processes. Systemic translocation of PM and/or oxidative/inflammatory mediators resulting from the interaction PM–lung cells can also cause adverse biological effects on remote organs. Therefore, we investigated the effects of ROFA (Residual Oil Fly Ash, a surrogate of air PM) exposure on the lung, heart, and vasculature, in a nutritional growth retardation (NGR) rat model *in vivo* and *ex vivo*. In order to achieve NGR animals, male weanling rats were fed a restricted 20% diet compared to *ad libitum* intake (control-C) for 4 weeks. NGR and C rats were intranasally instilled with either 1 mg/kg BW of ROFA or its vehicle. Alveolar macrophages (AM) were isolated and cultured 24 h post-exposure and cell viability, antioxidant response, and pro-inflammatory cytokine release were evaluated. Furthermore, histological and biochemical parameters such as oxidative metabolism and inflammation were assayed on lung, heart, and thoracic aorta tissues obtained from both animal groups. The aorta contractile function and vascular biomarkers were also analyzed. Cultured AM from NGR rats exposed to ROFA show diminished antioxidant response (Nrf2) and inflammatory mediators' production (TNF-α and IL-6). Histopathologically ROFA induced changes in the lung and was able to cause a rise in reactive oxygen species, always higher in C than in NGR animals. Even though ROFA exposure altered heart oxidative metabolism in NGR animals leading to lipid oxidative damage, no histological or biochemical changes were observed. Following ROFA exposure, the contractile capacity of the aorta declined and worsened in NGR animals. In addition, NGR rats presented a reduction in eNOS and the L-type calcium channel levels, proteins involved in the regulation of the vascular tone. In summary, our *in vivo* and *ex vivo* studies showed that undernutrition affects lung immune responsiveness to air pollutants and cardiovascular homeostasis. Therefore, we hypothesized that the undernourished children subpopulation, in scenarios of environmental pollution, would have a higher risk of cardiovascular diseases.

A12
ANIMAL HEALTH PROMOTES HUMAN HEALTH IN A FRIENDLY ENVIRONMENT

Vintiñi EO
Facultad de Agronomía y Zootecnia - LARIVENOA – UNT. E-mail: eovintini14@yahoo.com.ar

The close relationship between animal and human health is defined in One Health, which is an approximation to the understanding that there is an interconnection between humans, animals, plants, and the environment. Animal production cannot be considered in isolation from the environment. Regular evaluation is necessary to achieve an ecologically friendly use of the available resources and therefore it is essential to apply a holistic approach to prevent infectious and zoonotic diseases. In this sense, it is a priority to respect ecosystems, biodiversity, animal health, and human health in order to create a balance and safeguard the health of the planet. There are biological and chemical threats that affect our health by affecting the environment and highlight the problems caused by climate change to crops and animals, which has a direct impact on human health through the appearance of emerging infectious diseases. According to the United Nations, the world population will reach 9.6 billion in 2050, which will lead to an increase in the demand for food and, as a consequence, there will be a significant increase in the number of production animals to guarantee supply. In this respect, Animal Health will be a critical element in preventing zoonotic diseases, thus ensuring human health and safety, as 60% of human infectious diseases are of animal origin. Animal health is an essential component of animal welfare, which shows how the conditions of the environment in which animals are kept directly influence their physiology, behavior, and affective states. This is why animal welfare within a friendly environment in a production chain is considered a fundamental part of a successful sustainable system. The identification by molecular techniques of pathogens that influence the health of livestock production animals such as *S. gallinarum*, *S. pullorum*, *S. enteritidis*, *S. typhimurium*, *E. coli*, *Clostridium dificile*, *B. abortus*, *Mycobacterium bovis*, *S. suis*, *S. aureus*, *S. agalactiae*, *S. uberis*, and *S. dysgalactiae*, which can have serious consequences for human health, represent a valuable strategy to implement surveillance and control of these infectious agents and facilitate efficient decision making to apply holistic methodologies for zoonotic diseases. These bacteria are involved in outbreaks of foodborne diseases (FBD), through the consumption

of pork, chicken, partially cooked eggs, or milk and dairy products. In the case of brucellosis and tuberculosis, both zoonoses, are found in our country within the National Control and Eradication Plan. The work must be responsible and committed, integrating specialists from different fields, with a real political commitment at local, regional, national, and international levels. The goal is to achieve a more environmentally friendly production, taking care of animal welfare and natural resources; to highlight that Animal Health promotes human health in a friendly environment because we all share the same world, we all share the same health.

A13
BIOTECHNOLOGICAL STRATEGIES APPLIED TO ANIMAL PRODUCTION AND HEALTH

Liaudat AC[1], Capella V[1], Bonino R[1], Sosa E[1], Sommaro A[1], Gonzalez MA[1], Opizzo BA[1], Blois D[1], Morilla G[1], Fili A[1], Barbero C[2], Rivarola C[2], Bosch P[1], Rodriguez N[1]
[1]Instituto de Biotecnología Ambiental y Salud (INBIAS). [2]Instituto en Tecnologías Energéticas y Materiales Avanzados (IITEMA) Facultad de Ciencias Exactas, Físico Químicas y Naturales, Universidad Nacional de Río Cuarto–Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). E-mail: aliaudat@exa.unrc.edu.ar

Tissue engineering is an emerging interdisciplinary field which purpose is to replace or regenerate a damaged organ through the use of biomaterials. Poly-N-isopropylacrylamide (PNIPAM) hydrogels are synthetic polymeric materials with innate chemical and mechanical similarity to the extracellular matrix. Due to this, these scaffolds are being studied to be applied in tissue regeneration to support the growth of different cell types. Studies carried out in our laboratory evaluated the effects that PNIPAM and PNIPAM copolymers produce on immunological, fibroblastic, renal, and pulmonary cell biocompatibility. The results showed that hydrogels are biocompatible with all cell lines analyzed without activating an inflammatory immune response. In addition, PNIPAM hydrogels were used as sperm selection surfaces for livestock species, where it was possible to obtain a bovine and swine sperm selection system with high percentages of viability and motility. A new research area showed in preliminary results that the addition of calcitriol to sperm medium acts as a natural capacitation agent. The analysis of the biological characterization of bovine oocytes from slaughterhouses is also an important step to define its use in assisted reproduction techniques.

LECTURE:

A14
PUBLIC PERCEPTION AND COMMUNICATION OF SCIENCE AND TECHNOLOGY IN ARGENTINA: THE ROLE OF UNIVERSITIES AND RESEARCH CENTERS

Cortassa CG
Facultad de Ciencias de la Educación. Universidad Nacional de Entre Ríos. E-mail: carina.cortassa@uner.edu.ar

Long after it happened in other contexts, the public perception and communication of science and technology (S&T) have become topics of increasing political and institutional prominence in Argentina during the last two decades. Three milestones of this ongoing process are addressed in this talking: firstly, the inclusion of the issue in the agenda of S&T public policies; secondly, the role the scientific communities and institutions are expected to play towards the promotion of general scientific culture; finally, the evolution of some trends related with the people's knowledge, interests and attitudes over the course of the five National Surveys undertaken in our country since 2003. Policies devoted to improving scientific literacy were consolidated in developed countries during the second half of the 20th century, as a means of highlighting governmental efforts in S&T in order to ensure civil society's support for its expansion. This original purpose has been significantly broadened during recent decades to foster new goals. Among others, these include democratizing access to knowledge, stimulating the development of an innovative culture, promoting scientific vocations, and extending public participation in controversial issues. In Argentina, the earliest initiatives in this sense were the launch of the National Science Week and of the first Public Perception of Science National Survey, both in 2003. With the creation of the Ministry of Science and Technology, in 2007, the issue acquired a progressively higher profile, not only at the level of concrete actions but also in the frame of the latest National Plans of S&T –*Argentina 2020* and the brand new *Argentina 2030*–. On its part, local scientific communities and research institutions are following a slow but steady path into what has been called 'the communicative turn' in S&T organizations. This shift, which began to emerge in the 1990 decade and tended to widen and deepen since then, results from two different grounds: on the one hand, scientists and institutions are compelled to enhance their strategies of public communication due to intrinsic interests –the competition for credit, reputation and public prominence which, in turn, lead to ensuring the flow of resources–; on the other hand, they must do so to accomplish the requests of public policies and supporting agencies, which strongly encourages a more active

commitment on their part with the strengthening of the relationships between science and society. Even though some positive advances in this direction have been achieved during the last years, in fact, the communicative turn remains a major challenge for national scientific organizations –a task that needs to be tackled in a systematic, comprehensive, and sustained manner. Only in this way, Universities and research centers will be in a position to perform their duties in the expansion and consolidation of a critical scientific culture among the Argentinean population.

CLOSING LECTURE:

A15
EXTRACELLULAR VESICLES IN REGENERATIVE MEDICINE

Ezquer M
Facultad De Medicina, Instituto De Ciencias. Universidad Del Desarrollo. Santiago, Chile.

GENERAL, CELLULAR AND MOLECULAR BIOLOGY

A16
CLOCK'S DIFFERENTIAL TRANSCRIPTIONAL CONTROL ON OGG1 AND APE1 CIRCADIAN EXPRESSION

Castro Pascual IC[1], das Neves Oliveira A[2], Melendez M[2], Cargnelutti E[1,3], Altamirano FG[1], Ferramola ML[3], Lacoste MG[1], Delgado M[3], Anzulovich AC[1,3]
[1]Laboratorio de Cronobiología, IMIBIO-SL, CONICET-UNSL, San Luis, Argentina. [2]Centro de Pesquisa em Oncologia Molecular, Hospital de Câncer de Barretos, São Paulo, Brasil. [3]Facultad de Química, Bioquímica y Farmacia, UNSL, San Luis, Argentina. E-mail: *ivannacastropascual@gmail.com*

The circadian clock integrates external environmental changes with internal physiology. Different studies have described a role of clock molecular machinery in the regulation of DNA repair mechanisms. In this sense, other authors reported a clock-controlled modulation of DNA nucleotide excision repair. Accordingly, we previously reported evidence of circadian rhythmicity in the expression of genes involved in the DNA base excision repair (BER) system in 22-month-old rats. BER is a key mechanism to avoid oxidative and alkylative DNA damage, which predisposes to different diseases such as cancer or neurodegenerative disorders. Our objective was to elucidate the molecular mechanisms involved in the control of the circadian expression of the enzymes involved in the BER system. Through *in vitro* transient transfection studies in NIH-3T3 cells, we assayed the response of the regulatory regions of *Ogg1* and *Ape1* genes to de BMAL1:CLOCK heterodimer. Previously, our bioinformatics studies revealed 13 E-box-like (CANNTG) and 5 perfect (CACGTG) E-box sites in regulatory regions of *Ogg1* and *Ape1*, respectively. Subsequently, the bioluminescence assays showed that the BMAL1:CLOCK heterodimer exerted a differential regulation, activating the *Luc* expression driven by the regulatory region of *Ogg1* ($P < 0.001$), and repressing the *Luc* expression driven by the regulatory region of *Ape1* ($P < 0.01$). The ability to anticipate and repair cyclical DNA damage is essential for the protective functions of tissues, especially during aging; thus, our results would contribute to the growing evidence that circadian clocks may regulate the cellular response to DNA damage.

A17
NOREPINEPHRINE MODULATES DAILY CLOCK EXPRESSION IN *EX VIVO* SPLENIC MACROPHAGES

Cargnelutti E[1,2], Anzulovich AC[1,2]
[1]Laboratorio de Cronobiología (LABCRON), IMIBIO-SL-Universidad Nacional de San Luis-CONICET. [2]Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. E-mail: ecargnel@unsl.edu.ar

The time of day is critical to define the nature of the immune response since dysregulation of this mechanism can lead to inflammatory diseases or immunodeficiencies. In mammals, the central clock in the suprachiasmatic nucleus (SCN)

synchronizes cell-autonomous clocks to the sunlight. The splenic macrophages (MΦ) phagocytize and eliminate circulating pathogens and orchestrate the development of the specific acquired immune response. However, the central circadian regulation of these splenic cells has not been completely elucidated yet. Communication between SCN and spleen occurs by the sympathetic nervous system (SNS), through nerves that release norepinephrine (NE) in areas of MΦ cells. Previously, other authors reported daily oscillation of NE in the spleen. In order to study the role of NE in the regulation of the molecular clock of spleen MΦ, we have developed a rat model of local sympathetic denervation by guanethidine administration. Animals were maintained under 12 h-light:12 h-dark conditions and *ad libitum* food/water intake until the experiment. To analyze the NE temporal impact on molecular clock of splenic MΦ, ten days after injection of saline solution or guanethidine, control (N = 4/ZT) and sympathectomized rats (N = 3/ZT) were euthanized at different times during a 24-h period (ZT2, ZT6, ZT10, ZT14, ZT18, and ZT22), and the spleen was aseptically removed for *ex vivo* cultures. The BMAL1 and ACTIN protein levels were analyzed by Western blot from splenic adherent cells. Time-point data were computed by one-way analysis of variance (ANOVA) and followed by the Tukey post hoc test. Further, chronobiologic statistics were used for validating temporal changes as rhythms. Thus, each series of data were analyzed by the Cosinor method. Since BMAL-1 modulates some MΦ's functions through the direct control of *Rev-Erb α*, which in turn represses *Bmal-1* expression through the accessory loop of the molecular clock, the relative quantification of this gene was evaluated by q-PCR, using *s28* as reference gene. In this case, cDNA was obtained from *ex vivo* splenic adherent cells cultivated from control and sympathectomized rats, at ZT6, ZT14, and ZT 18. The Student's *t*-test was used for the comparison of data between both groups. The splenic MΦ from control rats showed a daily oscillation of BMAL1 (% rhythm: 71.8), with its acrophase occurring in the middle of the light period. Noteworthy, the *ex vivo* splenic MΦ from guanethidine-treated animals lost the 24-h oscillation of BMAL1 and showed significantly lower levels of this clock factor compared to the control. On the order hand, sympathectomized rats show a significantly higher *Rev-Erbα* expression, at the three analyzed ZTs ($P > 0.05$), compared to the control group. Our results would indicate that exists a SCN regulation on the molecular clock in splenic adherent cells through the NE sympathetic pathway.

A18
DAILY PATTERNS OF CLOCK NEGATIVE FACTORS ARE MODIFIED IN THE HIPPOCAMPUS OF Aβ-INJECTED RAT

Cortez M, Castro A, Cangiano B, Sanchez M, Alba M, Anzulovich AC, Gomez-Mejiba S, Navigatore Fonzo L
Laboratorio de Cronobiología, IMIBIO-SL, CONICET-UNSL, SL. E-mail: lorenavigfz@yahoo.com.ar.

Circadian disruption is prevalent in Alzheimer's disease (AD) and may contribute to cognitive impairment. At the molecular level, cellular oscillators consist of a network of interlocking transcriptional–translational feedback loops. The positive limb of the loop is represented by the transcription factors CLOCK and BMAL1, which heterodimerize and bind to E-box sites in the promoters of the clock negative factors, period (PER1-3), and cryptochrome (CRY1-2) genes. A negative feedback loop is achieved when the PERs and CRYs form heterocomplexes that translocate back to the nucleus and inhibit their own and other clock-controlled genes' transcription. RevErbα and RORα transcription factors, members of the retinoic acid-related orphan receptor (ROR) family, complete the molecular clock machinery. Previously, we found that an intracerebroventricular (i.c.v.) injection of Aβ(1-42) modified the daily rhythms of BMAL1 and RORα expression and cognition-related factors in the rat hippocampus. Taking into account those observations, the objective of this work was to investigate the effects of an i.c.v. injection of amyloid beta peptide (1-42) on daily rhythms of PER1, PER2, CRY1, and CRY2 expression, as well as Aβ protein levels, in the rat hippocampus. In this study, Holtzman male rats from control and Aβ-injected groups were sacrificed throughout a 24-h period, and hippocampus samples were isolated every 6 h. Daily rhythms of clock genes expression were analyzed by RT-PCR, and Aβ protein levels were analyzed by immunoblotting. Regulatory regions of clock PER1, PER2, CRY1, and CRY2 genes were scanned for E-box and RORE sites. We found that clock genes expression and Aβ levels displayed daily oscillations in the rat hippocampus. We found E-box and RORE sites on regulatory regions of clock genes. An i.c.v. injection of Aβ(1-42) modified daily rhythms of clock genes expression and Aβ levels. Therefore, elevated levels of Aβ peptides could modify the temporal patterns of clock negative factors and consequently could affect the transcription of genes related to cognition in AD.

A19
EFFECT OF AN I.C.V. INJECTION OF AGGREGATED BETA-AMYLOID (1-42) ON DAILY PROFILES OF Aβ-DEGRADING ENZYMES IN THE RAT PREFRONTAL CORTEX

Cangiano B, Cortez M, Sanchez M, Alba M, Anzulovich AC, Delgado M, Navigatore Fonzo L
Laboratorio de Cronobiología, IMIBIO-SL, CONICET-UNSL, SL. E-mail: lorenavigfz@yahoo.com.ar.

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the most common form of irreversible dementia, among the elderly. The accumulation of amyloid-β (Aβ) peptides in the brain of Alzheimer's disease patients is associated with cognitive deficit, loss of memory, and alterations in the circadian rhythms. Enzymes with Aβ-degrading activity include members of the zinc metalloendopeptidase family. Among them, the ones with the most physiological relevance

in the brain are endothelin-converting enzyme (ECE) and insulin-degrading enzyme (IDE). Brain-derived neurotrophic factor (BDNF) and its receptor (TrkB) play an important role in the synaptic plasticity underlying memory and learning. Previously, we observed Aβ and BMAL1 expression follow a daily rhythmic pattern in the prefrontal cortex of Aβ-injected rats. The objective of this work was to investigate the effects of an i.c.v. injection of aggregated beta-amyloid (1-42) on daily patterns of ECE and IDE expression, as well as on oscillating BDNF and TrkB mRNA levels, throughout a 24 h period, in the rat prefrontal cortex. Four-month-old male Holtzman rats were divided into two groups defined as control (CO) and Aβ-injected (Aβ) groups. Tissue samples were isolated every 6 h for 24 h. IDE, ECE, BDNF, and TrkB mRNA levels were determined by RT-PCR. We found that expression of Aβ-degrading enzymes and cognition-related factors varies on a daily basis in the prefrontal cortex and that an i.c.v. injection of Aβ aggregates modified these daily rhythms. Therefore, elevated levels of Aβ peptides could modify the temporal patterns of Aβ-degrading enzymes and consequently could affect the transcriptional activity of the endogenous cellular clock.

A20
EFFECT OF PTP1B PHOSPHATASE INHIBITION IN HER2-POSITIVE BREAST CANCER CELL LINES

Cordoba ME[1], Nadin SB[2], Cuello-Carrión FD[1], Fanelli MA[1]
[1]Laboratorio de Oncología, IMBECU-CCT-CONICET-Mendoza, UNCuyo. [2]Laboratorio de Biología Tumoral, IMBECU-CCT-CONICET-Mendoza, UNCuyo. E-mail: mcordoba@mendoza-conicet.gob.ar

The interference of the HER2 signaling pathway through monoclonal antibodies and tyrosine-kinase inhibitors constitutes a strategy in the treatment of HER2-positive (HER2+) breast cancer (BC). Our previous studies showed that HER2+ tumors with plasma membrane-associated β-catenin expression and without monoclonal antibody therapy (Trastuzumab, Tzb) had significantly better overall survival. The protein tyrosine phosphatase PTP1B plays an important role in BC and is also required for HER2/Neu-driven BC in mice. PTP1B binds to β-catenin keeping it located in the plasma membrane. We observed by copy number variation (CNV) analysis of β-catenin (CTNNB1) and PTP1B (PTPN1) from HER2 enriched BC patients using the TCGA database, that PTPN1 tended to be amplified in HER2+ patients while CTNNB1was downregulated. Also, these genetic changes were associated with PTPN1 expression levels. This study aimed to evaluate PTP1B role in cell migration, and spheroid growth in SKBR3 and MCF7 HER2+ cell lines treated with Tzb, heregulin (Hrg growth factor), and α-bromo-4-hydroxyacetophenone (PTP1B inhibitor). Cell migration was evaluated by wound healing assay and cell growth by 3D cultured spheroids. Concerning viability, SKBR3 cells overexpressing HER2 were more sensitive to Tzb than MCF7 cells in 2D cultures. For MCF7, the viability of 2D and 3D cultures with Tzb were similar while SKBR3 3D culture was more resistant than 2D culture. In SKBR3 cells, Hrg treatment promoted 3D culture growth and migration, while Tzb and the PTP1B inhibitor stopped the spheroids' growth and decreased cell migration. On the contrary, in MCF7 cells, none of the treatments affected spheroids' growth and cell migration. Cell growth arrest and decreased cell migration in SKBR3 cells by inhibition of PTP1B and blocking of the HER2 signaling pathway were observed. We conclude that PTP1B can be an interesting molecular target for HER2+ human breast cancer.

A21
HSP27 DOWNREGULATION THROUGH THE ATR/CHK1 PATHWAY INCREASES CISPLATIN SENSITIVITY IN HUMAN COLON CANCER CELLS

Cayado-Gutiérrez N[1], Redondo A[2], Cuello-Carrión FD[1], Lossino A[3], Fanelli MA[1], Vargas Roig LM[2], Nadin SB[2]
[1]Laboratorio de Oncología, IMBECU-CCT-CONICET-Mendoza, UNCuyo. [2]Laboratorio de Biología Tumoral, IMBECU-CCT-CONICET-Mendoza, UNCuyo. [3]Laboratorio de Bioquímica e Inmunidad, IMBECU-CCT-CONICET-Mendoza, UNCuyo. E-mail: snadin@mendoza-conicet.gob.ar

Hsp27 is a molecular chaperone with a widely described anti-apoptotic role. Hsp27 is overexpressed in many types of cancer and has been related to cisplatin (cPt) resistance. The heat shock proteins have also been implicated in DNA repair pathways. Previously, we reported that Hsp27 interacts with DNA mismatch repair (MMR) proteins in colorectal cancer cells. cPt has been reported to induce DNA damage response (DDR) through activation of the ATR/CHK1 pathway, whose function is to regulate cell cycle progression, to promote DNA repair and apoptosis. Accordingly, Hsp27 has become an attractive therapeutic target. This study aims to investigate Hsp27 and ATR/CHK1 pathway relationship in cPt-exposed human colon cancer cell lines: HCT116+ch2 (MMR deficient, MMR−) and HCT116+ch3 (MMR proficient, MMR+). Hsp27 was downregulated with OGX427 before cPt treatment and ATR was inhibited by VE-821 (VE). Cells were incubated with 10 μM cPt for 24 h and collected at time 0 (immediately after cPt treatment, T0), 3 (T3), 9 (T9), and 24 (T24) h post-treatments. DNA damage was evaluated by comet assay and the expression of Hsp27, pHsp27 (Ser78), pCHK1 (Ser345), γH2AX (Ser139), using western blot. Hsp27 nuclear colocalization with CHK1 was demonstrated in cPt-treated MMR−/+ cells. Combined therapy with cPt+OGX427 or cPt+VE reduced cell viability (CCK8), particularly after cPt+VE in MMR− cells ($P < 0.01$). High pHsp27 levels were detected in MMR− cells at T3. Interestingly, cPt+VE augmented γH2AX expression in both cell lines (T0), but it decreased during recovery ($P < 0.01$). Cleaved PARP1 and

activated caspase-3 were upregulated by cPt+VE, particularly in MMR+ cells, showing elevated DNA damage ($P < 0.01$). Conversely, DNA damage decreased ($P < 0.05$) and senescence was induced (T9, $P < 0.001$) especially in MMR− cells. Hsp27 modulation through VE combined with cPt could be a promising tool to improve cPt-chemosensitivity in MMR− tumors.

A22
APPLICATION AND MODIFICATION OF THE COMET ASSAY TO EVALUATE GENOTOXIC EFFECTS IN NATIVE FISH (*Cnesterodon decemmaculatus* AND *Jenynsia multidentata*)

Giménez Santobuono S[1], Pérez-Iglesias JM[1], Cortez A[2], Jorquera MA[2], Gonzalez P[1], Almeida CA[1]
[1]Laboratorio de Química Analítica Ambiental (LAQAA) – INQUISAL – UNSL. [2]Facultad de Química, Bioquímica y Farmacia. UNSL. E-mail: santobuonosol@gmail.com

The comet assay is a widely used test to detect *in vitro* or *in vivo* DNA damage caused by genotoxic agents in single cells. It is characterized by being a sensitive, fast, simple, low-cost method applicable to various types of eukaryotic cells. This technique described allows the magnitude of the damage to be analyzed according to the number of affected cells, the length of the tail, and the intensity of the fluorescence of the fragments. The objective of the following work was to fine-tune the kite assay methodology to be used in native bioindicator fish, making small modifications with respect to the original protocols in order to adjust them to each local species. In addition to this, the methodology was compared in two autochthonous species, *Cnesterodon decemmaculatus* (whose kite assay methodology is standardized) and *Jenynsia multidentata* (methodology fine-tuned). The methodology consisted of the extraction of blood from 10 individuals of each species. The samples were centrifuged to then prepare three layers of 1% agarose, with the 2nd layer containing 10 µL of sample, at this point modifications were made by diluting agarose in water or phosphate buffer. Then the lysis period was carried out and we modified the times between 1 and 24 h. Subsequently, the unwinding of the DNA and the electrophoresis run were carried out where the times were adjusted according to the species. Finally, as a last modification, different concentrations of DAPI were placed to make effective and reduce operating costs. The results were expressed as a percentage of the analyzed nucleoid category (type 0 and I, type II, type III, and type IV), as the mean number of damaged cells (sum of classes II, III, and IV), and the Genetic Damage Index (GDI) for each treatment carried out following the recommendations. The results were analyzed with a *t*-test for comparison between species and one-way ANOVA for comparison between cells. The same showed that the modifications in both species allow the correct visualization of the nucleoids. On the other hand, approximately 85% of healthy cells were observed for basal genetic damage in both species ($P < 0.05$) and no differences were observed in the modifications of the technique regarding the percentage of healthy/damaged cells ($P = 0.05$). In addition, the modifications were effective in demonstrating their application and utility for bioindicator species in the region, which suggests that this tool is important to apply in environmental biomonitoring. We emphasize that these studies provide important information to know the basal state of genetic damage in local species and protocolize this novel biomarker.

A23
EFFECT OF QUERCETIN ON THE LYSOSOME-DEPENDENT CELL DEATH PROCESS IN BREAST CANCER CELLS

Pereyra L[1], Peralta S[1], Vargas Roig L[2], Sosa MA[1,3], Carvelli L[1,3]
[1]IHEM-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina. [2]IMBECU-CONICET-Universidad Nacional de Cuyo, Mendoza, Argentina. [3]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina.E-mail: lorecarvelli@gmail.com

Breast cancer is the leading cause of death in women worldwide. Some tumor cells have shown increased lysosomal biogenesis, together with altered lysosomal integrity and/or functionality. Alterations in the lysosomal membrane permeability induce a release of proteases such as cathepsin D (CatD) into the cytoplasm, triggering apoptotic processes (lysosome-dependent cell death). Quercetin (Que) is a flavonoid highly used as an antioxidant, although it is known that, in some types of tumors, it has pro-oxidative effects. In breast cancer cells, Que induces cell death and upregulates lysosomal biogenesis, although its mechanism of action is still unclear. The aim of this study was to evaluate the effect of Que on the lysosome-dependent cell death process in MCF-7 human mammary tumor cells. Cell cultures incubated with Que (40 µM and 200 µM) for 8 and 24 h were processed for fluorescence microscopy and subcellular fractionation, followed by immunoblotting. In MCF-7 cells, Que treatments induced an apparent increase in the size of lysosomal compartments (labeled with LysoTracker™ Red DND-99) compared to untreated control cells. In turn, the effect of Que on lysosomal membrane permeability was evaluated by studying the CatD leakage to the cytoplasm. Immunoblots revealed that Que did not produce significant changes in the presence of CatD in the cytoplasmic fraction. Our results would indicate that Que does not trigger apoptosis through lysosomal membrane rupture in breast cancer cells, although other mechanisms involving lysosomes should not be ruled out.

A24
THE NEUROPROTECTIVE ROLE OF ESTROGEN IN A RAT MODEL OF PARKINSON'S DISEASE

*Meier G *[1,2], Bonaccorso MP*[,3], Carvelli L[2,4], Sosa MA[1,2,4], Cabrera R[3], Leiva N [1,2]*
*[1]Laboratorio de Histología e Embriología. Facultad de Ciencias Médicas-CONICET-Universidad Nacional de Cuyo. [2]Facultad de Ciencias Exactas y Naturales-Universidad Nacional de Cuyo. [3]IMBECU-CONICET-Universidad de Mendoza. [4]IHEM-CONICET- Universidad Nacional de Cuyo. Mendoza. Argentina. *Equal contribution.*
E-mail: nleiva@fcen.uncu.edu.ar

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra compacta and an intracellular accumulation of the protein α-synuclein, a cytosolic and presynaptic protein. A genetic study identified twenty-four loci associated with PD, one of which was related to autophagic-lysosomal pathways. Lysosomes participate in the degradation of macromolecules from endocytic processes. Epidemiological and clinical studies reveal a difference in the development of PD between genders, giving sex hormones a neuroprotective function and turning them into an interesting therapeutic proposal. The objective of this study was to determine the effect of estrogens on the expression of lysosomal proteins in rats with the PD phenotype. Two-month-old male Sprague-Dawley rats underwent stereotaxic surgery to deliver 6-hydroxydopamine (6-OHDA) or artificial cerebrospinal fluid (V) into the left striatum. After 7 days, they received chronic treatment for 10 days with 17-β-Estradiol (E) or V. The groups were made up of C (lesion V); E (lesion V + E); HP (6-OHDA lesion) and HPE (6-OHDA + E lesion). After the treatments, the animals were sacrificed and the left and right brain regions were extracted: substantia nigra, prefrontal cortex, and striatum. Samples were processed for immunoblotting using anti-cathepsin D (CatD) and anti-actin. Preliminary results show that chronic estrogen treatment in parkinsonian rats increases lysosomal enzyme CatD and actin expression in the substantia nigra, prefrontal cortex, and left striatum. Since CatD reduces the concentration of α-synuclein protein in PD, our results suggest that in animals with PD, estrogen exerts a neuroprotective effect through an increase in its lysosomal function. In turn, estrogens could also modulate the organization of the cytoskeleton, as a neuromodulation stage in these brain regions.

A25
THE ANTIPROLIFERATIVE ACTIVITY OF YERBA MATE EXTRACT IN PC3 AND MDA-MB-231 HUMAN CANCER CELLS

Santiano FE[1], Fernandez MA[2], Espino M[2], Ginevro P[1], Rabino D, Silva MF[2], Carón RW[1], López Fontana CM[1]
[1]Laboratorio de Hormonas y Biología del Cáncer, Instituto de Medicina y Biología Experimental de Cuyo (IMBECU), Universidad Nacional de Cuyo (UNCuyo), Consejo Nacional Investigaciones Científicas y Técnicas (CONICET), Mendoza, Argentina. [2]Instituto de Biología Agrícola de Mendoza (IBAM-CONICET), Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina. E-mail: fsantiano@mendoza-conicet.gov.ar

Yerba mate (*Ilex paraguariensis* -YM-) contains bioactive compounds that confer numerous benefits to human health and may delay tumor development. Traditionally, the extraction of plant metabolites is carried out with solvents that have negative effects on people's health and the environment. The extraction of natural compounds by natural deep eutectic solvents (NADES) is an innovative, natural method with high biodegradability, sustainability, the ability to solubilize and stabilize compounds of different polarity, and low or no toxicity. Therefore, the use of NADES becomes a priority to achieve safer extracts and enhance their bioactive properties. Our objective was to develop an extract rich in bioactive compounds with antitumor properties from YM using NADES and compare it with an aqueous extract. For this purpose, the extractive capacity of citric acid, glycerol, and water (CGH) NADES was tested and compared with aqueous extraction by HPLC-UV. In addition, we analyzed the direct effects of both extracts of YM on the proliferation and viability of human prostatic tumor epithelial cells (PC3) and human mammary tumor epithelial cells (MDA-MB-231). Both cell lines are not hormone-dependent and have high metastatic potential. The NADES had a greater performance in the extraction of theobromine, caffeine, rutin, chlorogenic, and caffeic acid, being chlorogenic acid and caffeine than the aqueous extract. When we compared the effect of the aqueous extract *vs.* CGH extract on the PC3 cell line, we observed that both reduced cell proliferation from a concentration of 1.87 *vs.* 0.39 mg/mL and viability from 15 *vs.* 3.12 mg/mL. In MDA-MB-231 cells, both extracts reduced proliferation and viability from low concentrations of 0.23 *vs.* 0.19 mg/mL. We can conclude that YM can delay prostatic and mammary carcinogenesis by reducing the viability and proliferation of the studied cells. Also, higher concentrations of the aqueous extract are needed to obtain similar biological effects to the NADES extract. The identification of an extract of natural origin that potentiates the benefits of YM could be beneficial for the treatment and/or prevention of prostate and mammary tumor development.

BIOCELL 47, Suppl. 4, 2023
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A26
T4 INDUCES THE PROLIFERATION OF HORMONE-SENSITIVE MAMMARY TUMOR CELLS

Cano R[1], Ferrando M[1], Zyla L[1], Bruna F[1], Santiano F[1], Pistone Creydt V[1], López Fontana C[1], Carón RW[1]
[1]Instituto de Medicina y Biología Experimental de Cuyo, Universidad Nacional de Cuyo, CONICET.
E-mail: rcano@mendoza-conicet.gob.ar

Each year, approximately 17,000 women are diagnosed with breast cancer (BCa) and 5,400 women die from this cause in Argentina. Mendoza, which has a slightly higher incidence of BCa than the national average, is considered an endemic goiter area. There is controversy about the relationship between thyroid disorders and BCa risk, and little molecular data based on *in vitro* studies is available. Hypothyroidism seems to be a protective factor against BCa but long-term exposure or overdoses of thyroid replacement therapy with thyroxine (T4) may increase BCa risk. In previous studies, we observed that hypothyroidism prolonged the latency of tumor appearance, reduced tumor incidence, and retarded tumor growth in rats. In addition, we demonstrated that T4 regulated mammary carcinogenesis by interacting with other hormone pathways. In the present study, we analyzed the biological activity of T4, alone or combined with other steroid hormones, on the proliferation, viability, and adhesion of human BCa cell lines. MCF-7 (REα+, REβ+, PgR+, TRβ1+) and MDA-MB-231 (REα−, REβ−, PgR−, TRβ1−) were treated with 10^{-9} M of T4; 17β estradiol (E2) and/or progesterone (P4) in DMEM/F12 with 1% of charcoal fetal bovine serum (FBSc) or DMEM/F12 with 1% FBSc as control. We evaluated proliferation and adhesion by MTT and viability by trypan blue. Statistical analysis was performed using ANOVA I and Bonferroni as post-test ($P < 0.05$). T4 induced cell proliferation of MCF7 compared to control after 24 and 48 h treatment ($P = 0.037$ and $P = 0.0011$, respectively). It also tended to increase the viability of this cell line. Moreover, the combination with E2 further increased the proliferation of MCF-7 after 48 h of incubation, while the co-administration with P4 decreased their proliferation. Finally, the mixture of the three hormones augmented, even more, the proliferation of MCF-7. However, none of the treatments modified the cell viability or adhesion of this cell line. On the other hand, no significant differences were observed in the proliferation, viability, or adherence of MDA after hormone administrations. In conclusion, T4 promotes the proliferation of hormone-sensitive breast tumor cells and enhances the proliferative effects of E2.

A27
STRUCTURAL CHARACTERIZATION OF FLAVONOIDS BOUND TO CYCLOOXYGENASE2

Carmona Viglianco NE[1], Rotelli AE[2], Pedernera AM[2], Maria AOM[2], Pelzer LE[2], Wendel GH[2], Gomez Barroso JA[1]
[1]Área de Biología Molecular, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. [2]Farmacologia, Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. [3]Farmacognosia, Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. E-mail: jagomez@email.unsl.edu.ar

Inflammation is a process present in different pathologies. Many substances are generated and involved in it, such as histamine, serotonin, prostaglandins, PAF, etc. The cyclooxygenase enzyme has two isoforms (COX-1 and COX-2) and is responsible for the generation of prostaglandins, which is why it is important in the inflammatory process. Flavonoids have shown gastric and intestinal anti-inflammatory and protective activity in different animal models. When testing different flavonoids, ligands with hydroxyl or oxymethyl groups at the 3'4' position on ring B were found to have higher activity. In previous work, the interaction of the flavonoids 7-O-methyleriodictiol, nepetin, 7-O-methylsudachitin, and quercetin with the COX-1 enzyme was determined by Autodock using indomethacin as a positive control. In that work, similar affinities to the active site were observed for all compounds and there was identified a second binding site. In this work, we intend to determine the possible interaction of the flavonoids 7-O-methyleriodictiol, nepetin, 7-O-methylsudachitin, and quercetin with the COX-2 isoform. We used the structural data of the human COX 2 protein (5IKR) deposited in the Protein Data Bank (PDB). Software Autodock Vina and Chimera were used to carrying out the *in silico* coupling assays of the compounds to the COX-2 enzyme. The binding compounds-enzyme was observed using the Pymol and Chimera programs. Our results suggest that the tested flavonoids bind strongly to the active site region and to alternative docking sites. The flavonoid 7-O-methyleriodictiol shows a higher binding affinity for the active site of COX-2.

A28
STRUCTURAL STUDY OF *Trypanosoma cruzi* PROTEINS FOR RATIONAL DRUG DESIGN

Carmona Viglianco N[1,2], Cabrera M[1,2], Zárate JM[1,2], Gomez Barroso JA[1,2]
[1]IMIBIO, Universidad Nacional de San Luis, CONICET. [2]Laboratorio de Biología Molecular Estructural, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. E-mail: jarturogomez@gmail.com

Trypanosoma cruzi is the etiologic agent of Chagas disease, which is a serious health problem in America. It has a high overall prevalence (6–8 million cases), and 65–100 million people are at risk of contracting this infection. Medications used for disease treatment show undesirable side effects, and currently, there are no available vaccines. The identification of new targets for chemotherapy is very important, and their three-dimensional structure resolution provides essential information. This project aims to solve the three-dimensional structure of proteins, mainly through X-ray crystallography and complementary bioinformatics tools. Different important proteins in the survival of the parasite, with structural peculiarities and differences with the human counterparts, are studied. We study different proteins, such as ribosomal P proteins, which form a pentameric complex: TcP0 and four small TcP proteins (TcP1α, TcP1β, TcP2α, and TcP2β). This complex has important functions, and it is related to the formation of autoantibodies in chagasic patients. In addition, we studied other proteins involved in high-energy phosphate transfer from trypanosomatids: nucleoside diphosphate kinases (NDPK1, NDPK2, NDPK3) and adenylate kinases (AdK1 and other AdKn). Finally, we also studied the Pap1 interaction factor (FIP1 type), the specific cleavage, and polyadenylation factor (CPSF-30) proteins involved in the mRNA maturation process. A homology model of TcP0 shows an N-terminal globular domain, an alpha domain, a disordered region, and a C-terminal negative tail. Homology patterns for all small TcP proteins show an N-terminal four-helix up-and-down bundle domain and a C-terminal disordered domain ending in an acid curve. Docking assays characterize the formation of dimers between small P proteins. We describe a quinary crystallographic structure for TcNDPK1 showing a multi-hexameric helix-like oligomer suggesting a model for enzyme regulation and storage. The TcAdK1 protein was crystallized, and the crystallization conditions for X-ray assays are being refined. A TcNDPK2 multidomain homology model was proposed. We characterized TcCPSF30 and TcFIP1-like proteins as intrinsically disordered proteins (IDPs). A homology model for the TcCPSF30-TcFIP1-like interface region was obtained, and the structural interaction was characterized. The three-dimensional structures and the bioinformatics results are important in the rational design of drugs and the use of natural compounds for the treatment of Chagas disease.

A29
DHL INDUCES ROS IN *Trypanosoma cruzi* AND COULD REDUCE ITS INFECTIVITY ON MAMMALIAN CELLS

Gomez J[1], Coll M[1], Cifuente DA[3], Sosa MA[1,2], Barrera PA[1,2]
[1]Instituto de Histología y Embriología (IHEM-CONICET), Facultad de Ciencias Médicas. Universidad Nacional de Cuyo (UNCuyo), Mendoza. [2]Facultad de Ciencias Exactas y Naturales (FCEyN), UNCuyo, Mendoza.[3]Instituto de Investigación en Tecnología Química (INTEQUI-CONICET, Universidad Nacional de San Luis, San Luis.
E-mail: jgomez@mendoza-conicet.gob.ar

Trypanosoma cruzi is a protozoan parasite of medical importance; this is the etiological agent of Chagas disease, endemic to Latin America. Its life cycle consists of three stages: epimastigote (non-infective), trypomastigote, and intracellular amastigote (infective). The establishment of infection and the development of the disease depend on the ability of the parasite to evade the host's immune response and survive the oxidative environment. *T. cruzi* has a highly efficient antioxidant system for the detoxification of reactive species. This system is composed of antioxidant enzymes and low molecular weight thiols, unique to trypanosomatids, making it an attractive molecular target. In our laboratory, we study the mechanism of action of dehydroleukodine (DhL), a natural compound that has an α-methylene group which could block the thiol groups of trypanothione or reducing enzymes and induce oxidative stress in the parasite. In this work, we study the mechanism of trypanocidal action of DhL focusing on the antioxidant defense of *T. cruzi*. We observed that DhL affected the growth of T. *cruzi* (IC50 4 µM), generated reactive oxygen species (ROS), and induced mitochondrial swelling (an indicator of oxidative stress). To determine if the methylene group is responsible for the trypanocidal activity, various chemical substitutions were made that affected this group, derivatives DC-X2 to DC-X11. The parasites were incubated with DhL, semisynthetic derivatives, DhL + a reducing molecule (glutathione), more active derivatives + glutathione. VERO cells were infected with *T. cruzi* to assess cytotoxicity and selectivity index. The effect of DhL on amastigotes was evaluated, for which VERO cells were infected and then treated with the compound (2 µM to 13 µM). The results showed that glutathione blocked the effect of DhL and the derivatives had a lower trypanocidal effect. Only DC-X6 and DC-X11 significantly affected proliferation (IC50 7.30 µM and 26.11 µM, respectively). DhL was shown to have low cytotoxicity on mammalian cells with a selectivity index of 3.34. Preliminary data showed that infected cells had fewer amastigotes and an increase in uninfected cells at 48 hours of treatment, in a dose-dependent manner. We conclude that the methylene group of DhL is important in the trypanocidal role, that its mechanism of action is associated

with the generation of ROS, and that it has chemotherapeutic potential for the treatment of Chagas disease. Finally, we expose that the antioxidant system of the parasite is an interesting target for the development of new trypanocidal drugs.

A30
THE INVASION OF *Trypanosoma cruzi* TO MAMMALIAN CELLS IS REDUCED BY OXIDATIVE STRESS

Coll M[1], Gomez J[1], Cifuente DA[3], Masone D[1,4], Sosa MA[1,2], Barrera PA[1,21]
Instituto de Histología y Embriología (IHEM-CONICET), Facultad de Ciencias Médicas. Universidad Nacional de Cuyo (UNCuyo), Mendoza. [2]Facultad de Ciencias Exactas y Naturales (FCEyN), UNCuyo, Mendoza. [3]Instituto de Investigación en Tecnología Química (INTEQUI-CONICET), Universidad Nacional de San Luis, San Luis. [4]Facultad de Ingeniería, UNCuyo.E-mail: mauro.coll16@gmail.com

We have demonstrated that the sesquiterpene lactone dehydroleucodine (DhL) is active against *Trypanosoma cruzi* with low cytotoxicity on mammalian cells (CC50:13 µM). We also confirmed that the trypanocidal action of DhL is a consequence of the generation of ROS. To assess the presumable interaction between DhL-glutathione and/or DhL-trypanothione we analyzed by conducting unbiased atomistic molecular dynamics simulations with Gromacs in the order of the microsecond. We conclude that DhL does not interact directly with glutathione or trypanothione. In order to identify possible molecular targets for DhL, we focused on the antioxidant enzymes. Therefore, we used *T. cruzi* epimastigotes that stably overexpress reducing enzymes such as mitochondrial tryparedoxin peroxidase (mTXNPx), cytosolic tryparedoxin peroxidase (cTXNPx), tryparedoxin II (Tc-TXN II), and glutaredoxin (Tc-GRx). We observed that the overexpression of mTXNPx exerted a protective effect against DhL, suggesting that the compound could interfere, directly or indirectly, with the activity of these enzymes. Accordingly, the parasites overexpressing mTXNPx produced less ROS in the presence of DhL. A protective tendency, although not significant was also observed in the cTXNPx overexpressing parasites. On the other hand, we evaluated the activity of TcTS on epimastigotes incubated with DhL by using Malachite Green and measuring the absorbance at 650 nm. We observed higher activity of TcTS on DhL-treated epimastigotes, indicating that the parasite is under oxidative stress. Finally, we consider that the oxidative stress induced on parasites could also occur in mammalian cells, but it would be a benefit for the cells. To demonstrate this, we designed an experiment where cells were pre-treated with the compound and then infected with trypomastigotes. Then the mammalian cells were stained with Hoechst, and the number of infected and uninfected cells (N = 100) was counted by fluorescence light microscopy. The pre-incubation with DhL avoids the invasion of *T. cruzi*. Further studies are necessary to confirm if DhL induces ROS in Vero cells.

DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

A31
STUDY OF THE EFFECT OF AMMONIUM TETRATHIOMOLYBDATE ON THE UTERUS OF MICE WITH INDUCED ENDOMETRIOSIS

Zapata F, Delsouc MB, Conforti RA, Zabala AS, Vallcaneras SS, Casais M
Laboratorio de Biología de la Reproducción (LaBiR), Universidad Nacional de San Luis (UNSL)/Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).E-mail: mbdelsouc@unsl.edu.ar

Endometriosis (EDT) is a complex estrogen-dependent disease that affects primarily pelvic tissues. It is characterized by the growth of tissue similar to the endometrium outside the uterus, which causes inflammation and the formation of adhesions. Therefore, EDT often causes intense pelvic pain, dysuria, dyspareunia, dysmenorrhea, and subfertility, affecting the quality of life of patients. There is no cure for this disease and new treatments are needed to control its progression. It was recently shown that ammonium tetrathiomolybdate (TM, copper chelator) inhibits the progression of experimental EDT. However, it is necessary to analyze the possible unwanted effects of the drug on the uterus. The aim of this work was to analyze if the oral administration of TM alters the histology and oxidative state of the uterine tissue of mice with EDT. Eighteen female C57BL/6 mice were divided into three experimental groups: Sham (placebo surgery), EDT, and EDT+TM. EDT induction was performed by autologous transplantation of uterine tissue to the intestinal mesentery. The EDT+TM group received 0.30 mg of TM/day in their drinking water from postoperative day 15. One month after inducing the pathology, intact uterine tissue samples were collected for histological analysis (hematoxylin-eosin stain) and oxidative stress studies (total antioxidant capacity, catalase activity, superoxide dismutase activity, and concentration of malondialdehyde and nitrites). Data were statistically analyzed using one-way ANOVA followed by Tukey's test ($P < 0.05$ was considered statistically significant). Interestingly, the analyzed factors did not show significant changes between the experimental groups. It is probable that the observation of possible changes in the uterus requires a

longer period of experimentation. However, it is not a minor detail that the administration of TM does not cause an adverse effect on the uterus, despite considerably inhibiting the development of endometriotic-like lesions in the same experimental model. These observations continue to support the study of TM as a possible innovative treatment for EDT.

A32
EFFECT OF HIGH-FAT DIET ON SPERM QUALITY EVALUATED BY HETEROLOGOUS BUNDUG ASSAY OF RABBIT SPERMATOZOA TO ZP-FREE MOUSE OOCYTES

Avena V[1], Funes A[1], Wetten PA[4], Klinsky OG[4], Monclus M,[1,2], Boarelli P[3], Saez Lancellotti E[1,2], Michaut MA [4,5,] Fornés M[1]
[1]Laboratorio de Investigaciones Andrológicas (LIAM), Instituto de Histología y Embriología de Mendoza (IHEM), UNCUYO-CONICET. [2]Laboratorio de Investigaciones de la Universidad del Aconcagua, UDA. [3]Laboratorio de Enfermedades Metabólicas, UMaza. [4]Laboratorio de Biología Reproductiva y Molecular (IHEM), UNCUYO-CONICET. [5]Facultad de Ciencias Exactas y Naturales, UNCUYO. E-mail: virginiaavenaihem@gmail.com

For sperm to fertilize an oocyte, it must undergo capacitation (Cap) and the acrosome reaction (AR); finally, it must penetrate the *zona pellucida* (ZP) before fusing with the oocyte to be incorporated into the ooplasm. Cholesterol loss is an essential step in sperm capacitation. In previous studies, we have observed that spermatozoa (sp) from hypercholesterolemic rabbits (HCR) generated by a fat diet display alterations in Cap and AR. To further explore the fertilization potential of HCR spermatozoa, we performed a binding assay using ZP-free mouse oocytes. For this purpose, control rabbits (NCR) and rabbits fed with 7% (½ HFDR) or 14% fat (HFDR) were prepared. Glycemia, TG, cholesterol, and HDL-Cho were monitored biochemically. Ejaculated samples followed three steps: (1) spermogram evaluation, (2) sperm selection by swim-up with BWW medium (0.5% BSA), and (3) incubation for 16 h with HTF medium (0.5% BSA) to promote Cap. In parallel, oocytes were collected from hormonally super-stimulated CF-1 mice (8 weeks old). ZP was removed by a brief incubation in Tyrode's acid and washed further in HTF medium. Sperm and oocytes were co-incubated for 1 h at 37°C and 5% CO_2 at a final concentration of 50,000 sp/100 µL. After co-incubation, the oocytes were washed in three drops of HTF medium to remove unattached sp. Oocytes with bound sperm were fixed with 2% PFA, washed in a blocking solution, and mounted on Vectashield containing permeable Hoechst to quantify the number of membrane-bound sp per oocyte. Spermatozoa from HFD-fed rabbits, which had abnormal spermograms, also gave poor binding rates (½ HFDR: 7.14 ± 3.39 and HFDR: 3.28 ± 2.10 sperm per oocyte) compared to control sp (NCR: 32.47 ± 16.16 sperm per oocyte). From these results, we conclude that the ingestion of the high-fat diet in this animal model is associated with lower sperm binding, which suggests another level of the fertilizing process in which HFDR sperm are altered.

A33
IODINE INTAKE ADEQUACY AND METABOLIC PARAMETERS IN POLYCYSTIC OVARY SYNDROME PATIENTS

Ortiz I[1], Ortiz G[2], Velez L[3], Altamirano J[4,5], Pelletán L[2], Martínez A[2], Gamarra-Luques C[1,5,6], Hapon MB[1,5]
[1]CONICET IMBECU CCT Mendoza. [2]Instituto de Medicina Reproductiva, Mendoza. [3]University of California, Department of Biological Chemistry, Irvine. [4]CONICET IANIGLA CCT Mendoza. [5]UNCuyo Facultad de Ciencias Exactas y Naturales. [6]UNCuyo Facultad de Ciencias Médicas. E-mail: bhapon@mendoza-conicet.gob.ar

Polycystic Ovary Syndrome (PCOS) is a multifactorial and frequent endocrinopathy in women. On the other hand, hypothyroidism is an endocrinopathy with a high prevalence in women, which impacts female reproduction and metabolism. Despite the high prevalence of thyroid disease in women with PCOS (10–30%), in our region, there is no permanent epidemiological surveillance, considering that it is an area of iodine deficiency. Scientific bases confirm that women with PCOS that later became hypothyroid may have adverse outcomes on metabolism and fertility. It is worth noting that this disorder is aggravated by iodine consumption deficiency, which correlates with a higher prevalence of hypothyroidism. In view of the preceding, this study hypothesized that women with PCOS have a higher prevalence of metabolic disorders associated with iodine intake. To corroborate this, the goal of this study was to perform a prospective study carried out from a cohort of 42 women from 18 to 42 years old (PCOS N = 11, Control N = 31), who attended for infertility consultation at the "Instituto de Medicina Reproductiva (IMR)" or volunteers from Mendoza province. The presence of PCOS was determined according to the Rotterdam criteria: oligo-or anovulation, clinical, and/or biochemical signs of hyperandrogenism, and/or polycystic ovaries on ultrasound. The following morphometric parameters were evaluated: age, height, and body weight. Besides, the biochemical profile was assessed by the serum determination of total, LDL and HDL cholesterol, triglycerides, glucose, and urine iodine. Statistical analysis was performed by means of GraphPad Prism 7 software. The analysis showed that PCOS have a higher body mass index than controls ($P = 0.0076$). With respect to the biochemical profile, PCOS patients showed lower levels of total cholesterol ($P = 0.038$) due to the decrease of HDL cholesterol ($P = 0.0218$). The glycemia, triglycerides, and LDL cholesterol were not affected. Urine iodine in PCOS women is lower than in controls ($P = 0.027$). It is also noteworthy that 27.3% of PCOS patients present

iodine insufficiency (< 100 µg/dL) compared to 7.1% of controls. To summarize, we can conclude that the presence of PCOS has adverse metabolic and fertility consequences, and especially in our region, it would be worsened by nutritional iodine deficiency. The progress in the understanding of the relationship between PCOS and iodine nutrition deficiency, that in turn promotes thyroid malfunction, will conduct to the improvement of health-promoting public strategies in terms of prophylaxis, assessment, and therapy. These policies are required to overcome the metabolic and reproductive disturbances in women's health in our region.

A34
LONG-TERM EFFECTS OF MATERNAL MILD HYPERTHYROIDISM ON THE DEVELOPMENT AND THE BEHAVIOR OF OFFSPRING IN ADULTHOOD

Michel MC[1,2,5], Sánchez MB[1,2], Neira F[1,2], Viruel LB[2], Soaje M[1,3], Pietrobon EO[1,3], Gomez SE[4], Jahn GA[1], Mackern-Oberti JP[1,3], Valdez SR[1,5]
[1]Laboratorio de Reproducción y Lactancia-IMBECU-UNCuyo. [2]Universidad Juan Agustín Maza. [3]Facultad de Ciencias Médicas-UNCuyo. [4]Laboratorio de Hormonas y Biología del Cáncer, IMBECU. [5]Facultad de Ciencias Exactas–UNCuyo. E-mail: cemichel@outlook.com

Abnormal levels of thyroid hormones (THs) are associated with alterations in anxiety and circulating hormones such as glucocorticoids (GCs) and prolactin affecting fertility and reproductive success. However, little is known about the long-term effects of maternal hyperthyroidism on the development and behavior of their offspring. For this purpose, eight pregnant Wistar rats were divided into two groups defined as Control (Euthyroid, N = 4) and HyperT (N = 4). Hyperthyroidism was induced with T_4 0.1 mg/kg/day, s.c. After delivery, on day 2 of lactation, the number of pups in each litter was standardized to eight, and the offspring were allowed to grow under standard conditions. Development parameters were recorded from birth to 42 postnatal days (PND). Adult female and male offspring (PND 85–100) were subjected to the Open Field Test (OFT) to evaluate locomotor activity using EthoWatcher computational tool. The parameters assessed were as follows: (a) rearing, (b) entries to areas of interest, and (c) total distance traveled. At PND 100–120, the adult offspring were sacrificed, trunk blood was collected for serum hormonal determinations, and adrenal glands (AG) were dissected for histological analysis using ImageJ program. We found that offspring from HyperT mothers (named HyperT pups) presented alterations in the postnatal development of some physical, sensory, motor, and reproductive parameters compared to offspring of euthyroid mothers (named Control pups). Body length at PND 1 was lower in HyperT pups ($P < 0.001$) while there were delays in the eye opening ($P < 0.01$), ear canal opening ($P < 0.05$), forelimb grasp ($P < 0.05$), auditory startle ($P < 0.001$), testicular descent ($P < 0.01$), vaginal opening ($P < 0.05$) in HyperT pups compared to Control pups. In the OFT, adult female and male pups showed different behaviors in locomotor activity and exploration: female HyperT pups exhibited greater distance traveled ($P < 0.01$), increased number of entries to areas of interest ($P < 0.01$) and of rearings ($P < 0.05$) compared to Control female pups. In addition, female HyperT pups showed increased entries and time spent in the center of the square ($P < 0.01$) and the number of rearings ($P < 0.05$) compared to male HyperT pups. At PND 100–120, we found an increased size of the AG *zona fasciculata* (GCs synthesis zone) in adult female HyperT pups compared to adult female Control pups (636.6 ± 40.01 *vs.* 451.0 ± 10 arbitrary units $P < 0.01$, while there were no differences between males of both groups. Also, at PND 100–120 the basal serum corticosterone levels were similar in both male and female Control and HyperT rats. These results show that maternal mild hyperthyroidism may have long-term effects on the physical and neurological development of the offspring triggering sex-dependent behavioral consequences in adulthood.

A35
EFFECT OF PRENATAL TREATMENT WITH D-AMPHETAMINE ON THE TESTIS OF MALE ADULT RATS

Pietrobon EO[1,4], Sanchez MB[1,6], Michel C[1,6], Rosales J[1], Neira F[1], Mackern-Oberti JP[1,5], Gomez S[7], Valdez SR[1,2] Bregonzio C[3], Monclus MA[4,5], Soaje M[1,5]
[1]Laboratorio de Reproducción y Lactancia-IMBECU-CONICET. [2] FCEN-UNCuyo Mendoza. [3]IFEC-CONICET. [4]IHEM-CONICET. [5]FCM-UNCuyo. [6]UJAM. [7]Laboratorio de Hormonas y Biología del Cáncer-IMBECU-CONICET.
E-mail: msoaje@mendoza-conicet.gob.ar

Prenatal amphetamine exposure (PEA) induces long-lasting changes that are evident even in adulthood. D-amphetamine (AMPH) is a stimulant of CNS, it reverses the action of monoamine transporters and blocks the reuptake and degradation of dopamine (DA) and noradrenaline (NA) increasing their availability. Evidence indicates that DA and NA participate in the regulation of gonadotrophin-releasing hormone (GnRH) neurons during development and migration. Also, it has been demonstrated that the intake of AMPH derivatives in adulthood alters the hypothalamic–pituitary–gonadal axis, producing low levels of testosterone and poor sperm quality. The aim of the present study was to evaluate the effects of prenatal AMPH exposure on the testis during adulthood, particularly on the development of the seminiferous epithelium structure. Female rats were treated daily with AMPH 2.5 mg/kg i.p or saline (SAL) during days 15 and 21 of pregnancy. On days post-natal 75–90, adult PEA and SAL treated male rats, were sacrificed by decapitation, and the testis and epididymides

were surgically removed and weighed. Blood samples were obtained for testosterone determination by chemiluminescence method. Testes were fixed for the histological analysis, and the epididymides were sectioned and incubated in PBS at 37°C for the release and posterior sperm count. Next, they were prepared in HM (no capacitating medium) and HMB (capacitating medium) for the later analysis of the acrosomal reaction. Data were analyzed using two-way ANOVA and Student's *t*-test. No difference in weight testes was observed in PEA rats compared with SAL treated rats. Serum testosterone levels diminished significantly in PEA rats ($P < 0.001$) and the sperm count resulted lower in PEA than in SAL male treated rats ($P < 0.03$). A significant diminution in the height ($P < 0.001$) and area of the seminiferous epithelium was observed in PEA compared with SAL rats ($P < 0.001$). However, the percentage of acrosomal reaction did not show significant differences between both groups ($P < 0.05$). Our results suggest that prenatal amphetamine exposure may affect the structure and development of seminiferous epithelium in adult males. These changes are followed by a decrease in serum testosterone levels and a low number in sperm count that might have some consequence on male fertility.

A36
- SERPIN F1 DIMINISHES MURINE SPERM ACTIVATION DURING IN VITRO ASSAYS

Conte MI[1,2], Arancibia Cabañez MR[2], Basaez Alonso MJ[2], Leporati JL[3], López ME[2], Sáez Lancelloti E[1,2], Fornés MW[1,2], Aguilera Merlo C[3], Monclus MA[1,2]
[1]IHEM – CONICET. [2]FCM – UNCUYO. [3]UNSL. E-mail: marinesconte@gmail.com

For proper fertilization, mammalian sperm need to suffer different steps after ejaculation inside the female tract – capacitation, acrosome reaction (AR), and hyperactivation– considered together as sperm activation. These processes are regulated by the loss of male "decapacitating" factors and the influence of female factors inside the female genital tract. A male decapacitating factor is a sperm head membrane molecule that allows capacitation after being removed such as SERPIN F1. This protein has been described recently by our group. It is expressed in an androgen-dependent manner in the Wistar rat male's reproductive tract. We have initiated the study with SERPIN F1 as a possible decapacitating factor in murine sperm. We preincubated in vitro mouse epididymal sperm with the anti-SERPIN F1 antibody (to block the endogen protein) followed by adding different concentrations of recombinant SERPIN F1 (100, 500, and 1000 nM) –as an exogen source. Then, we performed capacitation and AR triggered by calcium ionophore. We evaluated the AR by Coomassie Blue stain, and we noted a decrease in the percentage of AR with increasing concentrations of added Serpin. We also evaluated the AR triggered by progesterone using as experimental condition SERPIN F1 at 500 nM and the antibody, obtaining similar results. In another set of experiments, we incubated capacitated sperm with the antibody and the recombinant SERPIN F1 prior to AR stimulation with progesterone (to distinguish pre- or post-capacitation effect), obtaining a diminished percentage of AR. These results suggest that SERPIN F1 may affect sperm activation (measured as the percentage of AR) both before and after in vitro capacitation of mouse spermatozoa.

A37
EXPRESSION OF CYTOKERATIN 5 DURING THE POSTNATAL BLOOD–TESTIS BARRIER DEVELOPMENT

Pulcini G[1], Sosa MA[1,2], Aguilera AC[1,2], Morales CR[3], Carvelli L[1,2]
[1] IHEM-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina.
[2]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina.
[3]Faculty of Medicine, McGill University, Montreal, Quebec, Canada. E-mail: lorecarvelli@gmail.com

Cytokeratins (CKs), as part of the cytoskeleton, are important for the mechanical stability and integrity of epithelial cells and tissues. In keratinocytes, CKs 5 and 14 participate in the assembly of desmosomes that make up the epithelial barrier. In the testis, Sertoli cells participate in the formation and maintenance of the blood–testis barrier. Particularly, in mice fetuses, these cells express CKs 8, 18, and 19 until they differentiate (postnatal day 14). The expression of CK 5 in mouse Sertoli cells has not been described yet, although our preliminary studies demonstrated, for the first time, that these cells expressed this intermediate filament in adult mice. The aim of this study was to determine the relationship between CK 5 and the formation of the blood–testis barrier during postnatal testis development. Male mice (C57BL/6) of 1, 5, 18, and 90 days old were used. After sacrifice, the testes were removed and processed for immunocytochemical (IHC) and immunoblotting assays. Light microscopy–IHC observations of mice testis showed that in Sertoli cells, the CK 5 staining intensity is markedly increased in animals with a developed and functional blood–testis barrier. Moreover, by immunoblot analysis, an increased expression of CK 5 in adult mice testes was also noted. These preliminary results suggest that CK 5 could play an important role in the formation and maintenance of the blood-testis barrier in mice, providing new insights for this protein in the spermatogenesis process.

A38
GLYCOSAMINOGLYCANS ISOLATED FROM CAUDA EPIDIDYMAL FLUID IMPROVES SPERM FEATURES IN LIQUID-STORED PORCINE SPERMATOZOA

Baronian D[1], Ojeda G[1], Buttazzoni AC[2], Cantú DF[2], De Blas G[3], Sosa MA[3], Carvelli F[2,3], Aguilera AC[2,3]
[1]Facultad de Ciencias Veterinarias y Ambientales, UMAZA. Mendoza, Argentina. [2]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo. Mendoza, Argentina. [3]Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. CONICET. Mendoza, Argentina. E-mail: caguilera@mendoza-conicet.gob.ar

Artificial insemination (AI) is the most practiced technique in porcine reproduction. Since this practice requires the storage of spermatozoa in semen extenders, there is an increasing interest in improving the liquid-storage composition to preserve sperm quality. Glycosaminoglycans (GAGs) are a family of linear polysaccharides comprised of repeating hexosamine-containing disaccharides that are found in the reproductive fluids along the male and female genital tracts. Different studies showed that GAGs may be involved in sperm processes such as capacitation and acrosome reaction *in vivo*. The aim of this study was to investigate the effect of GAGs isolated from cauda epididymal fluid (G-EF) and seminal plasma (G-SP) on viability, membrane stability, and mitochondrial activity of boar spermatozoa stored in commercial sperm extenders. GAGs from EF and SP were isolated by protease digestion, lipid extraction, and by different precipitation conditions. Fresh boar sperm was diluted in commercial short-term extender (BTS) and long-term extender (GM) supplemented with G-EF and G-SP, for 3 and 7 days at 16ºC, respectively. Spermatozoa storage either with G-EF or G-SP showed a high percentage of membrane stability and viability assessed by flow cytometry using M540/YoPro ($P < 0.05$). Mitochondrial activity, assessed by Rhodamine123, showed a significant decrease in G-EF suggesting that GAGs could preserve mitochondrial function in liquid-stored spermatozoa ($P < 0.05$). To test the functional ability to undergo sperm capacitation, spermatozoa were incubated in TALP capacitation medium for 1 h at 38.5ºC in a humid atmosphere. No differences were observed in the capacitation status assessed by flow cytometry using M540/YoPro ($P < 0.05$). These results provide evidence that the supplementation of GAGs from EF and SP might have a protective effect on boar spermatozoa increasing membrane stability, reducing mitochondrial activity, and maintaining viability during liquid-stored sperm storage.

A39
- TESTOSTERONE MODIFIES OVARIAN ANTI-MULLERIAN HORMONE RELEASE IN RATS WITH POLYCYSTIC OVARY

Figueroa MF, Mendoza GV, Forneris ML
Lab. Biol. Reprod (LABIR). Fac. Qca. Bqca y Fcia (UNSL). Ejército de los Andes 950, San Luis, Argentina.
E-mail: mfigueroa@unsl.edu.ar

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, which is associated with increased androgens, antral follicle growth arrest, chronic inflammation, and oxidative stress. Anti-Mullerian hormone (AMH), a dimeric glycoprotein produced from the granulosa cells of pre-antral and antral follicles, is elevated in PCOS. It has been implicated in follicle dysfunction that leads to the development of the disease, but the exact mechanism of action of AMH in the ovary is not well understood. The purpose of this study is to evaluate the role of androgens on AMH release and its relationship with cytokines involved in ovarian steroidogenesis, in a PCOS-induced rat model. Polycystic ovary condition (PCO) was induced in adult female Wistar rats (60 days of age) via i.m. injection of estradiol valerate (2 mg/rat). PCO and control (C) ovaries were incubated with RPMI medium (basal value), T (10^{-6} M), or flutamide (an androgen receptor antagonist; 10^{-4} M) plus T (F+T), for 4 h in a metabolic bath. In ovarian conditioned media (supernatant), AMH and tumor necrosis factor alpha (TNFα) concentration were measured by enzyme-linked immunosorbent assay (ELISA) while nitric oxide (NO), as nitrites, were quantified by Griess reaction. The mRNA expression of androgen receptor (AR) and interleukin (IL)-1β were assessed in the ovary by RT-PCR. Serum AMH levels were higher in PCO than in C rats ($P < 0.05$). Compared to the basal value, the PCO ovary released more AMH than the C ovary ($P < 0.05$). The C ovaries responded with an increase of AMH release after T treatment in relation to basal values ($P < 0.05$). Instead, there was a significant reduction in AMH release in the PCO ovary exposed to T compared to the PCO ovary incubated in basal media ($P < 0.05$). This inhibitory effect on AMH release was partially reduced when the PCO ovaries were simultaneously incubated with flutamide ($P < 0.01$). Both AR and IL-1β mRNA expressions were down-regulated in C and PCO ovaries exposed to T. The NO and TNFα release pattern observed was the following: PCO+T > PCO > C+T ~ C ($P < 0.05$). The PCO ovary response differed from the C ovary. When the PCO ovary was exposed to a high androgen concentration, AMH was, at least locally, downregulated. Although, a possible synergistic effect of estradiol with T or interaction with mediators of inflammation cannot be ruled out. This mechanism may have implications for the understanding of PCOS pathophysiology.

A40
EFFECTS OF TESTOSTERONE ON ANTERIOR PITUITARY IN A POLYCYSTIC OVARY MODEL

Mendoza GV[1], Figueroa MF[1], Gornitz P[2], Forneris ML[1]
[1]Laboratorio de Biología de la Reproducción (LABIR). Facultad de Química, Bioquímica y Farmacia (UNSL). Ejército de los Andes 950. San Luis. [2]Laboratorio Gornitz, Villa María (Córdoba), Argentina. E-mail: gvmendoza@unsl.edu.ar

Polycystic Ovary Syndrome (PCOS) is a frequent hyperandrogenic condition that affects young women. Neuroimmunoendocrine alteration in the hypothalamic–pituitary–gonadal (HPG) axis has been reported. Previously, we demonstrated that splenic macrophage secretions affect the release of gonadotrophins, the expression of immune mediators, and the synthesis and release of nitric oxide (NO) in a rat model of polycystic ovary. The aim of this study was to investigate if testosterone (T) modulates luteinizing hormone (LH) release in PCO anterior pituitary (PCO-AP) and its relationship with NO, androgen receptor (AR), estradiol receptor (ER), IL-1β and macrophage colony-stimulating factor (M-CSF) mRNA expression. The PCO condition was induced in adult 60-day female Wistar rat with a simple dose of estradiol valerate (i.m. injection 2 mg/rat). PCO and control pituitaries (PCO-AP and C-AP) were incubated with RPMI medium (basal value), T (10^{-6} M), or flutamide (an androgen receptor antagonist; 10^{-4} M) plus T (F+T), for 4 h in a metabolic bath. The LH release was measured by electrochemiluminescence (ECLIA), and NO release (nitrites) was determined by the Griess reaction. The genetic expression of AR, ER, IL-1β, and MCSF, were analyzed by RT-PCR. The release of LH was lower in PCO than in C rats in basal conditions ($P < 0.01$). After T was added to PCO-AP, LH release increased significantly compared with the basal value ($P < 0.001$). This stimulatory effect on LH release was reduced to basal values in the presence of Flu. In basal conditions, the NO release was similar between PCO-AP and C-AP. In PCO-AP, T increased NO release ($P < 0.05$). In basal conditions, mRNA levels of AR were lower in PCO-AP in relation to basal C-AP ($P < 0.01$); with T, it was observed an increase in PCO-AP RA expression ($P < 0.05$). The RE mRNA levels were higher in PCO-AP than in C-AP ($P < 0.05$) in the basal condition. Despite no changes were observed in PCO-AP + T, it was evidenced an increase in RE expression in C-AP compared with basal values ($P < 0.05$). Interestingly, M-CSF and IL-1β mRNA levels increased in PCO-AP when T was added with respect to basal ($P < 0.05$). Our results showed that a hyperandrogenism environment could contribute, in PCO-AP, to disturb the complex feedback regulation system of the HPG axis, which involves crosstalk between gonadotrophic hormones, cytokines, and ovarian steroid hormones. In particular, we demonstrated that M-CSF is expressed in PCO-AP, and it participates in LH secretion. Taken together, these data provide a new perspective to understanding the molecular mechanism for PCOS development.

VETERINARY, ANIMAL ANATOMY, HISTOLOGY AND PHYSIOLOGY

A41
EFFECT OF FOOD RESTRICTION ON PLACENTAL AND PLASMATIC OXIDATIVE STRESS IN RATS DURING THE LAST THIRD OF GESTATION

Arabel R[1,2], Borghi D[1,3], Farías M[1,2], Binotti S[1,2]
[1]INBIAS- Instituto de Biotecnología Ambiental y Salud (CONICET). [2]Depto. Biología Molecular. Facultad de Ciencias Exactas, Fco-Qca y Naturales. [3]Depto. Anatomía Animal. Facultad de Agronomía y Veterinaria. Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba. E-mail: sbinotti@exa.unrc.edu.ar

The placenta serves as the critical intermediary between the maternal and fetal environments. Despite its transient role as the mediator of the exchange of nutrients, gasses, and waste between mother and fetus, the capacity of the placenta to adapt to environmental stresses has a lifelong impact on the health and well-being of the offspring. In this sense, maternal undernutrition is a common environmental stressor associated with an increased risk of postnatal health disorders. Moreover, endocrine response to undernutrition has been associated with an oxidative stress increase. In this study, the effect of food restriction on placental and plasmatic oxidative stress in pregnant rats, during the last third of gestation, was investigated. Three-month-old male and female Wistar rats were used. Pregnant rats were randomly divided into the control group (C): *ad libitum* food; and the food restriction group (FR): 40% reduction in daily food intake, during the last third of pregnancy. On day 21 of pregnancy, all rats were euthanized, and different samples were obtained. In plasma, malondialdehyde (MDA) and ferric reducing ability of plasma (FRAP) were determined. Placenta and fetus size and weight were measured for the placental efficiency determination. Also, placental MDA, carbonyls (CAR) levels, and superoxide dismutase (SOD) and catalase (CAT) activities were measured. No changes were observed in MDA plasmatic y FRAP levels. In response to food restriction, placental weight ($P \leq 0.02$) and diameter ($P \leq 0.00002$) decreased, when

compared to the C. On the other hand, non-statistically significant differences were observed in fetus weight and cephalocaudal length, between C and FR. As to oxidative stress markers, an increment in placental MDA ($P \leq 0.02$) and carbonyls ($P \leq 0.001$) was observed in the FR, while SOD and CAT activities showed no difference between groups. Oxidative damage observed in the placenta could be related to the stress situation caused by food restriction. It is known that stress increases corticosterone levels, resulting in a major production of reactive oxygen species. This increment, in addition to the basal antioxidant activity observed in FR, would lead to the placental oxidative stress found in the study. Thus, an increment in oxidative stress could result in a compromised placental function, predisposing to an increment in neonatal mortality or disease risk in adult life.

A42
EFFECT OF NUTRITIONAL RESTRICTION IN PREGNANT GOATS ON THE BODY DEVELOPMENT OF THEIR OFFSPRING

Mura N[1], Flores Bracamonte MC[2,3], Merkis CI[2], Díaz T[2], Bozzo A[2]
[1]Lab. de Biología Celular y Embriología, FAV-UNRC. [2]INCIVET-UNRC. [3]Becaria Doctoral CONICET.
E-mail: muranadya@gmail.com

The problems of the goat sector are integrative of social, productive, and environmental constraints that give the system exclusive particularities. Goats are a group with different demands than the rest of the ruminants because they have very low production rates. The intrauterine growth stage presents great plasticity, and when the fetus is subjected to inadequate nutrition, long-term deleterious organic changes may occur. The aim of this study was to evaluate the effect of maternal nutritional restriction during gestation on the body development of offspring. Ten primiparous pregnant goats were assigned to two dietary treatments during gestation. The control group (C, N = 5) was fed with an *ad libitum* diet that consisted of a 70/30 mixture of chopped alfalfa hay and ground corn grain (energy concentration of 2.4 Mcal/kg DM) and supplemented with a mineral vitamin core. The energy-restricted group (R, N = 5) was fed with 70% of the energy requirements of group C, from day 50 of gestation to term and supplemented with urea so that the restriction was not of the protein type. This group received a diet with an energy concentration of 1.68 Mcal/kg DM. The weight and morphometric variables such as height at the withers and shoulder length–ischial tuberosity, were recorded in the offspring, from birth and at 45 days. No statistically significant differences were observed in weight, height at the withers and shoulder length–ischial tuberosity between the groups C and R at both times studied. These results suggest that the nutritional restriction applied in pregnant goats had no influence on the morphological parameters determined in their offspring. Future research is needed on the impact of nutritional restriction on the number of muscle fibers and intramuscular adipose cells in offspring that will determine future performance in meat production.

A43
SACCHARIDE PATTERN IN GOAT PLACENTA DURING GESTATION

Flores Bracamonte MC[1,2], Gregori S[3], Mura N[3], Díaz T[1], Acuña F[4,5], Barbeito C[4,5], Bozzo A[1]
[1]INCIVET-UNRC. [2]Becaria Doctoral CONICET. [3]Lab. de Biología Celular y Embriología, FAV-UNRC.
[4]Cátedra de Histología y Embriología, FCV-UNLP. [5]CONICET. E-mail: cfloresbracamonte@ayv.unrc.edu.ar

In the province of Córdoba, goat production is an economic source for farming families that develop this activity with limited technological resources. To optimize their productivity, it is important to guarantee an adequate weight of the offspring at birth. The growth and survival of the fetus depend on the placenta, which is characterized by being cotyledonary and sinepitheliochorial with trophoblast giant cells that migrate from the chorionic epithelium and fuse with the endometrial epithelium to form syncytia. Cell surface carbohydrates are involved in the processes of cell recognition and adhesion between the trophoblast and the uterus. During placenta development, the composition of carbohydrates on the cell surface undergoes variations that have a fundamental role in the processes of cell recognition and adhesion between the trophoblast and the uterus. The placental pattern of carbohydrates has not been studied in this species. The aim of this study was to describe the pattern of carbohydrates in the goat placenta throughout gestation. Fifteen goats (*Capra hircus*) older than two years that had access to food and water *ad libitum* were used. They were sacrificed at 50, 100, and 135 days of gestation following the COEDI animal management recommendations Histological sections of placentas were obtained and the lectin histochemistry technique was performed with biotinylated lectins: GSL-I, WGA, SJA, SWGA, PSA, and LCA. The placental structures analyzed were glycocalyx (GC), binucleated cells (BN), mononucleated cells (MN), syncytia (S), and fetal (EF) and maternal (EM) endothelium. The intensity of binding to each lectin was qualitatively rated on a scale of 0 (negative), + (weak), ++ (moderate) to +++ (strong). Ten random fields were analyzed for each slice of placental tissue. At 50 days of gestation, the structures that possessed +++ binding intensity were the GC of mononuclear trophoblastic cells and EFs with GSL-I and EMs with WGA. At 100 days, the GC of mononuclear trophoblastic cells showed +++ binding intensity with GSL-I, the EF had +++ binding intensity with GSLL-I and WGA, and the EM showed +++ binding intensity with WGA. At 135 days, +++ GC binding of mononuclear trophoblastic cells was identified with GSL-I and EM and EF showed +++ binding intensity with GSL-I, WGA and LSA. The pattern of placental carbohydrates allowed evidence of remodeling in the different gestational stages studied.

A44

EXPRESSION OF HORMONAL RECEPTORS AND PROLIFERATING CELLULAR NUCLEAR ANTIGEN IN PARS DISTALIS CELLS: STUDY IN RELATION TO SEX

Rosales GJ[1,2], Filippa VP[1,2], Mohamed FH[1]
[1]Área de Morfología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. [2]Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET. E-mail: gabirosales87@gmail.com

Gonadal steroids are involved in the regulation of cellular activity of pituitary pars distalis (PD). Several studies carried out in some mammals have indicated that this regulation varies in relation to sex. The viscacha (*Lagostomus maximus maximus*) is a native rodent to Argentina with seasonal reproductive patterns. From February to April, nonpregnant females experience a massive poly ovulation phenomenon and they prepare for an extensive pregnancy, while the males are in their reproductive period. The aim of this work was to study and quantify the colocalization of androgen receptors (AR), estrogen receptors alpha (ERα), and proliferating cell nuclear antigen (PCNA) in LH-, FSH-, GH-, PRL- and folliculostellate (FS)-cells of pituitary PD of adult male and female viscachas. In each group, four pituitary glands were collected and processed for light microscopy. Colocalization of antigens in PD cells was detected by double-immunohistochemistry. These cells were quantified by morphometric and statistical analysis. The location of AR was mainly observed in GH-cells ($P < 0.01$) both males and females. In females, the ERα was mainly found in the PRL-cells ($P < 0.01$) and were numerous in FS cells compared with males ($P < 0.01$). Instead, in males the ERα was frequently observed in GH-cells ($P < 0.01$). In females, numerous GH-cells and LH-cells expressed PCNA ($P < 0.01$). While in males the expression of PCNA was mainly in FSH-cells and LH-cells ($P < 0.05$). The results show that AR are important to regulate the activity of somatotrophs in both sexes. The ERα regulate the activity of lactotrophs in females probably in preparation for pregnancy. In males, these receptors affect the somatotrophs who might be very active to maintain the metabolic conditions during the reproductive period. In females, cell proliferation is mainly in somatotrophs and gonadotrophs-LH, probably because these cells must maintain metabolism and particularly ovary for the reproductive process. In males, cell proliferation occurs basically in gonadotrophs indicating the great activity of the gonadotrophic axis at this time of the seasonal reproductive cycle. Finally, double immunohistochemistry provided morphological evidence for the existence of the specific regulation of the cellular activity in the different pituitary PD populations in relation to the sex of the animals. Further studies are needed to understand the mechanisms of regulation activated by gonadal steroids in the viscacha pituitary.

A45

REPRODUCTIVE ALTERATIONS IN MALE RATS EXPOSED TO THE HERBICIDE GLYPHOSATE IN A COMMERCIAL FORMULATION

Borghi D[1,3], Binotti S[1,2], Farias M[1,2], Cots D[1,3], Salinero C[1,4], Bonatto F[1,4], Aiassa D[1,4]
[1]INBIAS- Instituto de Biotecnología Ambiental y Salud (CONICET). [2] Depto. Biología Molecular. FCEFQyN. [3] Depto. Anatomía Animal. FAyV. [4] Depto. Ciencias Naturales. FCEFQyN. UNRC. E-mail: dborghi@ayv.unrc.edu.ar

Glyphosate is an organophosphate herbicide used to eliminate perennial annual grasses. Its action is probably due to the inhibition of the biosynthesis of aromatic amino acids, which are essential for plant growth and survival. In order to increase its effectiveness, other substances not specified in the labeling are added to glyphosate, thus enhancing the toxicity of the active ingredient. The objective of this work was to evaluate the effect of different concentrations of glyphosate (48%) from a commercial formulation on sperm viability in male Wistar rats. 8-week-old young albino male rats were used. A total of twenty-four rats were divided into four groups: (1) treated with drinking water (control), (2) 10 mg/kg body weight/day glyphosate diluted in drinking water, (3) 50 mg/kg of body weight/day of diluted glyphosate in drinking water, and group (4) 100 mg/kg of body weight/day of diluted glyphosate in drinking water. Body weight and water intake were measured. Sperm count and motility, frequency of malformations, plasmatic membrane capacity (HOST), and testosterone concentration were measured. No changes were observed in weight and water intake. A decrease in the number of sperm was found in groups 3 and 4 when compared to control ($P \leq 0.05$). A decrease in the capacity of the plasmatic membrane in groups 2 and 4 when compared to the control was observed ($P \leq 0.05$). Sperm motility showed no differences between the experimental and control groups, although a tendency to lower sperm motility was displayed. No differences were observed between the control and experimental groups and neither between experimental groups, regarding the number of live spermatozoa and frequency of malformations. Testosterone concentration was significantly lower in groups 3 and 4 when compared to the control ($P \leq 0.05$). Therefore, the alteration of some of the sperm viability parameters could be due to the testosterone levels decrease, which could result in reduced fertility potential.

A46

IN VIVO AND *IN VITRO ATRAZINE EVALUATION* ON DIGESTIVE ENZYMES OF EARED DOVE (*Zenaida auriculata*)

Manzanos NA, Bach NC, Cid FD, Chediack JG
Laboratorio de Biología Integrativa. IMIBIO–SL, Departamento de Biología, FQByF. Universidad de San Luis. CCT San Luis CONICET. San Luis–Argentina. E-mail: nazarenomanzanos@gmail.com

It has been observed that certain environmental pollutants can affect the enzymatic activity of the digestive system in some vertebrates. However, this effect is poorly studied in birds. Atrazine is a widely used herbicide in our country, and its negative effects on the physiology of some organisms have been described. In this sense, although atrazine has been shown to produce some adverse effects in birds, there is a lack of knowledge about its effect on digestive physiology. Thus, the main objective of this work was to elucidate the atrazine effects on digestive enzymes (intestinal and pancreatic) in an *in vivo* model of Eared Dove and the direct effect in brush border membrane vesicles (BBMV), *in vitro* model. To achieve our goal related to in vivo experiment, we established three independent groups of birds (N = 6 for each group), two groups were exposed for 15 days to the 25 mg/kg and 250 mg/kg atrazine concentrations. After exposure, body weight measure, blood extraction, and removal of the intestine, stomach, liver, and pancreas were performed at the same time (8:00 am), to avoid disturbance by circadian/daily variation. BBMV was obtained after intestinal lumen scraping using a polyethylene glycol (PEG) technique, then, BBMV were exposed to atrazine concentrations of 5 µM, 2.5 µM, 1 µM, and 0.5 µM, taking into account two controls, one with buffer and the other with ethanol, since atrazine is solubilized at 0.4% in ethanol. The intestinal enzymatic activities of sucrase, maltase, and aminopeptidase were determined in both *in vivo* and *in vitro* models, and the enzymatic activity of the pancreatic enzymes trypsin and chymotrypsin was determined *in vivo* model. Statistical analysis performed was RM-ANOVA to compare the proximal, medial, and distal portions of the *in vivo* model and for pancreatic enzymes. *In vitro* model was analyzed by one-way ANOVA, Tukey's post-hoc test ($P < 0.05$). We did not find any differences in the masses of the organs of the digestive system. We did not find an effect of atrazine on the intestinal enzymes or pancreatic enzymes. We found a classical pattern of intestinal enzyme activity reported for other bird species. Our results showed a non-significant direct inhibition of intestinal enzymes studied in BBMV exposed to different concentrations of atrazine. In conclusion, the doses assayed of atrazine do not affect the intestinal and pancreatic digestive enzymes in doves. *[Supported by CyT-UNSL PROICO 02-0820 and FONCYT PICT-201–0595]*

A47

S-100 PROTEIN EXPRESSION IN THE ADRENAL MEDULLA OF PREGNANT AND NON-PREGNANT VISCACHAS (*Lagostomus maximus maximus*): AN IMMUNOHISTOCHEMICAL AND MORPHOMETRIC STUDY OF SUSTENTACULAR CELLS

Gallol LE[1], Busolini FI[1 2], Dimenza Bujaldón JM[1], Vargas MR[1], Mohamed FH[1]
[1]Area de Morfología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. [2]Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET. E-mail: gallolezequiel@gmail.com

An accurate functional adjustment of the adrenal gland is key for a successful pregnancy. Its dysfunction is associated with premature births, low birth weight of offspring, psychomotor impairments, and diabetes, among others. In viscacha, a seasonal breeding rodent, pregnancy lasts approximately 154 days, and three stages can be defined: early (EP), mid (MP), and late pregnancy (LP). The purpose of this work is to analyze morphometric variations in the expression of S100 protein in the sustentacular cells (SC) of pregnant and non-pregnant viscachas by immunohistochemistry. We also aim to evaluate a probable relationship between medullar activity and pregnancy. Four adrenal glands (N = 4) per group were studied. The reproductive condition was assessed according to body weight and light microscope observations of ovaries. Additionally, uterine horns were examined to evaluate the presence of embryos and fetuses. The adrenal medullas were processed for light microscopy and the immune-positive percentage area (%IA) for the S100 protein was analyzed. Estradiol (pg/mL) and progesterone (ng/mL) serum levels were also determined by RIA. Statistical differences were evaluated by Kruskal–Wallis' test followed by Dunn's multiple comparisons test. A value of $P < 0.05$ was considered significant. Positive S100 nuclei were frequently triangular or elongated in shape. The cytoplasm and cytoplasmic processes of SC vary according to the pregnancy stage. In non-pregnant females (NP) the cytoplasm was scarce, and the processes were few, thin and well-defined surrounding some chromaffin cells. In EP, immunolabeling at the nuclear level decreased in intensity. The processes were mostly thin and well-defined, but they often did not surround chromaffin cells. During MP, nuclear and cytoplasmic labeling was significantly accentuated, and SC exhibited a more abundant cytoplasm; however, their cytoplasmic processes were fewer, shorter, and thicker. In LP, cytoplasmic processes were long, defined, and variable in thickness and frequently found surrounding chromaffin cells. Variations in the expression of the S100 protein related to serum hormone levels were found between pregnant and non-pregnant viscachas. In NP females, the %IA was the lowest (4.28 ± 0.20) compared with EP (6.03 ± 0.36), MP (5.36 ± 0.10), and LP (7.19 ± 0.18) values. In the biochemical study, variations in estradiol (NP: 18.01 ± 3.19, EP: 27.50 ± 2.50, MP: 75.02 ± 2.50, and LP: 24.25 ± 2.17), as well as in progesterone serum levels (NP: 0.72 ± 0.11, EP: 4.64 ± 0.95, MP: 51.02 ± 3.10, and LP: 18.61

± 2.25) were also reported in each pregnancy stage. Our results demonstrated that SC vary morphologically in relation to the levels of female sex hormones, indicating the participation of these hormones in the regulation of medullar activity in pregnant viscachas.

VEGETAL BIOCHEMISTRY, PHYSIOLOGY, PATHOLOGY AND PRODUCTION

A48
DEVELOPMENT OF SOYBEAN GERMPLASM (*Glycine max* (L.) Merrill) WITH NUTRITIONAL QUALITY FOR HUMAN CONSUMPTION

Bologna S[1], Rojas E[1], Soldini D[2], Gilli J[2], Pertusati L[1], Lucero V[1], Sartori L[1], Crozio E[1]
[1]Universidad Nacional de San Luis (UNSL), Facultad de Ingeniería y Ciencias Agropecuarias (FICA). Villa Mercedes, San Luis, Argentina. [2]Instituto Nacional de Tecnología Agropecuaria (INTA), Marcos Juárez, Córdoba, Argentina. E-mail: sbbologna@gmail.com, soldini.diego@inta.gob.ar

With the aim of generating variability for traits required for consumption of soy foods, such as non-GMO, high protein content, large grain size, light hilum color, reduced lipoxygenase enzyme activity, and antinutritional factors, in 2019, in the Marcos Juárez INTA experimental station, a biparental cross was carried out between the parents FICA 1513.2 (non-GMO genotype, triple null for lipoxygenases and for Kunitz trypsin inhibitor) and IA36 (non-GMO genotype, light hilum color, high protein content, and large grain size). In the INTA greenhouse, in the winter of 2020, the F_1 generation was advanced, and the F_2 population was harvested at the end of the year. In Villa Mercedes (San Luis), in the FICA experimental field on November 30th, 2021, the F_2 population was sown in bulk with the purpose of selecting individual plants and starting F_3 families. The following variables were registered: plant height at R8 (PHR8), number of nodes at R8 (NN), number of pods per plant (NPPP), number of seeds per plant (NSPP), hundred seeds weight (HSW), and hilum color (HC). The first field selection was made based on agronomic value, PH and NN, and individual plants were harvested. The second selection was made in the laboratory based on light HC and HSW. In order to characterize the variability generated, descriptive statistics and principal components analysis (PCA) were made. The mean values obtained for the variables were: PHR8: 79 cm, NN: 15, NPPP: 81, NSPP: 161, HSW: 15 g. The NPPP and the NSPP were the variables that showed the greatest variability (SD: 2.6 and 52.8, respectively). Regarding the HC, 74% of the plants selected displayed light color, 15% displayed dark color, and 11% were colorless. The existing 73% variability was explained by means of PCA, showing that the extreme genotypes 262 and 182 were the ones that contributed the most to said variability. The positive correlations between the variables with the greatest relevance were observed between PHR8 and NN, and between NPPP and NSPP, while HSW did not correlate with either NPPP or NSPP. Ten genotypes were identified, which were associated with the variables NPPP and HSW according to the projections about the Principal Component 1. The characterization of the developed germplasm allowed the identification and selection of genotypes that expressed the greatest variability for the traits of interest.

A49
INTA-FICA 5C k/lx: NEW SOYBEAN VARIETY (*Glycine max* (L.) Merrill) WITH DIFFERENTIAL QUALITY

Bologna S[1], Rojas E[1], Soldini D[2], Gilli J[2], Lucero V[1], Carrió A[2], Sartori L[1], Vicentin I[2]
[1]Universidad Nacional de San Luis (UNSL), Facultad de Ingeniería y Ciencias Agropecuarias (FICA). Villa Mercedes, San Luis, Argentina. [2]Instituto Nacional de Tecnología Agropecuaria (INTA), Marcos Juárez, Córdoba, Argentina. E-mail: sbbologna@gmail.com; soldini.diego@inta.gob.ar

One of the most important derivatives in soybean production is soybean meal destined for animal nutrition since it is one of the main protein sources in pig and poultry diets. The problem with soybean feed in monogastric animals is that protein availability is affected by the presence of antinutritional factors such as Kunitz (KTI gene), which inhibits trypsin and interferes with protein digestion, producing growth inhibition (Armour *et al*., 1998). On the other hand, acceptance and palatability of soybean derivatives are conditioned by the bitter and astringent flavor, which results from lipoxygenase enzymes (*Lx1, Lx2, Lx3* genes) which are part of the grain (Siedow, 1991). With the aim of obtaining soybean germplasm of differential quality and which expresses a reduced activity of Kunitz antinutritional factor and lipoxygenases, biparental crosses were carried out between the BRM92-6600 progenitor (triple null conventional genotype for lipoxygenases) and the PI 542.044 (L81-4590) progenitor (null conventional genotype for Kunitz trypsin inhibitor) in 2007 at INTA experimental station (Marcos Juárez, Cordoba). Segregating populations

were conducted by the Modified Single-Seed Descent Method at FICA-UNSL (V. Mercedes, San Luis). Molecular marker-assisted selection was performed using codominant functional markers for the *Kti* gene and the *Lx1/Lx2* linked genes (Sequin, et al., 2008) and the dominant marker which selects genotypes with at least one copy of the *lx3* allele (Sequin, 2009), which allowed identification of null families for Kunitz and lipoxygenases characteristics. A superior genotype with the proposed quality attributes was selected and the registration process was initiated in 2021 at the National Registry of Property of Cultivars under the name INTA-FICA 5C k/lx. It is a non-transgenic conventional variety with the biological characteristics required for pig feeding and the production of special meals. Its differential genetic characteristics are reduced activity of both Kunitz antinutritional factor and lipoxygenase enzymes since it has the *lx1*, *lx2*, and *lx3* recessive alleles for the three lipoxygenases and the *kti* recessive allele, which prevents accumulation of antinutritional factor in the seed, in addition to expressing an improvement in the content of protein and oil in the grain (41.5% of protein and 23% of oil on a dry basis). Through this newly obtained germplasm, value is added to national production and the consumer and agroindustry demands are met. It is the first variety developed by the public sector, which, by leveraging resources and efforts in both the educational and technological spheres, constitutes a contribution to improving the quality of national soybean production.

A50
INFLUENCE OF THE METEOROLOGICAL ELEMENTS IN THE PROTEIN CONTENT EXPRESSION IN SOYBEAN IN VILLA MERCEDES (SAN LUIS)

Sartori L[1], Rojas E[1], Bologna S[1], Gilli J[2], Soldini D[2], Lucero V[1], Pertusati L[1], Saber G[1], Perroni F[1]
[1]Universidad Nacional de San Luis (UNSL), Facultad de Ingeniería y Ciencias Agropecuarias (FICA). Villa Mercedes, San Luis, Argentina. [2]Instituto Nacional de Tecnología Agropecuaria (INTA), Marcos Juárez, Córdoba, Argentina.
E-mail: mlsartori@email.unsl.edu.ar; gilli.javier@inta.gob.ar

The soybean (*Glycine max* (L.) Merrill) is the oilseed par excellence. Argentina is the world's third-largest producer of this crop and leads the export of flour and oil. The Argentine industry has had difficulties to produce meals that meet the minimum international marketing standards due to the low protein content of soybeans produced in recent years. The potential content of protein and oil are determined genetically, these being quantitative characters strongly affected by the environment, it has been reported the protein content is four times more dependent on environmental conditions than on the variety. The moment in which the environment has the greatest effect on the determination of protein content (PR) is related to the stage in which the PR accumulates in the grain, this begins in the R3 phenological phase and decreases after the grain is fully developed (R6). The time of occurrence of these stages can be modified with management practices such as the choice of sowing date, which generates variation in the meteorological environment to which the plants are exposed. The objective of this work was to determine which meteorological elements best explain the protein content of soybean genotypes in Villa Mercedes (San Luis). In the experimental field of the Faculty of Engineering and Agricultural Sciences of the National University of San Luis, 160 genotypes corresponding to the National Soybean Genetic Improvement Program of the INTA Marcos Juárez, were sowing, in a Hill plot design. The phenological data were registered according to the scale of Fehr & Cavinnes (1977). From the automatic meteorological station of the University, the data that allowed calculating the meteorological variables for the phenological period R3-R6, average minimum temperature (T.MIN), average maximum temperature (T.MAX), average temperature (T.MED), accumulated radiation (RA) and accumulated precipitation (PP), was obtained. The protein and oil content (AC) of the grains was determined in the Laboratory of Industrial Quality and Added Value of Cereals and Oilseeds of INTA-EEA, Marcos Juárez (Córdoba). All statistical analyses were evaluated using InfoStat software. The principal component analysis (PCA) showed great variability for all variables evaluated. Regression analysis using partial least squares (PLS) showed T.MIN as the meteorological variable with the greatest inertia in relation to protein expression. Through this study, it was possible to gain a better understanding of the soybean protein in relation to meteorological variables of soybeans in Villa Mercedes (San Luis), tending to achieve a higher quality of this species.

A51
ACTION OF TEMPERATURE ON THE PROTEIN CONTENT OF SOYBEAN GRAIN IN VILLA MERCEDES (SAN LUIS)

Rojas E[1], Sartori L[1], Bologna S[1], Gilli J[2], Soldini D[2], Lucero V[1], Pertusati L[1], Perroni F[1], Saber G[1]
[1]Universidad Nacional de San Luis (UNSL), Facultad de Ingeniería y Ciencias Agropecuarias (FICA). Villa Mercedes, San Luis, Argentina. [2]Instituto Nacional de Tecnología Agropecuaria (INTA), Marcos Juárez, Córdoba, Argentina.
E-mail: erojas@email.unsl.edu.ar; gilli.javier@inta.gob.ar

Increasing the protein content is one of the main objectives of genetic soybean improvement (*Glycine max* (L). Merrill). Argentina is the world's third-largest producer of this crop and leads the export of flour and oil. The potential content of protein and oil are determined genetically, these being quantitative characters strongly affected by the environment. The moment in which the environment has the greatest effect on the determination of protein content (PR) is related to the stage in which the PR accumulates in the grain; this begins to happen rapidly between 12 and 28 days after flowering (R3

and R6, respectively), and then it declines. The moment of occurrence of these stages can be modified with management practices such as the choice of sowing date, which generates variation in the meteorological environment to which the genotypes are exposed. The main meteorological element that influences the determination of the protein is temperature, with a positive correlation between PR and cool environments. The objective of this work was to determine threshold values of temperature that allow for improving the current production scheme, tending to increase the nutritional quality of said species. In the experimental field of the FICA-UNSL (National University of San Luis), 160 genotypes were sowing, in a Hill plot design. The phenological data were registered according to the scale of Fehr & Cavinnes (1977). From the automatic meteorological station of the FICA, the data that allowed calculating the meteorological variable for the phenological period R3–R6, average minimum temperature (T.MIN) was obtained. The protein of the grains was determined in INTA Marcos Juárez (Córdoba). Using classification trees, a minimum temperature threshold (UT.MIN) was established from which all the genotypes were classified. Of these genotypes, 41 had their R3–R6 period with temperatures equal to or less than 13.8°C, being considered low protein genotypes (PRB ≤ 38%), and 116, whose temperatures were higher than said threshold, were grouped as high protein genotypes (PRA > 38%). The cluster analysis allowed us to separate four groups of genotypes, one of them being integrated by the two genotypes with the highest genetic potential for the character under study and whose T.MIN during R3–R6 was higher than the UT.MIN threshold. The genotypes that during their R3–R6 presented the lowest T.MIN of the trial, were not of high potential for PR and had the lowest mean content of said variable. This study allowed us to gain a better understanding of the changes in soybean protein levels in relation to meteorological variables and to establish what would be the optimal environmental conditions that would maximize the protein concentration of soybeans in Villa Mercedes (San Luis), tending to achieve a higher quality of this species as food.

A52
LONG-RANGE SYSTEMATIC OF THYMOL IN IMPERIAL SEEDLESS

Rosa MB[1], Acosta RJ[1], Reverendo AG[1], López L[2], Dambolena JS[3], Ravetti S[4,5], Kassuha D[1,6]
[1]Instituto de Ciencias Básicas (ICB), Universidad Nacional de San Juan. [2]Estación Experimental Agropecuaria INTA San Juan (EEA INTA San Juan). [3]Instituto Multidisciplinario de Biología Vegetal (IMBIV). Universidad Nacional de Córdoba. [4]Centro de Investigaciones y Transferencia Villa María (CIT VM). [5]Instituto Académico Pedagógico de Ciencias Humanas, Universidad Nacional de Villa María. [6]Instituto de Investigaciones en Ciencias Químicas. UCCuyo. E-mail: melisa_rosa88@hotmail.com

Grapevine production is one of the traditional factors in the economic development of San Juan province, so all types of pathologies that alter the grape yield and quality have fundamental implications for the economic development of the region. Internationally, the generic name “Grapevine Decay” or “Grapevine Wood Diseases” has been adopted to include those pathologies produced by ligninolytic fungi such as Eutypiosis, Esca, Petri’s Disease, BAD (Black Arm Death). Previous experiments in our laboratory demonstrated the significant *in vitro* antifungal effect of the monoterpene thymol against the following phytopathogenic fungi isolated and identified from Imperial seedless (*Vitis vinifera* L) in the San Juan Province: *Eutypella microtheca*, *Arambarria destruens*, *Lasiodiplodia theobromae*, and *Lasiodiplodia crassispora*. So, the objective of the present study was to determine the thymol systematic in Imperial seedless. In total, 18 grapevine plants were used in this study: 6 grapevine plants were treated by irrigation with a 0.1% m/v thymol solution, 6 plants were treated with a 0.5% m/v thymol solution, and finally, another 6 plants were not treated with thymol (control group). All plants were covered in the mid-zone to avoid contact with thymol. The plants were only exposed to thymol once (except the control plants) a day. Herb material extract was prepared from the plants’ middle parts after 3 days of treatment. The material was fractionated into 0.5 cm^2 small pieces and placed in containers with 50 mL of dichloromethane for 48 h. Each sample was filtered and concentrated under reduced pressure at the rotary evaporator. They were analyzed by gas chromatography (GC) coupled with mass spectrometry (MS). In total, 0.0032 and 0.0056 µmol per gram of plant were obtained from plants treated with thymol at 0.1% and 0.5% concentrations, respectively. The thymol-recorded uptake in treated plants, together with its antifungal effects in *in vitro* experiments, demonstrated the potential of thymol as an antifungal agent in diseased grapevine plants.

A53
SEED HEAT-PRIMING INDUCES CHANGES IN ANTIOXIDANT SYSTEM IN MAIZE SEEDLINGS

Eggel ML[1,2], Pérez Chaca MV[2], Pena LB[1,3]
[1]CONICET. Instituto de Química y Fisicoquímica Biológica (IQUIFIB). [2]Universidad Nacional de San Luis. Fac. Química, Bioquímica y Farmacia. [3]Universidad de Buenos Aires. Fac. Farmacia y Bioquímica. Dpto. Química Biológica. E-mail: luz.eggel@gmail.com

In addition to soil moisture, an abiotic factor that influences seed germination is temperature. Different priming techniques: osmo, hydro, chemical, hormonal, and nutrient techniques have been used to improve seed germination and crop yield. The increased levels of carbon dioxide (CO_2) trigger a rise in temperatures and an increase in drought episodes,

causing climate change. Seeds between harvest and sowing may experience temperature increases for short periods during storage. Our objective was to evaluate the redox state during post-germinative seedling development after heat-priming of maize seeds. Maize seeds (*Zea mays* L.) variety NS 7818 VIP3 from Nidera were subjected to pretreatment at 40 or 50 °C for 3 and 7 d. Seeds with and without heat-priming were superficially disinfected and germinated on cotton and paper towels saturated with distilled water at 28°C for 96 h. The determinations were made using the apical 2 cm of the roots. Ascorbate peroxidase (APX) and catalase (CAT) activities were determined as ascorbic and H_2O_2 consumed respectively. Lipid peroxidation was quantified by measuring the thiobarbituric acid reactive substances (TBARS). Root growth was induced by heat-priming at 40°C in maize seedlings. Pretreatments disrupted cellular redox balance, modifying antioxidant enzyme activities. All pretreatments induced at least 2-fold the APX activity significantly ($P < 0.01$); however, only heat-priming at 40°C with both times induced more than 4-fold the CAT activity ($P < 0.01$). On the other hand, pretreatment at 50°C, regardless of time, showed an increase of 200% of TBARS ($P < 0.001$). According to these results, we propose that seeds primed at 40°C have induced antioxidant defense systems that would limit oxidative damage to lipids in maize seedlings.

A54
- STUDY OF THE ALLERGENIC POTENTIAL IN GREENS SPACES AROUND THE NATIONAL UNIVERSITY OF SAN LUIS

Polak N, Isaguirre AC, Moglia MM
Facultad de Química, Bioquímica y Farmacia- Universidad Nacional de San Luis, Argentina.
E-mail: ac21isaguirre@gmail.com

Urban trees are a vital element in the afforestation of cities because they interact with citizens, providing them with well-being and indirectly increasing their quality of life. However, many of the afforestation plans do not take tree species into account, which causes the existence of a series of problems, among which is pollinosis. In this work, a novel index was used that contemplates biological and biometric parameters intrinsic to the tree species of the National University of San Luis. To carry out this work, a census of the tree species found inside and outside the University was carried out, their volume was calculated, and, in addition, the value of the allergenic potential of each species was taken into account. Thus, with the data obtained, the calculation of the index of potential allergenicity of urban green spaces (IUGZA) was carried out. Said index provides a standardized value between 0 and 1, establishing at 0.3 the threshold from which the flora of the green space can cause discomfort in the allergic population. For this study, four census zones were established, inside and outside the National University of San Luis (A, B, C, and D). Data on the pollination period were obtained from aerobiological studies carried out in the city using Lanzoni spore trap. In this sense, the following values were obtained: 0.12 (zone A), 0.031 (zone B), 0.106 (zone C), and 0.085 (zone D), in a total area of approximately two hectares. The results have shown that the areas studied do not exceed the threshold established as sufficient to cause allergy symptoms in the population. However, it can be seen that in zones A and C, which include the rear entrance of the rectory building and the Botanical Garden of the National University of San Luis, the IUGZA is close to the established threshold. Among the species cultivated in this area are several specimens of Cupressaceae, Fagaceae, and Oleaceae, with high allergenic potential (HPV). It is concluded that although IUGZA does not reach the critical thresholds in any of the zones, people sensitive to the pollens of these families should avoid going through zones A and C from the end of winter to the end of spring. Carrying out this type of study in other green areas of Argentina is important to reduce pollinosis problems in the population.

A55
- SALINITY EFFECTS ON *Atriplex lampa* (Moq.) D. Dietr. GERMINATION WITH SODIUM SALTS IN SAN LUIS PROVINCE – ARGENTINA

Arce AL[1], Videla AM[2], Sosa LR[1]
[1]Laboratorio de Fisiología Vegetal. Área de Ecología. Departamento de Biología. Facultad de Química, Bioquímica, y Farmacia. Universidad Nacional de San Luis (UNSL). San Luis, Argentina. [2]Área de Biología. Departamento de Biología. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis (UNSL). San Luis, Argentina.
E-mail: Arce.laurita@gmail.com.

Germination response of the halophyte species *Atriplex lampa* (Moq.) D. Dietr was analyzed using Na^+ salts. This study is important because the genus is widely used for the rehabilitation of degraded lands, forage, and fuel production in arid areas, and also since it is a native species of San Luis province. In order to carry out this work, seeds were collected in the surroundings of El Bebedero stream, next to national route 146 (33°43' Lat. S; 66°37' Long W). The assay consisted of exposing seeds to different sodium monosaline (NaCl, Na_2SO_4) and sodium bisaline (NaCl + Na_2SO_4), isosmotic solutions at osmotic potentials of 0.0 (control); −0.4; −0.8; −1.2; −1.5; 1.9 and 2.2 MPa and evaluating their effect on the germination percentage. Anionic effects were also evaluated. As a result, it was observed that, in the −0.4 MPa potential, both monosaline solutions of NaCl and Na_2SO_4, achieved the highest germination percentage reached being 47% and 20%, respectively, as well as in bisaline solutions of sodium, a germination percentage of 47% was reached. Germination

from −0.8 MPa did not exceed 15% with NaCl and 9% with Na_2SO_4, contrary to bisaline which registered 50% more germination at the same potential (33%). It was determined that in *A. lampa* the germination percentages were significantly affected by solution concentration, salt type, and the interaction between both factors. Obtained data suggest that *A. lampa* seeds are more sensitive to isosmotic monosaline solutions and SO_4^{2-} ions during the germination stage than bisaline solutions, where this toxicity is reversed.

A56
- EVALUATION OF THE BIOLOGICAL NITROGEN FIXATION OF *Adesmia bicolor* CONSOCIATED WITH *Festuca arundinacea*

Menichelli D, Novaira A, Malpassi R, Grassi E, Aguirre L, Castillo E, di Santo H, Gorjon J, Ganum MJ, Vega J, Basconsuelo S, Grossi F, Bianco L
Facultad de Agronomía y Veterinaria, Univ. Nac. de Río Cuarto, Córdoba. INIAB UNRC-CONICET (Instituto de Investigaciones Agrobiotecnológicas), Ruta 36 Km 601, (5800) Río Cuarto, Córdoba, Argentina.
E-mail: lbianco@ayv.unrc.edu.ar

In general, the introduction of legumes into grasslands leads to higher nitrogen (N) accumulation in the soil and higher productivity. Therefore, by growing associated with other species, legumes are able to increase the total nutritional value of non-fixing species and, in addition, increase their dry matter production. The objective is to evaluate the biological fixation of N of *Adesmia bicolor* (Fam. Leguminosae) growing consociated with *Festuca arundinacea* (Fam. Gramineae) in field conditions throughout its life cycle. The consociation trial was carried out in the Field of Teaching and Experimentation of the Faculty of Agronomy and Veterinary of the UNRC. Four treatments resulting from the combination of different ratios of legume *vs.* grass were evaluated: A1:F0 (Control I: *A. bicolor*, pure culture), A1:F1 (*A. bicolor* and *F. arundinacea* in similar proportions), A3:F1 (*A. bicolor* and *F. arundinacea* in proportions 3 to 1), and A0:F1 (Control II: *F. arundinacea*, pure culture). During the vegetative, flowering, and fruiting stages, three known surface areas (0.25 m^2) were selected for treatment, in which total dry biomass production, number of nodules in *A. bicolor*, and N content were determined through the Kjeldahl method, in both species. In the specie *A. bicolor*, higher total dry biomass production was observed in the pure crop (A1:F0) with respect to those consociated in all growth phases. In the specie *F. arundinacea*, higher production of total dry biomass was observed in the pure crop (A0:F1) in the vegetative and fruiting stage, while in the flowering stage, it was higher in the treatments consociated, although the differences were not statistically significant. In *A. bicolor* the highest nodule production was, in all treatments, in the vegetative stage and decreased throughout the growth phases. The N content in the two species was higher in the aerial part compared to the underground. The highest values in the N content were presented in the A3:F1 treatment. It is concluded that *A. bicolor* is effective to fix N in both pure and consociated cultivation, mainly in its first stages of life. Taking into account the total dry biomass values of *F. arundinacea* and the N content in both species, promising results were observed in the treatments consociated A1:F1 and A3:F1, for which it is suggested to continue with studies that allow to understand the behavior of this leguminous association – grass.

A57
EVALUATION AND COMPARISON OF VEGETATIVE CHARACTERISTICS IN POPULATIONS OF *Adesmia bicolor* (LEGUMINOSAE) OF THE CENTRAL REGION OF ARGENTINA

Gorjon J[1], Novaira A[2], Malpassi R[1,5], Grassi E[3,5], Aguirre L[3,5], Castillo E[3,5], Bianco L[1,5], di Santo H[3,5], Grossi F[3], Ganum M[4,5], Basconsuelo S[1]
[1]Morfología Vegetal. [2]Química. [3]Genética. [4]Sistema Suelo-Planta. Facultad de Agronomía y Veterinaria. Universidad Nacional de Río Cuarto. Ruta 36 km 601. Río Cuarto. Córdoba. [5]Instituto de Investigaciones Agrobiotecnológicas (INIAB). CONICET-Universidad Nacional de Río Cuarto. Ruta 36 km 601. Río Cuarto. Córdoba.
E-mail: jgorjon@ayv.unrc.edu.ar

In the central arid–semi-arid region of Argentina, faced with the shortage of winter pastures, the behavior of native autumn–winter growing legumes with forage potential is studied. Among them is *Adesmia bicolor*: herbaceous, perennial, stoloniferous, with indefinite growth, preferably winter. The objective was to evaluate and compare eight populations of *A. bicolor* from Córdoba (Populations 1 and 2), San Luis (Populations 3, 4, 5, 8, and 9), and Entre Ríos (Population 11) through morphometric vegetative characters. The trial was conducted at the National University of Río Cuarto (Córdoba). The design of the experiment was completely randomized with 4 samples matching the seasons. The number of stolons m^{-2}, length of internodes (cm), and length and width of leaflets (cm) were evaluated. The data obtained were analyzed by ANOVA and Fisher's LSD test. Populations 11 and 5 stand out for the highest records of quantified variables throughout the crop cycle, reaching average values in winter (the most critical season in forage availability) of 1740 m^{-2} runners (population 5), internode length 1.92 cm (Population 11), length and width of leaflets 0.53 and 0.26 cm, respectively (Population 11). In this way, both populations would be the most promising due to a greater dispersion capacity in the field providing greater perpetuity in time (Population 5) and greater production of aerial biomass

(Population 11). On the other hand, it is observed that the differences are not associated with the collection environment, but with the ability to adapt to the new environment presented by each of the populations. The results enhance the interest in continuing the study of these populations, integrating reproductive characteristics, carbohydrate mobilization, and total nitrogen.

A58
PHYSICOCHEMICAL PROPERTIES OF GUM EXTRACTED FROM THE FRUIT OF *Lithraea molleoides*

Becerra F[1,2], Garro MF[1], Filippa Mauricio[1], Masuelli M[2]
[1]FQByF-National University of San Luis, San Luis, Argentina. [2]INFAP-CONICET and FQByF-UNSL, San Luis, Argentina. E-mail: mfgarro@gamil.com

Lithraea molleoides (Vell.) Engl. (Anacardiaceae), popularly called “molle”, “drinking molle”, “white molle”, “sweet molle”, “chichita”. This plant is traditionally used in infusions as digestive and diuretic. This species develops in the phytogeographic region of southern Chaco, and specifically, our study focuses on the province of San Luis. In preliminary studies, we have observed that the *Lithraea molleoides* fruit shows diuretic and gastroprotective activity and is used as a natural sweetener in mate intake. It was collected in San Francisco del Monte de Oro, province of San Luis, on the banks of Route 2 (32°36'00" S 66°07'30" W / −32.6, 63 66.125), Voucher number, UNSL # 533. The objective of this study is to obtain polysaccharides that have applications as thickeners, biodegradable film formers, gelling agents, which allow absorption of the products groceries. The plant material for analysis was dried at room temperature, protected from light until reaching hygroscopic humidity, and then, it was ground to fine powder in a knife mill. In this study, we carry out the thermal extraction of polysaccharides at 80°C and then precipitate them with ethanol in a 70/30 ratio. This is dried at 60°C and finally ground. The rubber obtained exhibits absorbance at 276 nm, surface tension of 60 dyn/cm, 1.5° Brix for a solution of 0.04% by weight, whose optical activity is clockwise at 34.45°. The intrinsic viscosity is 22.83 mL/g using the Huggins method. Although these are preliminary data, this novel gum extracted from *Lithraea molleoides* has great potential in food science and possible applications in the food industry.

A59
FIELD EVALUATION OF *Bacillus velezensis* EM-A8, POTENTIAL BIOCONTROLLER OF MAIZE FOLIAR PATHOGEN *Exserohilum turcicum*

Grossi Vanacore MF, Sartori M, Barros G, Giordanino F, Nesci A, García D
Laboratorio de Ecología Microbiana. Depto. De Microbiología e Inmunología. Facultad de Ciencias Exactas Físico Químicas y Naturales. Universidad Nacional de Río Cuarto. Córdoba. Argentina. E-mail: mgrossi@exa.unrc.edu.ar

Maize is one of the most important cereals worldwide. Northern corn leaf blight, caused by the pathogen *Exserohilum turcicum*, is among the diseases that threaten maize yield. In order to find a biological alternative to traditional chemical management of the disease, two formulations with the antagonist *Bacillus velezensis* EM-A8 were developed. Previously, the antagonist ability of the bacteria was determined *in vitro*, and maize’s physiological response to the bacteria and the pathogen was evaluated in greenhouse conditions. To assess disease control ability under natural conditions, a trial was performed in the south of Córdoba province, Argentina. Biocontrol bacteria was applied in both nutritive broth (B) and enriched nutritive broth (EB) in late sowing maize at V_8 phenological stage, in natural incidence of the disease. Every ten days salicylic acid foliar concentration, proline foliar concentration, disease severity, and number of affected leaves were quantified, and yield and its components –number of rows per ear, number of kernels per row, and weight of thousand kernels (g)– were measured after physiological maturity. Visual estimation of severity and number of affected leaves resulted in high values in all the treatments, although the bio controller aided in maintaining lower values than the control. Proline (15.57 ± 3.96 $\mu mol.g^{-1}$ FW) and salicylic acid (32.04 ± 10.47 $nmol.g^{-1}$ FW) levels did not differ among treatments. However, yields were significantly superior in plots treated with EB (8711.88 ± 1790.29 $kg.ha^{-1}$) and B (8450.12 ± 2579.32 $kg.ha^{-1}$) compared to the control (7463.67 ± 1812.76 $kg.ha^{-1}$). The number of kernels per row was also higher in EB-treated maize (29.28 ± 4.64). Although b did not differ from the control in this trait (27.78 ± 4.89 and 27.52 ± 5.48, respectively), kernel weight was higher in B (216 ± 9.61 g) and EB (214.89 ± 23.17 g) treated plots compared to the control (194.67 ± 5.66 g). Further studies are necessary to determine the mechanisms by which *B. velezensis* EM-A8 enhances maize yield and clarify if this occurs due to its antagonistic activity or rather to its influence on the plant’s tolerance.

A60
BEHAVIOR OF THE FRUIT FLY PEST IN THE OASIS "C" OF THE PROVINCE OF SAN LUIS

Martinez Espeche ME[1], Lucero RA[1], Perez B[2], Monti F[1]
[1]Facultad de Ingeniería y Ciencias Agropecuarias, Universidad Nacional de San Luis. Ruta Provincial N° 55 S/N extremo Norte, Villa Mercedes, San Luis. [2]Asesor Externo. E-mail: mmartinezespeche@gmail.com

The pests *Ceratitis capitata* and *Anastrepha fraterculus* generate the greatest impact on fruit and vegetable production in the world. In the province of San Luis, the fruit sector must have knowledge of climatic factors, incidence of diseases and pests that affect the yield, and quality of the fruit. Provincial Law Nº. IX-0629-2008 promotes the plan to combat the eradication of the fruit fly. The oasis "C" includes the city of Villa Mercedes and surrounding areas. The objective of the work is to analyze the dynamics of the pest, regarding the phenology of fruit trees where it can cause damage. Data were taken from traps carried out from July to February in the 2020–2021 campaign, calculating the Fly/Trap/Day Index (MTD); the experimental fruit forest of the UNSL was used to record phenology. Climate data were extracted from the network of weather stations (REM). The fly population, during 21 weeks of trapping, takes values from 0.14 to 0.27 MTD; the temperature reaches values of 12.5º C (± 5.3ºC). From week 22 to 31, there is a greater presence of flies, obtaining MTD values between 2 to 6; the temperature in that period is 22.7°C (± 1.95°C). For the indicated stage of greater fly population, the peach, is susceptible to the attack of the fruits, because it is in the phenological states "H–I", fruit set and small fruit respectively aggravating when the conditions of temperature and humidity are optimal for the development of the pest (24–27 ºC). It was determined that the stage in which the population of fruit flies' increases is coincident with the critical phenological phases of the host, stimulated by the conditions of temperature and humidity. The management of the pest in early phenological phases of the crop would contribute to the curve of a greater population of flies, moving to phases of harvest maturity, reducing control treatments and damage levels.

A61
BEHAVIOR OF THREE VARIETIES OF ALMOND TREE IN THE QUINES – CANDELARIA REGION OF THE PROVINCE OF SAN LUIS – ARGENTINA

Lucero RA[1], Martinez Espeche ME[1], Monti F[1]
[1]Facultad de Ingeniería y Ciencias Agropecuarias, Universidad Nacional de San Luis. Ruta Provincial N° 55 S/N extremo Norte, Villa Mercedes, San Luis. E-mail: rolandolucero1962@gmail.com

The development of nut plantations in the province must be based on the selection of varieties that can produce commercial-quality fruits and that respond to the organoleptic requirements of current markets and consumers. In the context of the soil and climate characteristics of the eco-regions that exist in the Province of San Luis, the Quines–Candelaria area, according to the fruit map of the province, presents edaphoclimatic conditions and the possibility of irrigation for the commercial plantation of almond tree (*Prunus dulcis* Mill.) based on the requirements of this species. In field surveys of establishments in the area, 115 hectares of almond trees are currently implanted, which correspond to the varieties Felisia, Guara, and Penta, all with characteristics of late flowering and self-compatible pollen. For the characterization, the average flowering dates for each variety in each establishment and the occurrence of climatic events considered critically harmful to the development of the fruit were determined. From the analysis of the climatic data and flowering dates of the last 10 years (2012–2022), the minimum temperatures of damage recorded, developed between the end of May to mid-August, reaching −5.2°C, and 50% of the flowering, develops in the Penta variety around September 20, while in Guara it develops in mid-October, and for the Felisia variety, 50% of the flowering is concentrated around November 10. As a result, there are good possibilities for the development of these three varieties in the Quines–Candelaria region, capable of providing the market with quality fruits, the management of each forest and variety, in particular, must continue to be analyzed in order to evaluate the yields, as well as new varieties with similar characteristics.

A62
EVALUATION OF THE TOXIC EFFECT OF Zn/Cd ON THE ANTIOXIDANT RESPONSE IN *Glycine max* AND PROBABLE COMPETITION WITH Ca/Mg TRANSPORTERS

Gatica Aguilar CV[1,3], Gallardo LV[1], Ciminari ME[1], Casin N[2], Pacheco P[2], Pena LB[3,4], Gil RA[2], Pérez Chaca MV[1]
[1]*Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia – Proyecto 2-4318, San Luis, San Luis, Argentina, CPA-5700.* [2]*Instituto de Química de San Luis Dr. Roberto Olsina, Laboratorio de Espectrometría de Masas, San Luis, San Luis, Argentina, CPA-5700.* [3]*CONICET. Instituto de Química y Fisicoquímica Biológica (IQUIFIB).* [4]*Universidad de Buenos Aires. E-mail: caamigatica@gmail.com*

Heavy metals pollution has produced big soil problems all around the world. Cadmium (Cd) is a heavy metal without biological functions and also causes harmful effects at cellular and molecular levels. Zinc is an essential microelement for plants, but in excessive concentrations it causes alterations in physiological, biochemical, and molecular processes. There are soil ions that interact with Zn and possible pollutant Cd, such as calcium (Ca) and magnesium (Mg). Metals compete to get inside, transporting and utilization in the plant; because they have similar chemicals characteristics and also plants absorb them using the same transporters (IRT1-ZIP). The objective was to determine the competition between Ca and Mg transporters with Cd and Zn, in *Glycine max* leaves, and its effect on antioxidant and prooxidant parameters. Leaves were obtained after 10 days of plant development under hydroponics in Hoagland's nutrient solution conditions and subjected to contamination with two ions (Zn and Cd) for 6 days. The $ZnCl_2$ concentrations used in the study were: 0, 0.6, and 4.8 mM and 40 μM of $CdCl_2$, as a constant concentration. The measured parameters were photosynthetic pigments (Chlor) and carotenes (Car) and Ca and Mg endogen content. CAT and APX antioxidant enzymes, MDA, and H_2O_2 content were measured as oxidative response. Results showed a significant decrease in Car and Chlor in Zn (4.8 mM)/Cd ($P < 0.01$) treatments, and a Chlor increase in Zn (0 mM), Zn (0.6 mM) and Zn (4.8 mM) with Cd ($P < 0.01$). CAT activity decreased significantly in all treatments with respect to the control ($p < 0.05$, 0.01 and 0.001). APX activity increased in Zn (4.8 mM) treatments with Cd and without Cd ($P < 0.001$). MDA content rises significantly in Cd treatments both Zn (0.6 and 4.8 mM without Cd ($P < 0.001$). H_2O_2 content increased significantly in Zn (4.8 mM) whit and without Cd treatments ($P < 0.001$). The endogenous content of ions in the leaves showed that while Zn^{++} concentration increases, Cd^{++} absorption decreases significantly ($P < 0.001$) and vice versa ($P < 0.001$) in Cd presence. Ca^{++} and Mg^{++} diminished significantly with respect to control ($P < 0.01$) in all treatments, except for Mg^{++} in Zn (4.8 mM) without Cd treatment. According to these results we can conclude that Cd/Zn duet modifies essential ion (Mg and Ca) contents, probably due to transporters competition to enter the plant. The antioxidant activity as a prooxidant observed against high concentrations of Zn could be attributed to the plant response against toxicity originated by Zn/Cd.

A63
ISOLATES OF *Fusarium spp*. OF CORN GRAINS PRESENT IN PRODUCING AREAS OF THE PROVINCE OF SAN LUIS

Bravo MB[1], Guillin E[1], Calvente V[2], Salinas E[2]
[1]*INTA San Luis.*[2]*Facultad de Química, Bioquímica y Farmacia. UNSL. E-mail: salinaseloy@gmail.com*

In Argentina, the cultivation of corn (*Zea mays*) is one of the most important. It provides food to humans, animals, and it serves as raw material in the industry. Pathogens cause yield loss and feed toxicity through the production of mycotoxins. There is no information regarding the prevalence of mycotoxigenic species in San Luis, so sampling was carried out in four corn-producing areas, with two plots per site (Tilisarao, Comandante Granville, Buena Esperanza, and Candelaria). Healthy spikes, and those with disease symptoms, were collected. To obtain fungal colonies, different seeding techniques and culture media were tested. Healthy and diseased grains were sown on two media: Nash Snyder (H_2KPO_4, $MgSO_4$ 7 H_2O, peptone, agar, distilled water, PCNB (Terraclor 75%), streptomycin sulfate, neomycin sulfate) and Potato Glucosated Agar (PGA, Britania). Grain disinfection was washed in 70% alcohol, 2% sodium hypochlorite, and distilled water. Five grains for each Petri dish were seeded and added with dilutions obtained from solutions of healthy and diseased grains. Subsequently, a cultural and morphological characterization of the strains obtained was carried out. The color of the colony, texture, shape of the edges, and if it came from the grain or not, were observed. The morphological variables were typical reproductive structures of *Fusarium*. The largest number of strains was obtained by sowing the grains directly on the plate, prior to disinfection, but not with the dilutions. Diseased grains were not suitable for visualizing colonies due to the large amount of inoculum present. The preliminary results indicate that the highest percentage of *Fusarium* was found in the north of the province (Tilisarao) with 60% of colonies, then in Comandante Granville with 30%, followed by Buena Esperanza with 30% and Candelaria10%. The culture medium on which the greatest amount of the pathogen developed was PGA with 70.6 % *vs.* 24.13% NS. Light pink, dark pink, and violet colonies could be seen in the Petri dishes, with irregular edges with linear growth and a slightly cottony texture. Through the use of a light microscope, the characteristic fusiform macro and micro conidia, as well as monophyalides, could be observed. These results suggest the prevalence of *Fusarium* in the four representative sites of San Luis.

A64
EVALUATION OF THE PHENOLOGICAL BEHAVIOR AND YIELD OF THE CROP OF HOPS (*Humulus lupulus* L.) WITH APPLICATIONS OF ORGANIC FOLIAR FERTILIZER

Denegri A[1], Gutergoff A[2], Posadaz A[1], Ocaño S[1]
[1]Facultad de Turismo y Urbanismo UNSL. [2]Facultad de Ciencias Agropecuarias-UNC.
E-mail: andreadenegri211@gmail.com

The work sought to evaluate the phenological behavior and the yield of the hop (*Humulus lupulus* L.) crop with the management of commercial FFO organic foliar fertilizer with microbiological activity based on a consortium of total aerobic mesophilic microorganisms in a concentration of 1.5×10^5 CFU/mL *versus* management with irrigation and without any type of application. The tests were carried out in the town of Pampa de Olaen, Córdoba, Argentina, in an entisol-type soil, well supplied with organic matter and nitrogen, but with an abundance of calcium carbonate, which implies a reduction in the infiltration capacity of water and root development, added to limitations in the phosphorus content of the soil. We worked with two lines of 40 pods each of the Cascade variety, to which weekly applications of organic fertilizer were made to the soil for the first three weeks at a dose of 10 L/ha. and the following 6 weeks in foliar spraying at a dose of 5 L/ha. The results were analyzed with Infostat (V.2018), ANOVA analysis ($P < 0.05$) with *a posteriori* Tukey test. In the first year, the average height showed significant differences, with the treated plants being 44% higher (1.89 cm *vs.* 1.06 cm), as was the leaf area where it was observed that treatment presented an average of 24.85 cm^2 while the control showed values of 11.19 cm^2, 55% lower. Likewise, the sanitary state of the plants was considerably better in the treated plants that showed greater resistance to the attack of the spider might as well as greater vigor in the elongation of the guides and homogeneous coloration in their leaves. These results are promising when it comes to proposing, at least in small-scale production, treatments with products of biological origin, which favor plant development.

A65
ANTIOXIDATIVE RESPONSES OF *Adesmia subterranea* SEEDLINGS EXPOSED TO HEAVY METALS STRESS

Parera V[1], Gallardo L[2], Gatica CV[2] Pérez Chaca VM[2], Parera CA[3], Feresin GE[1]
[1]Instituto de Biotecnología-FI-UNSJ, Av. Libertador Gral. San Martin 1109 (O), 5400 San Juan, Argentina. CONICET. [2]Universidad Nacional de San Luis, Facultad de Química Bioquímica y Farmacia. [3]INTA- CR Mendoza-San Juan.
E-mail: victoriaparera.t@gmail.com

A prerequisite to beginning a successful phytoremediation program is the selection of adequate plant species capable to tolerate heavy metal stress. It will depend on the ability to cope with reactive oxygen species (ROS), by triggering the enzymatic and non-enzymatic antioxidant system. Fabaceae species are efficient in different phytoremediation processes. The aim was to evaluate the seedling establishment, the activity of the antioxidant enzymes, and the parameters of oxidative stress of *A. subterranea* under conditions of stress due to Cd and Hg. Plants were grown semi-hydroponically using vermiculite as substrates in solutions of different Cd (3, 4.5, and 6 ppm) and Hg (0.8, 1.2, and 1.6 ppm) concentrations and a control treatment (distilled water). Three replicates (25 seedlings) per level of treatment were tested in a growth chamber under control environmental conditions (T° 20°C day/night 12 h light/dark). Statistical analysis for enzyme activity was performed using ANOVA followed Tukey–Kramer Multiple Comparisons Test and generalized linear mixed-effects models with a binomial error distribution was used for seedling establishment. Results showed that the seedling establishment was greater than 70% in all the treatments and control. Under Hg treatments, the CAT activity (µmol H_2O_2/mg protein min) decreased as the concentrations increased, and it was significantly lower compared with the control. While the activity of APX (µmol ascorbate/mg protein min) and GSH (nMoles GSH/g PF) was similar to the control. Under Cd treatments, the CAT activity and GSH did not differ from the control. Contrarily, the APX activity was significantly higher in the 4.5 and 6 ppm treatments compared to the control. The application of HM treatments, a significant increase in membrane damage (TBARS), but no significant differences were found in the production of extracellular H_2O_2 with the control. These results suggest that the exposure of *A. subterranea* to HM induces lipid peroxidation, despite the activation of the enzymatic and non-enzymatic defense. The oxidative stress could be due to another type of ROS rather than H_2O_2. However, this species managed to establish itself and tolerate different concentrations of heavy metals and could be considered a potential species for phytoremediation.

A66

Medicago sativa L. *Var.* CW660 AND *Glomus intraradices:* GERMINATION AND RADICAL COLONIZATION UNDER SALINITY CONDITIONS

Achiary M[1], Chiroli CV[1], Zapico MG[2], Pacheco Insausti MC[1], Pedranzani HE[1]
[1]Laboratorio de Fisiología Vegetal, Facultad de Química Bioquímica y Farmacia. [2]Facultad de Ciencias Humanas. Universidad Nacional de San Luis (UNSL). E-mail: malenaachiary@gmail.com

Previous studies have shown that *Medicago sativa* L CW660 variety is sensitive to salinity. The objective of this study was to associate this sensibility with arbuscular mycorrhizae in order to study their effects on germination and root colonization under stress conditions. Seeds of *M. sativa*, var. CW660 were inoculated with 5 mL of commercial mycorrhizal solution containing *Glomus intraradices* (M) spores. Another group was the control without mycorrhizae (NM). Twenty seeds with three repetitions were sown in Petri dishes, irrigated with 5 mL of water in the control group, and solutions of 50, 100, and 200 mM NaCl in the treatment groups. They were placed in an oven at 25ºC and then germinative energy (GE) and germinative power (GP) were measured. Statistical analyzes were carried out for mycorrhizal (M) and non-mycorrhizal (NM), comparing the control group with different NaCl concentration treatments. For this purpose, a Levene's test for homogeneity of variances was performed ($P > 0.05$ in all cases), followed by an ANOVA test and next a multiple comparisons test. On the other hand, previously germinated seeds were sown in terrines with perlite/soil (1:1); before sterilization, they were separated into two lots: mycorrhized (M) and non-mycorrhized (NM). Control pots and pots treated with 100 and 200 mM NaCl were defined in each group. After 8 weeks, they were harvested, and the roots were stained and observed under an OLYMPUS CX31 microscope to identify vesicles, hyphae, and arbuscules at 40*X* and 100*X*. The GE of seeds (NM) decreased significantly at 200 mM NaCl with respect to the control and the other treatments. The M seeds showed GE and GP values higher than the NM in all treatments. In both inoculation conditions, GP at NaCl 200 mM decreased significantly with respect to all other treatments. The mycorrhization was successful and mycorrhizal vesicles and hyphae were observed in colonized plant roots, showing the same degree of infection in the control as in the salinized roots. Germination was increased in the seeds that were biostimulated.

A67

VERTICAL STRUCTURE AND DEFOLIATION FREQUENCY EFFECTS IN *Thynopirum ponticum* (Podp.) IN EARLY SUMMER (VILLA MERCEDES, SAN LUIS)

Bacha EF, Privitello MJL, Tello IM, Montenegro MA, Rodríguez MJ, Vetore OS.
Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Agropecuarias-Universidad Nacional de San Luis. Villa Mercedes. E-mail:efbacha@gmail.com

"Agropiro Alargado", *Thynopirum ponticum* (Podp.) Barkworth & Dewey, is a perennial grass with good capacity to produce in salinity conditions, which has shown phenotypic plasticity through the modification of morphogenic and forage quality parameters in response to applied defoliation regimes. The vertical structure of *T. ponticum* pasture was evaluated from distal leaf length: LFD, height of differentiated leaf sheaths: AVF, length of floral stems: LVF, and composition of structural fractions (sheaths: %VA, blades: %LA, and floral stems: %VF). The phenological state of the pasture was also determined and the percentual projected aerial cover. The effect of three frequencies of mechanical defoliation set at 30, 60, and 90 days, until December, was compared in a pasture implanted on lowland and slightly saline sector of the FICA UNSL experimental field (Villa Mercedes, San Luis). The data set was statistically analyzed by ANOVA or Kruskal Wallis test, with Infostat software. The LFD was higher in spring ($P < 0.01$), without exceeding 30 cm, with a more erect structure due to the reproductive state. While AVF, despite showing differences between dates, did not rise above 5 cm. LVF had a median of 52 cm, only generated under seasonal cuts. The %VA was low with little variation according to defoliation frequencies (2.6% and 4.5% in November, maximums close to 5.5% in reproductive stages in December). The fraction with the highest interest, %LA, was always the majority and 100% until October. In November, %LA for F30 was significantly higher ($P < 0.05$) than for F60, due to the phenological differentiation of the pasture. In December, no statistical differences ($P > 0.10$) of %LA of 70–75% are detected but considering the different accumulation periods from 30 to 90 days, the advance of reproductive structures generation is exposed. These values indicate that regardless of cutting frequency, "agropiro" maintains a high proportion of leaves and a low number of reproductive components until mid-December, at which time %VF increases regardless of defoliation frequency. In the evaluated period, aerial coverage varied between 75% and 100%. The variables evaluated reflect that for the year of study, the change to reproductive state began in November with the consequent variations in the constitutive fractions of the biomass. Although there is no marked effect of the established defoliation frequencies, seasonal cuts allow greater reproductive development of the pasture that would affect its quality (and accessibility precisely due to the characteristics of the species). In addition to the distal leaf height, it is necessary to consider in an integrated manner other variables such as the number of leaves, morphogenetic parameters, or productivity of the pasture to define the effects of the frequency of defoliation in "agropiro" pasture.

A68

AVAILABILITY AND FORAGE DISTRIBUTION OF AGROPIRO ALARGADO (*Thynopirum ponticum)* PASTURE, WITH DIFFERENT FREQUENCY OF SIMULATED DEFOLIATION

Bacha EF, Privitello MJL, Montenegro MA, Rodríguez MJ, Tello IM, Vetore OS
Departamento de Ciencias Agropecuarias. Facultad de Ingeniería y Ciencias Agropecuarias-Universidad Nacional de San Luis. Villa Mercedes.E-mail:efbacha@gmail.com

In the semi-arid region, there are few perennial alternatives to have quality forage for livestock during the winter period. *Thynopirum ponticum* (Podp.) Barkworth & Dewey, known as "agropiro alargado" or "agropiro" pasture, is a temperate species that adapts to conditions of low to moderate soil salinity, generates forage with a good balance of nutrients in the autumn–winter period, and can develop actively in spring. The frequency of defoliation can modify the expression of genetically determined growth, and it is necessary to know more about the productive behavior of this species in the area of Villa Mercedes, San Luis. Forage availability of dry matter, expressed as kg.MS.ha^{-1}, was determined, and estimated on "agropiro" pasture in the UNSL experimental field (Villa Mercedes, San Luis), subjected to different frequencies of simulated defoliation: 30, 60, and 90 days. The objective was to establish productive differences under fixed defoliation. The cut of the accumulated production and the estimates between February and December were made, simulating the desired intensity of grazing, to describe the distribution of the growth of the pasture for these frequencies. Since the data set did not present a normal distribution, it was analyzed using nonparametric statistics with Infostat software. No significant statistical differences ($P > 0.10$) were obtained for the total accumulated up to December, without effects of defoliation frequency on "agropiro" pasture forage accumulation. Forage availability varied around 1700 and 1850 kgDM.ha^{-1}. According to the accumulated for each cut-off date, the seasonal growth distribution was compared. It is observed that the lower frequency of defoliation stimulates the autumn–winter forage growth: considering the total production of the period, in the three conditions, the spring period concentrates from 70 to 90% approximately of the availability generated by the pasture. Under monthly defoliation, 20% of the total production is transferred to the autumn–winter period, while seasonal defoliations concentrate only 3% in the same period. It is necessary to continue with the evaluation of forage production in "agropiro" after December, and in order to generate adequate conclusions on pasture management, the information must be integrated with other variables (structural, morphogenetic and indicators of the perennially of the pasture). Although a good forage accumulation of the pasture is registered, the production is basically concentrated in spring, without the typical autumnal development of other environments. Pasture growth should be evaluated until the end of one or more cycles, to have more solid evidence on the distribution of growth and defoliation frequencies on forage accumulation.

A69

PREGERMINATING TREATMENT AND GERMINATION UNDER SALINITY AND HIDRIC STRESS OF BUFFEL GRASS (*Cenchrus ciliaris* L.)

Chiroli C[1], Achiary M[1], Zapico MG[3], Ruiz OM[2], Pedranzani HE[1]
[1]Laboratorio de Fisiología Vegetal. FQByF. [2]FICA. [3]FCH.PROICO 02-3318. Ciencia y Técnica. Universidad Nacional de San Luis. Ejército de los Andes 950. San Luis.E-mail: camilachiroli@gmail.com

Due to the increase in desertified areas in the province of San Luis, and the increase in infertile soils, *Cechrus ciliaris* L. (Buffel grass) is proposed as a species with adaptability and tolerance to extreme environments and as a soil improver for the recolonization of native species. The objective was to study seed dormancy and tolerance to saline and water stress. The seeds of Buffel grass cv. Texas were subjected to seven pre-germination treatments in order to select the most suitable for germination tests under stress conditions. The seven pre-treatments were: immersion in water for 24 h, potassium nitrate (KNO_3) 1000 ppm, cold stratification, mechanical and chemical scarification, hot/cold water alternation; gibberellic acid (AG3) 500 ppm. For each pretreatment, 50 seeds with four repetitions were used. For the stress study, the scarified seeds were sown and irrigated with 5 mL of water (control) and solutions of 100 mM and 200 mM NaCl (salinity treatment). Two levels of hydric stress were applied, 40% and 60% of humidity. In each treatment, 20 seeds were used, and five repetitions were made. The germinative energy (EG) was analyzed on the third day and the germinative power (PG) on the seventh day. For the statistical analysis of the pre-germinative treatments, the Mann–Whitney test ($P < 0.05$) was used, comparing each treatment with the control. For the stress study, the Kruskal–Wallis test ($P < 0.05$) and a post hoc test were applied. The results provided that mechanical scarification was the best with 40.77% germination. The GE showed a significant decrease against severe (31.5%) and mild (20%) hydric stress and severe saline stress (5%) with respect to the control (58%). PG decreased significantly only at 200 mM NaCl (20%). In conclusion, mechanical scarification was the best to eliminate physical dormancy in seeds and *C. ciliaris* showed tolerance to mild and severe water stress and mild salt stress.

A70

SALINITY EFFECTS ON *Atriplex lampa* (Moq.) D. Dietr. GERMINATION WITH DIFFERENT POTASSIUM SALTS IN SAN LUIS PROVINCE – ARGENTINA

Arce AL[1], Videla AM[2], Sosa LR[1]
[1]Laboratorio de Fisiología Vegetal. Área de Ecología. Departamento de Biología. Facultad de Química, Bioquímica, y Farmacia. Universidad Nacional de San Luis (UNSL). San Luis, Argentina. [2]Área de Biología. Departamento de Biología. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis (UNSL). San Luis, Argentina. E-mail: Arce.laurita@gmail.com

Germination response of the halophyte species *Atriplex lampa* (Moq.) D. Dietr was analyzed using K^+ salts. This study is important because the genus is widely used for degraded lands rehabilitation forage, and fuel production in arid areas, and also since it is a native species of San Luis province. In order to carry out this work, seeds were collected in the surroundings of the "El Bebedero" stream, next to national route 146 (33°43' S; 66°37' W). Before sowing to evaluate germination, experiments were carried out to determine the species' optimal threshing and scarification methodology. Seeds were exposed to different potassium monosaline (KCl, K_2SO_4) and bisaline (KCl + K_2SO_4), isosmotic solutions at 0.0 (control), −0.4, −0.8, −1.2, −1.5, 1.9, and 2.2 MPa osmotic potentials, and germination percentage and anionic effects were evaluated. As a result, KCl showed the highest germination percentage was 68% at −0.4MPa. Concerning K_2SO_4 germination was inhibited at −0.4 MPa with a 40% percentage. About potassium bisaline solution, 65% was obtained at −1.2 MPa. SO_4 anion exhibited a maximum of 40% germination obtained at −0.4 MPa, while Cl^- anion treatment led to 68% germination at −0.4 MPa. *A. lampa* germination percentages were significantly affected by solution concentration, salt type, and also the interaction between both factors. Obtained data suggest that *A. lampa* seeds are more sensitive to isoosmotic monosaline potassium solutions and the presence of Cl^- ions than other treatments, which would be affecting the germination process. In bisaline solutions, toxic effects would be mitigated by mechanisms of interaction or ionic antagonism.

ECOLOGY, ETOLOGY AND BIODIVERSITY

A71

EVALUATION OF NATIVE AND EXOTIC GRASSES IN GREEN ROOFS IN RIO CUARTO (CÓRDOBA)

Brandana AG[1], Estévez SJ[1], de Yong AD[1], Imhof L[2], Cáceres N[2], Núñez C[3]
[1]Cátedra de Espacios Verdes, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto. [2]IRNASUS (Universidad Católica de Córdoba – CONICET). [3]Cátedra de Botánica Sistemática, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto.E-mail: gbrandana@ayv.unrc.edu.ar

Cities advance in waterproofing surfaces; green roofs are an alternative to counter this situation, but they are subject to extreme conditions for vegetation. The objective was to evaluate the performance of plant communities. The trial (12 m^2) was carried out during 2020–2021, at U.N.R.C. A modular system (industrial model INPI UCC–CONICET) was used for extensive green roofs. Each treatment consisted of a community formed by a grass and creeping herbaceous (*Glandularia* × *hybrida*, *Sedum* spp., and *Phyla nodiflora*). Native grasses: *Eustachys retusa*, *Jarava ichu*, *Amelichloa caudata*, *Pappophorum pappiferum*; exotic grasses: *Pennisetum setaceum*, *Pennisetum allopecuroides*, *Pennisetum setaceum rubrum*, and control (*Sedum acre* and *Sedum confusum*). Plants were irrigated when symptoms of water stress were detected. Variables evaluated: survival, health status, and cover. Control treatment obtained the maximum cover value (85%) at 295 days after planting (DDP) and remained stable, but after the flowering period it decreased below 70%, and health status (wilting) scores also decreases. Between communities with native grasses, *Jarava Ichu* reached a maximum cover of 69.5% at 71 DDP and *Eustachys retusa* achieved maximum cover (71%) at 169 DDP, both communities maintained 100% survival values and high health status scores (healthy plants) towards the end of the trial. Between communities with exotic grasses, *Pennisetum setaceum* achieved maximum coverage (73%) at 325 DDP, and showed good performance, 90% of survival, and health status (healthy plants). In general, *Sedum* (control) takes longer to cover the surface but is more stable in coverage, health status, and survival, while communities with grasses decline in times under stress. Between grass communities, although they had similar trends among themselves, some of them showed better performance. Native grasses were *Eustachys retusa* and *Jarava ichu* and the exotic grass communities had *Pennisetum setaceum rupelli*. Despite they achieved lower coverage than the control, they reached acceptable values in less time.

A72

GRAZING OF *Artemia persimilis* PICCINELLI AND PROSDOCIMI, 1968 (CRUSTACEA, ANOSTRACA) IN A HYPERSALINE LAKE OF LA PAMPA (ARGENTINA)

Echaniz SA, Vignatti AM, Cabrera GC, Capecce C
Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa, Santa Rosa, La Pampa, Argentina.
E-mail: santiagoechaniz@exactas.unlpam.edu.ar

The anostracans of the genus *Artemia* are among the few animals that inhabit hypersaline lakes, given that they have physiological mechanisms to withstand osmotic stress. It is represented in Argentina by *Artemia franciscana,* an introduced species from North America, and *Artemia persimilis*, autochthonous and the only species that has been recorded in La Pampa. It has been indicated that *Artemia* species have an influence on ecosystems, since their grazing by water filtering, related to the size of the specimens, depresses the phytoplankton biomass and increases water transparency. Because the effect of *A. persimilis* on the lakes it inhabits is not known, the objective was to determine the filtering rates of this species in a hypersaline lake of La Pampa. Monthly samples were taken between October 2014 and September 2015 in East Lake of Parque Luro (64º17'W, 36º55'S). Water temperature and transparency were determined and samples were taken to determine the phytoplankton chlorophyll-*a*. Quantitative samples of zooplankton were taken and filtered with a 0.09-mm mesh net. The samples were anesthetized with CO_2 to avoid deformations of the specimens and were refrigerated until fixation. Specimens were removed in the laboratory, their sex and stage of development (grouped into nauplii, metanauplii, postmetanauplii, postlarvae, and adults) were determined, and their total length was determined with a Leitz micrometric eyepiece. The grazing rate (mL/day) was calculated using the formula that relates filtering to the length of the animal and to determine differences between stages, the Kruskal–Wallis test (H) was performed. Mean daily filtration rates were different between stages ($H = 9.19$; $P < 0.05$), very reduced those of nauplii (1.52 ± 0.21 mL/day) and higher in the case of postlarvae (100.99 ± 23.02 mL/day) and adults (159.99 ± 30.39 mL/day). The existence of previous information on the density of each group of stages and the approximate average volume of the lake during the study (~818000 m^3) allowed us to calculate the approximate daily filtration of the population, which varied between 202,243 m^3 in July (a quarter of the total lake volume) and 2,1514,180 m^3 in April (26 times the total volume of water in the lake), respectively. The largest volumes of water filtered coincide with the occasions in which higher densities of postlarvae and adults were recorded. The high volumes of water filtered by the population of *A. persimilis* would help explain the low concentrations of phytoplankton chlorophyll-*a* (4.84 ± 8.53 mg/m^3) in this lake.

A73

EDAPHIC CYANOBACTERIA IN *Geoffroea decorticans* (CHAÑAR) ISLETS AND NATURAL GRASSLANDS OFARGENTINA CENTRAL REGION

Ramos Irazola F[1], Manrique M[1], Fernandez Belmonte MC[1], Zitnik D[1], Denegri A[2], Sueldo R, Rauber R[3], Cendoya A[1]
[1]FICA–UNSL. [2]FTU–UNSL. [3]EEA INTA SAN LUIS
E-mail: floramos98@gmail.com

In arid and semi-arid regions, edaphic cyanobacteria presence plays an important role in ecosystems since they contribute to forming soil structure, improve the stability of aggregates, and provide the soil with atmospheric nitrogen. The objective of this work was to identify the cyanobacterial taxa in soils in the center and edge *Geoffroea decorticans* ("chañar") islets and natural grasslands of the region studied. To achieve this, samples were taken at 15 sites of four establishments, located in the region called "Dune area with grasslands and islets of *Geoffroea decorticans* (chañar)" in San Luis province, which were extracted from the first 5 cm of soil. Sample cultures were made in Petri dishes having Watanabe (1951) liquid medium and were placed in the FICA-UNSL Phycology Laboratory culture chamber under temperature (20–30ºC) and photoperiod (12 h light–12 h darkness) controlled conditions. Cyanobacteria observation and qualitative analysis were carried out using an Olympus BX 50 optical microscope, in order to measure their cellular and filamentous structures Species found classification was made: Non-Nitrogen Fixing (NF) and Nitrogen Fixing (NF), the latter in turn, in Heterocysts (HF) and Non Heterocysts (NHF). They were taxonomically determined with specific bibliography for the Cyanobacteria group. The NF genera found were *Oscillatoria*, *Phormidium*, *Synechocystis*, and *Chroococcus*, and HF genera were *Calothrix*, *Scytonema*, and *Nostoc*. The latter also appears in its NHF form. *Scytonema* genus was only found in one natural grassland site and also one of them on the islet edge. Sites corresponding to natural grassland presented greater generic diversity: 9 from NF and 6 from HF. In islet center and edge sites, 8 NF and 3 HF were found in both cases. Subsequently, the NF *Oscillatoria subbrevis* species appears in 6 natural grassland sites, in 9 edge islet sites, and in 3 sites in the islet center. An initial NF cyanobacteria growth was observed in all sites, followed by NHF and HF, such as it occurs in other studied sites. It is concluded that both arid and semi-arid environments cyanobacteria edaphic from the studied region contain various genera of NF, HF, and NHF. This group of microorganisms' importance presence lies in the fact that all of them would act effectively in the soil degradation recovery, therefore contributing to the productive systems sustainability.

A74
GERMINATION AND EARLY GROWTH PATTERNS OF *Leptochloa crinita* (LAG.) PARODI (POACEAE), UNDER DIFFERENT LEVELS OF SIMULATED PRECIPITATION

Silvestro L, Rodríguez Rivera M, Sosa L
PROICO 2-3918. Facultad de Química, Bioquímica y Farmacia - Universidad Nacional de San Luis. Argentina.
E-mail: ls10377@gmail.com

Leptoclhoa crinita is a natural component of the grasslands in San Luis province. *Leptoclhoa crinita* is interesting for the restoration activities of degraded environments due to its tolerance to defoliation, drought, and salinity. The germination and early growth processes of *L. crinita* under different levels of simulated rainfall were studied. The seeds were placed in pots with vermiculite as substrate. Each pot was irrigated with 10, 20, 40, 60, 80, and 100 mm of water (initial irrigation) in a random design. The number of germinated seeds was recorded and the germination percentage (PG) per treatment was calculated. When no more germination was recorded, the pots were watered up to field capacity to evaluate the PG of the non-germinable fraction (final irrigation). At the end of the test the length of the aerial part and root, fresh weight (FP), and dry weight (DS) of the seedlings were measured. The statistical treatment was performed by ANOVA, significant differences were found in the PG between the initial irrigation with 100, 80, and 60 mm (PG = 32, 18, and 20 %, respectively) with respect to the rest of the treatments. Length, PF, and PS of the root as aerial part were significantly higher for the 10, 20, and 60-mm treatments, and lower for 100 mm. It is concluded that the minimum amount of rainwater necessary to start the germination process in *L. crinita* seeds is 20 mm. The seeds of the fraction that did not germinate initially reach up to 52% germination when they were irrigated at field capacity. The early growth of length and weight of roots and aerial part is greater in those seedlings from initial treatments with lower levels of simulated rainfall.

A75
ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED TO LEAD ACCUMULATOR GRASS

Menoyo E[1], Flores T[2], Medero M[2], Shortrede S[2], Salazar J[3], Becerra A[3]
[1]Instituto de Matemática Aplicada San Luis (IMASL)-CONICET, Universidad Nacional de San Luis. San Luis, Argentina. [2]Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. [3]Instituto Multidisciplinario de Biología Vegetal (IMBIV)-CONICET, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba. Córdoba, Argentina.
E-mail: emenoyo@gmail.com

Microorganism–plant associations allow the establishment in harsh environments, including heavy metal contaminated areas. The studies with native organisms are very important in environmental remediation because they were adapted to local conditions, forming specific relationships that allowed to survive. The aim of this work was to study arbuscular mycorrhizal fungi (AMF) associated to the rhizosphere of *Jarava plumosa* (Spreng.) S.W.L. Jacobs & J. Everett, native lead accumulator grass. The study area is located in Bouwer, Córdoba, where the battery recycling factory is situated. The rhizosphere soil from *J. plumosa* was collected at six sites with different lead soil content (Pb: 14-2938 μg/g). The AMF spores were extracted through the decantation, wet sieving, and centrifuged in sucrose gradient. The spore density was determined under a stereomicroscope as the number of spores/100 g of dry soil. Also, the relationship between AMF density and Pb concentration in plant tissue was analyzed. The presence of AMF was determined in all study sites, density varied from 130 to 1524 spores/100 g soil, differed according to lead soil content. *Jarava plumosa* accumulated Pb in stem and root; this accumulation was greater at the site with high Pb. Bioremediations reduce the toxic effect of environmental pollutants through the use of plants and microorganisms. The application of this methodology is possible due to ability of some organisms to survive in contaminated soils. In environments highly contaminated with Pb, AMF-grass associations could allow the development of this plant community. Finally, highlight the importance of studies and applications of native species in restoration practices as determinant factor in local biodiversity reclamation.

A76
FECES PROCESSING TECHNIQUES FOR STUDY OF DIET THE OMNIVOROUS RODENT *Oxymycterus rufus* (RODENTIA-CRICETIDAE)

Lemanich Funes GO[1], Nuñez MB[2], Jofré LE[1]
[1]Área de Zoología, Universidad Nacional de San Luis (UNSL). [2]Área de Biología, Universidad Nacional de San Luis (UNSL). E-mail: georlemanich31@gmail.com

Studies of the diet of small mammals using their feces are particularly complex. On the one hand, due to the small sample size and, on the other hand, depending on the type of diet, it is sometimes necessary to combine macroscopic and microscopic techniques to analyze the nature of the food consumed. This study aimed to process the feces of the

omnivorous small mammal *Oxymycterus rufus*, using a combination of macroscopic and microscopic techniques to determine the presence of the different food components. Feces from 13 individuals of *O. rufus*, collected in La Florida, San Luis, were used. To determine the composition of the diet, the microhistological technique of Dacar & Giagnnoni (2001) was carried out with adaptations that included the separation of the sample to make macroscopic observations under a binocular loupe to determine the presence of arthropod fragments in the stool. The quantification of the remains found was carried out by applying the Relative Appearance Frequency (RF%) technique of the different trophic categories, with the Appearance Frequency being the number of feces containing a given item divided by the total number of feces analyzed by 100. The results obtained showed fragments of the Insecta Class with an FR% of 100% of the samples, observing remains of tegument, legs, and mandibles of insects. In the case of plant fragments, the RF% was 100%, observing fragments of the monocotyledonous and dicotyledonous epidermis and pollen grains. There are few dietary studies carried out on this species, but what was obtained in this work coincides with the results of other researchers. The little number of samples and their small size adds relevance to the need to fine-tune a highly efficient technique that makes it possible to take advantage of scarce material. For this work, the fact that there is a coincidence with other investigations is of the utmost importance to support the technique put to the test.

A77
ASSESSMENT OF THE TOTAL ENVIRONMENTAL IMPACT OF AGROCHEMICALS ON CORN AND SOYBEAN CROPS IN THE EL MORRO BASIN (SAN LUIS, ARGENTINA)

Martínez Espeche ME, Garbero MM
Facultad de Ingeniería y Ciencias Agropecuarias, Universidad Nacional de San Luis. Ruta Provincial N° 55 S/N extremo Norte, Villa Mercedes, San Luis. E-mail: mmartinezespeche@gmail.com

The expansion of agriculture in the "El Morro" basin has increased the use of pesticides associated mainly with direct seeding. The impacts of agricultural activities are usually associated with the use of pesticides. In the "El Morro" Basin, the crops of corn (*Zea mays* L) and soybeans (*Glycine max* L. Merr.) represent 98.06% of the Agricultural area. To obtain a global vision of agrochemical pollution in the basin, we worked with a scale that allows us to qualify the different characteristics of each substance in relation to various factors. They were (i) ecotoxicity, (ii) toxicity in humans, (iii) impact on environmental factors, and (iv) environmental aspects of the agrochemical. These characteristics were reflected in a qualitative matrix of Environmental Impact assessment with which the different substances were categorized according to their Total Environmental Impact (TWI) into: (i) Very High, (ii) High, (iii) Medium, (iv) Low, and (v) Very Low. In order to assess each attribute, the safety sheet of each chemical product (SENASA) and the Chamber of Agricultural Health and Fertilizers (CASAFE) were used. The most commonly used molecules in these systems correspond to Glyphosate, 2,4-D, Picloram, Dicamba, metolachlor, atrazine and flumioxazin, where Glyphosate, 2,4-D and Dicamba reached medium impact values, picloram low impact values and metolachlor, atrazine and flumioxazin reached very low impact values. However, 30 molecules are used in this basin in agricultural systems, which are below 5% of use. The repeated use of molecules with medium impact values could harm the sustainability of the agricultural systems that grow corn and soybean crops.

A78
CYANOBACTERIA PRESENT IN BIOLOGYCAL CRUSTS (BCs) AND THEIR RELATIONSHIP WITH INFILTRATION IN SOILS AFFECTED BY FOREST FIRES

Denegri A[1], Fernandez Belmonte C[2] Sueldo R[2], Zitnik D[2], Manrique M[2], Campitelli P[3]
[1]Facultad de Turismo y Urbanismo – UNSL. [2]Facultad de Ingeniería y Ciencias Agropecuarias – UNSL. [3]Facultad de Ciencias Agropecuarias – UNC.E-mail: andreadenegri211@gmail.com

One of the erosive effects with the greatest impact on soils affected by forest fires is caused by the generation of hydrophobicity and decreased infiltration capacity, a situation that worsens in soils with a weak structure and steep slopes. The infiltration rate serves to evaluate the impact of fires in terms of susceptibility to be eroded post-fire. The Cyanobacteria present in the biological crusts (BCs) play, among other roles, the fixation of particles and water retention, being able to reduce the risks of post-fire soil loss. The objective of this research was to analyze differences in infiltration in three soils affected by fires in the mountain areas of the central region of Argentina and to establish a relationship between them and the presence of different genera of Cyanobacteria present in the BCs of these soils. BCs samples were taken three days after the fire; they were cultivated in a specific medium and were observed under an optical microscope to identify the Cyanobacteria present, and later, classify them into fixers (heterocyst and non-heterocyst) and non-fixers. Infiltration tests were performed by the simple ring method. The three sites showed four common genera of Cyanobacteria, *Nostoc* and *Nodularia* (fixative), and *Oscillatoria* and *Phormidium* (non-fixative). In typical haplustol soils and high-intensity fires, the fixers decreased by 6.5% and the non-fixers by 23%; the infiltration rate was 10.3 mm/min in burned soil versus 18.2 mm/min in unburned soil. In soils classified as entisols affected by fire and by light-

intensity fires, fixatives showed an increase of 5.3% and non-fixatives decreased by 3.6%. The difference in infiltration rate was from 4.4 mm/min in control soil to 10.2 mm/min in fire-affected soil. These results allow us to conclude that fires affect the infiltration rate of the soils under analysis; this depends on the type of soil, the intensity of fire, as well as the condition of fixative and non-fixative cyanobacteria present in the BCs.

A79
USE OF BIODIVERSITY DATABASES AND PROPOSED COLLABORATIVE APPROACH AS TOOLS FOR THE STUDY OF EXOTIC ARTHROPODS IN CUYO

Verteramo GR[1], Ripa RR[2,3], Lapadula WJ[4], Werenkraut V[2]
[1]Facultad de Química, Bioquímica y Farmacia (UNSL). [2]Instituto de Investigaciones en Biodiversidad y Medioambiente (CONICET–UNComa). [3]Instituto de Evolución, Ecología Histórica y Ambiente (CONICET). [4]Instituto Multidisciplinario de Investigaciones Biológicas de San Luis (CONICET). E-mail: ilgino15@gmail.com

Invasive alien species represent a growing concern due to the environmental and economic problems they cause. Although arthropods represent a large part of the world's exotic fauna, they have received less attention than other taxa, leading to a lack of knowledge of the number and identity of introduced species in many regions. Here we used biodiversity databases to compile and update the existing information about Exotic Arthropods (EA) for Cuyo. We found a high number of AE recorded for the region, with 41 species belonging to three classes: Insecta (27 spp), Arachnida (12 spp), and Malacostraca (2 spp). This constitutes approximately 53% of the 75 species of AE reported in databases for Argentina. In the national context, Cuyo has more species reported than NOA (33) and NEA (31) regions and fewer species reported than Centro (58) and Patagonia (50) regions. Considering that many of the other species of AE in Argentina are present in localities neighboring Cuyo, they could be present in the region and still not have been detected. Within the region, the greatest number of species was detected in the province of Mendoza (39 sp.) followed by San Luis (16 spp), San Juan (15 spp), and La Rioja (14 spp). By using these databases, we improved our knowledge of introduced biodiversity in Cuyo and detected provinces and groups that were under-studied. The differences found in the number of AE could be due to different factors that affect invasiveness such as: economic development, commerce, agriculture, competition with local species, etc. However, the low number of EA in certain provinces and regions could also be due to the absence of specialists conducting invasion ecology studies on arthropods. Thus, we do not know if the observed patterns are real or if they are due to data absence. Focusing on the use of methodologies such as "horizon scanning" and considering that we do not have a reliable list of exotic species for the region, we propose to enrich the knowledge about the presence of AE in Cuyo, through the coordination of a collaborative assessment with specialists of different arthropods groups.

A80
CONTRIBUTION TO THE KNOWLEDGE OF AN URBAN LAKE PHYTOPLANCTON

Quaife ES[2], Daruich GJ[1,2], Ray AM[3], Algozino C[2], Muñoz Carbajal D[2], Almeida C[2], Gonzalez P[2]
[1]Área de Biología, [2]Área de Química; Universidad Nacional de San Luis. [3]Facultad de Filosofía Humanidades y Artes (FFHA). Instituto de Ciencias Básica (ICB) Universidad Nacional de San Juan.E-mail: elbasquaife@gmail.com

Algae are widely present in freshwater environments, such as streams, lakes, and rivers. Although relatively inconspicuous, they have major importance in the freshwater environment, in ecology terms, and in relation to human use of natural resources. Phytoplankton is an important primary producer; it is the basis of the whole autotrophic food web in the aquatic ecosystem. This work is part of an overall Project that includes the study of the algal communities in aquatic ecosystem dynamics in the Cuyo region. In this first stage, we analyze the algal and cyanobacterial communities of samples collected during the 2022 winter season. The study area is located in "Parque de Mayo", in the urban lake "Parque de Mayo" of San Juan province. The methodology used in the collection followed the standard parameters for lentic environments. Samples were collected with phytoplankton net opening of 10-µm mesh. Physico-chemical variables (T°C, pH, conductivity, nitrites, nitrates, dissolved oxygen, among others) were obtained using portable sensors and subsequent laboratory techniques. These parameters provided an autecological characterization of the species studied. Identification was performed using an optical microscope with 400× and 1000× magnification. Preliminary results show 24 taxa, of which 50% corresponds to Chlorophyta (Green algae), 37 % Bacillariophyta (diatoms), and 12 % Cyanobacteria. Low diatom diversity, presence of *Nitzschia* and *Crucigenia* microalgae resistant to eutrophication processes, evidenced urbanization impact. Microscopic analyses of water samples provide information on the algal species diversity and density which could potentially be useful as early warning signs of deteriorating conditions.

A81
STIPEAE SPECIES IDENTIFICATION KEY OF SAN LUIS (ARGENTINA) BASED ON VEGETATIVE AND REPRODUCTIVE CHARACTERS

Mercado SE[1], Prina, A[3], Aostri CA[1], Genovese C[2], Pérez D[1], Salguero A[1]
[1]Facultad Ingeniería y Ciencias Agropecuarias. Universidad Nacional de San Luis. [2]Facultad de Turismo y Urbanismo, Universidad Nacional de San Luis. [3]Facultad de Agronomía. Universidad Nacional de La Pampa
Fac de Ing. y Cs. Agropecuarias. UNSL, 5730 San Luis.E-mail: seocampo@email.unsl.edu.ar

The tribe Stipeae Martinov is distributed in temperate to warm-temperate grasslands of the world (America, Eurasia, and Australia) and rarely in tropics. Stipeae are perennial C_3 herbs with 1 floret spikelets, without rachilla extension and a single awned lemma, specialized for anemochory. Due to recent molecular and morphological studies carried out by several authors, *Stipa* s.l. genus was segregated into several American genera, considering *Stipa* only an EuroAsian genus. To date, the tribe under study comprises 9 genera present in Argentina: *Aciachne*, *Amelichloa*, *Anatherostipa*, *Jarava*, *Nassella*, *Ortachne*, *Pappostipa*, *Piptatherum*, and *Piptochaetium.* The aim of this work was to build a genera and species identification key based on morphological characters easily to recognize in laboratory or in the field. Considering the importance of proper identification, herbaria plant collections were checked, and new wild specimens were collected. The material was identified by traditional botanical methods, then kept in the herbaria Ciencias Agropecuarias (VMA) and EEA INTA San Luis (VMSL). As a result, to date 26 botanical entities distributed in 5 genera were identified: *Amelichloa ambigua*, *Amelichloa brachychaeta*, *Jarava ichu*, *Jarava juncoides*, *Jarava plumosa*, *Jarava pseudoichu*, *Pappostipa vaginata*, *Piptochaetium medium*, *Piptochaetium montevidense*, *Piptochaetium napostaense*, *Piptochaetium ruprechtianum*, *Piptochaetium stipioides*, *Piptochaetium stipioides* var. *echinulatus*, *Nassella longiglumis*, *Nassella cordobensis*, *Nassella filiculmis*, *Nassella hunzikeri*, *Nassella hyalina*, *Nassella megapotamia*, *Nassella neesiana*, *Nassella nidulans*, *Nassella niduloides*, *Nassella poeppigiana*, .*Nassella pseupampagrandensis*, *Nassella sanluisensis*, *Nassella tenuis*, *Nassella tenuissima*, *Nassella trichotoma*. So far 28 species distributed in 5 genera of this tribe have been identified.

A82
GERMINATION TRIALS OF FOUR NATIVE SPECIES FROM THE UPPER MENDOZA RIVER BASIN WITH POTENTIAL USE IN ECOLOGICAL RESTORATION PROGRAMS

Frete JF[1], Duplancic MA[1,2], Ontivero M[1,3], Bonjour L[1,3]
[1]Grupo de Investigación Geobotánica y Fitogeografía – IADIZA. [2]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo. [3]CCT CONICET–Mendoza.E-mail: aduplancic@gm.fcen.uncu.edu.ar

In July 2019, nearly 8000 ha of the Mendoza River basin in Potrerillos suffered severe degradation by fire. SumáNativas is a collaborative project developed in the area, as an initiative coordinated between public and private sectors, scientific agencies, and organizations dedicated to natural environment and biodiversity conservation, with the aim to restore damaged areas by revegetating them with native plants, favoring natural regeneration. To achieve this, it is necessary to have high numbers of seedlings multiplied in nursery. Four native species' germination capacity was evaluated under nursery conditions: *Chuquira garuscifolia, Berberis empetrifolia, Dysphania ambrosioide*s, and *Tetraglochin alatum*, through the application of pre-germinative treatments. Tests were carried out in a Petri dish with filter paper kept permanently saturated with drinking water. Three replicates of 30 seeds were made for each treatment and their controls. Germination counts were made daily for 45 days. The number of germinated seeds was determined, and the following indexes were calculated: mean germination percentage (%G), mean germination time (MT, mean time in days to reach maximum germination of the seed lot), and the germination time coefficient of variation (CVt) as a synchrony measurement. Pretreatment application improved germinative parameters. In *C. ruscifolia* the application of a fungicide solution increased %G, both in *B. empetrifolia* stratification in refrigerator and in *D. ambrosioides* imbibition, decreased MT. While in the latter the highest germination percentage was in the control, without pretreatment, in *T. alatum* mechanical scarification allowed germination, being null in the control, and else scarified seeds incubation at a higher temperature decreased MT and increased synchrony. The maximum mean percentages reached with the best treatment for each species were: *Chuquira garuscifolia* 75.67% (SD 11.87), *Berberis empetrifolia* 70.00% (SD 6.67), *Dysphania ambrosioide*s 95.56% (SD 5.09), and *Tetraglochin alatum* 54.44% (SD 13.47). Different seed species have their own attributes that condition germination, seedling establishment, and therefore their possibility of being used in restoration. Each species, depending on these attributes, responded differently to pre-germinative treatments. Those that optimize each species' germination parameters were detected, to facilitate their production in the nursery. Likewise, all the proposed pre-treatments can be carried out in a simple way and without expensive resources, expected to be used by the community nursery in the project area, assuming that knowledge transfer is effective and efficient.

PHARMACOLOGY AND TOXICOLOGY

A83
GALANTAMINE AND DONEPEZIL COMBINATION AS CHOLINESTERASES INHIBITORS IN ALZHEIMER'S DISEASE THERAPY

Adarvez-Feresin C[1], Zaragoza-Puchol D[2], Parravicini O[1], Garro A[1], Enriz D[1], Feresin GE[2]
[1]IMIBIO-SL CONICET, Facultad de Química, Bioquímica y Farmacia, UNSL, Ejército de Los Andes 950, San Luis, Argentina, CP: 5700. [2]Instituto de Biotecnología. UNSJ, CCT CONICET SAN JUAN. Av. Lib. Gral. San Martín 1109(O), San Juan, Argentina, CP: 5400.E-mail: camilaadarvezferesin@gmail.com

Alzheimer's disease (AD) is the most common cause of dementia affecting the elderly population. Current drug-based treatments that may temporarily ease symptoms or slow down the progression consist of cholinesterase inhibitors (iChE) designed to increase acetylcholine levels to maintain the cholinergic signal. There are six treatment FDA-approved: iChE including galantamine (GAL), donepezil (DON), and rivastigmine; N-methyl-D-aspartate antagonists, donepezil–memantine combination therapy; and recently approved monoclonal antibodies (aducanumab). The pharmacological interaction between a combination of drugs results in synergistic (increases), antagonist (decreases), or indifferent effects. This study aimed to perform a kinetic analysis study to determine (1) the *in vitro* interaction of the GAL (a natural product of Amaryllidaceae) and DON (synthetic drug) combination against AChE and BuChE cholinesterases, and (2) to corroborate these results by a molecular modeling study employing docking techniques, molecular dynamics simulations, and QTAIM (Quantum Theory of Atoms in Molecules) studies. The results from the kinetic plots showed that when GAL and DON are combined over a range of concentrations around the IC_{50}, the best inhibition of AChE occurred with the mixture containing the highest concentration of GAL and the lowest of DON. Evidence from molecular modeling indicated the existence of co-occupancy of the ligands in both enzymes. In the AChE result it was important that GAL must first occupy the active site before DON. Moreover, it is important to highlight that in BuChE, the two ligands are easily located due to the larger size of the active site of this enzyme, obtaining six possible poses. On the other hand, from the simulations of the complexes for AChE, three possible poses were shown. The present results may indicate that the combined use of GAL and DON may represent a starting point to reduce the dose and avoid hepatotoxic and gastrointestinal side effects. It also lays the foundation for further studies on the interaction of other compounds isolated from natural sources that inhibit cholinesterase.

A84
Prosopanche americana: STUDY OF ACUTE TOXICITY IN MICE

Rodríguez S[1], Paredes J[1], Barcia CS[2], Petenatti E[3], Wendel G[1]
[1]Farmacología, [2]Bromatología, [3]Farmacognosia, Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. E-mail: jdparedes@unsl.edu.ar

Prosopanche americana (R. Br.) Baillon (Family Hydnoraceae), commonly known as "guaycurú santiagueño", "huaycurú", "huáchar", "guacharo", "flor de fierro", "flor de la tierra" o "espinazo de lagarto". This plant is a perennial hemiparasitic plant distributed in Argentina (Córdoba, Santiago del Estero, Mendoza, La Rioja, San Luis). The rhizomes are used in folk medicine as vulnerary, homeostatic, expectorant, and cardiac disorders. Infusion of the rhizome (Del Vitto LA & EM Petenatti 560, UNSL) was prepared, separated by filtration, and the aqueous extract was concentrated and lyophilized to preserve it. The *P. americana* lyophilized extract (PALE) was studied for acute toxicity, as per revised OECD guidelines Nº 423: Acute Toxic Class Method. Albino mice (20–25 g) of both sexes were randomly divided into five groups of six animals each (3 males and 3 females). Mice were fasted for 4 h and given oral increasing doses of PALE (5, 50, 300, and 2000 mg/kg, respectively). The fifth group served as control and was treated only the vehicle (distilled water). Animals were observed daily for 14 days. The parameters studied were weight and macroscopic analysis of the vital organs: heart, lungs, liver, spleen, and kidneys. The Irwin observation test was used to evaluate the effects of PALE on behavior and physiological function. The oral doses of 2000 mg/kg of PALE did not produce any sign of acute toxicity in the animals (male and female). Over the 14 days following the oral administration of PALE, none of the animals died, and no significant changes in organ weight were observed through the end of this period ($P > 0.05$). No gross lesions were noted in any mice on necropsy. There were no signs on symptoms of ataxia, catalepsy, excess curiosity, scratching, restlessness, respiratory distress, urination, diarrhea, convulsions, and coma. Oral doses of PALE up to 2000 mg/kg produced no mortality and visible signs of delayed toxicity 14 days post-treatment. These results ensured the continuance of pharmacological studies on this species using the oral route and motivated us to proceed with the biological assays. The highest dose did not induce noticeable signs of toxicity. In conclusion, under the present experimental conditions, PALE had not presented signs of toxicity.

A85
EVALUATION OF GASTROPROTECTIVE EFFECT OF *Jodina rhombifolia* (Hook. & Arn.) Reissek

Duarte SR[1], Paredes JD[2], Teves MR[2], Wendel GH[2]
[1]Enfermería, Facultad de Ciencias de la Salud. [2]Farmacología, Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. San Luis, Argentina.E-mail: gwendel@unsl.edu.ar

Jodina rhombifolia (Hook. & Arn.) Reissek (Santalaceae) is a small perennial hemiparasitic tree, popularly known as "peje", "quebrachillo", "quebracho flojo", "sangre de toro", "sombra de toro". This species is utilized in Argentine folk medicine for a great diversity of health problems such as anti-alcoholic, digestive/stomach, anti-ulcer, gastritis, antidiarrheic, anti-inflammatory, hepatoprotective, and hypotensive, among others. The major chemical constituents of the leaves of *J. rhombifolia* are phenolic compounds, organic acids, tannins, flavonoids, steroids, gums, and mucilage; the extract of its leaves revealed the presence of C-glycosyl flavonoids. The aim of this study was to assess the gastroprotective effect of the *J. rhombifolia* leaves lyophilized aqueous extract (JRLE) on experimental ulcers and mechanisms in rats. The medicinal plant was collected in the "Los Chañares" establishment, in the Fraga locality, San Luis Province. Infusion to 10% was prepared following the methodology outlined in the VI Ed Argentine National Pharmacopoeia and then lyophilized to preserve it. The JRLE was redissolved in distilled water just before oral administration. In all protocols (Approved protocol Nº F-348/20, F-357/21, and F-360/21), Wistar rats (180–200 g, N = 6–8) fasted 24 h prior to treatments. JRLE was investigated by using various *in vivo* ulcer models. The stomachs were removed and inspected for lesions in the glandular portion. A scanner examined the specimens, and the scanned images were analyzed by using a program developed by the National Institutes of Health (ImageJ 1.46r). The role of prostaglandins (PG), sulfhydryl groups (SH), and nitric oxide (NO) were evaluated. Oral pretreatment with JRLE (250, 500, and 1000 mg/kg) produced a significant decrease in the intensity of gastric mucosal damage induced by ethanol ($P < 0.001$ *vs*. ethanol); damage inhibition (%): 44.08, 59.28, and 90.41 (dose-dependent). Moreover, JRLE prevented damage induced by other ulcerogenic agents: HCl 0.6 N, NaCl 25% and NaOH 0.2 N ($P < 0.001$ *vs*. controls). The pretreatment with the SH blocker NEM (N-ethylmaleimide, s.c., 10 mg/kg) did not reduce the mucosal protection observed with JRLE treatment. These findings suggest that endogenous SH is not involved in the protective effect of JRLE. The inhibitory effect of JRLE on ethanol-induced ulcerogenesis continued even after the inhibition of NO following pretreatment with NO synthase inhibitor (L-NNA, i.p., 40 mg/kg), The antiulcerogenic JRLE protection was only partially blocked by pretreatment with indomethacin (inhibitor of PG, i.p., 10 mg/kg), ($P < 0.05$ *vs*. control). Present findings suggest that JRLE gastric protection depends, at least partially, on a possible mechanism related to the modulation of endogenous PG. Several flavonoids are reported for their gastroprotective activities. These compounds could be responsible for gastroprotective activity. These results contribute to the scientific validation of the digestive, anti-ulcer, and gastritis indications of this botanic species in Argentine folk medicine.

A86
DIURETIC EFFECT OF *Jungia polita* IN RATS

Cabello F[1], Contreras Fernández M, Moyano F[1], Príncipe V[2], Petenatti EM[2], Wendel GH[1]
[1]Farmacología. [2]Farmacognosia. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. Chacabuco y Pedernera. 5700. San Luis. E-mail: mfmoyano07@gmail.com

Jungia polita Griseb. (Asteraceae–Multisieae) is popularly known as "zarzaparrilla" or "viña". This shrub species is used in Argentine folk medicine as a diuretic, anti-sclerotic, hypotensive, for skin affections; antihyperlipidemic, bradycardic, and depurative. Infusion (10%) of the aerial parts (Del Vitto LA & EM Petenatti s.n., III-2007 (UNSL # 835)) was prepared, separated by filtration, and the aqueous extract was concentrated and lyophilized to preserve it. Oral administration of *Jungia polita* up to 2 g/kg produced no mortality and visible signs of delayed toxicity 14 days post-treatment. This study was designed to determine the diuretic activity of the *J. polita* lyophilized extract (JPLE). The test was performed as described by Lipschitz *et al*. The experiments were approved by the local Committee CICUA (Protocol F-405/22). Wistar rats (150–180 g) were employed. The animals, randomly assigned into groups (N = 6–8), were deprived of food for 18 h before starting the experiments and had free access to water. The test groups were administered with different doses of JPLE (250 or 500 mg/kg, orally). The reference group received Furosemide (10 mg/kg, intra-peritoneal). The control group received only the vehicle (50 mL/kg, orally). Immediately after administration, the rats were paired and placed in metabolism cages. At the end of the experiments, the animals were euthanized by inhalation of carbon dioxide. Urinary volumetric excretion (UVE) and urine chemical parameters were measured in 3-h diuresis. All values were expressed as the mean ± SEM. Graph Pad Prism was used for the statistical analysis and *P*-values less than 0.05 were considered statistically significant. Student's *t*-test was performed to evaluate the differences between the control and the experimental samples for each time point. The lot treated with JPLE (500 mg/kg) showed diuretic activity between 45 min (UVE: 20.06 ± 8.04 *vs*. control: 4.84 ± 1.69; $P < 0.01$) and 180 min (UVE: 82.95 ± 8.92 *vs*. control: 52.74 ± 6.03; $P < 0.001$). The urine samples presented normal chemical parameters in all the cases: urinary density and pH were similar to controls. The data reported in this work indicate that the infusion of *J. polita* showed diuretic activity

(0.59), compared to furosemide, a potent loop diuretic. This diuretic activity could be due, in part, to the presence of flavonoids in this plant. Further investigations are necessary prior to their recommendation for use as a diuretic.

A87
SUB-LETHAL EFFECTS OF A GLYPHOSATE-BASED HERBICIDE ON *Daphnia spinulata* Birabén, 1917 AT DIFFERENT SALINITIES

Lares BA[1], Vignatti A[1], Echaniz SA[1], Cabrera GC[1], Giacomino V[1], Cora Jofré F[1], Gutiérrez MF[2]
[1]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa, Santa Rosa, La Pampa, Argentina. [2]Instituto Nacional de Limnología, CONICET-UNL, Paraje El Pozo, Ciudad Universitaria UNL, Santa Fe, Argentina. E-mail: aliciavignatti@exactas.unlpam.edu.ar

Cereal and oilseed production is one of the most important economic activities in the Argentine Pampas region and it involves the use of large amounts of glyphosate (N-(phosphonomethyl) glycine) for crop protection. Part of the residues of this herbicide can reach nearby water bodies and put non-target aquatic organisms at risk, including *Daphnia spinulata* (suborder Cladocera), an endemic species of shallow, subsaline lakes in the region. The present study's objective was to evaluate the effects of an herbicide (Panzer Gold®) on the life cycle parameters of this species. Two treatments were carried out, with 0.11 mg/L (T_1) and 3.75 mg/L (T_2) of herbicide in two concentrations of salts, 1 g/L (S_1) and 2 g/L (S_2), plus their respective controls. Neonates less than 24-h-old were placed individually in containers with 25 mL of solution and kept until death under a light/darkness regime of 16:8 and a temperature of 22 ± 1 °C. Molts and offspring were measured and removed every 48 h; the medium was changed, and the specimens were fed with *Chlorella vulgaris*. Significant differences ($P < 0.05$) were registered between both saline concentrations for every measured parameter, indicating the lower performance of this species at higher levels of salinity. For S_1, significant differences ($P < 0.05$) in longevity were seen between the control (mean = 17 days) and both treatments (mean = 14 days). Likewise, for S_2, longevity was significantly higher ($P < 0.05$) in the control (mean = 13 days) compared to both treatments (mean = 8 days). For S_1, the mean number of offspring in the control solution was 10 individuals, and no difference was seen in the treatments. For S_2 however, a mean of 8 individuals was recorded in the control solution compared to only 2 individuals in T_1. This demonstrates an increase in the effect of the herbicide at higher levels of salinity. Although the number and size of the molts were higher in both controls compared to the treatments, the differences were not statistically significant. Large-sized filter-feeding cladocerans, such as *D. spinulata*, play a key role in water bodies by depressing phytoplankton biomass and increasing water transparency. Therefore, the decrease in the size of its populations due to glyphosate-based herbicides could alter the characteristics of the ecosystems it inhabits.

A88
GASTRIC CYTOPROTECTIVE ACTIVITY OF ACACIAIN PEPTIDASE IN RATS

Villegas Gabutti C[1], Quiroga GH[2], Paredes J[1], Barberis S[2,3], Wendel G[1]*
[1]Laboratorio de Farmacología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina. [2]Laboratorio de Control de Calidad y Desarrollo de Bromatología, Universidad Nacional de San Luis, San Luis, Argentina. [3]Instituto de Física Aplicada (INFAP) - Centro Científico Tecnológico (CCT) San Luis - CONICET, San Luis, Argentina.E-mail: cmville@gmail.com

Acacia caven (Mol.) Molina is an arid and semi-arid area native tree of South America, which belongs to the Mimosaceae (Leguminosae) family. It is a very widespread species in Argentina, Chile, Bolivia, Uruguay, Paraguay, and Brazil. Non-wood forest products, defined as "goods of biological origin other than wood, coming from forests", have experienced a sustained increase in their consumption in recent decades, especially foods and medicinal species, valued for their condition as goods of wild, natural and/or organic origin, with a significant impact on health and in the context of healthy eating. The objective of this work was to evaluate the anti-ulcerogenic effect in rats and the role of prostaglandins in this effect of the purified proteolytic extract of *Acacia caven* (Mol.) Molina pollen. The crude extract and the purified proteolytic fraction (acaciain peptidase) of *A. caven* pollen were obtained according to Barcia et al. (2019), using an FPLC unit (Akta Prime Plus, General Electric), and concentration and vacuum drying (SpeedVac™ Vacuum Concentrator SPD1030/2030). The anti-ulcerogenic effect of acaciain peptidase was evaluated using Wistar rats (180–200 g; N = 6–8) of both sexes and fasting for 24 h. The rats were grouped into an ulcer control group that was administered 1 mL of the necrotizing agent (absolute ethanol) and experimental groups that were administered the extract (16.2, 32.5, and 65 mg/kg) 60 min before absolute ethanol. Indomethacin (10 mg/kg, s.c.) was used to evaluate the role of prostaglandins (PG). The animals were euthanized with CO_2 after 60 min of the necrotizing agent administration. The stomach scanned images were analyzed using ImageJ software (NIH). Ethanol caused gastric ulcers in all animals, while pretreatment with the extract at doses of 32.5 and 65 mg/kg prevented the formation of gastric lesions induced by ethanol ($P < 0.001$ *vs.* ethanol). The damage inhibition percentages were: 61.1% and 82.1% for the 32.5 and 65 mg/kg doses, respectively. The effect elicited by the lyophilized extract at 65 mg/kg was not attenuated by pre-treatment with indomethacin (10 mg/kg, s.c.), a prostaglandin synthesis inhibitor. Further assays will be carried out to suggest its use as

a new food or pharmaceutical ingredient. These results suggest that the gastroprotective mechanism of action of acaciain peptidase does not involve prostaglandins at the dose assayed.

A89
SPECIATION ANALYSIS OF ADSORBED ATMOSPHERIC ARSENIC IN POLLEN AND AEROBIOLOGICAL SAMPLES BEFORE AND DURING THE COVID-19 PANDEMIC

Escudero DR[1], Isaguirre A[1], Moyano F[1,2], Fourcade G[2], Hasuoka P[3], Pacheco P[3], Moglia MM[1]
[1]Facultad de Química, Bioquímica y Farmacia- Universidad Nacional de San Luis, Argentina. [2]Hospital San Luis, San Luis, Argentina.[3]Instituto de Química de San Luis (INQUISAL-CONICET-Universidad Nacional de San Luis), Argentina.E-mail: danielarocioescudero@gmail.com

Arsenic (As) is a toxic element for humans, which can be released into the environment naturally or by anthropogenic activities. Pollen grains have the ability to adsorb and transport heavy metals such as As; these have become indicators for contamination by As. The toxicity of As depends on the chemical form in which it is present, the inorganic form being more toxic than the organic forms. Therefore, to assess the risk of exposure to As, the individual concentrations of each species must be determined rather than a total elemental analysis. The objective of this research is to evaluate the effects of vehicular traffic on the concentration of As in the atmosphere, for this aerobiological samples were collected in pre-pandemic and pandemic periods in the city of San Luis, Argentina, in the vicinity of the San Luis Regional Hospital. Subsequently, total As was determined in the mentioned samples. Similarly, for a more comprehensive study, As speciation was performed on fresh pollen samples from *Vachellia caven*. Firstly, an elution of atmospheric As species adsorbed on pollen samples was performed, which was optimized by using 1% v/v acetone, 15 min of ultrasonic bath, and centrifugation. The eluted As species were determined by LC-ICP MS, with As (III) and As (V) concentrations between 0.08 and 0.62 µg/g and 0.33 and 0.89 µg/g, respectively; these concentrations correspond to the year 2021. Analyzing 0.05 g of pollen, the method reaches a detection limit and quantification limit of 0.01 and 0.04 µg/g for As (III); and 0.01 and 0.06 µg/g for As (V), respectively. A microscopic analysis of the aerobiological samples was also carried out in order to determine the most abundant pollen species in the periods studied, which was identified as Chenopodiaceae-Amaranthaceae, with a concentration of 38 grains of pollen m^{-3} of air in 2019 and 36 grains of pollen m^{-3} of air in 2021. The concentration of this type of pollen, in the years studied, is in the moderate category of the Manual of the Spanish Network of Aerobiology. In addition, other types of pollen were recorded during these periods, such as Poaceae, *Plantago, Artemisia,* and other Asteraceae, but in the "low" categories established by REA. The results obtained in this study showed significant variations in the concentration of total As, in aerobiological samples, which suggests that traffic increases the concentration of atmospheric As.

A90
EVALUATION OF ACUTE ORAL TOXICITY OF *Kosakonia radicincitans* IN RATS

Possetto P, Calvo J, Sansone G, Calvente V, Teves MR, Wendel GH
Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis.E-mail: papossetto@unsl.edu.ar

Several species of the *Kosakonia* genus act as beneficial microorganisms that growth-promote and carry out biological control of pests in plants. Inside this genus, *K. radicincitans* has been reported as a growth-promoting of radish, tomato, and maize plants. In addition, we have reported on the ability of this bacterium to promote the growth of lettuce plants (*Lactuca sativa* L.) under greenhouse conditions and biological control agent of pathogenic fungi. Regarding the interaction of this bacterium with humans, there are bibliographic records that indicate, to date, only two case reports (United States 2016 and Austria 2020) where some strain of *K. radicincitans* acted as a human pathogen. The objective of this work was to evaluate of acute oral toxicity of *K. radicincitans* in rats, for providing data about the toxicity at the preclinical level of this new growth-promoting agent. Wistar rats of both sexes (180–200 g) from the Central Bioterio of the National University of San Luis were used. The potential acute toxicity of the native strain *K. radicincitans* was tested in accordance with the provisions of guideline No. 423 of the Organization for Economic Co-operation and Development. A negative control group (saline solution) and an experimental group for each of the doses of *K. radicincitans* [10^6, 10^8, 10^{10}, and 10^{12} CFUs (Colony Forming Units)/kg weight of animal; *p.o.*] were established. Each animal group was constituted of three female rats and three male rats. After administration, it was registered the animal's motor activity with an actograph. The animals were observed periodically during the first 24 h (with special attention during the first 4 h), and then daily during the 14 days of the study to observe their overall behavior and evidence of any toxicity signs or death. The animals and food were weighed at the start, seventh, and fourteenth days of the study. On the fourteenth day, the animals were euthanized with CO_2. The heart, spleen, lungs, liver, kidneys, and testicles/ovaries were removed, which were macroscopically examined and weighed individually. The weight of each organ was expressed as a percentage of weight relative to the weight of the whole animal. The experimental results showed that the administration of a single dose of *K. radicincitans* did not cause mortality or visible symptoms of toxicity in either male or female rats at the doses tested. In none of the animals were observed symptoms of restlessness, respiratory depression, convulsions, or death. The

analysis by one-way ANOVA indicated that the experimental procedure did not produce statistically significant modifications either in the relative weight of examined organs or in the rest of the analyzed parameters when compared to records of the negative control group. The results demonstrated that the under the experimental conditions and based on the toxicological records evaluated, there were no observed changes of toxicological significance in female and male Wistar rats, so this study provides some important information with respect to the use of native strain *K. radicincitans* as growth promote/biological control agent of vegetables used in the human alimentation.

A91

COMPARATION OF THE ACUTE TOXICITY BY GLYPHOSATE (COMMERCIAL FORMULATION) IN TWO SPECIES OF PLANKTONIC MICROCRUSTACEANS: *Boeckella poopoensis* MARSH, 1906 AND *Daphnia spinulata* BIRABÉN, 1917

Cabrera GC[1], Vignatti AM[1], Echaniz SA[1], Lares BA[1], Gutiérrez MF[2], Giacomino V[3]
[1]Grupo de Ecología y Fisiología del Zooplancton (GEFZoo), Depto. de Ciencias Biológicas, FCEyN, UNLPam. [2]Instituto Nacional de Limnología (CONICET-UNL) [3]Depto. de Química, FCEyN, UNLPam.
E-mail: gabrielacabrera@exactas.unlpam.edu.ar

In the central region of Argentina, there is a significant number of wetlands and shallow lakes, most of them located in basins where agricultural production is developed. This means that a large number of herbicides are used each year to control weeds; the most widely used being commercial formulations based on glyphosate (N-(phosphonomethyl) glycine). Given that these agrochemicals can be transported to surface water bodies by leaching and runoff, the aim of this study was to determine and compare the effect of glyphosate on two species of microcrustaceans that are frequent in the zooplankton of shallow lakes in the center of the country, the copepod *Boeckella poopoensis* and the cladoceran *Daphnia spinulata*. Acute bioassays (48 h duration, without medium renewal or feeding) were carried out with nauplii of *B. poopoensis* and neonates of *D. spinulata* with increasing concentrations of glyphosate (commercial formulation Panzer Gold): 0 (control), 1.3, 2, 3, 4.5, 6.75, and 10.13 mg/L. The media used were mesocosm water (previously sterilized) where a stable population is maintained for the former and distilled water with 1 g/L of NaCl (optimum medium for its culture) for the seconds. 25 specimens were used for each concentration (one per 25 mL transparent bottle). The photoperiod was 8/16 h (darkness/light), and the temperature was 22 ± 1 °C. As an indicator of death, the immobility of the specimens was considered, and the Probit method was used to calculate the LC50. *Boeckella poopoensis* was more sensitive to the herbicide since its LC50 was 5.1 mg/L while that of *D. spinulata* was 7.5 mg/L. The greater tolerance recorded in cladocerans could be due to that their postembryonic stages develop protected within the females breeding chamber, unlike copepods which, due to external postembryonic development, their nauplii larvae (with their organ systems and resistance mechanisms still immature) are directly exposed to environmental conditions as soon as the eggs hatch.

A92

OXIDATIVE STRESS BY GLYPHOSATE (COMMERCIAL FORMULATION PANZER GOLD) IN *Boeckella poopoensis* MARSH, 1906 (CRUSTACEA, COPEPODA)

Cabrera GC[1], Vignatti AM[1], Echaniz SA[1], Negro L[2], Gutiérrez MF[2], Cora Jofré F[3]
[1]Grupo de Ecología y Fisiología del Zooplancton (GEFZoo), Depto. de Ciencias Biológicas, FCEyN, UNLPam. [2] Instituto Nacional de Limnología (CONICET-UNL). [3]Depto. de Química, FCEyN, UNLPam.
E-mail: gabrielacabrera@exactas.unlpam.edu.ar

Glyphosate (N-(phosphonomethyl) glycine) is the most widely used herbicide worldwide for controlling weeds. In South America, particularly in the Pampean region, where an important agricultural production is developed, large quantities are used each year. Although its toxicological risk is disputed, various studies have shown its harmful effects on aquatic organisms. Given that *Boeckella poopoensis* is a frequent and representative calanoid of the saline lakes of the Neotropical region, the aim of this work was to evaluate the effect of this herbicide on the antioxidant mechanisms of the species. An experiment was developed to evaluate the enzymatic activity of catalase (CAT) and glutathione S-transferase (GST) and the levels of hydrogen peroxide (H_2O_2). For 15 days, copepodites IV and V males and females, separately, were exposed to four concentrations of glyphosate (commercial formulation Panzer Gold): 0 (control), 0.58, 1.16, and 2.32 mg/L. For each treatment, four replications were carried out with 15 individuals each, and transparent bottles of 100 mL were used. Every 48 h, the medium was renewed (mesocosm water, previously sterilized, where a stable *B. poopoensis* population is maintained), and *Dunaliella salina* was fed. The photoperiod was 8/16 h (darkness/light), and the temperature was 22 ±1 °C. After exposure, a significant difference ($P < 0.05$) was recorded in CAT levels between males and females since, in the former, the enzyme increased as glyphosate concentrations increased, whereas, in females, a decrease was observed, although these differences were not significant when compared to their respective controls. Since the function of CAT is to catalyze the decomposition of H_2O_2 into water and molecular oxygen, its behavior in this study could explain why males maintained H_2O_2 levels similar to the control, unlike females in which an increase was observed. Although

no significant differences were found in GST, imbalances in the oxidant/antioxidant balance, recorded especially in females, would show that glyphosate has a toxic effect on *B. poopoensis*.

A93
EVALUATION OF THE ACUTE TOXICITY OF IVERMECTIN FORMULATION AND CADMIUN IN AMPHIBIANS

Pistone C[1,], Sanchez E[2,3,*], Cianci Romero A[1], Alvarez SM[2,3] , Enriz RD[1,3], Giannini FA[1]*
*[1]Laboratorio de Bioensayos, Cátedra de Química General e Inorgánica, Universidad Nacional de San Luis. [2]Laboratorio de Nutrición, Medio Ambiente y Metabolismo Celular, Cátedra de Fisiología, Universidad Nacional de San Luis. [3]IMIBIO-SL CONICET. *Equal contributions.E-mail: emysilsanchez@gmail.com*

Amphibian populations are decreasing globally. Environmental contamination is one of the mayor factors that contribute to amphibious population declines. However, there is a great disagreement about if amphibious are good sentinels of ecological contamination or not, due to a lot of ecotoxicological studies indicating that amphibians are generally less sensitive than fish and invertebrates to different groups of contaminants. Ivermectin is a broad-spectrum antiparasitic used as a treatment in humans and mainly in animals. It also recently gained importance in the framework of the 2020–2022 Covid-19 pandemic produced by the SARS-CoV-2 virus as a therapeutic alternative. On the other hand, cadmium (Cd) is a pollutant of global concern due to its high toxicity at low concentrations. Various modern industrial activities cause serious Cd contamination because of its wide use in fields such as batteries, pigments, coatings, plating, and plastics. Our working group has studied, over the years, the toxicity of a commercial formulation of Ivermectin for humans and the toxicity of Cd. Previously, it was carried out the evaluation of the toxicity of both substances using two species of fish (*Poecilia reticulata* and *Danio rerio*) as experimental models. Herein, we present a study using amphibian larvae as an experimental model. To evaluate these compounds, we use the technique recommended by the U.S. Fish and Wildlife Service (Johnson and Finley 1980) modified by our working group. The toxicity was evaluated in amphibian larval stages (V–VI according to Martin et al.) of *Rhinella arenarum* obtained by collection in the month of November in San Luis City. Ten specimens were exposed in duplicate to different concentrations (5–0.625 mg/L for Cd and 0.250–0.032 mg/L for ivermectin) for a period of 96 h. The LD_{50} was determined according to the ratio of mortality in 96 h of exposure using an online software that develops "Probit analysis". The results obtained showed LD_{50} = 2.3 mg/L for Cd and LD_{50} = 0.122 for ivermectin. Considering our previous studies in fish (Cd DL_{50} = 2–3 mg/L and ivermectin LD_{50} = 0.0039 mg/L) amphibians have greater resistance than fish in the case of ivermectin but similar to both fish species in Cd acute intoxication. Our results highlight the importance of testing the distinct nature and mechanism of toxicity of contaminants individually to be able to come to any conclusion on the relative toxicological sensitivity of amphibians and further investigate the sensitivity and consequences of chronic exposure to these axenics.

BIOTECHNOLOGY AND GENETICS

A94
PNIPAM AND PNIPAM-co-3% APTA HYDROGELS ARE BIOCOMPATIBLE IN AN *IN VIVO* ANIMAL MODEL

Sommaro A, Capella V, Zabaldano L, Rodriguez N, Liaudat AC
Instituto de Biotecnología Ambiental y Salud (INBIAS), Universidad Nacional de Río Cuarto (UNRC)-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). E-mail: aliaudat@exa.unrc.edu.ar

The design of matrices to guide the regeneration of different tissues requires the creation of a scaffold and the desired cell type culture to achieve the functional unit to be transplanted. Previous studies carried out in our laboratory have shown that poly-N-isopropylacrylamide (PNIPAM) and PNIPAM-co-3% (3-((acrylamidopropyl) trimethyl-ammonium chloride), APTA) hydrogels are scaffolds that allow adhesion and development of epithelial, renal, pulmonary, and immune cells of different species with the absence of cytotoxicity. The aim of this study was to analyze the *in vivo* biocompatibility of PNIPAM and PNIPAM-co-3% APTA hydrogels with 3D architecture in male Wistar rats. PNIPAM or PNIPAM-co-3% APTA hydrogel half-discs of 0.5 cm width were implanted in subcutaneous pockets of three rats per treatment (control, PNIPAM, and PNIPAM-co-3% APTA) for 30 days. The healing process was evaluated in all animals during the first 10 days after surgery. After 30 days of exposure to the different hydrogels, the rats were sacrificed according to the UNRC animal care protocol. Blood samples were taken for hematological and biochemical analysis, in addition to kidneys, liver, and spleen for further histopathological evaluation. The results showed the absence of alterations in the healing process and in the post-surgery recovery time in all the animals evaluated. The values of the

hematological parameters (number of erythrocytes, leukocytes, platelets, hematocrit %, leukocyte formula, and hemoglobin concentration) and liver enzyme activities (GPT, GOT, and ALP) of different hydrogels implanted animals were similar to the control group. Histopathological studies remained unchanged in all experimental groups. In conclusion, PNIPAM and PNIPAM-co-3% APTA hydrogels are biocompatible in a murine model, maintaining hematological, biochemical, and histological parameters without alterations.

A95
CLASSIC CYTOGENETICS IN ADVANCED TRICEPIRO LINES

González V[3], Castillo E[3,5], Grossi F[3], di Santo H[3,5], Aguirre L[3,5], Malpassi R[1,5], Bianco L[5], Gorjon J[1], Novaira A[2], Ganum M[4,5], Basconsuelo S[1], Grassi E[3,5]
[1]Morfología Vegetal. [2]Química. [3]Genética. [4]Sistema Suelo-Planta. Facultad de Agronomía y Veterinaria. Universidad Nacional de Río Cuarto. Ruta 36 km 601. Río Cuarto. Córdoba. [5]Instituto de Investigaciones Agrobiotecnológicas (INIAB). CONICET-Universidad Nacional de Río Cuarto. Ruta 36 km 601. Río Cuarto. Córdoba.
E-mail: ecastillo@ayv.unrc.edu.ar

Interspecific hybridization is used to incorporate desirable traits into germplasm of agronomical interest. The ploidy level was analyzed in three advanced tricepiros lines obtained from the crosses between the triticales Cayú-UNRC, Yagán-INTA, and Yavú-UNRC (2n = 6× = 42) used as female progenitors, and the two trigopiros Don Noé-INTA (2n = 8× = 56) and SH16-INTA (2n = 6× = 42) employed as male progenitors. The assessed lines were Cayú × SH16, Yavú × SH16, and Yagán × Don Noé. The number of bivalents in stem cells of pollen grains meiosis (II/SCP) was determined and the cytological stability was quantified through an index based on the count of tetrads with absence/presence of micronuclei (MI) and an index considering the number of microspores with micronuclei per tetrade (IxM). Comparisons were made through nonparametric tests. The ploidy level of the lines resulted in 6× = 2n = 42, proving the trend in these Triticeae to stabilize at the hexaploid level. There were no significant differences in the MI indexes, and the mean values were: Cayú × SH16 51.12 ± 32.1; Yavú × SH16 44.50 ± 26.3, and Yagán × Don Noé 32.58 ± 28.2. Contrary to expected based on the ploidy level, the cross involving Don Noé appeared to be the most stable line according to the MI index. No significant differences were found among lines for the IxM index either. Mean values were Cayú × SH16 1.88 ± 0.9; Yagán × Don Noé 1.65 ± 0.8, and Yavú × SH16 1.44 ± 0.7; the latter with the least number of microspores with micronuclei per tetrad. These materials were superior in agronomical traits in comparative yield trials, and the results in this study contribute to their description and characterization according to INASE standards.

A96
EVALUATION OF TWO TECHNIQUES FOR APPLICATION OF *Kosakonia radicincitans* AS BIOLOGICAL CONTROL AGENT FOR BLUE MOLD IN APPLES

Rodríguez Rivera M, Navarta G, Calvo J
Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. E-mail: mfrodri@gmail.com

The fungus *Penicillium expansum* causes blue rot affecting apples. Mechanical damage caused by harvest and post-harvest handling is one of the main causes of the entry of pathogenic fungi. Apples are usually stored after harvest. During cold storage, losses of economic importance are produced by decays due to fungal rot. The bacterium *Kosakonia radicincitans* showed antifungal activity against *P. expansum* and is considered a biological control agent (ACB). The objective of this work was to evaluate the efficiency of two application techniques of one aqueous formulation of ACB against *P. expansum* in stored cold apples. For the biocontrol assays, Red delicious apples were obtained from a commercial orchard. Surface apples were disinfected by immersion for 1 min in a dilute solution of sodium hypochlorite (1% active chlorine), washed two times by immersion in distilled water, and left in a dry place to remove excess water off the surface. Then, fruits were wounded (3 × 3 × 3 mm^3) in two places (midway between the calyx and the stem end) with a punch. Two treatments were carried out: (T1), immersed for 1 h in a suspension of *K. radicincitans* (UFC = 7×10^6/mL), and (T2) sprayed with the same solution. Then both groups were inoculated with 20 µL of the phytopathogen (3×10^5 conidia /mL). The group control was inoculated only with the pathogen (T3). After 32 days of storage at 4°C, the wounds were examined, and the number of infected wounds was counted and expressed as the percentage of disease incidence (DI % = (number of wounds that develop disease/total number of wounds treated) × 100), DI value may range from 0% (total effectiveness) to 100% (no antagonist effect). Diameters and depth rots were measured. Firmness (kg/cm^2) and sugars (°Brix) of apples were evaluated at the beginning and end of the trial. Ten apples constituted a single replicate, and each treatment was replicated two times. Data analyzed with ANOVA showed that the number of infested wounds in T1, treated with immersion in an aqueous formulation of *K. radicincitans*, was significantly lower (DI 44%) than in T2 and T3 (DI 100%). The average rot diameter was significantly larger in T3 (33.0 mm) compared to T2 (21.0 mm) and T1 (20.2 mm). The depth of rotting was lower in T1 (11.0 mm), compared to T2 (21.0 mm) and T3 (22.0 mm). The average firmness of the apples at the beginning of the trial (11.4 kg/cm^2) was significantly decreased in T3 (6.55 kg/cm^2) compared to T2 (9.33 kg/cm^2) and T1 (10.39 kg/cm^2), while the initial sugars (14°Brix) showed no difference between treatments at the end of the trial. The results would indicate that the apple immersion technique for the application of a

suspension of *K. radicincitans* was effective in reducing the effects caused by *P. expansum* under cold storage conditions. The optimization of the application will be achieved by studying other variables of interest.

A97
TRICEPIRO: SEED PRODUCTION IN STABILIZED LINES DERIVED FROM DIFFERENT TRIGOPIROS

Lanzetti A[1], Aguirre L[1,2], Vega DJ[2], Plevich A[1], Rovere M[2], Grossi Vanacore MF[1], di Santo H[1,2], Castillo E[1,2], Kaufman E[1], Ferreira A[1,2], Ferreira V[1], Grassi E[1,2]
[1]Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto, Ruta Nac. 36 km 601, Río Cuarto, Córdoba.
[2]Instituto de Investigaciones Agrobiotecnológicas, Ruta Nac. 36 km 601, Río Cuarto, Córdoba.
E-mail: egrassi@ayv.unrc.edu.ar

Tricepiro is a winter annual forage crop derived from the intergeneric hybridization of three species (wheat, rye, and *Thynopirum ponticum*) with high potential for climate and soil-limited agricultural systems in the central region of Argentina. The combination of different genomes causes fertility issues in the hybrids, particularly in early generations. In order to evaluate reproductive traits, 22 stabilized tricepiro lines previously selected by agronomical traits were used. These lines originated from crosses between triticales (6×) and three trigopiros. The trial was sown in August 2021 at the National University of Río Cuarto. Biomass production (DW/m^2), number of tillers (Till/m^2), number of spikes (Spi/m^2), grain production (GP/m^2), percentage of fertile tillers (FT), and harvest index (HI) were measured at physiological maturity. Previous studies indicate that the tested lines were stabilized at hexaploid level (6×) with wheat and rye genome retention and differential introgression of *T. ponticum* in their genome. Mean values of DW/m^2, Till/m^2 and GP/m^2 were 965.7 ± 357.2 g/m^2, 242.8 ± 91.0 Till/m^2, and 318.0 ± 127.7 g/m^2, respectively. ANAVAs suggest that HI, Till/m^2 and FT traits were statistically different between genotypes. ANAVAs and PCA showed that lines derived from the male HOR performed better in the measured traits, except for FT, a trait in which the lines from male octoploid trigopiro (DN) had improved behavior. It was proved that the stabilized lines do not present fertility problems that affect normal grain production and the contribution of trigopiros used as progenitors could be differentiated.

A98
ISOLATION AND CHARACTERIZATION OF MICROORGANISMS FROM A LANDFARMING OF THE PROVINCE OF SAN LUIS WITH POTENTIAL CAPACITY FOR USE IN BIOAUGMENTATION

Salinas A[1], Lijteroff R[2], Delfini C3, Villegas L[3,4]
[1]Área de Biología. Departamento de Biología. Facultad de Química, Bioquímica y Farmacia. UNSL. [2]Área de Educación en Ciencias Naturales. Departamento de Biología. Facultad de Química, Bioquímica y Farmacia. UNSL. [3]Instituto de Química de San Luis (INQUISAL). UNSL-CONICET. [4]Área de Bromatología. Departamento de Farmacia. Facultad de Química, Bioquímica y Farmacia. UNSL. E-mail: asalinaskenny@gmail.com

The final disposal of industrial effluents with organic or inorganic compounds is a concern for companies and governments. Consequently, in recent decades, different methodologies have been used to reduce them, without causing damage to the environment, among which are bioremediation processes. Landfarming is an ex-situ bioremediation treatment process that is carried out in the upper zone of the soil. In San Luis Province there is a landfarming that receives effluents from the chemical industry and has been used for more than 10 years. The aim of this work was to isolate microorganisms from landfarming soil with the capacity to tolerate the environmental stresses which can favor increasing and accelerating landfarming processes. The samples were collected in two stages, during the winter period of 2022: the first was taken from the supernatant of the effluent after its discharge; the second from the mud generated in the mixture of soil and effluent, after five days of discharge. The sampling points were three at different distances from the discharge of effluents. Two approaches were utilized to isolate microorganisms from the landfarming soil: a direct isolation approach in EG medium following the streaked-on agar plate and an enrichment approach in liquid medium incubated a 30°C, 150 rpm for 72 h. While, using a direct isolation approach only 12 morphotypes were isolated, using the enrichment approach 15 morphotypes were isolated. To characterize the isolates, the characteristics of the colonies, the cells, and the Gram coloration in the case of bacteria were taken into account. The isolates will be molecularly identified. It is well established that only approximately 1% of microorganisms on Earth can be readily cultivated *in vitro*. But this work aims to use microorganisms in the bioaugmentation process, so it only focused on culturable microorganisms. These preliminary results show that landfarming has an active microbiota, and it is feasible to isolate microorganisms that can be used in bioaugmentation processes with native strains.

A99
EVALUATION OF BIOLOGICAL AND MICROBIOLOGICAL QUALITY PARAMETERS IN AN AMENDED SUBSTRATE FROM ALPERUJO'S ANAEROBIC DIGEST

Quiroga V[1], Zarsavilla F[1], Bueno J[2], Martín M. L[1], Gil R[1], Rodríguez L[1]
[1]Instituto de Biotecnología, Facultad de Ingeniería, U.N.S.J., San Juan. Argentina. [2]Departamento de Biología, Facultad de Ciencias Exactas, U.N.S.J., San Juan, Argentina.E-mail: laurirodriguez@gmail.com

Anaerobic Digestion (AD) process is based on a series of metabolic reactions, mediated by different groups of microorganisms that produce biogas (methane) and a digested substrate (DS). The DS has been defined as a semisolid fraction from this AD that can be used in both liquid and solid form. It contains organic material and considerable amounts of minerals (nitrogen, phosphorus, potassium) with a low C/N index, which makes them interesting as fertilizers or soil amendments. In previous work, using the germination seed method, no phytotoxicity was found when digest concentrations were 10 and 50 %. What's more, root elongation and germination index were higher than blank test, so the aim of this work was to analyze biological and microbiological quality parameters such as microorganisms and enzymes as responsible agents in vegetable-promoting activities. The essays consist of 12 samples with three repetitions. They were carried out using 500 g pots with fertile soil and digest addition in 25 t/ha dose with 1/1, 1/10, and 1/100 dilutions. A witness sample (without digested) and commercial fertilizer Fertifox® were included among the samples. Tomato seeds (*Solanum lycopersicum*) were cultivated at moderated temperature, indirect solar exposure, irrigated on days 0 and 15 with a diluted fraction digested and then with 75 mL of water tap. After 28 days, plants were harvested. Parameters regarding plant growth were measured (fresh and dry weight, number of leaves, aerial part, and root elongation, among others). Also, there were analyzed the presence of the main microbial group (molds, yeast, and bacteria) using a selective culture method and the enzyme activities (laccase, β-glucosidase, amylase, and cellulase) responsible for nutrient assimilation. In terms of plant growth parameters, some negative effects (less dry and fresh weight) were observed when digest was added without dilution. Regarding microbial groups, CFU was higher for mold and bacteria when dilution 1/100 was applied. On the contrary, Yeast CFU was lower. In terms of enzymatic activities, all samples show less activity, between 70–80% of the witness sample. B-glucosidase was the only one that show higher activity when dilutions 1/10 and 1/100 were tested. Finally, it is concluded that the addition of the diluted fraction of the digested increases microbial group population, which favors plant nutrient assimilation. Future essays will include the determination of other compounds such as humic acids to set synergy effects.

A100
SIMULTANEOUS DETERMINATION OF ZINC AND COPPER IN WATER SAMPLES BY POTENTIOMETRIC STRIPPING ANALYSIS

Biasi AM[1], Valverde AM[2], Takara EA[3], Scala-Benuzzi ML[4], Messina GA[4], Gómez NN[1]
[1]Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO-CONICET-UNSL). [2]Facultad de Química, Bioquímica y Farmacia (FQBF-UNSL). [3]Instituto de Física Aplicada (INFAP-CONICET-UNSL). [4]Instituto de Química de San Luis (INQUISAL-CONICET-UNSL).E-mail: anto.mbiasi@gmail.com

The detection of metal ions, such as copper and zinc, is of great interest in the environment and health fields. As is widely known, water is one of the essentials that support all forms of plant and animal life. Among the wide diversity of contaminants affecting water resources, metals receive particular concern considering their toxicity even at low concentrations. Therefore, the determination and monitoring of these metals in water have become necessary. For years, several methods using traditional techniques, such as spectrometry, have been developed. These methods are available for the determination of trace amounts of heavy metals with sufficient sensitivity for most applications. However, these methods are more expensive and time-consuming compared to electrochemical methods. Anodic stripping voltammetry (ASV) is a powerful analytical technique due to its high sensitivity and reproducibility. Mercury-based electrodes have traditionally been used in stripping techniques, but due to their strong toxicity, extensive research efforts have been devoted to finding alternative electrode materials. An interesting approach in electrode modification is the use of carbon-based conductive materials, such as graphite and graphene, which are ideal for sensor applications since they possess unique physical and chemical properties. Nevertheless, they have a great drawback, their agglomeration and adhesion to the electrode surface. To overcome these disadvantages and to improve the stability of modified electrodes, polymers are usually incorporated. In the present work, a new nanocomposite containing graphene oxide/graphite (GO/GRA) and polyethylenediimine (PEI) was prepared and characterized. The interactions and morphology of GO/GRA/PEI were studied by infrared spectrophotometry and SEM, respectively. The modified electrode was characterized by cyclic voltammetry and electrochemical impedance spectroscopy. Also, the simultaneous determination of Zn(II) and Cu(II) based on square wave anodic stripping voltammetry (SWASV) was investigated. The determination was carried out at pH 4.5 at a deposit potential of −1.2 V and an accumulation time of 200 s. The obtained limits of detection for Zn and Cu were 12 and 4.5 nM, respectively. The results revealed that the newly developed sensor has high practical applicability, reproducibility, and stability in the simultaneous detection of Zn and Cu.

A101
DEVELOPMENT AND OPTIMIZATION OF AN AFFINITY COLUMN FOR PROTEINS' ISOLATION AND PURIFICATION

Torres CF[1,2], Gonzalez UA[2], Gómez A[1], Ferraris MP[1,2]
[1]Laboratorio de Biología Estructural, Área de Biología Molecular. [2]Laboratorio de Procesos Separativos con Membranas. Facultad de Química, Bioquímica y Farmacia - Universidad Nacional de San Luis, Av. Ejército de los Andes 950, CP 5700, San Luis, Argentina.E-mail: pilarferraris05@gmail.com

The most effective affinity purification technique has been affinity chromatography, which combines conventional column chromatography with affinity interactions. The objective of this work was to develop methodologies to be applied in protein purification using affinity chromatography with the Cell–Cibacron macroligand immobilized on solid supports. Affinity macroligands were prepared from yeast cells modified by chemicals and with the Cibacron Blue F3GA ligand molecule immobilized to the wall cell by a covalent bond. The amount of ligand immobilized on the wall cell was determined by the spectrophotometric method. Agarose–macroligand cubes were prepared using 4% agarose dissolved in 1× PBS buffer, pH = 7.6, using a 2×2 mm red metallic. A fixed-bed column system with immobilized agarose–macroligand cubes was prepared. Bovine Serum Albumin (BSA) adsorption on Affinity Agarose–macroligand was investigated by means of adsorption isotherms using BSA. Adsorption experiments were carried out in batch cultures. The chromatographic column was studied by Human Serum Albumin (HAS) and BSA adsorption from human and bovine serum. The selectivity of the separation process was compared using the agarose–macroligand affinity column with Blue-Sepharose TM 6 Fast Flow (Ge Healthcare) commercial chromatographic resins. The purity of HSA and BSA was assayed by gel electrophoresis (SDS–PAGE). The maximum attachment of ligand on the wall cell was 212 µmol of Cibacron dry/g of dry cell. Adsorption values such as Kd = 9.35 $\times 10^{-4}$ M and qm = 135 mg BSA/g adsorbent were calculated by the linear transformation of Langmuir. HSA and BSA were purified with high purity (more than 80%) with the affinity chromatography column system using agarose–macroligand cubes. The comparative selectivity showed that the purity of the BSA obtained with the agarose–macroligand affinity column is similar to that obtained with the commercial column. It is an easily reproducible system, which also demonstrated an adequate sample processing speed. There was no occlusion or blockage of the affinity column during the separation process.

A102
SCREENING AND SELECTION OF VARIABLES FOR OPTIMAL OBTAINING OF ENZYME INVERTASE FROM *Aspergillus niger* AND ANALYSIS OF ENZYMATIC ACTIVITY USING THE 3,5-DINITROSALYCIC ACID METHOD

Ramos Gomez PD[1,2], Ferraris MP[1], Bustos Crescentino D[2], Campderrós ME[1], Gonzalez UA[1]
[1]Facultad de Química Bioquímica y Farmacia - Universidad Nacional de San Luis. Instituto de Investigaciones en Tecnología Química (INTEQUI) - CONICET. Av. Ejército de los Andes 950, San Luis. [2]Instituto de Ciencias Básicas (ICB), Facultad de Filosofía, Humanidades y Artes - Universidad Nacional de San Juan.
E-mail: paoladanielaramos27@gmail.com

The enzyme invertase, also known as β-D-fructofuranosidase catalyzes the hydrolysis of sucrose producing a mixture of its two monomers glucose and fructose called invert sugar. This product, sweeter than sucrose, is mainly used in the food and pharmaceutical industry. It can be obtained using a chemical or enzymatic method, the enzymatic is much more efficient because undesirable products are not obtained. Invertase is produced by a wide variety of organisms that can use sucrose as a carbon source. It can be found in invertebrates, vertebrates, green algae, bacteria, vegetables, and fungi. The genus *Aspergillus* has been shown to be a good producer of this enzyme. The aim of this work is to obtain the enzyme invertase at a laboratory scale from the fungus *Aspergillus niger* and study the influence of experimental parameters that affect the production and purification process of this enzyme. We worked with *A. niger* and analyzed the enzymatic activity of invertase using 3,5-dinitrosalicylic acid. To determine the concentration of invertase in the samples, a glucose calibration curve was performed, and absorbances of the samples were measured at 540 nm. A high glucose concentration is indicative of high enzyme activity. A screening of different variables such as culture medium, time, pH, and temperature was performed. A higher biomass was obtained working with Sabouraud dextrose liquid medium (SDLM) supplemented with sucrose (SDLM) than Sabouraud dextrose liquid medium (SDL) or Potato dextrose liquid medium (PDL) in all the cases. The highest values of glucose concentration were 65.22 and 64.51 g/L corresponding to biomass obtained in SDLM supplemented with sucrose 20 and 30 g/L, respectively, and then suspending it in a 10 g/L sucrose solution before obtaining the enzymatic extract. The fungus was suspended in sucrose solution at 10 g/L in order to increase the production of more enzyme Invertase in the presence of more substrate. These values were obtained working at pH 5, at a temperature of 28°C.

A103
BIOAUGMENTATION OF BIOMIXTURES WITH ACTINOBACTERIA FOR ATRAZINE REMOVAL: OPTIMIZATION OF INOCULUM CONCENTRATION

González SK[1], Maldonado LM[1], Benimeli CS[1,2], Polti MA[1,3], Saez JM[1,3]
[1]Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), S. M. de Tucumán, Argentina.
[2]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca (UNCA), S. F. del V. de Catamarca, Argentina. [3]Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán (UNT), S. M. de Tucumán, Argentina. E-mail: jsaez@conicet.gov.ar

Biopurification systems (BPS) or biobeds are bioprophylaxis systems to prevent pesticide point-source contamination, whose efficiency relies mainly on the pesticide removal capacity of the biomixture, the majority component of a BPS. The microbial metabolic abilities of the biomixture could be improved through bioaugmentation with microorganisms with specific degrading capacities, like actinobacteria. In this sense, *Streptomyces* sp. M7 is a previously selected actinobacterium with well-known pesticide-degrading abilities. The aim of this work was to optimize the concentration of *Streptomyces* sp. M7 (M7) inoculated in organic biomixtures for atrazine (ATZ) removal. For this purpose, the biomixtures B1 and B2 were formulated with soil, peat, and sugarcane bagasse and filter cake, respectively, inoculated with three concentrations of M7 (2, 4, and 8 g/kg), and contaminated with atrazine (50 mg/kg). The residual concentration of ATZ and different microbial groups were determined along a 28-day assay. In general terms, at the end of the assay, an increasing trend was shown in the microbial developments of the different groups studied in both contaminated and bioaugmented biomixtures for the three concentrations of inoculum used. In B1, the microbial counts were significantly higher with 8 g/kg inoculum, with respect to lower inoculum concentrations: total heterotrophic microorganisms, total bacteria, fungi, and actinobacteria reached counts of 3.2×10^7; 2.7×10^7; 1.7×10^5, and 1.5×10^7 CFU/g, respectively. In B2, total heterotrophs, total bacteria, and actinobacteria were significantly higher for the 4 g/kg inoculum concentration, reaching 7.9×10^7; 1.1×10^8, and 5.9×10^7 CFU/g, respectively; fungal counts did not show significant differences. ATZ removal showed no significant differences in B1 and B2 between the three concentrations of M7 inoculum evaluated. The concentration of 2 g/kg of M7 was selected for further studies considering lower costs and optimum removal efficiency.

A104
TOWARDS THE DESIGN OF STABLE AND EFFECTIVE DYE-DECOLORIZING CONSORTIA OF EDIBLE FUNGI

Domínguez FG[1], Pacheco MS[1], Torres MA[1], Nieto Peñalver CG[1,2], Pajot HF[1,3]
[1]PROIMI CONICET CCT NOA SUR. Avda Belgrano y Pje Caseros, SM de Tucumán. [2]Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán. [3]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca. E-mail: hipolitopajot@conicet.gov.ar

Synthetic dyes are used in textile industries due to their advantages over natural dyes. However, textile effluents cause irreparable damage to water bodies because of the proper toxicity of textile dyes or by reducing the penetration of visible light leading to the eutrophication of rivers and lakes. Aerobic biodecoloration is an interesting treatment alternative for these effluents. However, no microorganism is capable of degrading all existing dyes, making the use of microbial consortia mandatory. Wood White Rot fungi produce enzymes such as laccase, manganese peroxidase, or lignin peroxidase that have been widely used for the degradation of textile dyes. The objective of this work was the selection of compatible edible fungi for the formation of consortiums capable of degrading different colorants. For this, we selected ten *Pleurotus*, *Psilocybe*, *Ganoderma*, and *Lentinula* strains. Fungi were maintained in Petri dishes with 20 mL of solid YM medium (glucose: 1%, soy peptone: 0.5%, yeast extract: 0.3%, malt extract: 0.3%, agar: 1.8%), incubated at 25°C. Media were inoculated with 5-mm diameter plugs obtained from growths in solid YM medium, preincubated for 7 days at 25°C. The laccase production of the different fungi was evaluated in solid media using eight substrates: 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), catechol, 2,6-dimethoxyphenol, 1-naphthol, benzidine, syringaldazine, 3,4-dimethoxybenzylalcohol, and guaiacol. Assays in which oxidized substrates produced haloes of different colors around the wells were considered positive. To carry out the compatibility tests, the ten strains were confronted with each other on different plates with YM solid medium in duplicate and incubated for 7 days at 25°C. Four modes of interaction were observed: inhibition haloes, zone with a dark line (in the contact of one strain with the other the line is produced), overgrowth (invasion of one strain on the other), and growth without inhibition. For the evaluation of the bleaching capacity of each strain, four industrial textile dyes were used: Black Vilmafix® B-V, Blue Vimafix® RR-BB, Red Vilmafix® 7B-HE, and Orange Procion® HER, which were seeded in four wells made in the plates of each strain forming halos of each color. After 24 h of incubation at 25°C, different levels of degradation were observed around the haloes depending on the structure of the dye. The results show that the tested fungi could be employed in the design of effective dye-decolorizing consortia.

A105
ASSESSING THE TEXTILE-DYE DECOLORIZATION POTENTIAL OF PSYCHROPHILIC AND PSYCHROTOLERANT YEASTS FROM 25 DE MAYO/KING GEORGE ISLAND (ANTARCTICA)

Pacheco MS[1], Domínguez FG[1], Torres MA[1], Martorell MM[2], Ruberto LAM[2], MacCormack WP[2], Martinez LM[2], Pajot HF[1,3], Nieto Peñalver CG[1,4]
[1]PROIMI CONICET CCT NOA SUR. Avda Belgrano y Pje Caseros, SM de Tucumán. [2]Instituto Antártico Argentino, Av. 25 de Mayo 1147, Villa Lynch, Provincia de Buenos Aires. [3]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca. [4]Instituto de Microbiología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán.E-mail: hipolitopajot@conicet.gov.ar

Antarctic environments typically present low temperatures, high solar radiation, and low nutrient availability, being one of the harshest environments on Earth. Psychrophilic and psychrotolerant yeasts from Antarctic soils and cryoconites have adapted to such conditions, being an interesting source of new enzymes with great biotechnological potential. This work intends to prove the textile dye decolorizing potential of 139 yeasts isolated from soil and cryoconite samples from 25 de Mayo/King George Island, Antarctica. Isolates were cultivated in YM, and NDM (Normal Decolorization Medium) media at 15, 20, and 25°C, and classified into psychrophilic and psychrotolerant according to their growth profiles. Textile dye decolorization was evaluated in the same two media, plus 200 mg/L of one of four commercially available reactive azo dyes: Vilmafix® Blue RR-BB (CI, Reactive Blue 221), Vilmafix® Red 7B-HE (CI, Reactive Red 141), Vilmafix® Black B-V (CI, Reactive Black 5), or Vilmafix® Green RR-4B (CI, Reactive Green). Plates were incubated for 72 h at 15, 20, or 25°C, depending on the yeast isolate. Dye decolorization haloes and colony dyeing were recorded daily and used to select the most promising isolates. Of 139 isolates, 34% were classified as psychrophilic (growing only at 15°C) while the remaining 66% were classified as psychrotolerant (growing at all the assayed temperatures), irrespective of the medium assayed. Forty-five isolates produce haloes in at least one of the tested dyes in NDM, while only 40 yeast produced haloes in YM. Fifteen isolates were selected for further studies. Isolates A075 and Y28D produced intense haloes without colony dyeing; isolates Y67, Y70, and Y75 produced intense haloes and got dyed only at the edge of the colonies, while the remaining ten isolates, A092, A099, A104, A105, A106, A107, Y6, Y59, Y73, and Y84, produced neat haloes with a significant colorization of the entire colonies. The obtained results prove the biotechnological potential of Antarctic yeasts, raising the possibility of designing more efficient dye-decolorizing methods at low temperatures.

A106
INDIRECT HEAVY METALS DETERMINATION IN WATER SAMPLES BASED ON ALKALINE PHOSPHATASE INHIBITION USING A NOVEL MODIFIED SURFACE BY LASER-INDUCED FLUORESCENCE DETECTION

Piguillem Palacios SV[1], Jofre F[1], Scala-Benuzzi ML[1], Gomez G[2], Raba J[1], Bertolino FA[1], Pereira SV[1], Messina GA[1]
[1]Instituto de Química de San Luis (INQUISAL-CONICET-UNSL), San Luis, Argentina. [2]Instituto de Investigación en Tecnología Química (INTEQUI – CONICET - UNSL), San Luis, Argentina.E-mail: sofiapiguillem@gmail.com

During the last few years, the need for fast, sensitive, specific, automated, and in situ detection methodologies has led to the development of easy-to-use and inexpensive devices. Paper has attracted great interest as a potential support material for sensors in analytical chemistry and clinical chemistry due to its versatility, abundance, and low cost. Its fibrous and porous structure generates a high surface-to-volume ratio that increases immobilization capability compared to traditional materials for the fabrication of sensor devices. Considering these advantages, the development of Paper-based Analytical Devices (PADs) has had great growth. This work is based on the modification of paper through the impregnation of porous materials that provide greater advantages. In this sense, Metal–Organic–Frameworks (MOFs) are one of the new materials with major advantages that have been synthesized in the last decades. Among them, we can mention their defined crystalline structure, simple synthesis, and high capacity to increase the surface area through functionalization with reactive groups. The problem of certain metal ions, such as copper, mercury, and lead, is growing, as their detection corroborates environmental contamination. Various sources of water samples are of great interest in this study, as multiple life forms depend on the toxicity found in water. Consequently, one of the main objectives in water quality monitoring is the determination of metal concentration. For this purpose, a paper biosensor modified with UiO-66-NH2 was developed. This support was characterized by several techniques to determine the crystalline structure, morphology, and different immobilizations on it (SEM, EDS, XRD, FTIR). Metals were indirectly determined by measuring the enzymatic response of the enzyme alkaline phosphatase (ALP) because the activity of this enzyme decreases in the presence of the mentioned metal ions (Cu^{2+}, Hg^{2+}, and Pb^{2+}). Serial decreasing concentration solutions of the metals under study were evaluated, starting from a solution of 2 mg/mL. The evaluation of the enzymatic response was performed by measuring the fluorescence generated by the activity of FAL against its substrate 4-methylumbelliferyl phosphate (MUP), which is converted into methylumbelliferone. Detection was performed by laser-induced fluorescence using a solid-state laser (λ excitation 430 nm, λ emission 458 nm). The optimum concentration of FAL was 0.005 mg/mL in DEA buffer 0.1 M, pH = 8. A decrease in its fluorescent response was found to be proportional to the increase in the metal ion solutions concentrations.

MICROBIOLOGY AND IMMUNOLOGY

A107
ISOLATION AND CULTIVATION OF THE GREEN MICROALGAE *Desmodesmus* sp

Muñoz Carabajal DML, Ferrari SG.
Área Microbiología e Inmunología. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. San Luis. Argentina.E-mail: danacarabajal1@gmail.com

Different anthropogenic activities generate wastewater and effluents that, when discharged into natural water bodies, can cause serious damage to the environment and the health of people. Different methods of purification have been used for contaminant removal; however, they present economic limitations. Thus, a technique of removal by microalgae is a promising and low-cost alternative. Microalgae are a group of photosynthetic organisms that can use the components present in wastewater to grow and, furthermore, they are useful for studying the toxicity of aquatic contaminants. The goal of this work was to isolate and cultivate the autochthonous microalgae *Desmodesmus* sp. for future use in bioassays. For the isolation of this microalgae, two culture media were used (BBM and BG-11) added with 1% agar-agar. Subsequently, biomass was inoculated in liquid media and incubated at 25 ± 2°C with continuous illumination (2500 lux) and orbital agitation (150 rpm) for 10 days. Growth was determined by DO at 580 nm measurements, chlorophyll *a* and *b* and cell counting in the Neubauer chamber. Cell morphology was also studied by optical microscopy. Biomass production at the end of the culture was higher in BBM medium (OD_{580}= 0.462) than in BG-11 (DO_{580} nm = 0.398). However, the highest specific growth rate was observed in the BG-11 medium (0.0339 h^{-1}), which can be attributed in part to the different composition of the culture media since the N/ P ratio is 37 times higher in BG-11 than in BBM. The isolate showed characteristic features of the genus *Desmodesmus* with coenobia of 2–4 ovoid cells showing a parietal chloroplast with a single pyrenoid and spiny projections. In BBM, coenobia of 4 cells predominated: 28 ± 0.25 µm wide and 20 ± 0.25 µm long while in BG-11 the measures were significantly lower ($P < 0.5$) 20 ± 0.25 µm wide and 16 ± 0.25 µm long. The final cell count in the Neubauer chamber of 9.75x106 cells/mL was greater than in BG-11. However, with the exception of cell sizes, the differences in the parameters studied were never significant ($P > 0.5$). For this reason, we conclude that both culture media are suitable for the isolation and growth of *Desmodesmus* sp and would allow the use of this native microalga in water bodies monitoring tests in the province of San Luis.

A108
MODULATION OF INNATE IMMUNE RESPONSE IN BOVINE MAMMARY GLAND EPITHELIAL CELLS BY *Minthostachys verticillata* ESSENTIAL OIL

Arsaute S[1], Montironi ID[2], Cecchini ME[1], Reinoso E[1], Cariddi LN[1]
[1]Laboratorio de Inmunología, Instituto de Biotecnología Ambiental y Salud (INBIAS), Universidad Nacional de Río Cuarto (UNRC)/Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Río Cuarto, Córdoba, Argentina. [2]Cátedra de Farmacología, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto (UNRC), Río Cuarto Córdoba, Argentina. E-mail: sofi.arsaute@gmail.com

Minthostachys verticillata is an autochthone medicinal plant and our research group has demonstrated that its essential oil (EO) has the ability to modulate the immune response. The aim of this work was to evaluate whether EO modulates the synthesis of proinflammatory cytokines in bovine mammary gland epithelial cells (MAC-T) in the presence or absence of a *Staphylococcus aureus* strain (Sa) isolated from cows with mastitis. The cells were treated with EO (25, 50, and 100 µg/mL), Sa (5×10^6 CFU/mL) and pretreated with EO, and then challenged with Sa at different times (2, 6, 24, and 48 h). Cells without stimulation were used as control. IL-1β and IL-6 cytokines were quantified in the cell supernatant by sandwich ELISA. MAC-T cells stimulated with Sa produced higher levels of IL-1β and IL-6 than untreated cells ($P < 0.001$) up to 48 h. MAC-T cells also responded to EO stimulation (25, 50, and 100 µg/mL) with increased IL-1β levels between 2 and 6 h ($P < 0.05$) and increased IL-6 synthesis between 6 and 48 h ($P < 0.01$ and $P < 0.05$) compared to untreated cells. A decrease in both cytokines in cells treated with EO was observed between 24 and 48 h without differences compared to untreated cells. In cells pretreated with EO and then challenged with Sa, increased levels of both cytokines were observed in the first hours. However, after 6 h a decrease in IL-1β was observed compared to cells treated with Sa alone ($P < 0.01$) being the lowest values at 48 h ($P < 0.001$). After 24 h, a decrease in IL-6 was observed compared to cells treated with Sa alone ($P < 0.05$), being the lowest values at 48 h ($P < 0.01$, $P < 0.001$, and $P < 0.001$). These results suggest that after 24 h, EO would stimulate the production of anti-inflammatory cytokines, and these would inhibit the production of IL-1β and IL-6 in cells pretreated with EO and challenged with Sa. Anti-inflammatory cytokines will be quantified in further assays.

A109
EVALUATION OF CHEMICAL STABILITY AND TOXICITY OF *Minthostachys verticillata* ESSENTIAL OIL

Arsaute S[1], Montironi ID[2], Cecchini ME[1], Reinoso E[1], Cariddi LN[1]
[1]Laboratorio de Inmunología, Instituto de Biotecnología Ambiental y Salud (INBIAS), Universidad Nacional de Río Cuarto (UNRC)/Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Río Cuarto, Córdoba, Argentina. [2]Cátedra de Farmacología, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto (UNRC), Río Cuarto Córdoba, Argentina.E-mail: sofi.arsaute@gmail.com

Minthostachys verticillata (Griseb.) Epling is an aromatic plant characteristic of Argentina. Our research group has characterized the effect of its essential oil (EO) on the immune response of animals. However, to achieve a marketable bioproduct it is necessary to evaluate its stability and toxicity. The aim of this work was to evaluate the chemical stability of EO after storage at −20°C for 12 and 24 months and to determine its toxicity in bovine mammary gland epithelial cells (MAC-T). Leaves of the species *M. verticillata* from Villa Larca, San Luis, obtained commercially in April 2019, were used. The EO was obtained by hydrodistillation, and the identification and characterization of its metabolites was carried out by gas chromatography coupled to mass spectrometry (GC-MS). Six compounds were identified, being pulegone (77.2%) and menthone (8.6%) the majority, followed by limonene (1.5%), among others. After 12 months of storage at −20°C, GC-MS was performed again, and a variation in the relative percentage of pulegone (58.87%), menthone (18.91%), and limonene (1.47%) was observed. After additional 12 months (total of 24 months) of storage at −20°C, the percentages of pulegone, menthone, and limonene (57.94%, 22.22%, and 4.49%, respectively) remained stable. No organoleptic changes were observed in the EO and the chemotype (pulegone/menthone) was not affected. The cytotoxic effect of EO on MAC-T cells was determined by MTT assay. Different concentrations (10, 25, 50, 100, 250, 500, 750, 1000, and 2000 µg/mL) were evaluated at different exposure times (6, 24, and 48 h). EO did not alter cell viability up to 1000 µg/mL at the times tested. The results obtained are important for the future development of a bioproduct applicable to animal health.

A110
CONTROL OF INFECTIONS CAUSED BY *Alternaria alternata* WITH *Trichoderma harzianum* AT DIFFERENT STAGES OF TOMATO CULTIVATION (*Solanum lycopersicum*)

Autran VA[2], Guiñazú LB[1,3], Pastor NA[3], Pretti A[1], Torres AM [3], Andrés JA[1,31]Laboratorio de Microbiología Agrícola, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto.
[2]Laboratorio de Morfología Vegetal, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto.
[3]Instituto de Micología y Micotoxicología (IMICO) UNRC-CONICET. E-mail: jandres@ayv.unrc.edu.ar

Tomato cultivation is one of the most widespread in Argentina. This is affected by various diseases, including those caused by the fungal genus *Alternaria.* It includes pathogenic species that can infect crops from planting and even cause significant post-harvest damage. The use of phytopathogen biocontrollers is a promising tool that gained a strong boost thanks to technology and coupled with the paradigm shift in society's consumption habits. In this work, we evaluated the effectiveness of *Trichoderma harzianum* ITEM 3636 as an antagonist of *A. altermata*. *In vitro* evaluation tests of the antifungal activity of *T. harzianum* against *A. alternata* were carried out, and subsequently, it was evaluated on tomato seedlings germinating on agar and contemplating inoculation with *A. alternata* and co-inoculation of *T. harzianum and A. alternata.* In addition, post-harvest tests were carried out in a culture chamber, trying to determine the protective action of *T. harzianum* against *A. alternata* in fruits at commercial maturity. The statistical analysis of the data obtained was performed with the INFOSTAT software. The r^2 was 0.74, which indicates that 74% of the differences between the plates are due to the treatments used. The *P*-value was < 0.0001, which shows a positive action of the biocontrol microorganism. In the test with seedlings several parameters were evaluated and in the analysis of variance a correlation of $r^2 = 0.52$ and a *P*-value < 0.0001 were obtained, which indicates statistically significant differences between the treatment inoculated only with *A. alternata* and the co-inoculated with *A. alternata + T. harzianum*. Finally, in the post-harvest determinations on ripe tomato fruits, it was observed that the application of *T. harzianum* delays the decomposition of the fruits, in addition to maintaining the diameter of the wounds delimited with significant differences. This work provides validity for the use of biological control agents, indicating that they constitute an effective and friendly alternative to the environment in an attempt to reduce the use of chemical products.

A111

THE PROTECTIVE ROLE OF *Pseudomonas* sp. PCI2 ON POST-HARVEST DETERIORATION OF TOMATO CAUSED BY *Alternaria alternata*

Guiñazú LB[1, 3], Pastor NA[3], Autrán VA[2], Cejas LJ[3], Torres, AM[3], Andrés JA[1, 3].
[1]Laboratorio de Microbiología Agrícola, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto.
[2]Laboratorio de Morfología Vegetal, Facultad de Agronomía y Veterinaria, Universidad Nacional de Río Cuarto.
[3]Instituto de Micología y Micotoxicología (IMICO) UNRC-CONICET.E-mail: jandres@ayv.unrc.edu.ar

The high moisture content and water-soluble nutrients in tomato fruits make them perishable and susceptible to a number of fungal pathogens that cause postharvest rots. The *Alternaria* genus, widely distributed in nature, includes pathogenic species that can infect field crops or cause significant post-harvest damage, behaving as a facultative pathogen that is favored by stress, maturity, and senescence of the host. In the tomato fruit, the conidia of the fungus germinate and penetrate through wounds and the infection remains latent until maturity when tissues weaken. *A. alternata* infection is visualized as dark brown to black, smooth, slightly sunken, firm-textured lesions that can reach several centimeters in diameter. The application of fungicides is a common strategy used in an attempt to minimize post-harvest losses; however, this practice has caused environmental problems due to its residual toxicity, stimulated the appearance of strains resistant to active principles, and generated concern for human and animal safety. A sustainable alternative is the development of products based on biological control agents. Among them, bacteria of the *Pseudomonas* genus have been reported as efficient against various fungi. In this work, the effectiveness of *Pseudomonas* sp. PCI2 in suppressing diseases caused by *A. alternata* in tomato fruits was evaluated. To establish the antagonistic activity of the bacterial strain against the fungal strain, commercially ripe tomato fruits were superficially disinfected by immersion in a suspension of 2% sodium hypochlorite for 2 min, rinsed with sterile distilled water, and then they were dried by a stream of sterile air in a laminar flow chamber. Incision wounds were made on the fruits, 3 mm deep and 3 mm in diameter in the equatorial region. Immediately, 20 µL of an aqueous suspension of *Pseudomonas* sp. PCI2 were applied to each of the wounds, evaluating different concentrations (10^9, 10^7, and 10^5 CFU/mL). Three hours later, 15 µL of a suspension containing 10^4 conidia/mL of *A. alternata* was applied to each wound. The fruits were kept in a chamber at 20ºC and 95% humidity for 7 days in plastic containers protected with plastic wrap. Inoculation with the *Pseudomonas* sp. PCI2 strain showed a significant decrease in the symptoms of the disease, with an average reduction of 50% in the area of the lesions, a result that was observed with all bacterial concentrations, suggesting the use of the lowest. Therefore, this microorganism can be considered a promising tool in the biological control of *A. alternata* and suitable for the design of an effective strategy for the conservation of fruits.

A112

SECRETED PROTEINS BY *Candida albicans* SHOW ANTIGENIC SIMILARITY WITH PROTEIN FRACTIONS OF *Larrea divaricata* Cav. (JARILLA)

Urquiza Martínez MP, Dávila S, Isaguirre AC, Mattar Domínguez MA
Facultad de Química, Bioquímica y Farmacia- Universidad Nacional de San Luis, Argentina
E-mail: urquizapaulina@gmail.com

Heterologous immunity or cross-reactivity are fundamental attributes of adaptive immunity. *Candida albicans* presents virulence factors that favor adhesion or penetration and consequently, modify its role as a commensal to become a pathogenic microorganism. In our laboratory, it has been shown that proteins from *Larrea divaricata* Cav. ("jarilla") are inducers of cross-reactive antibodies against cellular proteins of *C. albicans*. However, there are no studies on cross-reaction against the exoproducts (EP) of this yeast, which have significant proteolytic activity. The objective of this work was to demonstrate the molecular mimicry between jarilla proteins and EP of *C. albicans*, through specific antibodies against *L. divaricata* proteins. For this work, proteins from a crude jarilla extract obtained by different purification methods were used. Balb/c mice were subcutaneously immunized. In the first dose, antigens obtained from protein extracts precipitated with ethanol or ammonium sulfate were used. In a second dose, antigens obtained from the extract with or without prior washing treatment of jarilla leaves with acetone were used. Two doses of the antigen were applied, separated from each other by 21 days. Mice were bled 15 days after the last dose. *C. albicans* was cultured in MMO medium (modified MacDonald/Odds) to induce EP with proteolytic capacity. The supernatants were obtained at different culture times, 72 and 96 h. The cross-reaction of anti-jarilla antibodies against *C. albicans* EP was tested by ELISA assays. For these assays, the following sensitizing antigens were used: jarilla proteins and *C. albicans* culture supernatants concentrated and partially purified with 10 kDa cut-off membrane concentrators (Amicon Ultra – 0.5 mL 10 K). Our results demonstrated the importance of choosing the methodology used to obtain jarilla proteins as immunogens. On the other hand, it was observed that heterologous anti-jarilla protein antibodies recognized *C. albicans* EP in a proteolytic activity-inducing medium, thus demonstrating that antigenic similarity exists between both proteins, at different growth times. Our findings encourage further study on the ability of specific antibodies to neutralize some *C. albicans* virulence factors involved in host-pathogen interaction. These antibodies are obtained from plant proteins. Because this methodology is considered friendly to the environment, it is intended to collaborate from this study with promising pharmacological targets through a possible prophylactic and/or protective action of *L. divaricata* proteins. From the point of view of human health, this approach could contribute to possible future biotechnological developments.

A113

Yersinia enterocolitica OUTER PROTEIN P (YopP) INHIBITS NITRIC OXIDE PRODUCTION THROUGH SIALIC ACID INTERACTION BY A POSSIBLE EXTRACELLULAR MECHANISM

Garay JA[1], Silva JE[1,2], Jofre BL[1,2], Di Genaro MS[1,2], Davicino RC[1,2]
[1]Area de Microbiología e Inmunología, FQByF-UNSL. [2]IMIBIO-CONICET. E-mail: davicino521@gmail.com

Previous studies of our laboratory demonstrated that the food-borne pathogen *Yersinia enterocolitica* (Ye) inhibits nitric oxide production (NO) in murine peritoneal macrophages (Mϕ) through Ye outer protein P (YopP) by a carbohydrate-dependent manner. However, the knowledge about the glycan motifs involved in the YopP- Mϕ interaction is underdeveloped. The aim of this study was to explore YopP activity as lectin and identify the possible N-glycans used as target. Mϕ were obtained by intraperitoneal lavage in C57BL/6 mice under aseptic conditions, cells were plated and Mϕ were purified by incubation for 2 h at 37°C in supplemented DMEM medium in an atmosphere of 5% CO_2. Mϕ (1×10^6 cells/mL) were incubated with 5 IU/mL of PNGase F or 2 IU/mL of α-2,6-syalidase in DMEM medium without fetal bovine serum, washed and cultured overnight in supplemented DMEM medium. Subsequently, Mϕ were infected with Ye serotype 0:8 (pYV^+, WA-314) (Ye wt) or Ye WA-314 deficient in YopP (pYV^+, WA-C pYVNalrKanr) (Ye Δ*yopP*) at MOI 10:1. To elucidate possible targets, we performed competition tests with five lectins: PNA, SNA, LEL, ECL, or MAL II. After infection, Griess, lactate deshydrogenase (LDH), and urea tests were performed, and YopP expression was determined by Western Blot. Hemagglutination tests were carried out with Ye wt, Ye Δ*yopP*, purified Yops or periodate treated Ye. We identified YopP presence in the supernatants of Ye wt-infected Mϕ, while LDH assay showed that experimental conditions did not induce cell lysis. These results suggest a possible YopP secretion by Ye. Yops hemagglutinin results suggest a lectin property of Yops. Moreover, deglycosylated Mϕ (Md) showed increased urea production and decreased NO secretion. Urea production was not modified after infection, while NO production was modulated by YopP. In this regard, the presence of YopP inhibited NO generation in Mϕ meanwhile stimulated NO in Md. SNA is a vegetable lectin with Neu5Ac (alpha2-6) Gal (GalNAc or syalic acid) affinity. We found that SNA did not affect NO production by Ye wt-infected Mϕ while inhibited NO production in Ye Δ*yopP*-infected Mϕ. Interestingly, Ye wt-infected Mϕ treated with syalidase showed a significant increase in NO production, suggesting that syalic acid-containing motifs plays a critical role in YopP-mediated effects. Further studies are required to consolidate the importance of a possible extracellular role of YopP collaborating with injected intracellular YopP in regulating the NO production.

A114

ANTIMICROBIAL ACTIVITY MODULATION OF *Bacillus velezensis* SL-6 BY THE PRESENCE OF *Yersinia enterocolitica*

Climent FV, Cozzolino ME, Iriarte HJ, Ferrari SG, Silva PG
Área Microbiología e Inmunología, Facultad de Química, Bioquímica y Farmacia, UNSL.
E-mail: marianacozzolino@gmail.com

Some *Bacillu*s species, safe for industrial use, are commercially available as biocontrol agents to control crop pests. Also, some of them have been proposed as probiotic bacteria to regulate human and animal health by their secretion capability of ribosomal and non-ribosomal antimicrobial metabolites, among others. Emergent research studies propose that co-cultivation and biotic additives such as heat-killed cells are novel strategies for the enhancement of secondary metabolite synthesis. The present work describes the influence of *Yersinia enterocolitica* W1024 (*Ye*) on the antibacterial activity of *B. velezensis* SL-6 (*Bv* SL-6), a strain with a broad inhibitory spectrum against pathogenic microorganisms. *Bv* SL-6 was grown for 24 h at 30°C with orbital agitation (200 rpm) in a liquid synthetic medium under two different induction conditions. In the co-culture experiments, both bacteria were inoculated at 2% v/v from a fresh cell suspension prepared in saline solution. In addition, batch cultures in the presence of thermally killed cells (autoclaved for 30 min) of *Ye* at 10% v/v were performed. A pure culture of the SL-6 strain was included as a control. Cell-free supernatants (CFSs) were obtained by centrifugation and filtration. The antibacterial activity was determined by the well-diffusion method against *Y. enterocolitica* W1024, *Escherichia coli* ATCC 25922 (*Ec*), *Listeria monocytogenes* (local isolate), and *Staphylococcus aureus* ATCC 43300 (MRSA), and inhibition zone diameters were measured using a digital caliper. Furthermore, the serial two-fold dilution method was used to determine the antimicrobial titer expressed in arbitrary units per milliliter (AU/mL). The CFS obtained from co-culture, increased the diameter inhibition zone against *Ye* (4.9%), while a minimal reduction (2.9%) in antagonism against *Ec* was observed ($P < 0.05$). The inhibition zones against gram-positive bacteria did not show a statistically significant difference. Moreover, *Ye* heat-inactivated cells reduced the antagonistic activity against both gram-negative bacteria. The antibacterial activity against *Ye* showed a 10.3% diminution in the inhibition zone diameter, with a substantial reduction of antibacterial titer in AU/mL (54.3%), while the antagonism against *Ec* dropped to 11.3 and 44.7% for both parameters, respectively ($P < 0.05$). *Ye* showed poor induction in modulating the antagonistic response of *Bv* SL-6 against the indicator bacteria tested, with a predominance of the inhibitory action on the antimicrobial activity.

A115
ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OBTAINED FROM *Eupatorium buniifolium*

Centorbi HJ[1], Aliendro OE[1], Gambluch Negre XY[1], Kurina-Sanz MB[2], Mattana CM[1], Satorres SE[1]
[1]Área Microbiología. Facultad de Química Bioquímica y Farmacia. Universidad Nacional de San Luis. [2]Instituto de Investigaciones en Tecnología Química (INTEQUI), Facultad de Química Bioquímica y Farmacia. Universidad Nacional de San Luis, CONICET. San Luis, Argentina. E-mail: 768centorbi@gmail.com

Essential oils (EOs) are aromatic and volatile mixtures of secondary metabolites obtained from different parts of plants. *Eupatorium buniifolium* Hook. et Arn. (Asteraceae), popularly known as "romerillo" or "romerillo colorado" is part of the native flora of the Province of San Luis, Argentina. EOs were isolated by hydrodistillation techniques from the aerial part. The *in vitro* antibacterial activity of EOs was evaluated against *Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, and *Listeria monocytogenes* CLIP 74904 strains. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the micro-well dilution assay method, in trypticase soy broth (TSB) with 0.01% (W/V) addition of 2,3,5-triphenyltetrazolium chloride as a visual indicator of bacterial growth. Suspensions of 10^6 CFU/mL for each strain were used. EO was dissolved in DMSO, and then serial twofold dilutions were made in a concentration range from (µg/mL) 5,000 to 2.44. The 96-well plates were prepared by dispensing into each well 100 µL of TSB, 100 µL of EO serial dilutions and 5 µL of the inoculum. In addition, TSB, strains and EO controls were also included. The plates were incubated at 37°C for 24 h under aerobic conditions. The MIC was defined as the lowest concentration of the EO in the medium, in which there was no visible growth after incubation (no red color). The MBC was determined by subculturing on trypticase soy agar (TSA) from the last three wells that showed no visible bacterial growth. The experiments were performed in duplicate and then replicated at least twice. *E. buniifolium* EO showed inhibitory activity against *S. aureus* ATCC 43300 (µg/mL), MIC 1,250-MBC 1,250; *L. monocytogenes* CLIP 74904, MIC 312-MBC 625; and no activity against *P. aeruginosa* in the studied range (MIC-MBC > 5,000 µg/mL). In this work, we observed that EO resulted to be more active on Gram-positive than on Gram-negative bacteria. However, *S. aureus* was less affected, if compared with *L. monocytogenes*. The effect observed for *P. aeruginosa* is probably due to the hydrophilic lipopolysaccharides contained in the outer membrane, which create a barrier against the hydrophobic compounds of the EOs. Antibacterial activity of EOs presents an increasing interest in the last years since they were shown to be effective even on multidrug-resistant strains. Further studies are needed in order to find the proper ways to deliver these active natural compounds.

A116
EFFECT OF *Yersinia enterocolitica* O:8 INFECTION ON GLUCOSE HOMEOSTASIS

Velazquez M[1,2], Pelegrina M[1], Di Genaro MS[1,2]
[1]Universidad Nacional de San Luis, Argentina. [2]Instituto Multidisciplinario de Investigaciones Biológicas de San Luis, CONICET, Argentina. E-mail: velazquez0104@gmail.com

The relationship between metabolism and the immune response to bacterial infection on the organismal level remains to be completely elucidated. Glucose is the key metabolite for immune cells and plays a critical role in the outcome of the infection. Here, we examined the relationship between sickness-induced anorexia and glycemia with cytokine production during the course of *Yersinia enterocolitica* (Ye) infection. C57BL/6 (WT) or TNFR1-deficient (*TNFR1*$^{-/-}$) mice were orally infected with Ye O:8 by gavage. Body weight, food intake, glycemia, and serum cytokine (TNF, IL-17) levels were evaluated during the course of infection. Both Ye-infected WT and *TNFR1*$^{-/-}$ mice exhibited significant weight loss ($P < 0.05$ and $P < 0.001$ for WT and *TNFR1*$^{-/-}$, respectively) and a decrease in food intake ($P < 0.001$ compared with non-infected control mice). Moreover, Ye infection induced significant serum IL-17 on day 3 ($P < 0.05$) followed by TNF production on day 5 after infection ($P < 0.001$). WT mice developed hypoglycemia as early as 24 h post-infection with Ye ($P < 0.01$). Although slightly later (3 days after infection), TNFR1 deficiency did not reverse the hypoglycemia in the mice. We observed that the weight loss correlated with hypoglycemia upon Ye infection ($P < 0.01$). In addition, TNF levels correlated with anorexia in mice after acute Ye infection ($P < 0.05$). Our results show that Ye infection impacts glucose homeostasis, suggesting immune-metabolism interactions during this enteric infection. Further studies are necessary to elucidate the virulent factors and immune mediators involved in the regulation of glucose metabolism after Ye infection.

A117

EVALUATION OF THE EFFECT OF THE CELL-FREE SUPERNATANT FROM *Lactococcus lactis* sl40 ON THE BIOFILM FORMED BY *Yersinia enterocolitica*

Giorggi TA, Benito Rodriguez PG, Gaido Riso N, Mitjans NM, Stagnitta PV
FQBYF, UNSL, San Luis, Argentina. E-mail: *pvstag@unsl.edu.ar*

Bacterial biofilms are aggregates of microorganisms, which are in a sessile state, immersed in a matrix of extracellular polymeric substances preventing the action of antimicrobial agents. Bacterial biofilms produced by human pathogens are related to chronic infections due to their ability to resist therapeutic treatment by forming biofilms on indwelling medical devices, including implanted artificial heart valves, catheters, and joint prosthetics. Furthermore, in food factory environments some biofilm-forming species are human pathogens. These pathogens are able to develop biofilm structures on different artificial substrates common in the food industry. The purposes of this study were to evaluate *Yersinia enterocolitica*'s capacity to form biofilms and the effect of cell-free supernatant of *Lactococcus lactis* sl40 isolated from raw goat milk, on biofilm formed by *Y. enterocolitica*. For biofilm formation, Petri dishes containing sterile coverslips were used in which *Y. enterocolitica* was incubated in tryptone soya broth (TSB) medium at 37ºC. At 24 h, a mature *Y. enterocolitica* biofilm was obtained. The coverslips with biofilm were washed and placed in sterile Petri dishes. The effect of cell-free supernatant (CFS) and neutralized cell-free supernatant (NCFS) of *L. lactis* sl40 on the biofilm formed was evaluated by incubation for 24 h at 37ºC. The biofilms used as controls were incubated with MRS medium. Later, coverslips were washed with sterile distilled water and to study the planktonic cells, samples were taken from Petri dishes. Quantification of planktonic cells and sessile cells, viability of sessile cells, optical microscopy (OM), and scanning electron microscopy (SEM) tests were performed for control and treated with CFS and NCFS biofilms. The quantity of cells that remained attached to SLC-treated biofilms decreased significantly when compared to controls, as well as cell viability. By quantification, a reduction of planktonic cells was verified, being more pronounced by treatment with CFS. For both treatments, notable changes in the morphological integrity of the biofilm cells were observed by SEM. These results allow us to conclude that CFS from *L. lactis* sl40 was able to reduce both the number of sessile cells in the *Y. enterocolitica* biofilm and the viability of attached cells. In addition, the morphology of *Y. enterocolitica* cells in biofilm was modified. Besides, it was possible to establish a decrease in the quantity of planktonic cells after both treatments, with a greater effect of CFS. These data provide promising information to combat the formation of bacterial biofilms, which represents a problem in the food industry, biotechnology, medicine, and other industrial sectors.

A118

MICROBIOLOGICAL ANALYSIS OF VINEYARD SOILS FOCUSED ON THE SEARCH FOR PLANT GROWTH PROMOTERS

Lambrese Y, Navarta G, Calvo J, Possetto P, Sansone G, Calvente V
Área de Tecnología Química y Biotecnología, Universidad Nacional de San Luis, Facultad de Química, Bioquímica y Farmacia.E-mail: mgsanso@unsl.edu.ar

The excessive use of fertilizers and agrochemicals causes the deterioration of agroecosystems. For this reason, eco-friendly alternatives are being investigated, such as the use of biofertilizers (beneficial microorganisms that stimulate crop growth and productivity). The soil microbiota is a natural source of microorganisms, many cultivable, that can be isolated and tested for their ability to promote plant growth. The mechanisms through which plant growth-promoting microorganisms can carry out their beneficial effect are phosphate solubilization, nitrogen fixation, production of siderophores, phytohormones, and biological control, among others. The objective of this work was to carry out a study of the soil microbiota of vineyards in order to analyze the presence of microorganisms capable of promoting the growth of *Vitis vinifera* plants. Methodology: 40 soil samples of 11 grapevine farms located in Valle de Uco and Luján de Cuyo, Province of Mendoza were used to isolate microorganisms. The samples were transported refrigerated, and, in the laboratory, they were preserved in the refrigerator and processed in less than 48 h. Three culture media were used: base medium with soil extract, Sabouraud glucose medium, and medium for phosphate solubilizers. Serial dilutions (from 10^{-2} to 10^{-6}) of the samples were inoculated, and the results were expressed in CFU/g of soil. Similar values were obtained in the counts of total heterotrophic microorganisms (from the medium with soil extract and medium phosphate solubilizers) from the same farm (between 10^7 and 10^8 CFU/g of soil) in most of the samples. On the other hand, the percentages of phosphate-solubilizing microorganisms ranged from less than 1% to 10%. The counts of filamentous fungi showed lower values (between 10^6 and 10^7 CFU/g of soil). The results obtained allow us to conclude that the analyzed soils present a healthy microbiota with good counts of bacteria and yeasts and low counts of filamentous fungi (possible pathogens). In addition, the presence of phosphate-solubilizing microorganisms allows us to assume that these microorganisms would have the capacity to promote the growth of *V. vinifera* plants and could be used as a biofertilizer. In the future, these isolated phosphate-solubilizing microorganisms will be tested in other biofertilizer capacities such as nitrogen fixation, the production of siderophores, and phytohormones.

A119
STUDY OF THE DISINFECTANT POWER OF PRODUCTS USED FOR CLEANING AND SANITATION IN CRAFT BREWERY

Venturini V[1], Sánchez Peterle MB[1], Sansone G[1], Palumbo L[2], Calvente V[1], Fernandez JG[1]
[1]Grupo de Investigación, Desarrollo y Asistencia al Sector Cervecero. (GIDACER). Facultad de Química, Bioquímica y Farmacia, UNSL, San Luis. [2]Facultad de Agronomía. UNCuyo. Mendoza. Monteño Insumos. Mendoza
E-mail: jgastonfernandez@gmail.com

Beer is one of the most widely consumed beverages in the world, which reaches all social statuses. Craft breweries in Argentina are an innovative industrial sector that has experienced explosive growth in the last few years. The growing demands for craft beers have driven quality controls. Cleaning and sanitation of breweries is a critical point in quality. Sanitizing agents are used to reduce the number of microorganisms to acceptable levels in brewing. With the aim to generate more information for craft breweries, it was studied the disinfectant power of commercial products used for cleaning and sanitation in craft beer production. Two commercial products were studied (ES and OP). To determine the inhibitory power, a test was designed based on the UNE-EN 1040 protocol (test for the evaluation of the basic bactericidal activity of chemical antiseptics and disinfectants). 100 µL of bacterial inoculum (beer contaminants) of 0.5 McFarland standards ($1-2 \times 10^8$ CFU/mL) were placed in an Eppendorf tube, then 900 µL of each treatment was added. The tubes were vortexed and kept at 27 + 2°C for 5 min. Next, was inoculated 200 µL in plates with YGM medium agar. The inoculated plates were incubated at 27 + 2°C for 24 h. The colonies were then counted. The treatments were ES and OP (concentration of 1%, indicated by the seller). The products were neutralized as control (ESN and OPN). Sterile water was used as a negative control. Assays were performed in duplicate. The results indicated that in the treatment with ES and OP, there was no evidence of bacterial growth in the cultures, in 100% of assays, while ESN and OPN showed bacterial growth, at an average concentration of approximately 10^6 CFU/mL in both cases, after incubation. The negative control also showed growth, at a concentration of approximately 10^6 CFU/mL. In conclusion, the results showed that both commercial products inhibit the bacterial growth of beer contaminants when they are used at the recommended concentration, and according to UNE-EN 1040 protocol, are considered as disinfectants. In future studies it will be necessary to study different concentrations or combinations of these, to give more information to the craft beer sector.

A120
COMPARATIVE STUDY OF THE ANTIMICROBIAL ACTIVITY OF KEFIR GROWN UNDER DIFFERENT SUBSTRATE

Amieva MI[1,2], Delfini CD[1], Quiroga E[2,3], Villegas LB[1,2]
[1]Instituto de Química de San Luis (INQUISAL). CONICET-UNSL San Luis. [2]Área de Bromatología. Departamento de Farmacia. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. [3]Instituto de Instituto de Física Aplicada (INFAP) - CONICET-UNSL San Luis.E-mail: tati.amieva.81@gmail.com

Kefir is an ancient beverage, slightly acidic and alcoholic fermented that originated in the Caucasian region of Asia. Kefir is a naturally fermented product comprised of a probiotic bacteria and yeast complex that coexist in symbiotic association. Kefir consumption has been associated with many advantageous properties to general health, including as an antioxidative, anti-obesity, anti-inflammatory, anti-microbial, and anti-tumor moiety. Generally, kefir may be identified depending on the types of substrates used for fermentation, which are dairy and non-dairy kefir. The different manufacturing conditions of kefir (agitation, the inoculum concentration, as well as the fermentation time and temperature) may alter the original characteristics of the microbial composition, hence affecting their health-giving properties. Therefore, this study aims to compare the antimicrobial activity of kefir grown under different substrate. Kefir drinks were prepared from three different substrates: 0% milk fat (4.9 g% carbohydrates), water (4.8 g% Muscovado sugar), and LB lactose (4.5 g% lactose). A total of 3 g of kefir grains were inoculated in 30 mL of each substrate (10% w/v). Erlenmeyer flasks were incubated at 28°C and 100 rpm. Samples were taken at 24 (T1), 48 (T2), 72 (T3), 120 (T4), and 168 h (T5). The supernatants were obtained by centrifugation at 10,000 × *g* for 10 min. Antimicrobial activity was determined by diffusion in agar on Petri dishes containing the LB for bacteria and potato-glucose agar for fungi. The target strains used were *E. coli*, *Staphylococcus aureus*, *Fusarium* sp., *Aspergillus* sp., and a fungus isolated from bread without being identified yet. The antimicrobial activity varied according to the type of kefir and the fermentation time and was found after T3. The supernatants of water kefir presented the best results of microbial activity, followed by the milk kefir, while the kefir that grew in LB-lactose did not show activity. The water kefir supernatant inhibited four of the five target strains. The most sensitive microorganisms to it were *E. coli*, followed by *Aspergillus* sp., the mold isolated from bread, and *Fusarium* sp. *Staphylococcus* was not inhibited by any of the supernatants. The pH of the three types of kefirs decreased as the incubation time increased. The antimicrobial activity of milk and water artificially acidified with acetic acid and lactic acid was evaluated as a control, but no inhibitory activity was obtained for any of the target strains. Therefore, supernatants from kefir could be attributable to antimicrobial metabolites in supernatants rather than the low pH. Further research is necessary to study the compounds responsible for these functional properties and their stability for their use as food additives.

A121
OPTIMIZATION OF PURIFICATION AND IDENTIFICATION METHODS OF IGY FROM HEN EGGS

Poggio L, Isaguirre A, Dávila S, Mattar Domínguez MA
Área Microbiología e Inmunología. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. E-mail: mattardominguezmariaaida@gmail.com

Egg yolk immunoglobulin (IgY) biotechnology has many advantages over mammalian antibodies and is used in Veterinary Medicine for passive immunization against bacterial infectious diseases. The method of obtaining IgY guarantees animal welfare and produces a high concentration of antibodies. Like IgG, IgY is a compound antibody with two heavy chains of between 67–70 kDa and two light chains of 25 kDa, linked by disulfide bridges, with a molecular weight of ~150 kDa. IgY does not activate complement, does not bind to proteins A and G, does not bind to mammalian antibodies, reducing the risk of obtaining false-positive reactions in immunoassays, and does not bind to the cell surface of the Fc receptor. All these differences have allowed the application of IgY in different methods in research areas such as diagnosis, medicine, and biotechnology. The aim of this work was to optimize the protocols for the separation, purification, and identification of IgY in our laboratory. The high lipid content of egg yolk interferes with purification steps. It is for this reason that IgY purification requires an initial delipidation by centrifugation at 8000 × *g* for 12 min at 4°C. The lipid-free supernatant was stored at −20ºC overnight to eliminate proteins sensitive to freezing. The fractionation was carried out with ammonium sulfate. The sample was separated by chromatography using ion exchange columns (HiTrapDEAE FF) and Tris buffer solutions (pH = 8) with different NaCl molarities (from 0 mM to 250 mM). The proteins collected were subjected to 10% and 8% polyacrylamide gel electrophoresis under non-reducing and reducing conditions. For identification, ELISA and Western Blot assays were performed. For ELISA the plates were coated with the different eluids of IgY and subsequently, they were confronted with different dilutions of a conjugated specific anti-IgY rabbit antibody. For WB analysis, protein samples of electrophoresis were transferred to nitrocellulose membranes and subsequently, they were marked with different dilutions of a conjugated specific for subsequent determination by chemiluminescence. In SDS-PAGE, the bands corresponding to both whole molecule-IgY (141–148 kDa) and their heavy (64–73 kDa) and light (23–29 kDa) chains were obtained. The presence of IgY could be determined by ELISA and identified by WB in the eluted obtained at the different NaCl molarities tested. The eluted at the different molarities gave a significant difference compared to the negative controls in an order of 10^{-6} with the 1/5.000 conjugate, by ELISA. In WB, better results were obtained with the 1/20.000 conjugate at the different molarities. The three techniques allowed for determining the presence of IgY in the sample. These findings will be important in the evaluation of the antigenicity of plant proteins used as immunogens that provide protection against opportunistic pathogens, being a promising biotechnological product.

A122
ACTINOBACTERIA RESISTANT TO CONTAMINANTS OF EMERGING CONCERN

Gonzalez Holc VG[1], Aparicio JD [1,2], Raimondo EE [1,2], Costa Gutierrez SB [1], Polti MA[1,3]
[1]Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), S. M. de Tucumán, Argentina. [2]Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán (UNT), S. M. de Tucumán, Argentina. [3]Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán (UNT), S. M. de Tucumán, Argentina. E-mail: marta.polti@conicet.gov.ar

The Contaminants of Emerging Concern (CECs) are synthetic or naturally occurring chemicals that are not commonly monitored in the environment but have the potential to enter the environment and cause adverse ecological and/or health effects. A promising technology to clean up environments contaminated with CECs is bioremediation using actinobacteria, which are microorganisms with great metabolic diversity and the ability to detoxify different organic and inorganic compounds. In this context, the objective of the present work was to select actinobacteria resistant to CECs of regional relevance. The resistance to CECs of 12 actinobacterial strains, previously isolated from contaminated environments, was qualitatively evaluated. The CECs studied were diclofenac (DIC), sildenafil (SIL), and ivermectin (IVE). These CECs were selected because they were detected in several domestic and hospital effluents in the northwestern region of Argentina, and they belong to different chemical groups. The qualitative screening assay was carried out in Petri dish plates containing 20 mL of casein starch agar medium (CSA). For the DIC and SIL assays, rectangular troughs (1.5 × 6 cm) were cut in the center of plates and filled with 1 mL of the solution to be tested. For the IVE assays, due to its insolubility, the solution was added directly to the CSA medium. The concentrations tested were: 1, 5, and 10 mg/mL. The strains were inoculated perpendicular to the rectangular troughs (DIC, SIL) or equidistant (IVE). Plates were incubated at 30ºC for 7 days. Control plates were also performed, using sterile distilled water instead of CECs. For each strain, growth, spore formation, and pigment production were evaluated in comparison to that observed on control plates. For DIC and SIL, two strains, different for each CEC, were able to grow, form spores, and produce pigments, at levels comparable to their corresponding controls. For IVE, 12 strains were able to grow and produce pigments, but only 9 of them formed spores. None of the strains studied showed tolerance to the three CECs, although several strains showed tolerance to two CECs. These results demonstrate the great potential of actinobacteria to grow in

the presence of several types of CECs, also indicating that the metabolic pathways involved in each type of tolerance may be different. The strains with the highest resistance to each CEC were selected for degradation tests in liquid culture media, in order to evaluate their ability to use the CEC as the only source of carbon and energy.

A123
THE VEGETATION IN THE SURROUNDINGS OF THE RAMÓN CARRILLO HOSPITAL AND ITS POTENTIAL ALLERGENIC VALUE

Gambluch Negre X[1], Marchevsky KE[1,2], Moyano[2] FM, Moglia MM[1,2]
[1]PROICO 03-1320- (FMN-UNSL). [2]PEIS 02-0320- (FQByF-UNSL). E-mail: mmmoglia@unsl.edu.ar

The presence of green areas favors the physical and mental health of the population. However, some plants can also cause health problems. In this sense, the management of urban green spaces is of great importance, especially in the vicinity of health centers. Many trees, and also some shrubby and herbaceous plants, can cause pollen allergies. Among the herbaceous area The Chenopodiaceae–Amaranthaceae, which include plants common in disturbed areas such as quinoa, Russian thistle, and morenita, implicated in summer and autumn allergies, and Poaceae such as *Lolium* spp. and *Cynodon dactylon*, which cause spring and summer allergies. The objectives of the work were to carry out an evaluation of the Value of the Allergenic Potential (VPA) of the species, both cultivated and spontaneous, from the surroundings of the East zone of the Ramón Carrillo Hospital, where its main entrance is located, and to make suggestions regarding the types of plants used and their management. The methodology included: (a) the exploration of the area through satellite images, using Google Earth Pro, for the delimitation of differentiated zones of vegetation, (b) carrying out censuses of species *in situ* and identifying specimens in the laboratory, and (c) calculation of the VPA. The results indicated that the woody plants used in afforestation, both shrubs and trees, are for the most part appropriate since they have low VPA. Among them are *Robinia pseudoacacia* (VPA: 4), *Brachychiton acerifolius* (VPA: 2), and *Albizia julibrissin*, among others. However, male specimens of *Acer buergerianum* (VPA: 8), *Fraxinus excelsior* (VPA:18), and *Salix* sp. (VPA: 18), with moderate to high VPA, were also recorded. On the other hand, the disturbed areas of the circuit that surrounds the hospital are invaded by Chenopodiaecae–Amaranthaceae, and species of the *Lolium* genus were used as lawns in the landscaping near the hospital entrance (VPA: 27). Finally, it is recommended to avoid the cultivation of allergenic plants detected in the area and to carry out appropriate management practices for established species, both cultivated and spontaneous.

A124
POTENTIAL ALLERGENICITY OF TREES IN THE SAN LUIS HOSPITAL GREEN SPACES

Polanco MN, Moglia MM
Proyecto de investigación: PROICO 03-1320- (FCFMyN-UNSL). E-mail: mpolanco@unsl.edu.ar

The identification of the city's green areas vegetation and its allergenicity, especially in health centers, is of great importance for people who suffer from allergies to pollen. The objective of the present work was to calculate, through the Index of Allergenicity of Urban Green Areas (IUGZA), the areas with different pollinosis exposure risks of the San Luis Hospital (Argentina). The methodology involved calculating the Allergenic Potential Value (APV) for each of the tree species registered in each of the four homogeneous areas that were delimited in the studied area. The values obtained varied from a minimum of 0.007, in zone D, to the NE of it, and 0.022 in zone B, located to the south, to a maximum of 3.7 in zone C in the extreme NW. The Addictions Care Center (ACC) is located in this area. This last IUGZA value is considered high, exceeding the threshold value of 0.3 and, therefore, sensitized people should avoid driving through it during the pollination season of the allergenic species. Among these, the main ones recorded included individuals from the Ulmaceae, Cupressaceae, and Pinaceae families. From the results, it is concluded that most of the studied area has low IUGZA below the threshold. The recommended accesses to the hospital for sensitized patients are those at the extreme NE (zone D) and south (zone B), the least suggested being the entrance through the ACC (zone C). These results, together with those obtained from the previous analysis of water quality, made it possible to characterize the water and air conditions in the San Luis Hospital area.

HEALTH AND NUTRITION

A125
NUTRITIONAL COMPOSITION OF *Pleurotus Ostreatus* HARVESTED FROM SUBSTRATES WITH DIFFERENTS AGRO-INDUSTRIAL BY-PRODUCTS, CHILECITO, LA RIOJA

Leal NIA[1], Lucero RE[1], Cantón NV[1,2], Rojas MY[1], Varas MM[1,2]
[1]Departamento de Ciencias Básicas y Tecnológicas, [2]Instituto de Ambientes y Regiones Áridas. Universidad Nacional de Chilecito. 9 de Julio Nº 22. 5360. Chilecito. La Rioja. Argentina.E-mail: mvaras@undec.edu.ar

In Chilecito, La Rioja, the main industries are based on walnut, olive, and grape production, generating large volumes of agro-industrial by-products. Mushroom cultivation provides, for the agro-industrial by-products, alternative employment and helps to avoid the associated environmental problems of your accumulation. Many studies have been conducted to test the ability of *Pleurotus* to grow on different agricultural wastes. These mushrooms have the ability to colonize and degrade a wide variety of lignocellulosic wastes with a relatively short cycle of production. The growing consumption interest of the oyster mushroom is increasing largely due to its taste, medicinal, and nutritional properties. *Pleurotus ostreatus* demands few environmental controls, diseases and pests do not often attack their fruiting bodies, and they can be cultivated in a simple and cheap way. All this makes *Pleurotus ostreatus* cultivation an excellent alternative for the production of mushrooms when compared to other mushrooms. The objective of this study was to research the nutritional composition of *Pleurotus ostreatus* in substrates enriched with different agro-industrial by-products. There were evaluated substrates based on pine sawdust, with the aggregate of walnut shell, olive pruning remains, or vine pruning remains. We used the technique of cultivation of fungi in plastic bags. Tested substrates: (A) pine sawdust (PS, control), (B) PS + walnut shell, (C) PS + olive pruning remains, and (D) PS + vine pruning remains. The experimental design was completely randomized, with four treatments and five repetitions per treatment. The protein content varies between 17.7 and 22.5 g/100g dry matter (%), and the results showed that the protein content of the mushroom harvested was higher when vine pruning residues were added to the control. The crude fiber content depends on the substrate on which *Pleurotus* is produced. Specifically, the values obtained vary between 22.8% and 12.9%. From these results, we can notice that the lowest crude fiber content is obtained with *Pleurotus* cultivated in group C (with olive pruning remains, 1.6%), compared to those harvested from PS (3.4%). On a dry basis, the carbohydrate content was higher in *P. ostreatus* grown on pine sawdust substrate with olive pruning residues (53.2%) in relation to the control treatment (44.4%). There was a significant difference in values of ash content of *Pleurotus* harvested from the different substrates. The use of vine pruning residues increases the values of ash in relation to pine sawdust (9.9% *vs* 6.4%). This study showed that there is variation in the nutritional composition of the *P. ostreatus* grown and harvested from different substrates, which could be attributed to the nutritional composition of the substrate where these were cultivated.

A126
RABBIT QUALITY MEAT: EFFECT OF DEHYDRATED OLIVE OIL WASTE IN RABBITS DIET

Müller N[1], Martínez P[2], Almirón RS[2], Ortega MG[3, 4], Varas MM[2,5]
[1]Colegio Nacional Agrotécnico "Julio César Martínez". [2]Departamento de Ciencias Básicas y Tecnológicas, Universidad Nacional de Chilecito. [3]Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. [4]IMBIV-CONICET. [5]Instituto de Ambientes y Regiones Áridas. Universidad Nacional de Chilecito. 9 de Julio Nº 22. 5360. Chilecito. La Rioja. Argentina.E-mail: mvaras@undec.edu.ar

At present, the importance of rabbit meat in human nutrition is growing, for its nutritional and dietary properties. Feeding has been the most important component of rabbit production representing 70% of the costs. Diets based on commercial balanced feed increase the costs of meat production. This fact demonstrates the importance of looking for cheap alternatives and feedstuffs to replace traditional foods and reduce costs. The olive production industry in La Rioja, Argentina, produces relatively big amounts of waste generally known as olive by-products. The use of these by-products in animal feed can be of considerable economic importance. The aim of this work was to study the effect of different percentages of feeding with dehydrated olive oil waste on chemical parameters in rabbit meat. Thirty-two rabbits of the French Hyplus hybrid breed, weaned at 25 days of age, and housed in individual cages, were used. The rabbits were randomly distributed at a rate of 8 animals per treatment, and a completely randomized block experimental design (blocking based on weight) with 4 treatments and eight (8) repetitions was obtained. The treatments were: T1 = 100 % commercial balanced feed (BC); T2 = 97.5% BC + 2.5% dehydrate olive oil waste (DOW); T3 = 95% BC + 5% DOW, and T4 = 90% BC + 10% DOW. Experimental diets were offered *ad libitum* for 10 weeks until the slaughter. After dissection, fresh rabbit meat was obtained for analysis. It was determined: dry matter, proteins, fats, and ashes, as a physical parameter the pH of meat was measured. Evaluation of all results we found statistically significant differences ($P < 0.05$) between the groups, in dry matter, proteins, and fats. Dry matter in T1 was 27.3 g/100 g dry matter (%), in T2

was 51.2%, in T3 was 48.9%, and, in T4, 55.8%. The protein content varies between 25.6% and 31.6%, and the results showed that the protein content was lowest when higher percentages of DOW were added to the diet (T4). On the contrary, when higher levels of DOW were added to the diet, the content of fat in the meat was higher (T1 = 3.2% *vs.* T4 = 4.1%).

A127
MICROBIAL PHYTASE INFLUENCE IN MONOGASTRIC ANIMALS' NUTRITION

Piguillem SN[1,2], Salinas E[1], Toledo MF[4], Ciminari ME[1], Filippa MA[3], Gómez NN[1,4,5]
[1]Laboratorio de Morfofisiología, FQByF – UNSL. [2]Facultad de Ciencias de la Salud, UNSL. [3]Laboratorio de Química Física, Facultad de Química, Bioquímica y Farmacia-UNSL. [4]Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO)-SL/(CONICET), San Luis, Argentina. [5]Facultad de Ciencias Médicas, UCCuyo.
E-mail: spiguillem@gmail.com

Phytase (3-phytase EC 3.1.3.8 and 6-phytase EC 3.1.3.26) is an enzyme present in microorganisms such as fungi, yeasts, and bacteria that hydrolyze phytic acid (6-phosphate inositol or myoinositol) releasing phosphate ions in solution. These enzymes are used as food supplements in monogastric animals, which do not have this enzyme, counteracting the phytate anti-nutritional effect that decreases the other minerals' availability required for animals' development. This work's purpose was to learn about microbial phytase effects in monogastric animals. The enzyme is extracted from yeasts (*Saccharomyces cerevisiae*). It is obtained at the end of the fermentation process and then it is cold centrifuge at 15,000 rpm and the supernatant is frozen for storage and subsequent use in food. To prepare the feed supplemented with phytase, a dilute enzyme solution is applied and sprayed on the dry and ground food, left to act for a period of 24 h at 45°C, then the food is dried, and the pellets are reconstituted to their original form. We used Wistar rats (females and males; N = 14), separated into four groups: control (females FC and males MC) fed with common food and females and males fed with food+phytase (FF and MF). Different biochemical parameters were analyzed during two months of treatment, body weight was controlled once a week, and blood pressure was monitored too. Female rats supplemented with phytase presented lower body weight (121.32 ± 10.34) than control rats (144.33 ± 15.98) ($P < 0.0001$). Hematological and glycemic parameters did not show significant differences after two months of treatment, although glycemia exhibited a trend to decrease and neutrophils to increase in phytase females. After 60 days of treatment, diastolic pressure did not exhibit significant differences, but it displayed a trend to increase in phytase treatment (117.34 ± 25.38) compared to controls (83.47 ± 19.76). Perhaps, blood pressure increment especially in FF could be explained by a rise in muscle tone. Phytase addition in monogastric animal's diet optimizes energy utilization and minimizes nutrient excretion; therefore, body mass decreased in FF could be due to fat tissue loss and muscle gain, which is also consistent with the tendency to lower blood glucose.

A128
EXTRA VIRGIN OLIVE OIL AMELIORATES HIGH-FAT DIET-INDUCED LIVER ALTERATIONS BY MODULATING THE CHOLESTEROL PATHWAY IN RABBITS

Funes AK[1], Avena MV[1,2], Agüero R[1], Boarelli PV[2], Conte MI[1], Monclus MA[1,3], Fornés MW[1,3], Saez Lancellotti E[1,3]
[1]Laboratorio de Investigaciones Andrológicas de Mendoza (LIAM), IHEM, Universidad Nacional de Cuyo, CONICET. [2]Laboratorio de Enfermedades Metabólicas (LEM), Universidad Maza. [3]Consejo de Investigaciones de la Universidad del Aconcagua (CIUDA), Universidad del Aconcagua. E-mail: abikarenina@yahoo.com.ar

Hepatic cholesterol (chol) accumulation induced by lipid overload is a major public health problem worldwide, and natural products such as Extra Virgin Olive Oil (EVOO) have proven benefits, but the mechanism remains unclear. Sterol regulatory element-binding protein 2 (SREBP2) leads intracellular chol metabolism as a transcription factor and is sensitive to dietary fat intake. Our aim was to test the effects of EVOO addition to a high-fat diet (HFD) on the expression of hepatic chol metabolism pathway molecules using rabbits as an experimental model of hypercholesterolemia (HC). New Zealand rabbits were fed a commercial pellet (control), an HFD (14% bovine fat, HC rabbits), or an HFD plus EVOO (HFD 7% + EVOO 7%: protected rabbits) for up to 12 months. Hepatic chol accumulation was characterized by the specific marker filipin III. The expression of SREBP2, HMGCR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase), and LDLR (low-density lipoprotein receptor) was studied by western blot and PCR. Our results show that hepatic chol increased in HC rabbits but decreased in protected animals. SREBP2 mRNA was not modified by HFD although protein expression decreased in the short term and raised under a long-term HFD. When EVOO was added, in both cases the expression increased significantly. HMGCR expression did not vary significantly with HFD, but it increased with the addition of EVOO. LDLR mRNA and protein showed an increase with both diets. These results indicate that fat intake deregulates SREBP2 expression, leading to lipid accumulation in rabbit hepatocytes. The addition of EVOO prevented fat diet-induced lipid increase despite rising HMGCR and LDLR expression. The former needs further research as it involves many post-translational regulators, and the LDLR increase is reasonable as the hepatocyte is the main cell involved in the removal of plasma cholesterol through LDLR activity. The improvement in hepatic lipid accumulation is probably related to other mechanisms such as bile production. Finally, all the molecules analyzed here

were sensitive to EVOO supplementation, although specific studies are needed to determine the exact mechanism of protection.

A129
DAILY VARIATION OF METABOLIC PARAMETERS ARE MODIFIED IN AN EXPERIMENTAL MODEL OF NUTRITIONAL OBESITY IN ADULT RATS

Alfonso JO, Salinas E, Gutiérrez Aguero A, Lopez M, Delgado SM, Perez Chaca MV, Ciminari ME, Biaggio V, Gomez NN, Navigatore Fonzo L, Anzulovich AC. Laboratorio de Cronobiología, IMIBIO-SL, CONICET-UNSL.
E-mail: javieroscaralfonso@gmail.com

It is well known that the cause of obesity (OB) is multifactorial, including genetic, environmental, and dietary factors, among which, high-calorie diets play a central role in the development of the disease. On the other hand, several investigations have related altered metabolic homeostasis and circadian parameters with Alzheimer's disease. As part of the IMIBIO-SL institutional project (P-UE 013), which studies OB as a predisposing disease to the development of chronic age-associated diseases and the search for early biomarkers with predictive potential, we obtained a nutritional model of OB in adult rats and analyze potential early biomarkers of Alzheimer's disease (AD). In this framework, our objective here was to evaluate the daily variation of metabolic parameters in our model of OB in adult rats. For this, male Wistar rats were weaned at 21 days of age, and fed a normocaloric diet (NC), containing 366 kcal of lipids/kg diet, then, at the 2-mo-age, they were randomly separated, and fed: one group, with the NC diet (control, CO, group) and the other, with a high-fat diet (HFD, 1570.7 kcal of margarine/kg diet, OB group), for the next 14 weeks. The animals were kept under 12 h-light:12 h-dark and 22–24°C conditions, with water and food *ad libitum*. For chronobiological studies, at least four (4) animals from each group were euthanized every six (6) hours, at the zeitgeber times (ZT): ZT2, ZT8, ZT14, and ZT20. All the experiments were performed following national and international guides for the care and use of laboratory animals and were approved by the CICUA (UNSL). Metabolic parameters such as glucose (G), triglycerides (TG), total cholesterol (TC), HDLc, and LDLc + VLDLc levels, were determined in the serum of both CO and OB groups, using commercial kits. Statistical differences throughout the 24-h period were analyzed by one-way ANOVA, followed by a post-hoc test, to confirm statistical differences between ZTs within each group; chronobiological statistics were used to confirm the presence of rhythm and Student t test to compare rhythm's parameters (acrophase, mesor, and amplitude) between groups. We found G, HDLc, and TG levels vary significantly and rhythmically throughout a day in the serum of the CO group, with rhythms' acrophases occurring at the beginning of the light period. Unexpectedly, TC and LDLc+VLDLc do not display a rhythmic variation throughout a 24-h period. Noteworthy, the HFD abolished the rhythmic patterns of daily G and TG levels and induced oscillating patterns of TC and LDLc+VLDLc levels, with the TC rhythm's acrophase at the beginning of the day and the lipoproteins' peak at the second half of the night. Furthermore, daily means of G, TG, and LDLc+VLDLc levels increased in the OB group ($P < 0.05$, $P < 0.01$, and $P < 0.05$, respectively), while HDLc mesor decreased ($P < 0.01$). Most of the changes observed in circulating G, TC, and lipoproteins have been linked to the pathogenesis of AD, thus, our results would highlight potential early chronobiological and metabolic biomarkers for AD in an experimental model of OB in adult rats.

A130
INTERMITTENT FASTING AND LOW-CARB, HIGH-FAT DIET IN OVERWEIGHT ADULTS

Garro Bustos JV [1], Bonfiglioli MJ[2], Biasi AM[1,3,5], Gómez NN[3,4,5]
[1]Facultad de Ciencias de la Salud, Universidad Nacional de San Luis (UNSL). [2]Hospital San Luis. Servicio de Nutrición y Alimentación. [3]Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO)-SL/ CONICET. [4]Facultad de Ciencias Médicas (UC de Cuyo). [5]Laboratorio de Morfofisiología, FQByF - UNSL, Argentina.
E-mail: vanigarrob@gmail.com

Overweight and obesity are currently an epidemic and represent a global public health problem. A high body mass index is related to a greater probability of developing chronic diseases such as coronary heart disease, diabetes, and arterial hypertension, among others, which are increasing and represent a significant burden for the health system given the morbidity and mortality they entail. Intermittent fasting (IF) and the low-carbohydrate, high-fat diet (LCHFD) have gained considerable scientific and popular repercussions; and are postulated as effective practices for the treatment of excess weight and its comorbidities. The objective of this study was to determine the impact on weight, changes in body composition, and biochemical parameters such as triglycerides and total and LDL cholesterol, in overweight and obese people with IF treatment (16/8 protocol) and LCHFD. A longitudinal, correlational study was carried out. 80 individuals of both sexes between 18 and 59 years old (young and mature adults) participated for ten months. The sample was made up of 32.5% (N = 26) men and 67.5% (N = 54) women. 96% (N = 77) of the individuals registered weight loss. 80% (N = 64) of the sample showed a decrease of more than 25% in weight with respect to their initial weight, the remaining percentage (16%; N = 13) decreased from 10 to 25%. Regarding the percentage of body fat, 55% (N = 44) began with a diagnosis of "very high", and at the end of the analyzed period, this percentage was reduced to 30% (N = 24). 75% (N =

60) began with a diagnosis of abdominal waist circumference (WC) of "very high risk", after treatment, this percentage changed to 55% (N = 44). Regarding total cholesterol, 40% (N = 32) decreased its value with respect to the initial value ($\bar{x}$: 280 to 187 ± 20 mg/dL), the rest did not change. Regarding LDL, 85% (N = 68) decreased the value to normal parameters ($\bar{x}$: 150 to 98 ± 15 mg/dL); and in relation to triglycerides, 95% (N = 76) positively modified their values ($\bar{x}$: 180 to 78 ± 10 mg/dL). The IF and the LCHFD promote weight loss, decrease in abdominal fat mass, decrease in WC, and positively influence the biochemical parameters of the overweight and obese people analyzed, to a greater extent in the male sex and in young adults ($P < 0.05$).

A131
IMPACT OF DRYING METHOD ON THE POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY OF THE *FLAME SEEDLESS* RAISINS

Ponce Pozo FB[1], *Zaragoza-Puchol JD*[1], *Espíndola R*[2], *Feresin GE*[1], *Luna LC*[1]
[1]*Instituto de Biotecnología. Facultad de Ingeniería. Universidad Nacional de San Juan. San Juan. Argentina.*
[2]*Estación Experimental Agropecuaria INTA San Juan. E-mail: lunalorenac@gmail.com*

The grape and its products are widely consumed, especially the red ones, because they represent a rich source of nutritionally beneficial compounds, such as carotenoids, vitamins E and C, and polyphenols, among the bioactive compounds. Raisins, within dried fruits, are the ones with the highest concentration of phenolic compounds, recognized for being the main responsible for antioxidant activity. In recent decades, they have shown a relevant role in consumption, especially those obtained from seedless grapes, since they allow the full use of raisins; they are incorporated easily into other processed foods such as yogurts, bakery products, cereal bars, granola, etc. In addition, they have a longer shelf life, which facilitates the availability of the product throughout the year. The grape drying methods used around the world are diverse; the economic benefits and feasibility of each method used are continuously studied, but it is also important to know how each process affects the functional conditions of this food. The objective of this work is to evaluate how the content of total phenolic (TPC) and antioxidant activity (DPPH assay) varies in the raisins obtained by different drying methods. Four repetitions per sample, the TPC (Folin-Ciocalteu method) and the antioxidant activity by means of the DPPH radical discoloration assay were analyzed. The raisin extracts were obtained by seven drying methods: (TI) in tall structure without rain, (TII) on transparent perforated plastic with slope, (TIII) on black perforated plastic without slope, (TIV) on black plastic without perforation with slope, (TV) on black plastic without perforation without slope with rain, (TVI) on gravel and (TVII) Dry On Vine. The extracts of the raisin samples were obtained by ultrasound-assisted extraction for 1 h, with ethanol: water (1:1). Among the results obtained the extracts: (TIV) presented 490.97 ± 18.20 mg GAE/100 g raisins and 8.39 µg/mL of EC_{50} of discoloration of the DPPH radical; (TVI) showed 397.87 ± 14.26 mg GAE/100 g raisins and EC_{50} 6.75 µg/mL; (TVII) showed 365.71 ± 7.82 mg GAE/100 g raisins and EC_{50} 6.7 µg/mL. Those dried raisins on black plastic without perforation with slope, on gravel and by DOV; presented the best functional properties (content of bioactive compounds and antioxidant capacity). These results would indicate that the drying methods affect the evaluated parameters differently. Furthermore, such drying methods would require low investment costs for the producer, allowing him to obtain a product with a positive impact on nutritionally beneficial properties.

A132
EFFECTS OF INTERMITTENT FASTING ON PHYSICAL, BIOCHEMICAL AND COGNITIVE PARAMETERS IN A D-GALACTOSE-INDUCED AGING MODEL IN RAT

Ferramola ML[*,1,2], *Ponce IT*[*,1,2], *Pérez CM*[2], *Godoy MP*[2], *Fernandez G*[2], *Anzulovich AC*[1,2], *Lacoste MG*[1,2]
[1]*Laboratorio de Cronobiología, IMIBIO, Universidad Nacional de San Luis, CONICET.* [2]*Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. San Luis, Argentina. *Equal contribution.*
E-mail: lacostemariagabriela@gmail.com

Aging is a multifactorial process that leads to the gradual deterioration of physical and mental abilities. Currently, there are no pharmacological treatments that modify the course of aging, so it is of great interest to find interventions that can delay and/or reduce the deleterious effects of this process. Due to the relationship between dietary intake and health throughout life, different nutritional interventions are being considered as possible anti-aging strategies. Intermittent fasting (IF) is a dietary intervention that alternates periods of feeding and prolonged fasting. Our objective was to investigate the effect of IF as a preventive strategy for cognitive dysfunctions associated with the aging process. Wistar rats were randomly divided into three groups receiving daily: (1) physiological saline solution (CTL) via intraperitoneal injection (IP), (2) D-galactose 150 mg/kg (DGAL) via IP, and (3) D-galactose 150 mg/kg via IP + IF protocol (DGAL+IF) for a period of eight weeks. The IF protocol consisted of access to food *ad libitum* for 24 h that was alternated with 24 h without food. We evaluated the physical aspect of the animals, biochemical parameters in serum, and cognitive tests such as the Barnes Maze (BM) and the Novel Object Recognition (NOR). At the end of the treatment, we observed that the DGAL group presented yellowish and opaque hair with darker regions. This was in contrast with the DGAL+IF rats,

which presented whiter and brighter hair, similar to the CTL group. Although there were no significant differences in body weight between CTL and DGAL animals at the end of treatment, weight gain in DGAL was greater ($P < 0.01$). On the contrary, body weight gain was significantly lower in the DGAL+IF group, in comparison to the CTL and DGAL ($P < 0.0001$). Glycemia in DGAL rats was higher than in CTL ones ($P < 0.05$), while there were no differences in cholesterol and triglyceride levels. Interestingly, in DGAL+IF animals, the blood glucose decreased significantly, resembling the CTL group. Also, triglycerides were significantly lower in the DGAL+IF relative to the other groups ($P < 0.01$). There were no significant differences in cholesterol levels between DGAL+IF and the other animals' groups. In relation to cognitive tests (BM and NOR), we found a high degree of individual variability within each group under study, which is frequently observed when working with a small number of animals (in our case, N = 5 for each group), so a greater number of individuals should be evaluated to accurately compare the performance between the groups. The findings of this preliminary study suggest that IF has positive effects on physical and biochemical parameters in aged rats. Future research is required to assess the effects of IF on cognitive performance in this model.

A133
DEVELOPMENT OF A COMPUTER APPLICATION TO ESTIMATE THE RISK OF ZINC DEFICIENCY

Ramírez MM[1], Biasi AM[2,3,4], Agüero WF[1], Pérez Chaca MV[5], Gómez NN[2,4,5]
[1]Universidad Nacional de Villa Mercedes (UNVIME). [2]Instituto Multidisciplinario de Investigaciones Biológicas (IMIBIO)-SL/ CONICET. [3]Facultad de Ciencias de la Salud, Universidad Nacional de San Luis (UNSL). [4]Facultad de Ciencias Médicas (UC de Cuyo). [5]Laboratorio de Morfofisiología, FQByF - UNSL, Argentina.
E-mail: mmramirez@evirtual.unvime.edu.ar

Subclinical zinc deficiency, that is, one that does not present symptoms, represents a serious public health problem and can generate health complications or aggravate different clinical conditions. A computer application that estimates the risk of mineral deficiency is very useful for early detection of this problem and to be able to implement actions to prevent or reverse it. The objective of this study is to design a computer application to estimate the risk of zinc deficiency in adults from 19 years of age (adults and older adults). Computer software was designed to evaluate the amount and frequency of zinc intake/ per day. To do this, the foods to be evaluated were defined (high bioavailability zinc source foods: meat, eggs, and dairy), the average portions of each food, the images of each portion, and the zinc contribution of each one of them. With this data and the amount and frequency of food consumption by the respondent, the application calculates the total amount of zinc consumed in the diet. The application compares the amount of zinc consumed, with the recommended daily amount, and in this way estimates if the respondent may be at risk of deficiency of the micronutrient. This computer tool allows information to be recorded using electronic devices (computers, tablets, or cell phones, with an Internet connection and Android platforms) allowing the collected data to be exported to a document with an xls extension (spreadsheet), for later analysis in statistical programs. In addition, this computerization produces significant savings in paper, helping to preserve the environment and thus contributing to sustainability. The development of computer tools with practical applications in the health area is of great benefit for large-scale population and epidemiological studies. In particular, the evaluation of zinc intake in susceptible adult populations is an ideal opportunity to identify risk factors and implement measures to prevent such nutritional deficiency in the future. In the case of older adults, the improvement in dietary quality contributes to metabolic compensation and preservation of antioxidant activity, among other functions.

A134
EATING HABITS AND NUTRITIONAL STATUS ACCORDING TO: ECONOMIC INCOME OF PREGNANT WOMEN ATTENDING "DR. TERESITA BAIGORRIA" MATERNITY

Piguillem SN[1,2], Ciminari ME[1], Zambrano G[2]
[1]Laboratorio de Morfofisiología, FQByF – UNSL. [2]Facultad de Ciencias de la Salud, UNSL.
E-mail: spiguillem@gmail.com

During pregnancy occur physical and metabolic changes, among others. Bearing in mind that there is a large percentage of impoverished people in Argentina and that this is a major factor (although not the only one) that influences food acquisition, we are interested in knowing eating habits and nutritional status, taking into account economic incomes, of 2nd-trimester pregnant women that attend pre-natal medical checkups at the provincial maternity hospital. A descriptive, cross-sectional study was carried out, with qualitative and quantitative variables in pregnant women between weeks 13 and 27 of gestation who attended the "Dra. Teresita Baigorria" maternity, during March–July 2022. From the surveys, it was possible to obtain the following data: age, current employment status and stability, the number of home members, food intake, and Body Mass Index (BMI). The final sample was made up of 36 pregnant women, older than 18 years, 86.11% (N = 31) between 18 and 34 years, and only 13.89% (N = 5) were older than 35 years. 58% are employed, and 42% are unemployed. It was observed that 97% consume meat and only one is vegetarian and 94% consume oils and

bakery products (N = 34). 89% (N = 32) consume tubers, legumes, and sugar. Regarding economic income, most of them earn more than $60.001 in their household, in second place incomes less than $25.000, followed by those who earn between $40.001 and $60.000 and between $25.001 and $40.000. 21 respondents reported that this was not enough for living. It was concluded that evaluating the ENNyS (2007) reports in Argentina, low weight prevalence was 24.9%, normal weight 31.1%, overweight 19.7%, and obesity 24.4% according to anthropometric nutritional value state of pregnant women between weeks 10 and 43 of gestation. Therefore, the data obtained from this work are not consistent with the trend of ENNyS, considering that obesity was less prevalent and there have been notoriously fewer pregnant patients with low weight.

A135
DETERMINATION OF PHENOLS AND ANTINUTRIENTS OF HEALTHY CANDIES MADE FROM AMARANTH SEEDS AND JELLY

Piola HD, Riveros L, Morales I, Sturniolo H
Laboratorio de Bromatología, Facultad de Química, Bioquímica y Farmacia, Universidad de San Luis, San Luis, Argentina.

The consumption of unhealthy diets and a sedentary lifestyle are responsible for metabolic diseases or congenital errors of metabolism (CEM), reflected in numerous scientific publications, which confirm a change in trend among the main causes of mortality: infections and problems congenital children are losing weight, at the same time cardiovascular diseases (CVD), cancer and diabetes are increasing. Also, the World Health Organization (WHO) reveals that malnutrition is the main problem for public health in this century. Called Noncommunicable Diseases (NCDs), there is a Global Action Plan for the Prevention and Control of these diseases, the objective is to achieve new goals by 2025. Our project is dedicated to contributing to the solution of this global problem, through the preparation of popular consumption foods such as the one that has been developed: a healthy gummy, based on amaranth seed and gelatin. A healthy food has been developed, a gummy based on amaranth seed and gelatin, which is nutritionally different from commercial gummies, due to the fact that they provide a low amount of kcal, simultaneously increasing the protein content, total fat, and dietary fibers. The energy intake is reduced by 330 kcal at 207 kcal in 100 g; carbohydrates have decreased from 81% to 28.4%; proteins increase from 1.8% to 20.2%; total fats increase from 0 to 1.4% and dietary fibers increase from 0 to 1.7%. These nutritional benefits are mainly given by amaranth. The objective of the present work was to determine the variation that occurs in the content of total phenols and antinutrients when applying the processes for making gummies. Methodology: Total phenols were determined by the Folin-Ciocalteu method and the presence of saponins was investigated through hemolytic activity and foam index. With respect to antinutrients, the antitriptic activity, the oxalic acid content, and phytic acid were measured. The results obtained confirm an increase in antioxidant properties and at the same time a decrease in the content of antinutrients in the final product.

A136
BROMATOLOGYCAL CHARACTERIZATION OF *Citrullus lanatus FRUIT*

Nesteruk RI, Riveros L, Amieva MI, Barcia CS, Sturniolo HL
Área de Bromatología, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. San Luis, Argentina. E-mail: rineteruk@gmail.com

Citrullus lanatus commonly known as sandilleja, wild watermelon, chayote, cayota, or citron, is a creeping plant native to Africa and is an important weed in the central region of Argentina. It has a large fruit, with an approximate size of between 20 to 50 cm long and a diameter of 10 to 20 cm. The rind of the immature fruit is dark green alternated with light green spots, and when it matures it completely turns pale yellow. The flesh of the sandilleja is white yellowish, with olive-green seeds. In this work, *C. lanatus* fruit was studied from its bromatological and physicochemical properties to promote its use in the food industry. Ripe fruits of *C. lanatus* were collected in the capital of San Luis province. Immediately, these whole fruits were washed, a sample was taken for the humidity determination and the rest was dried, labeled, and stored for other determinations. All analyses were carried out in triplicate. Proximal analysis showed a moisture, ash, fiber, fat, protein, and carbohydrate content of 96.7 g, 0.4 g, 0.3 g, 0.3 g, 0.2 g, and 2.2 g, respectively, and its caloric value was 12 kcal/50 kJ (Atwater factors for caloric value by difference (proteins: 4 kcal/g – fats: 9 kcal/g – carbohydrates: 4 kcal/g)). Also, the fruit was evaluated in its pH, acidity, and total solids presenting a pH of 5.29, acid index of 0.15g malic acid/100 g, 1.8° Brix. The moisture content was carried out by gravimetric method in an oven at 105ºC. The amount of ash was determined by incineration at 550ºC in a muffle. The protein content was determined by the Kjeldahl method using the conversion factor of 6.25. Fats were determined by extraction by the Soxhlet method with petroleum ether. Crude fiber was determined by the acid and basic hydrolysis method. Carbohydrate content was calculated by difference: %carbohydrates = 100 − (%moisture + %ash + %proteins + %lipids + %crude fiber). pH was measured using a pH meter, titratable acidity was determined by titration with sodium hydroxide, Brix degrees, soluble solids direct reading on refractometer. The results obtained are characteristic of the genus *Citrullus*: the low caloric intake comes especially from carbohydrates and the main component of this fruit is water, so it can be considered a good option

for the hydration of the body. The results also allow us to infer that the fruit of *C. lanatus* presents a composition that allows it to be used in the food industry in its traditional fresh form or in the development of new food products such as jams, considering it an alternative in the population's diet, allowing its inclusion in the Composition Tables, and preserving and revaluing its consumption.

HUMAN CLINICS AND ODONTOLOGY

A137
DISCORDANT LIPID PATTERN IN PATIENTS WITH NON-COMMUNICABLE DISEASES. IMPORTANCE OF REMNANT CHOLESTEROL

Flores MY[1,2], Moreno MS[2], Gallo CB[2], Muñoz J[2], Figueroa MF[1], Forneris M[1]
[1]Curso de Bioquímica Clínica. FQByF-UNSL. [2]Hospital Juan G. Vivas (Juana Koslay. San Luis).
E-mail: yamiflores@hotmail.com

Hypertension, type 2 diabetes, and dyslipidemia are established risk factors for cardiovascular diseases internationally. Remnant cholesterol (RC) is the cholesterol content of triglyceride-rich lipoproteins that consists of very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), and chylomicron remnants. The RC is highly atherogenic, because of its lesser size and high cholesterol content, and increased residence period in the blood, which may not be reflected by the levels of low-density lipoprotein cholesterol (LDL-C). In this work, we aimed to identify the discordant/concordant pattern between LDL-C and RC in patients with hypertension, type 2 diabetes, or both. A total of 335 subjects (192 females and 143 males) with a mean age of 53.6 years (CI: 44-64) who attended in the area of chronic diseases at a primary care hospital (Juana Koslay City, San Luis) during April 2019 to May 2022 were evaluated. Of these, 28 subjects had diabetes (DB), 100 subjects had hypertension (HT), 102 subjects had diabetes and hypertension (DB/HT), and 105 were healthy control subjects (C). Informed consent was obtained from all participants. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Fasting serum lipids were measured by enzymatic colorimetric method (autoanalyzer CM250 Wiener). LDL-C was calculated using the Friedewald formula if triglycerides (TG) were < 200 mg/dL. Otherwise, it was measured directly. RC was estimated as total cholesterol minus LDL-C minus HDL-C. We selected 100 mg/dL and 30 mg/dL as the cut-off points for the LDL-C and RC, respectively. Hence, we divided all the participants into four groups (Group 1: low LDL-C/low RC, Group 2: low LDL-C/high RC, Group 3: high LDL-C/low RC, and Group 4: high LDL-C/high RC). RC, but not LDL-C, was statistically lower in the C group compared with HT, DB, or HT/DB ($P < 0.001$). Although many individuals had concordant levels of LDL-C and RC, the prevalence of lipid discordance was 49.1%. Compared with patients in Group 3 (high LDL-C/low RC), those in Group 2 (low LDL-C/high RC) had higher BMI, glucose, and TG values and lower HDL-C levels ($P < 0.05$). The prevalence of HT was higher in Group 3 (53.6%) than in Group 2 (45.4%). While Group 2 and Group 3 were more common in DM patients, group 1 (low LDL-C/low RC) and Group 4 (high LDL-C/high RC) were more common in HT/DM patients. Since remnant lipoproteins may increase the expression of inflammatory proteins, adhesion molecules, and coagulation factors, promoting the formation of foam cells, it would be benefit expand the strategies in primary prevention to evaluate the cardiovascular risk using not only LDL-C levels but also RC. When discordant with LDL-C exist, RC may identify individuals who may benefit from more comprehensive lipid modification.

A138
OPPORTUNITY FOR *Chlamydia trachomatis* SCREENING DURING THE STUDY OF BALANCE OF VAGINAL CONTENT IN PRIMARY HEALTH CARE SETTING

Godoy DL[1], Mendieta GH[1], Flores MY[1,2], Figueroa MF[2], Forneris M[2]
[1]Hospital Juan G. Vivas (Juana Koslay. San Luis). [2]Curso de Bioquímica Clínica. FQByF-UNSL.
E-mail: yamiflores@hotmail.com

Chlamydia trachomatis (CT) is the most common etiological agent of bacterial sexually transmitted infections worldwide. Most infected women remain asymptomatic, which facilitates the spread of the pathogen and may lead to the development of chronic infection such as cervicitis, pelvic inflammatory disease, and even infertility. Although CT is of public health significance, the prevalence of CT infection in the general population is variable. Considering that vaginal dysfunction increases the likelihood of sexually transmitted diseases acquisition, this study was performed to establish the frequency of CT and its association with basic vaginal states (BVS) in women attending gynecologic outpatient service in Public Hospital (Juana Koslay City, San Luis) during 2021. The detection of *N. gonorrhoeae*, *T. vaginalis*, and yeasts was also incorporated. Exclusion criteria: actual antibiotics treatment, pregnancy, and recent parturition. All the patients answered

a specific questionnaire, which included information concerning obstetric history and contraceptive practices. Written informed consent was obtained from the participants. Endocervical samples from 146 symptomatic or asymptomatic women (31.9 ± 10 years) were assayed for CT using polymerase chain reaction (PCR) (Roche Molecular Diagnostics, USA). Simultaneously, samples of cervicovaginal smears were evaluated by wet mount, Gram and Giemsa stains, according to the Balance of the Vaginal Content (BAVACO) methodology. Five BVS can be recognized: normal microbiota (NM), NM associated with a vaginal inflammatory reaction, intermediate microbiota, bacterial vaginosis, and nonspecific vaginitis (NVI). Contingency tables were used for categorical variables, and statistical significance was determined using the Chi-Square test. A $P < 0.05$ was considered significant. The patients were divided into four groups according to age: ≤25, from 26 to 35, from 36 to 45, and ≥46 years old. Altogether, a significant frequency of alterations in vaginal function (78.76%) and CT infection (10.3%) were detected. The prevalence of CT infection was higher in the group of ≤25 years than in those of >25 years (28.3% *vs.* 2.0%, $P < 0.001$). Of all samples, 19% were positive for *C. albicans*, and 6.9% were positive for *T. vaginalis*. No cases of gonorrhea were detected. The distribution of positive cases among BVS was different: women presenting with NVI had a significantly higher prevalence of CT infection ($P < 0.01$). The most commonly used contraceptive method among women ≤25 years old was oral pills (30%) followed by subdermal implants (24%) and condoms (15%). The high prevalence of CT infection and the alteration of the normal vaginal microbiota may be a consequence of the lack of condom use and the lack of periodicity in the gynecological examination. This study highlights the importance of CT screening among the population younger than 25 years.

A139
ENTEROPARASITES IN A PRIMARY CARE HOSPITAL IN SAN LUIS CITY, ARGENTINA

Camargo PG[1], Lopresti RA[2,3], González LE[2], Lapierre AV[2]
[1]Hospital Cerro de la Cruz. [2]Parasitología y Micología, UNSL. [3]Hospital San Luis, Argentina.
E-mail: alicia.lapierre@gmail.com

Enteric parasites are common causative agents of infections in many parts of the world and are an important cause of morbidity and mortality, especially in developing countries, where they are considered a serious public health issue. This study was conducted in a primary care hospital in San Luis City and aimed to investigate the occurrence of parasitic infection in fecal samples corresponding to individuals of both sexes from 0 to 65 years old. The study comprised 645 subjects (45.8% male, 54.2% female). In total, 1290 samples that were two per patient (serial parasitological and anal brushes, preserved in 10% formalin) were collected over a period of five years from 2017 to 2021. Some of the patients live in places without access to drinking water or sewers. All individuals were made to sign an informed consent. The serial parasitological samples were analyzed for parasites using Carles-Barthelemy's enrichment technique, and the anal brushes were tested by Graham's method. After that, sediments were stained with iodine and observed under a microscope at low magnification. Enteroparasites prevalence was 33.33% (N = 215). Of the 215 positive cases, *Enterobious vermicularis* was the most frequent of the helminths (74.97%), followed by *Blastocystis hominis* (61.76%) and *Giardia* sp. protozoan (25.69%). There was no difference in prevalence by sex ($P > 0.05$); however, it was higher in individuals less than or equal to 10 years old. If we analyze the positivity data according to the years, we see that the highest percentages of positives were observed in 2018 (42.62%) and 2017 (38.1%), followed by the years 2019, 2020, and 2021 with percentages of 29.49%, 36.16%, and 19.82%, respectively. We compare our results with other studies, and we can deduce a similar prevalence in comparison with data from other authors. Effective treatment of infected patients and improved sanitary habits is advocated.

A140
IMMATURE OVARIAN TERATOMA: CASE REPORT AND REVIEW

Cassone ER[1], Baracchini ME[2], Catania ME[2], Colque Huailla AF[2], Kungis JE[2], Lopresti Cortegoso S[2], D'Angelo CR[2], Penissi AB[1], Lopresti EF[3]
[1]Facultad de Ciencias Médicas – Universidad Nacional de Cuyo. [2]Facultad de Ciencias Médicas – Universidad del Aconcagua. [3]Hospital Central.E-mail: ercassone98@gmail.com

Immature ovarian teratoma is a rare tumor representing less than 1% of all ovarian malignant tumors and is the second most frequent malignant ovarian germ cell tumor. It is found either in pure form or as a component of a mixed germ cell tumor, occurring primarily during the first two decades of life. These neoplasms are typically represented by immature/embryonic-like neural tissue. It is the only ovarian germ cell neoplasm that is histologically graded; the grade is based on the proportion of tissue containing immature neural elements. We present a case of a 15-year-old female patient who consults for oppressive abdominal pain of five months of evolution, localized in hypogastrium, associated with signs of virilization. Abdominal ultrasound (US) and magnetic resonance imaging (MRI) confirmed the presence of a voluminous mass of 27.5 × 12.5 × 10 cm extending from epigastrium to hypogastrium. Serum values of cancer antigen-125 (CA-125), α-fetoprotein, and testosterone were elevated. Surgical resection was performed. The anatomopathological examination revealed a grade 3 immature ovarian teratoma with omentum implants. After 6 months, the patient presented

a recurrence of the tumor and a second surgery was performed, with adjuvant chemotherapy (bleomycin, etoposide, and cisplatin). MRI performed six months later showed no signs of recurrence. Combined treatment of surgery plus adjuvant chemotherapy can achieve remission in more than 90% of cases.

A141
PHYSICAL ACTIVITY AND DIABETES RISK IN A RURAL POPULATION

De Pauw MC[1], Salinas E[2]
[1]Facultad de Ciencias de la Salud. [2]Facultad de Química, Bioquímica y Farmacia. UNSL.
E-mail: salinaseloy@gmail.com

Worldwide, the incidence and prevalence of diabetes mellitus 2 (DM2) are increasing due, among other factors, to the increase in obesity and physical inactivity. Physical inactivity and a sedentary lifestyle have a high prevalence, exceeding 70% of the population. In America, 41.4% of the population spends 4 h or more per day sitting down; this sedentary time is independent of moderate or vigorous physical activity (PA). PA is defined as any bodily movement produced by muscles that result in energy expenditure above baseline levels. This broad concept includes exercise, sports, and physical activities carried out as part of daily life, occupation, leisure, and active transport. The risk of DM2 can be measured with various simple scales to identify subjects with undiagnosed DM or at risk of developing it in the next 10 years; one of them is the FINDRISC (*Fin*nish *D*iabetes *Ri*sk *Sc*ore), developed by the Finnish Diabetes Society. Our objective was to identify the level of physical activity, during the usual occupation, transportation, sports or exercise, sedentary time, and the risk of DM2 in a population aged 18 or over in a rural area. A descriptive, cross-sectional, observational study was carried out using a survey and physical examination of the rural population grouped in the towns of Zanjitas, Cazador, Alto Pelado, and Beazley, Juan M. de Pueyrredón department, San Luis province. The risk of DM2 was identified with the FINDRISC test. PA is expressed as METs (multiples of resting metabolic rate)-minutes/week, which represent the energy expenditure of activity as low-inactive (< 600 Mets), moderate (600–1500 Mets), or high (> 1500 Mets). Results (%): Sex: female, 68.14; male, 31.86. Age distribution: 18–26 years, 22.57; 27–59 years, 52.21; and 60 or more years, 25.22. DM2 risk: low, 23.89; slightly high, 31.86; moderate, 23.01; high, 19.91; very high, 1.32. AF during the transfer: low-inactive, 97.79; moderate, 1.77; and high, 0.44. AF during the usual occupation: low-inactive, 2.65; moderate, 7.08; and high, 90.26. AF by sport/exercise: low-inactive, 68.58; moderate, 15.48; and high, 15.93. Total physical activity: low-inactive, 1.33; moderate, 3.1; and high, 95.6. Sedentary time: up to 4 h sitting, 83.62; more than 4 h, 13.28; NC, 3.09. PA level according to DM2 risk: low with moderate risk, 0.44; and with high risk, 0.89; moderate with low risk, 0.88; with slightly moderate risk, 0.44; and with high risk, 1.78; high with low risk, 23; with slightly moderate risk, 31.42; with moderate risk, 22.57; with high risk, 17.25; and with very high risk, 1.33 The population studied was predominantly female and adult and had a high level of PA, much of it performed during their usual occupation. They mostly presented a low level of a sedentary lifestyle. Half of the population with high physical activity presented a risk of DM2 in the next 10 years between low and slightly moderate, almost a quarter a moderate risk, and approximately a fifth a high to very high risk. Although the benefit of PA in reducing the risk of DM2 has been proven, there are other factors such as diet, obesity, sedentary time, and genetic load that have an important weight as risk factors.

A142
BIOCOMPATIBILITY AND GENOTOXICITY STUDIES OF SPORE SUSPENSIONS AND SURFACTIN EXTRACTS OF *Bacillus* spp. WITH POTENTIAL APPLICATION IN ANIMAL HEALTH

Di Giacomo AL, Azcurra LN, García GR, Dogi CA, González Pereyra ML
[1]Departamento de Inmunología, Facultad de Ciencias Exactas, Fisico-Químicas y Naturales, Universidad Nacional de Río Cuarto. Argentina. [2]Instituto de Ciencias Veterinarias–Consejo Nacional de Investigaciones Científicas y Técnicas (INCIVET-CONICET-UNRC). E-mail: mlgonzalezpereyra@exa.unrc.edu.ar

Bacillus species include beneficial strains that are used as probiotic additives to improve animal production. Thermo-resistant spores can be easily administered in water or feed to germinate in the gut and exert their beneficial effect through immunomodulation, reducing inflammation, and protecting against enteric pathogens. Surfactin (SF) is a cyclic lipopeptide (LP) produced by *Bacillus* species with demonstrated anti-inflammatory, anti-microbial, anti-tumoral, and immunomodulatory activities. The aim of the present study was to isolate native SF-producing *Bacillus* spp. strains and to study the biocompatibility of live bacteria and their LP extracts (LPE) with animal systems *in vivo* and *in vitro* to determine their potential to be included in products of veterinary use such as feed additives and/or health-improving treatments. *Bacillus* spp. were isolated, and six isolates were randomly selected. Cell-free culture supernatants of each were extracted 1:1 v/v with n-butanol, and the SF content of LPE was quantified by HPLC. On the other hand, *Bacillus* spp. endospores suspensions (ES) (1×10^8 UFC/mL) were obtained from solid medium culture. ES, purified SF, and different dilutions (1:10, 1:50, 1:100, 1:500, and 1:1000) of LPE were tested for biocompatibility *in vitro* on the Caco-2 cell line using the MTT colorimetric assay. ES genotoxicity was tested *in vivo* on BALB/c mice (N = 6) administered 0.2 mL of the spore suspensions (10^8 spores) orally for 10 days. Animals were sacrificed and bone marrow samples were

collected for the bone marrow erythrocyte micronuclei assay. All isolates produced between 15.96 and 239.96 µg/mL SF. ES suspensions containing 10^8 UFC/mL resulted as non-cytotoxic to Caco-2 cells showing viability percentages (%V) over 70%. Likewise, SF and all dilutions of LPE showed no cytotoxicity over Caco-2, demonstrating not to harm intestinal cells. Only MFF 1.11 isolate showed %V < 70 in all dilutions tested. However, the SF concentration of this extract was within the safe range that showed no toxicity for SF (from 10 ng/mL to 500 µg/mL), suggesting that another harmful compound was produced by this isolate. SF concentration of non-cytotoxic LPE dilutions tested varied between 0.01 and 239.97 µg/mL. ES of the three tested isolates (MFF 2.2, MFF 1.11, and TC 12) did not show genotoxicity or cytotoxicity *in vivo*. The present study allowed us to select the safe *Bacillus* spp. isolates and SF concentrations to test their beneficial and immunostimulant properties and their potential to be used in animal production and health.

A143
SAFETY ASSESSMENT AND EFFECT OF SURFACTIN AND *Bacillus* spp. LIPOPEPTIDES ON MICROBICIDAL CAPACITY OF RAW 264.7 MACROPHAGES

Di Giacomo AL, Azcurra LN, García GR, Dogi CA, González Pereyra ML
[1]Departamento de Inmunología, Facultad de Ciencias Exactas, Fisico-Químicas y Naturales, Universidad Nacional de Río Cuarto. Argentina. [2]Instituto de Ciencias Veterinarias -Consejo Nacional de Investigaciones Científicas y Técnicas (INCIVET-CONICET-UNRC).E-mail: mlgonzalezpereyra@exa.unrc.edu.ar

Bacillus species include beneficial strains used as probiotic additives to improve animal production for their beneficial effect through immunomodulation, reducing inflammation, and protecting against enteric pathogens. Surfactin (SF) is a cyclic lipopeptide (LP) produced by *Bacillus* species with demonstrated anti-inflammatory, anti-microbial, anti-tumoral, and immunostimulant activities. The aim of the present work was to study the biocompatibility of SF and LP extracts (LPE) from native *Bacillus* isolates and their effect on the phagocytic and microbicidal capacity of RAW 264.7 murine macrophages. Concentrations ranging from 10 ng/mL to 10 µg/mL of a purified SF standard and LPE containing the same concentrations of SF (10, 50, 100, and 500 ng/mL; 1 and 10 µg/mL) were tested for cytotoxicity on RAW 264.7 cells with the MTT assay. Afterward, non-cytotoxic concentrations were selected to perform the phagocytic and microbicidal activity tests. Macrophages were cultivated on 96-well plates and pre-treated with SF or LPE for 1 h; then, 1×10^8 UFC/mL *Salmonella* spp. were added and incubated for 4 h. Cells were lysed, and the remaining UFC/mL were counted on McConkey agar to determine microbicidal capacity. SF did not alter cell viability percentages (%V) in any of the tested concentrations. TC.12 and MFF 2.2 extracts resulted non-cytotoxic to RAW 246.7 cells, showing %V over 70%. MF 1.11 and TC 2.5 extracts in the highest concentration resulted cytotoxic, showing V < 50%, which differed significantly from controls ($P < 0.05$). None of the treatments altered the microbicidal capacity of RAW 264.7 cells, and a microbicidal activity of 99% was observed. In conclusion, SF and LP extracted from native *Bacillus* spp. are non-cytotoxic for animal macrophages and do not alter their microbicidal capacity or their function as phagocytic cells; therefore, the immunomodulating properties of these compounds can be studied to determine their potential to be included in products of veterinary use to improve animal health and productivity.

BIOCHEMISTRY, PHYSIOLOGY AND NEUROCHEMISTRY

A144
GLIAL-DERIVED NEUROTROPHIC FACTOR REGULATES THE EXPRESSION OF TREK2 IN RAT PRIMARY SENSORY NEURONS LEADING TO ATTENUATION OF AXOTOMY-INDUCED NEUROPATHIC PAIN

Peralta ED, Messina DM, Acosta CG
Laboratorio de Neurobiología del Dolor, Instituto de Histología y Embriología de Mendoza, FC Médicas, UNCuyo peraltaemanuel0@gmail.com

TREK2 is a member of the 2-pore domain family of K^+ channels (K2P) preferentially expressed by unmyelinated, slow-conducting, and non-peptidergic isolectin B4-binding ($IB4^+$) primary sensory neurons of the dorsal root ganglia (DRG). $IB4^+$ neurons depend on the glial-derived neurotrophic factor (GDNF) family of ligands (GFLs) to maintain their phenotype. In previous work, we demonstrated that seven days after spinal nerve axotomy (SNA) of the L5 DRG, TREK2 moves away from the cell membrane resulting in a more depolarized resting membrane potential (E_m). Given that axotomy deprives DRG neurons of peripherally derived GFL, we hypothesized that they might control the expression of TREK2. Using a combination of immunohistochemistry, immunocytochemistry, western blotting, *in vivo* pharmacological manipulation, and behavioral tests, we examined the ability of the GFLs (GDNF, neurturin, and artemin)

and their selective receptors (GFRα1, GFRα2, and GFRα3) to regulate the expression and function of TREK2 in the DRG. We found that TREK2 correlated strongly with the three receptors normally and ipsilaterally for all GFRs after SNA. GDNF, but not NGF, neurturin, or artemin, upregulated the expression of TREK2 in cultured DRG neurons. *In vivo* continuous, subcutaneous administration of GDNF restored the subcellular distribution of TREK2 ipsilaterally and reversed mechanical and cold allodynia seven days after SNA. This work is the first to demonstrate that GDNF controls the expression of a K2P channel in nociceptors. As TREK2 controls the E_m of C-nociceptors affecting their excitability, our finding has therapeutic potential for treating chronic pain.

A145
EFFECT OF A PPARγ SYNTHETIC AGONIST ASSOCIATED WITH VALPROIC ACID ON THE 24-HOUR RHYTHMS OF INSULIN-DEGRADING ENZYME IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

Sanchez M, Cangiano B, Cortez M, Golini R, Alba R, Garraza M, Anzulovich A, Navigatore Fonzo L
Laboratorio de Cronobiología, IMIBIO-SL, CONICET-UNSL. UNViMe.E-mail: lorenavigfz@yahoo.com.ar

Alzheimer's disease (AD) is the most common form of age-related neurodegenerative disorder. Numerous studies have shown that an imbalance between the production and clearance of amyloid-β (Aβ) peptides in the brain results in the accumulation of Aβ. It is known that insulin-degrading enzyme (IDE) plays a crucial role in the clearance of Alzheimer's amyloid-β (Aβ). Numerous studies have shown that pioglitazone (Pio), a PPAR-γ agonist, possesses antioxidant properties and improves cognitive deficits in AD. Valproic acid (VPA), a multifunctional drug, plays important roles in promoting the release of neurotrophic factor and improving memory deficits. In addition, evidence shows that the molecular clock function depends on the cellular redox state. Previously, we found that the treatment of Pio/VPA reestablished rhythmicity of oxidative stress parameters in the hippocampus. Taking into account these observations, the objective of this study was to evaluate the effect of Pio/VPA on the 24-h rhythms of Aβ, IDE, BDNF and its receptor in the hippocampus of Aβ-injected rats. Four-month-old male Holtzman rats were divided into three groups: (1) control, (2) Aβ-injected, and (3) Aβ-injected treated with Pio/VPA. Rats were maintained under 12 h-light:12 h-dark conditions and received water and food *ad libitum*. Hippocampal samples were obtained every 6 h during a 24-h period. Transcript levels of insulin-degrading enzyme and cognition-related factors were determined by RT-PCR, and Aβ protein by immunoblotting. We found that injection of Aβ (1-42) phase-shifted Aβ, IDE and BDNF/TrkB rhythms. Remarkably, Pio/VPA reestablished rhythmicity of those temporal patterns. Thus, combination therapy with pioglitazone and valproic acid ameliorates pathologic changes observed in an experimental model of AD and might represent a potential treatment approach for AD.

A146
ASSOCIATION BETWEEN RENIN–ANGIOTENSIN SYSTEM GENE POLYMORPHISMS AND HYPERTENSION IN A SAN LUIS POPULATION

Correa MM, Arce ME, Fuentes LB
Laboratorio de Farmacología - Facultad de Química, Bioquímica y Farmacia - Universidad Nacional de San Luis; IMIBIO-SL, CONICET. E-mail: mariamilagroscorrea@gmail.com

Arterial hypertension (HTA) is a polygenic disorder resulting from the interaction of several genetic and environmental factors. Renin angiotensin system (RAS) gene polymorphisms influence risk of developing HTA. The study of RAS polymorphisms remains controversial, and its identification in HTA patients is required. The aim was to investigate a possible association between RAS gene polymorphisms and hypertension in a San Luis population. A case-control study was performed in 397 subjects, 230 hypertensive (HTA) and 167 healthy (control), selected at Juana Koslay Hospital. Blood samples were obtained and polymorphism angiotensinogen (AGT M235T), angiotensin-converting enzyme insertion/deletion (ACE I/D) and angiotensin II type 1 receptor A1166C (AT1R A1166C) genotypes were performed by Polymerase Chain Reaction combined with Restriction Fragment Length Polymorphism (PCR-RFLP). Anthropometric, clinical, and biochemical parameters were evaluated by standard methods. Blood pressure and body measurements were recorded. Mean age (years): 54.2 ± 9.3 HTA and 39.2 ± 13.7 control ($P < 0.0001$), Body mass Index (kg/m^2): 31.7 ± 5.3 HTA and 27.1 ± 4.8 control ($P < 0.0001$). Systolic and diastolic blood pressure (mm Hg): 152.4 ± 15.3 / 90.1 ± 9.9 HTA and 118.0 ± 11.2 / 71.0 ± 9.4 control subjects ($P < 0.0001$). We found Hardy–Weinberg equilibrium in all groups studied ($P > 0.05$). No significant difference was found in genotype frequency of M235T: MM 12.1%, MT 48.5%, and TT 39.2% in HTA patients, and MM 15.5%, MT 51.7%, and TT 32.7% in controls. The allele frequency was M 0.36 and T 0.63 in HTA and M 0.41 and T 0.58 in control subjects. Chi square analysis found a statistically significant difference for T allele in HTA patients ($P < 0.0002$). Carriers of T allele had an increased risk of hypertension (Odds Ratio (OR) = 2.47, 95%CI: 1.55–3.92; $P < 0.0002$). Significant ACE I/D genotypes differences between HTA and control were found: II: 28.2% *vs*. 24.5%, ID: 50.0% *vs*. 39.5%, DD: 21.7% *vs*. 35.9%, $P < 0.008$. There was an association between ID and DD genotypes and hypertension (ID *vs*. II + DD, OR = 1.53, 95%IC: 1.01–2.30, $P < 0.04$; and DD *vs*. II + ID, OR = 0.49, 95%IC: 0.31–0.77, $P < 0.002$). Significant differences in OR between allele D *vs*. I (OR = 0.69, 95%IC: 0.52–0.92, $P < 0.01$) in HTA

patients and controls were found. A significant increase in ACE DD and allele D was detected in hypertensive women. No significant differences in AT1R A1166C genotypes and their allele frequencies were found. AGT M235T and ACE I/D polymorphisms could impact on genetic susceptibility to develop essential hypertension in San Luis population.

A147
BRAIN ANGIOTENSIN II IN A CHRONIC EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

Arce ME, Ochoa Munafo SG, Pulcini G, Sanchez SI, Ciuffo GM.
Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. IMIBIO-SL CONICET. San Luis.
E-mail: earce.arce7@gmail.com

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the selective death of dopaminergic neurons of the *substantia nigra* (SN), loss of striatal dopamine, glial activation, and development of α-synuclein (α-Syn) aggregates. It is well known that the brain renin–angiotensin system regulates multiple physiological functions, activating angiotensin II (Ang II) AT_1 and AT_2 receptors. It has been demonstrated the existence of both Ang II receptor subtypes in the SN, which are considered involved in neurodegenerative processes. In this work, we performed an immunohistochemical analysis in an experimental animal model of PD. As was demonstrated previously by our group, rotenone-loaded PLGA microparticles allow a slow delivery of the neurotoxin rotenone and, thus, a long-term effect following a single-dose subcutaneous administration. Immunohistochemical staining for Ang II receptors, tyrosine hydroxylase (TH), and α-Syn were performed in brain tissue sections (at the SN level) from control and rotenone-treated rats. In agreement with our previous results, we confirmed the presence of both Ang II receptors in the SN of treated rats. We found a loss of dopaminergic neurons and decreased immunoreactivity against anti-TH antibodies in these animals. Furthermore, we observed many nigral cells with α-Syn positive aggregates. These findings contribute to a better understanding of the potential role of brain angiotensin II in neurodegenerative diseases.

AUTHOR INDEX

A

B

C

D

E

F

G

N

O

P

Q

R