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LECTURES

Opening Lecture (Cuyo Biology Society)

A1
MELATONIN IN MITOCHONDRIA: PROTECTING AGAINST CLEAR AND PRESENT DANGERS

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The circadian melatonin rhythm plays an essential role in reducing the development of diseases such as solid tumors which adopt cytosolic aerobic glycolysis (Warburg effect) to support their enhanced metabolism. Experimental data shows that solid mammary tumors depend on aerobic glycolysis during the day but likely revert to mitochondrial oxidative phosphorylation at night for ATP production. This conversion of diseased cells during the day to a healthier phenotype at night occurs under control of the circulating melatonin rhythm. When the nocturnal melatonin rise is inhibited by light exposure at night, cancer cells function in the diseased state 24/7. The ability of melatonin to switch cancer cells as well as other diseased cells, e.g., Alzheimer disease, fibrosis, hyperactivation of macrophages, etc., from aerobic glycolysis to mitochondrial oxidative phosphorylation may be a basic protective mechanism to reduce several different pathologies. When diseased cells prevent the product of glucose metabolism, pyruvate, from entering the mitochondria, it deprives the mitochondria of the ability to generate acetyl Co-A, which normally feeds the citric acid cycle and supports oxidative phosphorylation. Since acetyl Co-A is also a necessary co-factor for the synthesis of melatonin, which occurs in the mitochondria of normal cells, cancer cells also cannot synthesize melatonin, a molecule with significant anticancer activity.

Lecture (Tucumán Biology Association)

A2
REGULATION OF EXTRACELLULAR VESICLES RELEASE BY AUTOPHAGY

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Autophagy and extracellular vesicles (EVs) are components of the cellular proteostasis network that keeps misfolded proteins in check and ensures they do not accumulate in the cell or trigger deleterious effects. In the Central Nervous System, all cell types release EVs that are taken up by neighboring cells or are released into the cerebrospinal fluid and blood. EVs carry amyloidogenic proteins and potentially spread the disease from one cell to another. There is growing evidence of intense crosstalk between autophagy and EVs. Our lab has studied the regulation of EV secretion by autophagy in the context of cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases, specifically the transmission of amyloid beta peptide (Aβ). We developed a protocol to label EVs in vivo in cultured cells using the fluorescent lipid DiI, and we analysed cell-derived EV preparations, virtually unprocessed, by Imaging Flow Cytometry (IFC) and nanoparticle tracking analysis (NTA) as reliable methods to understand EV secretion regulation. We demonstrated that blockade of autophagic flux causes a significant increase in EV release. Extensive autophagic-lysosomal pathology in Alzheimer's disease (AD) brain contributes to disease pathogenesis, although the underlying mechanisms are not well understood. Using the reporter mcherry-GFP-LC3 we found that $A\beta_{42}$ blocks autophagic flux in cultured cells and mice in vivo. Thus, we examined if Aβ42 increases EV secretion and promotes its own cell-to-cell transfer. Our data, using IFC and NTA analysis indicate that Aβ-induced autophagy blockade increases EV secretion and favors cell-to-cell transfer of EVs, however we could not detect any significant increase of Aβ spreading. Our results are important in the general context of EV release regulation and in neurodegenerative diseases since reversing autophagy dysfunction in AD is considered a valuable innovative therapeutic strategy.

Lecture (Cuyo Biology Society)

A3
COVID-19: MELATONIN AS A SOLE OR ADJUVANT TREATMENT

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Regulation of the melatonergic pathways, both pineal and systemic, may be an important aspect in how viruses drive the cellular changes that underpin their control of cellular function. Viral, or preexistent, suppression of pineal melatonin disinhibits neutrophil attraction, thereby contributing to an initial "cytokine storm", as well as the regulation of other immune cells. Melatonin induces the circadian gene, Bmal1, which disinhibits the

pyruvate dehydrogenase complex (PDC), countering viral inhibition of Bmal1/PDC. PDC drives mitochondrial conversion of pyruvate to acetyl-coenzyme A (acetyl-CoA), thereby increasing the citric acid cycle, oxidative phosphorylation, and ATP production. Pineal melatonin suppression attenuates this, preventing the circadian "resetting" of mitochondrial metabolism. This is especially relevant in immune cells, where shifting metabolism from glycolytic to oxidative phosphorylation switches cells from reactive to quiescent phenotypes. Acetyl-CoA is a necessary cosubstrate for arylalkylamine N-acetyltransferase, providing an acetyl group to serotonin, and thereby initiating the melatonergic pathway. Virus- and cytokine-storm-driven control of the pineal and mitochondrial melatonergic pathway therefore regulates immune responses. The focus of this presentation will be the role of melatonin, an immune regulator, and its treatment implications for COVID-19 and other viral infections

Lecture (Argentinean Biology Society)

A4
MOLECULAR MECHANISMS OF ADAPTATION TO HYPOXIA IN *Drosophila melanogaster*

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Animal cells have the capacity to adapt to low oxygen levels (hypoxia), through several different mechanisms that depend on changes in their transcription profile. The Hypoxia Inducible Factor (HIF) is the master regulator of transcriptional responses to hypoxia conserved throughout the animal kingdom. It is a heterodimer composed of two subunits that belong to the bHLH-PAS family of transcription factors, where the beta-subunit is constitutive, and the alpha-subunit is regulated by oxygen levels. In normoxia, one or more HIFα prolyl residues are hydroxylated in a reaction catalyzed by specific prolyl-4-hydroxylases. Hydroxylated HIF prolyl residues can be then recognized by the Von Hippel Lindau (VHL) tumor suppressor factor, which is part of an E3 ubiquitin ligase multimeric enzyme that marks HIFα for proteasomal degradation. In hypoxia, hydroxylation of the prolyl residues does not take place, HIFα stabilizes, forms the heterodimer with the β-subunit, and the transcription factor can induce expression of hundreds or thousands of genes that mediate adaptation of the organism to hypoxia. In our lab, we utilize *Drosophila melanogaster* as a model system to get insights into new mechanisms of HIF regulation, and adaptive responses to hypoxia. We have performed an RNAi-based genome wide screen in *Drosophila* S2 cultured cells, aimed at identifying new HIF regulators required for activation of adaptive responses to hypoxia. Thirty-one genes scored as positives in the screen, 11 of which corresponded to HIF regulators that had been previously characterized, while the other 20 hits were genes that had not been linked before to hypoxia biology. One of the new HIF regulators was Argonaute-1, a key player in miRNA biogenesis, suggesting that one or more miRNAs could be required for optimal HIF activity. Thus, we performed a new genetic screen, this time in transgenic flies, in which we overexpressed all the miRNAs encoded in the *Drosophila* genome with the aim of identifying one or more miRNAs that can enhance HIF-dependent transcription. We identified miR-190, which in turn, we found that inhibits prolyl-4-hydroxylase translation, thereby enhancing HIF-dependent transcription. Another hit of our original screen performed in S2 cells is the translational repressor Musashi, that had been previously identified as a Notch repressor. We demonstrated that Musashi can bind HIFα mRNA, repressing its translation in normoxia. Finally, another hit of the screen corresponded to the chromatin regulator Tip60. We showed that Tip60 is part of a multiprotein complex with histone acetyl transferase activity, necessary for HIF transcriptional activation. In summary, we have utilized *Drosophila melanogaster* as a genetic model to explore new mechanisms of HIF regulation, finding novel elements that control this transcription factor essential for adaptation to hypoxia.

Lecture (Córdoba Biology Society)

A5
NON-VISUAL OPSINS AND PHOTORECEPTOR CELLS IN THE INNER RETINA OF DIURNAL ANIMALS. IS THIS A POSSIBLE CAUSE OF PHOTOPROTECTION?

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Most living organisms have generated, throughout evolution, photoreceptor cells specialized in the detection of light. In vertebrates these cells are mainly located in the retina and constitute the visual photoreceptors "rods and cones" responsible for daytime (color) and nighttime (black/white) vision, respectively. They contain photosensitive molecules made up of an "opsin" and a vitamin A-derived chromophore, "retinaldehyde", capable of photoisomerizing from 11-*cis*-retinal to all-*trans*-retinal and triggering the nervous responses that lead to vision. However, at present it is known that other cells of the inner retina (retinal ganglion cells (RGCs), horizontal cells (CHs) and glial Muller cells) express non-visual photopigments such as melanopsin (Opn4), encephalopsin (Opn3), neuropsin (Opn5) and RGR photoisomerase, and respond to light stimulation. Recent evidence suggests that these photoreceptors participate in various non-image formation (NIF) functions, such as the setting of the biological clock, the pupillary light reflex, the lateral interaction between visual photoreceptors and CHs, the glia-neuron interaction, and the retinoid recycling to form the active photopigment. Among these opsins, melanopsin is the most studied opsin. In non-mammalian vertebrates, there are 2 Opn4 genes, the mammalian ortholog (Opn4m) and the *Xenopus* one (Opn4x) which are differentially expressed in intrinsically photosensitive RGCs (ipRGCs) and CHs of the chicken retina. These non-visual photoreceptors appear very early in development. The pioneer work of this laboratory was the first in

characterizing the biochemical nature of the Opn4x-mediated phototransduction cascade. This cascade in ipRGCs involves the Gq protein, activation of phospholipase C, mobilization of Ca^{2+} and later depolarization; cascade that also involves GABA release in CHs. Furthermore, these non-visual photopigments (Opn3, Opn4 and Opn5) respond to blue and /or UV light, which confer the capacity of detecting light in a broader light spectrum, complementing that for visual photoreceptors, and thus regulating a significant number of NIF functions. We can conclude that "a constellation of photoreceptor cells and molecules are present in the inner retina of vertebrates, from early developmental stages, even before any sign of vision may occur", strongly suggesting the possibility of photo-resistance and –photo-protection in the inner retina mainly of birds and other diurnal animals.

"Miguel Lillo" Lecture (Tucumán Biology Association)

A6
MEMBRANES, WHERE LIPIDS AND PROTEIN MEET

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Membrane proteins have central roles in a vast number of vital cellular processes. A structural feature that most membrane proteins have in common is the presence of one or more hydrophobic helices with which they interact with the lipid bilayer. Because of the interaction with the surrounding lipids, the organization of these helices will be sensitive to lipid properties like fluidity and hydrophobic thickness. The helices may adapt to the lipids in different ways, which in turn can influence the structure and function of the intact membrane protein. In this talk, I will focus on how the lipid environment governs the signaling state of a transmembrane protein and in how the lipid bilayer influences the catalytic and substrate channeling role of a peripheral protein.

Lecture (Córdoba Biology Society)

A7
MOLECULAR IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN RESPONSE TO ABIOTIC STRESS IN TROPICAL PLANTS WITH ECONOMIC VALUE

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We have combined different methodologies from several subjects in the biological sciences. This in order to develop a multidisciplinary platform to understand better the gene products that participate in a coordinated matter between them. This to understand the mechanism of response and tolerance to particular abiotic stress until the point of plant death due to wilt. These genes can be used to develop new biotechnological products with a potential not to affect the performance in the commercial cultivars regardless of the climate changes that have been caused by global warming. Our research has been focused in defining the different elements in signaling implicated in the molecular mechanism in response to abiotic stress in plants with economical value for Mexico. This has been done using advanced "omics" technology (Metabolomic, genomic and proteomic). Therefore, the experiments in physiology and biochemistry helped us to id the different limits that a plant can have in a time wise presence of abiotic stress. In order to establish the relevant information to carry out massive sequencing and using bioinformatics to obtain and assemble small sequences to define complete genes and create the network of genes involved in the response to stress. With the id genes and comparison with other species and their gene information we can establish the correlation in different species and again with a set of novel bioinformatic tools it helps predict all the ancestral lines and evolution history of multigenic families. Even if the complexity of the family involves polyploidy or gene redundancy. We then use the genes that have a preestablished function by their *in-silico* analysis and use molecular biology techniques to edit the genomes (CRISPR/Cas9). With the new genotypes can be established until getting pure transgenic lines from either Agrobacterium or bio-ballistic transformation. Results and discussion. In this talk we will show some results that will describe how we develop the multidisciplinary platform using different methodologies that allowed us to get international patents, as well as the human resources with a multi-disciplinary background that have resulted from the work shown when developing this type of research.

SYMPOSIA

Symposium 1: Córdoba Biology Society

A8
MEMBRANE FUNCTIONALITY IN ADAPTATION TO STRESSING FACTORS IN RHIZOBACTERIA OF AGRONOMIC IMPORTANCE

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An environmentally friendly alternative is the inoculation of legumes such as *Medicago sativa* (alfalfa) with rhizobia that can fix atmospheric nitrogen in symbiosis. To establish an effective symbiosis, the first step is a strict chemical signaling between the plant and the bacteria that involves the incorporation by the bacteria of the inducing flavonoid exuded by the alfalfa roots (luteolin), its binding to the NodD1 protein. and then the binding of the complex to the promoter of the box *nod*, which will allow the expression of the genes necessary for the Nod factors to be produced. Temperature is one of the most variable environmental factors and can cause important changes in the cell envelope of bacteria, which can then be translated into changes in their ability to signal and establish symbiosis with alfalfa. Recently, our working group characterized the response of outer and inner membrane of *Sinorhizobium meliloti* 1021 to cyclical temperature changes (10ºC–40ºC–10ºC), evaluating the biophysical state, the composition of phospholipids and fatty acids. The main findings indicate that the outer and inner membranes exhibited different biochemical and biophysical responses that cannot be predicted from whole cell data. The change in the degree of unsaturation of the esterified fatty acids in the most abundant phospholipids (phosphatidylcholine and phosphatidylethanolamine) seems to be the predominant mechanism for controlling the fluidity of both membranes. These changes in the bacterial envelope caused by temperature influenced the incorporation of luteolin, registering at 40ºC values well below those observed at 28ºC (48 ng/mg and 78 ng/mg of biomass, respectively), most of which was located in the outer membrane (77%). Cells exposed to 40ºC showed a delay in the ability to form infection threads in alfalfa roots. These results lay the biochemical bases for a successful and rapid rhizobia-legume interaction, taking into account the biochemical characteristics that the bacteria bring to the inoculant.

A9
MYCORRHIZAL FUNGI IN AGRICULTURAL, SALINE, FOREST AND POLLUTED ENVIRONMENTS. ITS POTENTIAL CONTRIBUTION TO ECOLOGICAL RESTORATION

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One of the most important soil microorganisms associated with most plants is the arbuscular mycorrhizal fungi (AMF). They form the most common type of mycorrhiza, arbuscular mycorrhizae, present in all terrestrial ecosystems. The fungus colonizes the roots, without damage, developing an intraradical mycelium –symbiotic interface–, an extraradical mycelium –a network of hyphae that extend and branch in the soil– and spores. The extraradical mycelium grows beyond the zone of nutrient depletion surrounding the root, forming mycelial networks that allow the connection and exchange nutrients between different plant species and contribute to the formation and maintenance of the soil structure. Among the benefits that AMF confer on host plants are the improvement in mineral nutrition, acquiring mainly phosphorus and other macro- and micro-nutrients, protection against radical pathogens (like nematodes), improved water uptake and tolerance to drought, salinity, and toxic metal pollutants. These effects improve growth, establishment, and survival of the host plant in stressful environments. Furthermore, AMF have a potential economic importance as biofertilizers and bioindicators in agricultural practices. The objective of this presentation is to show the results obtained regarding the arbuscular mycorrhizal symbiosis associated with plants growing in different types of environments: agricultural, saline, forest and polluted. Studies show that AMF play a major role in plant growth, survival, and restoration of degraded ecosystems.

A10
CAPACITY OF BACTERIA OF THE GENUS *Bacillus* TO INDUCE THE DEFENSE RESPONSE AGAINST FUNGAL PATHOGENS OF PEANUT

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The diseases caused by fungi represent an important limitation in the production of peanut in the province of Córdoba. One strategy for diseases control is the use of microorganisms and/or compounds of its metabolism released into the growth culture medium. In addition, the combined inoculation of bacteria capable of promoting plant growth by different mechanisms constitutes an alternative or complement to the use of agrochemicals. Studies carried out in the research group have shown that the native strain *Bacillus* sp. CHEP5 induces systemic resistance (ISR) in peanut plants, protecting them against the phytopathogen *Sclerotium rolfsii* in plant culture chamber assays, guaranteeing the physical and temporal separation between the biocontrol bacteria and the phytopathogen. Peanut plants also interact with *Bradyrhizobium* sp. SEMIA6144, establishing a symbiotic association for nitrogen fixation. In plants inoculated with both bacteria, the defense response (percentage of diseased plants and plant

growth parameters as indicators of incidence and severity, respectively, and the determination of peroxidase activity and production of phenolic compounds as markers of "priming" of plant defense response) was not affected and both in the absence and in the presence of the pathogen, the symbiotic behavior (number and dry weight of nodules, percentage of red nodules) was positively affected. These same results were obtained in field trials, particularly showing a protective effect against *Thecaphora frezii*, the causal agent of peanut smut. On the other hand, it was determined in the growth culture medium supernatant of *Bacillus* sp. CHEP5 the presence of the lipopeptide surfactin, elicitor of the systemic defense response in peanut. Moreover, the bacterial growth culture medium supernatant was shown to induce the defense response in peanut against *S. rolfsii*. However, co-inoculation of the microsymbiont and the biocontrol growth culture medium supernatant did not reverse the negative effect on nodulation in plants challenged with the phytopathogen. Considering the importance of crops such as peanut in our country, it is of interest to consider the simultaneous interaction of biocontrol bacteria and/or their metabolites released to the growth culture medium with symbiotic nitrogen-fixing bacteria, not only to understand the ecological role of such interaction but also for the biotechnological application of these microorganisms in the promotion of peanut growth.

Symposium 2: Córdoba Biology Society

A11
TYPE 1 DIABETES MELLITUS: EFFECT OF NARINGIN ON TISSUES REGULATING THE EXTRACELLULAR CALCIUM HOMEOSTASIS

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The extracellular Ca^{2+} homeostasis is controlled by tissues such as the intestine, bone and kidney. At intestinal level, the cation absorption is mainly regulated by calcitriol; in bone Ca^{2+} accumulates and exchanges with the medium according to the physiological needs under parathyroid hormone and calcitriol control, whereas in the kidney Ca^{2+} is also reabsorbed and/or excreted under hormonal control. Ca^{2+} homeostasis is altered in the type 1 Diabetes mellitus (type 1 D.m.), leading to development of bone disease, a complication characterized as a secondary osteoporosis or intense osteopenia that is not prevented or cured with insulin. In our laboratory, we induced type 1 D.m. in male Wistar rats aged two months by using a unique dose of streptozotocin (STZ), drug that destroys pancreatic β cells. After a month, the animals show oxidative stress (OS) in different tissues. Since NAR is a natural antioxidant, we evaluated the effect of NAR on different parameters of OS and derived processes in tissues regulating the Ca^{2+} homeostasis in order to know whether these complications could be attenuated or avoided with this treatment. Different doses of NAR (40 and 80 mg/b.w.) were used for 30 days. In the intestine, NAR avoided the inhibition of the intestinal Ca^{2+} absorption caused by STZ, as well as the loss in bone mass and the alterations in the renal functions. NAR improved the quality in bone mass through enhancing the number of osteoblasts and reducing the number of osteoclasts and adipocytes. In the three tissues, NAR blocked the enhancement in the OS. The data suggest that the adjuvant use of NAR could be a useful tool to prevent alterations in the extracellular Ca^{2+} homeostasis, which lead to the bone disease associated with type 1 D.m.

A12
FORMATION OF EXTRACELLULAR VESICLES AND THEIR POSSIBLE ROLE IN THE PATHOGENICITY AND DRUG RESISTANCE OF THE PARASITE *Giardia lamblia*

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A defining characteristic of cells is the ability to communicate with each other through different mechanisms including direct cell-cell contact or the transfer of secreted molecules. Recently, a third intercellular communication mechanism was found that involves the release of extracellular vesicles or EVs, carriers of biologically active molecules with defined biological functions that are a conserved mechanism present in Bacteria, Archaea, and Eukarya. In our laboratory, we discovered that *G. lamblia* trophozoites are capable of producing small VEs similar to exosomes of other cell types in terms of size, shape, density, and presence of typical proteins. The results obtained also showed that, despite the lack of typical multivesicular bodies in this parasite, exosome-like vesicles (tExo) are form in the endo-lysosomal peripheral vacuoles (PVs) of *G. lamblia*. We also found that the ESCRT-associated protein Vps4a, the small GTPase Rab1, and ceramide are essential for the formation of intraluminal vesicles in PVs and for obtaining tExo in the extracellular medium. Furthermore, we carried out the first proteomic analysis of the extracellular vesicles of two pathogenically different assemblages and found that both microvesicle-type and exosome-type vesicles could be selecting the content of conserved and specific *G. lamblia* proteins. Differences in the composition, origin, and release of EVs from the protozoan parasite may reveal functional and structural properties of EVs and thus may provide information on cell-to-cell communication and survival mechanisms.

A13
ALPHA-2 MACROGLOBULIN RECEPTOR/LRP1 EXPRESSION IN PERIPHERAL BLOOD MONOCYTES: IMPLICATIONS IN THE ATHEROSCLEROSIS

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Atherosclerosis is a chronic inflammatory process of the arterial wall. In general, the evolution of this disease occurs as a subclinical form (SCAT) and is characterized by early endothelial dysfunction, dyslipidemia, arterial hypertension, and sub-endothelial accumulation of low-density lipoproteins. Endothelial dysfunction also facilitates adhesion and extravasation of peripheral blood monocytes (PBMo), which at the level of the vascular intima undergo differentiation processes to macrophages and foam cells that exacerbate the inflammatory condition and atheroma formation. The alpha-2 macroglulin receptor/LRP1 (for Low-density lipoprotein Receptor-related Protein 1), through cellular processes of endocytosis and intracellular signaling, mediates various events of cell adhesion, migration, proliferation and differentiation, remodeling of the extracellular matrix and control of the pro-inflammatory response. LRP1 is expressed in macrophages and vascular smooth muscle cells where it plays a key role in atheroma formation. Although LRP1 is also expressed in PBMo, its functional relationship with the development of SCAT is unknown. Through an observational clinical study, the expression of LRP1 in PBMo subpopulations (classical/$CD14^{++}CD16^{-}$, intermediate/$CD14^{++}CD16^{+}$ and non-classical/$CD14^{+}CD16^{++}$) was analyzed (N = 227; 20-59 years) in individuals enrolled under informed consent at the Hospital Privado Universitario de Córdoba (CRI-HP 4-178). Through anthropometric, biochemical, and imaging data (Doppler ultrasound of Carotid and Coronary Calcium Score-Agatston Score), three study groups were classified: low risk (LRG; N = 21; 15 males; 6 females), intermediate risk (IRG; N = 82; 29 males; 53 females) and ATSC (SCATG; N = 124; 46 males; 78 females). By flow cytometry measured LRP1, CD36, CD11b and CD11c in different PBMo subpopulations. LRP1 showed a very significant decrease in total (P = 0.0004) and classical (P = 0.0002) PBMo and a moderate decrease in intermediate (P = 0.0083) and non-classical (P = 0.0206) PBMo in individuals with SCAT compared to the other study groups. No significant changes in the other monocyte markers were observed between the study groups. By quantitative PCR, a decreased level of mRNA for LRP1 was established in classical PBMo in the SCATG compared to the other groups (P = 0.0002). In these individuals a significant increase in mRNA levels for IL-1β (P = 0.0014), TNF-α (P = 0.0121), CCL2 (P = 0.0069) and CCR2 (P = 0.0429) were found in classical PBMo. In conclusion, the expression of LRP1 in association with the pro-inflammatory profile of classical PBMo constitutes a potential diagnostic tool for atherosclerosis.

A14
EDITING AND REMODELING OF LIPIDS DURING STRESS RESPONSE IN PLANTS

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Plants are continuously confronted with a great diversity of chemical and physical signals during their life cycle. To cope with these variable environmental conditions, they have developed sensing and signalling mechanisms. Glycerolipids are the main constituent of cellular membranes, and their remodelling plays an important role in how plants adapt to stress. Modifications in the lipid composition of cellular membranes during chilling are essential to maintain their integrity, fluidity, and recovery. In general, and under stress conditions, the remodelling of membrane lipid composition comprises two phenomena. The first one involves changes in the proportions of the polar lipid head group while the second includes changes in the saturation level and in the chain length of fatty acids forming glycerolipid non-polar tails. We previously described phosphatidic acid (PA) is a minor phospholipid but an important signal lipid. It is also known as a key precursor for the synthesis of major glycerophospholipids and galactolipids. PA is structurally the simplest type of glycerophospholipids, although it comprises a number of molecular species, which differ in acyl chain length, number of acyl double bonds, and acyl linkage at the sn-1 and sn-2 positions. In this work, we used a lipidomic approach to show tissue-specific differences associated with the response to chilling and recovery. Lipids from leaves and roots grown under control conditions and submitted to chilling treatment were quantitatively profiled using electrospray ionization-triple quadrupole mass spectrometry (ESI-MS/MS). The data provided information on phospholipids and glycolipids at the level of the head group and the number of carbon atoms and double bonds present in the acyl chains. This ESI-MS/MS-based profiling approach identified and quantified 150 glycerolipid molecular species. These included plastidic lipids (MGDG, DGDG and PG), extraplastidic phospholipids (PA, PI, PC, PG, PS, PE) and lysolipids (LPC, LPE, LPG). We found that the level of phospholipids and galactolipids was higher in roots than in leaves, although the phospholipid to galactolipids ratio was similar for both tissues. Compared with plants grown at 25 °C, the chilling treatment produced contrasting responses in phospholipids and galactolipids. We also found that structural and signalling lipid species changed their abundance during chilling and recovery periods. Although the cold-acclimation-responsive lipids were globally returned to non-acclimated levels by short and long recovery, several representative phosphatidic acid molecular species as (34:2, 34:3, 36:4, 36:5, 36:6) cold-acclimation-responsive molecular species tended to remain at remodelled during de-acclimation process. We propose that the rearrangements during chilling and recovery of phosphatidic acid represents a key component in the stress response in dynamic environments.

Symposium 3: Tucumán Biology Association

A15
BIOPROSPECTION OF NATURAL PRODUCTS WITH THERAPEUTIC POTENTIAL

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Nature has been a source of drugs since the beginning of history and in many cultures it still is. WHO estimates that 80% of the developing population base health care on traditional medicine; the remaining 20%, in more than 25% of cases, use drugs that have been derived from natural products. The path a substance from nature takes to becoming a new drug is long, laborious, and generally very expensive. The most important steps along this path are the isolation of the natural source, the structural elucidation of the isolated metabolites, their testing in different biological systems until reaching the clinical phases and, finally, their approval. The United States Food and Drug Administration (FDA) between 1981 and 2014 approved 1,562 drugs of which 64 (4%) were unaltered natural products, 141 (9%) were phytopharmaceuticals, 320 (21%) were drugs derived from natural products and 61 (4%) were synthetic drugs in which the pharmacophore was of natural origin. The success of natural products lies in the fact that they are compounds that have already been validated by evolution and have been biosynthesized, degraded, and transformed by enzymatic systems. Therefore, when it comes to interacting with the target molecules, they will do so in a privileged way. For example, in recent years, the prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruits, vegetables or infusions rich in natural antioxidants. There are a large number of studies that suggest that a higher intake of these compounds is associated with a lower risk of mortality from these diseases, which also include high blood pressure, atherosclerosis and diabetes mellitus. In this context and with the appearance of new infectious diseases, illnesses that still have no treatment or the emergence of drug resistance that threatens the efficacy of current medication, our work group systematically searches biodiversity, new sources of more effective chemical compounds and herbal complexes with fewer side effects for the development and solution of new therapeutic options. Biodiversity must be conserved in order not to lose natural products that are yet to be discovered. Each species that becomes extinct is something unrepeatable in history and conserving them is a very important task that we must carry out.

A16
ANTIFUNGAL AGENTS FROM NATIVE PLANTS OF NORTHWEST ARGENTINA: USEFULNESS IN THE CONTROL OF FUNGI RESPONSIBLE OF FOOD ROT

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Cereal grains and other agricultural food products suffer fungal rots which annually generate losses for millions of dollars not only before harvest but also during storage and other steps of food chain processing. When fungi involved are toxigenic fungi, the economical losses translated as minor yields are also accompanied with the mycotoxigenic risk. Comsuption of mycotoxins can generate intoxications in humans, cattle, and farm animals. One of the most common strategies to fight against phytopathogenic and/or mycotoxigenic fungi is the application of antifungals in the form of fungicides before harvest or food preservants during storage. The intensive use of the current antifungals available led to several problems including appearance of fungal resistance, induction of mycotoxin accumulation at sublethal doses, modifications in organoleptic features and intoxication of organisms that are not control targets. This lecture presents the advances performed in the LABIFITO, in the search of antifungals from native plants of Northwest Argentina able to control food rot fungi, particularly fungal species of *Fusarium* and *Aspergillus*. It also analyzes the main problems faced in the identification, isolation, and characterization of biological activity of these compounds, and in some cases, it is depicted their possible uses in agriculture and agri-food.

A17
NEUTRALIZING EFFECT OF PLANT EXTRACTS ON *Bothrops diporus* SNAKE VENOM

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In Argentina, ophidian accidents constitute a serious public health problem. Among the poisonous snakes of greatest medical importance in our country, *Bothrops* species are responsible for 98% of accidents, particularly *Bothrops diporus* accounts for 80% to this percentage of accidents. In the search for alternative treatments for snake bites of this genus, several natural products, and their derivatives (phenols, sesquiterpenes, alkaloids, among others) are evaluated for their inhibitory properties on the toxic effects of snake venoms. The plant species studied were selected taking into account the ethnobotanical background, on the one hand, but also based on the chemical composition. The alexiteric activity was evaluated using *in vitro* biochemical techniques as a tool (SDS-PAGE, inhibition of indirect hemolysis, coagulation, proteolysis), both for the study of its physiological effect as well as to determine the effect of certain natural products on venom composition. Aqueous and hydroalcoholic extracts were obtained from red quebracho sawdust (*Schinopsis balansae*), which were lyophilized and chemically characterized (total phenols and total tannins). A pool of *B. diporus* venom was used, its minimum hemolytic dose was determined, the inhibition of its hemolytic activity was evaluated with different doses of extracts and SDS-PAGE of the treated venoms was performed. At a venom: extract ratio (1:10), the total inhibition of the *in vitro* hemolytic effect of the venom was observed. The sawdust extracts presented between 11% and 30% tannins. SDS PAGE assays showed changes in the venom protein

profile when they are treated with the extracts. Low-dose red quebracho sawdust extracts were active, particularly in inhibiting the hemolytic activity of yarara venom. The variations in the protein profile of the venom suggest the interaction between tannins and the venom proteins. Although further studies, both *in vitro* and *in vivo*, remain to be carried out, the data revealed so far indicate that the tested extract would show promise in mitigating part of the local tissue damage induced by the venom.

A18
SESQUITERPENOIDS WITH INSECTICIDAL EFFECTS

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Sesquiterpene lactones (SL) are chemical agents with innumerable effects on different biological models. Pure SL obtained from *Vernonanthura squamulosa* and *Cyrtocymura cincta* (Vernonieae) was evaluated on two insect pests. SL **1a, 1b, 2a** and **2b** were obtained from *C. cincta* and were tested separately at a dose of 100 µg SL/g of diet on *Sitotroga cerealella*, a lepidopteran stored grain pest. A single sesquiterpene lactone *GA* was obtained from *Vernonanthura squamulosa* and tested on *Spodoptera frugiperda* (Lepidoptera) at the same dose. *S. cerealella* and *S. frugiperda* were raised in the laboratory, under controlled conditions (26 ± 2 ºC; 50–70% RH and 14/10 h L/O). *S. frugiperda* was fed with artificial diet impregnated with an acetonic solution of GA and *S. cerealella*, was fed with wheat grains that were soaked with acetonic solutions of each sesquiterpene lactones obtained from *C. cincta* separately. Effects on life cycle, fecundity and fertility of 1st generation were evaluated in both species. On *S. cerealella* a mating protocol was followed, crossing females treated with control males, males treated with control females, males and females treated with each SL separately, and control males and females. The results obtained for this species were: lengthening of the life cycle which resulted in mortality in all states and malformation of surviving adults for all the SLs tested. Sesquiterpene lactone 1b decreased fecundity by 68.47% with respect to the control and the fertility of those eggs was 36%; the SL 2a decreased the oviposition by 57.42% and the fertility of those eggs was 41%. On *S. frugiperda* SL GA produced a lengthening of the life cycle, larval and pupal mortality, and adult malformations. Oviposition decreased a 50% and fertility of eggs was 40%. Pure SL of *C. cincta* and *V. squamulosa* produced lethal and sublethal effects on the populations of both pests, which contributed to a marked decrease in their populations. Pure SL of both botanical species can be evaluated in strategies of control of these insect species.

Symposium 4: Rosario Biology Society

A19
BILIRUBIN: MORE THAN JUST A PIGMENT

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Bilirubin (BR) is an endogenous bile pigment generated from heme degradation, due to the sequential action of enzymes heme-oxigenase 1 and biliverdin reductase. Historically, BR has been considered not only a waste product, but also a potentially toxic compound. However, this view began to change by the late past century, owing to the work of Roland F. Stocker *et al*, and today BR is regarded as a potent endogenous antioxidant agent with cytoprotective properties. *In vitro*, antioxidant effects of BR comprise the scavenging of singlet oxygen and superoxide anion, as well as of peroxyl radicals, thus explaining its protective effects against membrane lipid peroxidation. *In vivo*, BR has been found to protect rodents against both OS-induced retinal degeneration and diabetic nephropathy, and it also shows anti-genotoxic effects in humans and animal models. More recently, BR has also been shown to bear immunomodulatory, anti-inflammatory, anti-apoptotic and anti-mutagenic effects. Various studies carried out until now have demonstrated the potential utility of BR in the treatment of cardiovascular, pulmonary, and metabolic diseases, as well as of post-transplantation ischemia-reperfusion and the immune response. Our research group has demonstrated that unconjugated BR exerts an important protective effect on the oxidative stress-induced biliary secretory failure, even at physiological concentrations. Later, we demonstrated that heme-oxygenase 1 induction and the consequently elevated endogenous BR levels completely prevent oxidative stress-induced acute cholestasis in the whole animal model. Following our hypothesis that hepatic diseases bearing an oxidative background would have a worse outcome in terms of hepatobiliary function in the absence of BR, and that the modulation of endogenous BR levels would have a beneficial effect on oxidative cholestatic diseases, we are now devoted to the study of the mechanisms through which BR exerts its hepatoprotective effects. In line with this, we are carrying out an invaluable pre-clinical evaluation aimed at the future establishment of anti-cholestatic treatments based on the modulation of the endogenous levels of BR.

A20
FOOD DESIGN MORE BENEFICIAL TO HEALTH

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In recent years, more attention has been paid to the development of healthier diets and, therefore, there is a demand for food products with reduced fat content, sugar, and salt. However, these compounds contribute significantly to the organoleptic properties and palatability of food products. Therefore, the acceptability of low-fat, low-sugar, and low-salt products is compromised. The first step in the food intake and metabolism process is oral processing, which contributes to sensory perception and appreciation of food. Thus, it is important to understand the oral food processing related to its microstructure and its disintegration. During the same, a cohesive bolus is formed as the food particles stick together by saliva and fluid released by the product. The amount of the latter is related to the product microstructure, the greater the porosity of the food, the greater the release of fluid. The intensity of the perceived taste is significantly higher when there is a greater release of whey. The challenge, then, is to reduce the salt and sugar content of semi-solid and soft foods without compromising taste. The microstructure of a semi-solid food can be designed to maximize the release of a sweet or salty flavor from the food matrix into the oral cavity during oral processing. This is achieved by managing the relative proportion of proteins and polysaccharides in the food. This approach allows the sugar and salt content in foods to be significantly reduced while maintaining sweetness and salinity.

A21
THE ORAL TRANSMISSION OF CHAGAS DISEASE: A NEW CHALLENGE IN THE AMERICAS

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Chagas disease is an anthropozoonosis caused by the parasite *Trypanosoma cruzi*. The disease is endemic in Latin America, affects approximately 6–8 million people and generates an average of 14,000 deaths per year. The natural transmission routes of this disease are vector, congenital and oral; while transmission by transfusion, organ transplants and laboratory accidents has also been reported. Oral Chagas disease is caused by the ingestion of food contaminated with the parasite. This route of infection has been little studied. In our country there are few reports of this type of transmission, dating from the last century. Currently, there are numerous outbreaks that have occurred in different Latin American countries, such as Brazil, Colombia, Peru, and Bolivia. This form of infection tends to have an acute phase that is much more symptomatic and more lethal than the vector and congenital forms. There are few clinical studies on this form and numerous studies are currently being carried out in experimental models to deepen its study. The objective of this presentation is to review the data currently available on Oral Chagas Disease.

A22
CHARACTERIZATION AND EPIDEMIOLOGY OF *Xanthomonas arboricola* PV. *juglandis* IN WALNUT (*Juglans regia*) IN THE CENTER REGION OF ARGENTINA

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The purpose of this research was to characterize the performance of *Xanthomonas arboricola* pv. *juglandis* in the Argentine central region and to develop an epidemiological model to its management. The specific objectives were: (1) To corroborate the identity of *X. arboricola* pv. *juglandis* in Walnut. (2) To determinate molecular variability of *X. arborícola* pv. *juglandis* isolations from different locations of Argentine humid region. (3) To evaluate the severity of Walnut Blight through time on Franquette, Tulare, Chandler, and Davis varieties. (4) To relation to link severity with meteorological factors to develop an epidemiological model. (5) To study hibernation strategies and pathogen dispersion through buds. The essay was performed in the Field of FCA UNR (Zavalla, Santa Fe) on Franquette, Chandler, Tulare and Davis varieties (6 11 plants per variety) with injuries on leaves and fruits, and also leaves from Inriville, Corral de Bustos and Oliveros. Leaves pieces were cultivated on Petri dishes with BS medium (Brilliant Cresyl Blue Starch). Bacterial isolations were cultivated on agar tripteina soya médium. Of this culture DNA was extracted. Two reactions of PCRs (REP-PCR and ERIC PCR) were used for the DNAs typification. Foliar severity dates were recorded since November to February of 2010–2011, 2011–2012 and 2012–2013, and progress curves were made. With values of severity and the meteorological conditions a predictive model was developed. In Autumn–Winter 2010–2011 and 2012 buds of each variety were collected and cultivated in BS medium, and incidence was evaluated. Also pollen grains of 50 catkins of each variety were collected and studied in Laboratory to count number of colonies. Pieces of leaves developed yellowish and mucous colonies on Petri plates. PCR analysis showed similar DNA patterns for each location and different patterns between locations. Franquette variety showed the lower severity values during the 3 years (19.51%). The period with lowest severity value was 2011/12 (17.43%) and the highest severity value was in 2012/13 (79.58%). Proc Logistic of SAS program adjusted the predective model (Ec1 = –2,189 + 1,024 * DPr > 9 + 0,609 * DMojro + 1,7147 * sus) that includes two metheorological variables and one discrete binary variable that explains variety performance (sus = 1 and sus = 0). The bud incidence of disease was between 76% and 96%. High level of infection in buds is the inoculum for the next cycle.

Symposium 5: Argentinean Biology Society

A23
NUTRITION AND EARLY DEVELOPMENT: UNDERSTANDING THE LINK BETWEEN VITAMIN E DEFICIENCY AND NEURAL TUBE DEFECTS

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Neural tube closure defects (NTD) are congenital malformations caused by the abnormal development of the embryonic structure that originates the brain and spinal cord. NTDs are among the most common birth defects that cause infant mortality and severe disability, with a prevalence of 0.5–1/10,000 in human pregnancies. Non-genetic factors involved in the etiology of NTDs include maternal metabolic and nutritional abnormalities (e.g., diabetes and obesity) and inadequate intake of micronutrients such as folate (vitamin B9). During the closure of the neural tube (during weeks 2–3 of human pregnancy and gestational days 8.5–9.5 in mice), nutrients from uterine secretions are transported to the embryo via extra-embryonic tissues, such as the yolk sac (YS). This structure, formed by giant trophoblasts and the visceral endoderm, expresses nutrient transporters including the HDL receptor SR-BI, which takes up lipids such as cholesterol and fat-soluble vitamins from lipoproteins. We demonstrated that SR-BI KO embryos generated from heterozygous intercrosses exhibit NTD (incidence 1:3 in males, 2:3 in females) and develop exencephaly/anencephaly, a malformation that results in incomplete brain development and is lethal perinatally. SR-B1 KO embryos show undetectable levels of vitamin E (VE) and elevated reactive oxygen species (ROS), suggesting that SR-B1-mediated VE transport regulates embryonic oxidative state. In addition to its antioxidant activity, VE modulates gene expression and activates intracellular signaling pathways in different cell types. For the last years, our laboratory has focused on understanding the mechanisms that link defects in maternal-embryonic metabolism and transport of EV and NTDs. We demonstrate that maternal VE supplementation reduces ROS significantly and completely prevents NTDs in SR-B1 KO embryos. However, the VE intervention does not normalize embryonic VE levels, suggesting an indirect effect of VE on the YS. In line with these results, KO YS from dams fed VE show high levels of AKT phosphorylation, a pathway shown to regulate multiple biological processes in trophoblasts. At the embryonic level, we detected different expression profiles between morphologically normal KO and KO with NTDs that may explain the divergent phenotype in embryos of identical genotype. We recently studied the impact of excess malnutrition in our model. We observed that consumption of a high-fat high-sugar diet in heterozygous dams hinders VE uptake by the YS and results in NTD in WT (1:5) and heterozygous (1:3) embryos, in addition to SR-B1 KOs (1:2). This malformation is completely prevented by maternal supplementation with VE in embryos of all the genotypes (1:20, $P < 0.0001$ χ^2-test). Our results propose EV as a micronutrient involved in neural tube closure. This information is relevant to aid in the prevention of NTD in human populations with sufficient folate intake, in obese and diabetic women and in patients with recurrent NTD while consuming folic acid supplements. *Funding: Regular FONDECYT # 1141236 and # 1180347 (DB), CONICYT Doctoral Scholarship # 21130444 (NS) and # 21170306 (AQ).*

A24
BEHAVIORAL AND HORMONAL ASPECTS OF AGGRESSIVENESS AND REPRODUCTION IN FISH

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Social animals usually have hierarchical domination systems and are susceptible to changes in their environment: both natural and anthropogenic. Interactions with their conspecifics can greatly affect individual behavior and reproductive success. Throughout this talk I will show how social behavior modulates gonadal steroidogenesis and gametogenesis in African and Neotropical cichlid fish with different social systems and how the aggressiveness of the dominant regulates the reproductive capacity of the subordinates. Our investigations are focused on both males and females. Social behavior and aggressiveness are strongly linked to the synthesis of sex steroids, glucocorticoids, and various neuropeptides such as GnRH, serotonin, arginine, vasotocin. The challenge hypothesis holds that behavioral interactions increase androgen levels in response to social instability. On the other hand, there is little evidence regarding what would happen with estradiol levels in the face of any effect that produces social instability. We have recently shown that in males of the chanchita *Cichlasoma dimerus*, a neotropical cichlid fish, the challenge hypothesis could also be extended to estrogens. In the case of females of this same species, the challenge hypothesis is not fulfilled for either androgens or estrogens, although females with higher estrogen levels are usually the winners of the female *vs.* female contests in a neutral arena. In *C. dimerus*, the dominant males have a higher gonadosomatic index than the subordinate ones. The percentage of spermatocytes and spermatids is higher in the subordinates while the dominant ones show a higher percentage of sperm. The same has been observed in other model species of African cichlids: subordinate males, socially suppressed, are not reproductively incompetent and maintain some activity at all levels of their reproductive axis. The reactivation of the axis in social ascent (passage of male subordinate to dominant) is physiologically similar to the onset of puberty in mammals or to the reactivation of the brain-pituitary-gonad axes observed in animals with seasonal reproduction. To conclude, it will be shown how the study of behavior is an important and relatively low-cost tool to evaluate the welfare in fish.

A25
ENVIRONMENTAL CHEMICALS AND THEIR INFLUENCE ON MAMMARY GLAND DEVELOPMENT

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The mammary gland is a hormone-dependent organ that is characterized by reaching its maximum development postnatally. This characteristic allows to analyze, at different stages of its development, how exposure to chemical compounds with hormonal activity can affect the growth and differentiation of the mammary gland; processes that occur in the rat in both female and male animals. One of the main functions of the mammary gland is to provide the necessary nutrients for the maintenance of the offspring. Any interference in the mammary development of the female can influence the synthesis and secretion of milk. In this sense, we have shown that exposure *in utero* and during lactation to bisphenol a (BPA) modifies the quality of milk produced by exposed animals when they have to feed their own pups. Exposure to BPA delays alveolar maturation during secretory activation of the mammary gland and modifies the synthesis and secretion of beta-casein (CSN2) and of fat globules at the beginning of lactation. As lactation progresses, the proportion of CSN2 and lipids present in milk changes, with a decrease in CSN2 and an increase in lipids being observed when comparing exposed *versus* unexposed animals. Epigenetic changes induced by exposure to BPA may explain some of these observed modifications. On the other hand, it becomes increasingly important to analyze the effects of exposure to pesticides with potential hormonal action. The mammary gland of the male rat is a good experimental model for evaluating these compounds. The mammary growth and differentiation in males are different from that of in females, and in adult animals the mammary structure presents sexual dimorphism that is not observed in young animals. Using different routes and periods of treatment, we evaluated whether exposure to a glyphosate-based herbicide (HBG) altered mammary gland development in the male rat. Our results show that early postnatal exposure to low doses of HBG produces increased mammary gland development in prepubertal animals that is accompanied by changes in proliferation and expression of the alpha estrogen receptor (ESR1) after puberty. When animals are exposed *in utero* and during lactation to HBG, no morphological changes occur in prepubertal animals. However, decreased mammary growth accompanied by decreased ESR1 expression is observed in post-pubertal animals. These results indicate that the route and/or moment in which the mammary gland is exposed to this type of compound can modify the response obtained, and it is necessary to take it into account when analyzing the effects produced by these substances. The mammary gland is a sensitive organ, and exposure to chemical compounds with hormonal activity at different times in life can condition its development and differentiation in both sexes.

A26
BIOINDICATION OF HEAVY METALS, URANIUM, AND TRIBUTYLTIN IN FRESHWATER BODIES USING A LABORATORY MODEL ORGANISM

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Agricultural practices and industrial emissions have driven the development of techniques to detect the effects of metal pollutants on ecosystems. Biological monitoring has the advantage of retaining a "memory" of pollutant emissions, thus overcoming the difficulty of detecting contaminants that are irregularly emitted and quickly diluted in large water volumes such as lakes and streams. *Pomacea canaliculata* is a freshwater snail native to Argentina that has emerged as a model organism due to its ease of cultivation and handling, its short life cycle, its adequate morphology, and the growing availability of biomolecular data that allows studies at different levels of biological organization. Furthermore, this snail has established a symbiotic association with an intracellular organism, plausibly a cyanobacterium, that lives within the digestive epithelial cells. Here, we will review the extraordinary capacity of different snail's tissues and its symbiont to generate "inventories" of different elements toxic for human health, when these elements are incorporated at permitted concentrations in drinking water, such as Hg (2 µg/L), As (10 µg/L), and U (30 µg/L). A comparative analysis between the three elements studied allows us to affirm that: (1) the kidney accumulates mainly Hg, while the digestive gland accumulates As and U; (2) the elemental distribution in the symbiont is ~71%, ~48 %, and ~11% for U, Hg, and As, respectively; (3) tissue elemental depuration is variable between 8- and 16-weeks post-exposure. At week 16, the tissue depuration of U is the highest (digestive gland = 92%; kidney = 80%), while that of Hg was the lowest (digestive gland = 51%; kidney = 53%). At week 16, As shows a differential pattern of tissue depuration (digestive gland = 23%; kidney = 88%). Symbiotic detoxification of the three elements in the feces is rapid between weeks 8 and 10, and slower afterward. New questions about the accumulation process of As has been possible through the ^{76}As (V) radiotracer with high specific activity. Finally, we will comment on the peculiar effect of compounds containing tin (TBT and TPT), on the growth stimulation of the vestigial reproductive system of adult females *of P. canaliculata*. Symbiotic detoxification of the three elements in the feces is rapid between weeks 8 and10 and slower thereafter. New questions about the accumulation process of As has been possible through the development of a ^{76}As (V) radiotracer with high specific activity. Finally, we will comment on the peculiar effect of compounds containing tin (TBT and TPT), on the growth of the vestigial reproductive system of adult females of *P. canaliculata* and the hypothetical molecular mechanism involved.

Symposium 5: Chilean Society of Reproduction and Development

A27
EPITHELIAL–MESENCHYMAL TRANSITION/STEMNESS AXIS IN METASTASIS AND RESISTANCE IN PROSTATE CANCER

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There is increasing evidence that an Epithelial–Mesenchymal Transition (EMT)–Stemness axis seems to be a critical process related to cancer relapse, metastasis, and resistance. We have developed a modified orthotopic model of human prostate cancer (PCa) in NOD/SCID immunocompromised mice establishing a suitable and reproducible system of metastatic progression model. Metastasis and therapy resistance are complex and related processes that involve a cooperative action of different cancer cell subpopulations in which cancer stem cells (CSCs) and mesenchymal cancer cells would be responsible for invading, colonizing pre-metastatic niches, initiating metastasis, and evading treatments response. The aim of this work was to characterize the role of main stemness and EMT genes on metastasis progression and resistance in PCa. PCa cell cultures were obtained from several passages of primary cultures derived from tumor samples. All protocols were approved by institutional Ethical Committees. Cells were cultured under non-adherent conditions favoring CSCs spheres formation. Stemness markers CD44 and CD133, pluripotency genes SOX2, KLF4 and c-MYC and EMT gene ZEB1 were knocked down using specific shRNAs within lentiviral vectors. Metastasis progression and hormone resistance were evaluated in the pre-clinical orthotopic model of human PCa. CSCs knocked down for these genes showed chemotherapeutic drugs sensitization, increased apoptotic rate, and decreased clonogenic and invasive abilities. SOX2-knocked down CSCs decreased tumor growth rate and completely inhibited metastasis in the orthotopic NOD/SCID model for PCa. Also, knocking down ZEB1 induced an important decrease of stemness markers CD44 and CD133, pluripotency gene SOX2, clonogenic capacity and prostatosphere formation of CSCs. The pluripotency genes analyzed have a relevant role in maintaining the stemness functional signature of CSCs from PCa, promoting anti-apoptotic, invasive, resistance, clonogenic, tumorigenic and metastatic characteristics. An EMT/stemness axis seems to regulate CSCs formation involving SOX2 and ZEB1 genes. SOX2 have a determinant role in metastatic progression and might be considered as a suitable therapeutic target for PCa.
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A28
AN ALTERNATIVE IRRIGATION SYSTEM IN AN OVARIAN CANCER MODEL

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Vasculogenic mimicry (VM) is an alternative irrigation pathway for tumors in which cancer cells establish tubular structures in an endothelial cell-free manner. Although associated with reduced patient survival, the mechanisms by which a cancer cells can create a self-generated irrigation system are still not understood. An already established *in vitro* model of ovarian cancer cell line demonstrated that these cells require to be grown upon Matrigel, an *in vitro* approximation of the extracellular matrix (ECM), for the process of VM to occur. However, the nature of this matrix and the signaling pathway involved in this process are unknown. Using an established *in vitro* cancer model of HEY-Green cells we tested varying culture conditions, matrix components and utilized pharmacological inhibitors and gene silencing to elucidate the conditions and signaling pathways required for VM. Differently to what observed in the presence of Matrigel, VM did not occur when cancer cells were cultivated on plastic, glass or heat denatured Matrigel (3 min at 63°C). Using exclusively Collagen 1 or Laminin matrix to mimic the extracellular matrix we observed than only in the presence of Laminin could VM formation occur. Laminin is secreted and deposited by HEY cells and constitutes a part of the luminal lining. Silencing of integrin β1, but not β3, by siRNA prevents this process. Chemical inhibition of PI3K pathway and metalloproteases (MMP) activation demonstrate that these pathways are also essential. RNAseq analysis suggests that this process has minimal dependence on *de novo* transcriptional activity. We have shown that VM only occurs when cells are seeded on Matrigel but not on plastic, glass or heat denatured Matrigel, suggesting that this phenomenon is susceptible to substrate/matrix rigidity. Furthermore, we identified Laminin as the essential matrix protein secreted and deposited by cancer cells to allow for VM assembly. Its interaction with integrin β3, and the consequent regulation of MMP activity may lead to the remodeling of the ECM. As VM is strongly associated with poor patient survival, understanding the formation of this alternative irrigation system may deliver new druggable targets.

A29

EFFECT OF ALLOPREGNANOLONE, A PROGESTERONE METABOLITE, ON HUMAN OVARIAN CANCER CELL LINES PROGRESSION: POTENTIAL USE AS THERAPEUTIC TOOL

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Ovarian cancer is one of the most common cause of gynecologic cancer death. Allopregnanolone (ALLO), a progesterone (P4) metabolite, modifies ovarian physio-pathological processes. The effects of progesterone, its precursors and derivatives have been extensively studied in breast cancer. Progesterone and the 5α derivatives are known to have a protective effect, the 4- pregnenes have the opposite effect. While in ovary very little is known about its actions. Changes in ALLO levels during estrous cycle or under stress situations can generate several alterations in ovarian development. We reported the first evidence that ALLO induces ovarian morpho-physiological changes altering proliferation, apoptosis, and angiogenesis. Epidemiologic and *in vitro* studies have shown controversial information about P4 effects in cancer. The effect of P4 metabolites over ovarian cancer is relevant and require more deep trials due to it could be involved on cancer progression. Then, we first investigated biological behavior of culture cells in proliferation, apoptosis, clonogenic capacity and migration experiments in two human ovarian cancer cell lines IGROV-1 and SKOV-3. Two cell lines were exposed to increasing concentrations of both drugs, from physiological, stress and pharmacological concentrations (10^{-11} to 10^{-5} M) for 72 h. Proliferation was analyzed by MTT and Ki67 expression. Apoptosis was measured by immunocytochemistry of cleaved caspase 3. Clonogenic capacity was evaluated by counting colonies. Migration was analyzed by wound assay. ALL increased proliferation and Ki67 expression respect to control on IGROV-1 cells, while expression of cleaved caspase 3 did not change in any cell line studied. In IGROV-1 clonogenic capacity was also increased by ALLO treatment. Both steroids, P4 and ALLO, increased IGROV-1 migration in a concentration dependent manner. None of the steroids modified SKOV-3 biological behavior. This is the first evidence that ALLO affects biological process that can affect tumor development of human epithelial ovarian cancer. The regulation of progesterone and allopregnanolone steroideogenesis and their molecular mechanisms of action could be considered as potential therapeutic tools in ovarian cancer. In conclusion, ALLO significantly increase malignant proliferation in human epithelial ovarian cancer, then a pharmacological inhibition of ALLO cyclic elevation could be a potential anti-cancer tool.

POSTER PRESENTATIONS

GENERAL, CELLULAR AND MOLECULAR BIOLOGY

A30

EFFECT OF BOTULINUM NEUROTOXINS FROM MENDOZA OF *Clostridium botulinum* STRAINS ON CYTOSKELETAL PROTEINS OF MAMMARY TUMOR CELLS

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The botulinum neurotoxin serotype A (BoNT A) produced by *Clostridium botulinum*, which causes botulism, is used for the treatment of multiple neurological diseases and its therapeutic action against cancer is currently being evaluated. In previous studies, we have shown that BoNT A from autochthonous soil strains (Su) have different properties than the reference A Hall strain. Among these, its molecular structure, its enzymatic activity against brain SNARE proteins and its greater specific toxic activity (AE) stands out. In cells from human mammary carcinoma (MCF-7) treated with BoNTs for 45 min, we found a marked effect on the expression of cytoskeletal proteins. Therefore, in this work, we delve into the study of the action of autochthonous BoNTs A and prototype A Hall on the distribution of actin and tubulin in these cells. Native forms of autochthonous BoNT (Su strains 1935 and 1891, Tupungato) and prototype A Hall were purified by saline precipitation. Their AE values (LD_{50}/mg protein) were established, and their electrophoretic characteristics were evaluated under non-denaturing conditions. 250 LD_{50} of the BoNTs were incubated to MCF-7 cell cultures for 10 or 25 min. Later, the cells were fixed and processed for indirect immunofluorescence with the use of specific antibodies that recognize tubulin or actin. The samples were visualized by fluorescence microscopy. At the two times evaluated, the three types of BoNTs produced a marked redistribution of the actin cytoskeleton, patch form, on areas coinciding with the plasma membrane. Tubulin was redistributed to multiple areas with high signal density at 10 min of incubation only in the presence of BoNT 1891. At 25 min of incubation, the cells treated with BoNTs 1891 and 1935 showed this effect, while in those incubated with A Hall, the distribution of these proteins was not modified. The notable alterations in the distribution of components of the tumor cell cytoskeleton by BoNT from native strains of Mendoza soils open new perspectives for therapy against solid tumors.

A31
IDENTIFICATION OF POTENTIAL PROTEINS INVOLVED IN ANGIOGENESIS ASSOCIATED WITH CERVICAL CANCER USING PROTEOMICS AND BIOINFORMATICS APPROACHES

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Angiogenesis is the growth of blood vessels from the existing vasculature and is essential in the progression of cervical cancer (CC), the fourth most common tumor in women worldwide. This process studies in search of potential biomarkers and therapeutic targets since the endothelial cells that form the abnormal tumor vasculature are characterized by changes at the protein level when are regulated by tumor and microenvironmental factors. The objective of this work was to identify potential proteins involved in the response of endothelial cells to soluble factors released by tumor cells derived from CC, using proteomics and bioinformatics approaches. We previously observed that treatment with conditioned media from CC HeLa cells (TCMs) for 24 h increases the number of endothelial HMEC-1 cells. In this work, the proteome response of HMEC-1 cells was studied under these experimental conditions, performing a Label-Free quantitative (LFQ) mass spectrometry (MS) at the CEQUIBIEM Proteomics Center. Proteins were identified and quantified with the Proteome Discoverer software and the Uniprot database. Also, a more in-depth statistical study was performed using the Perseus software. Proteomic analysis revealed 26 proteins with increased expression levels in endothelial cells treated with TCM ($P \leq 0.05$). Then, to evaluate the biological characteristics of these proteins, they were classified using the PANTHER analysis tool, according to their molecular function and biological processes. As a result of this study, catalytic activity was the most represented molecular function (11/26), followed by binding (4/26). Respect to biological processes, proteins were mainly classified into cellular processes (12/26) and energy metabolism (11/26). This analysis suggests that factors released by tumor cells mainly increase the expression of proteins involved in metabolic processes in endothelial cells. Within these proteins, the probable ATP-dependent RNA helicase DDX47 showed the greatest magnitude of change (> 2). DDX47 is related to rRNA processing and ribosome biogenesis, which are processes associated with cell proliferation and cancer progression. Furthermore, ribosomal activity is also a critical regulator of metabolism. These results highlight the use of Label-Free spectrometry and bioinformatics approaches in an initial phase of discovery of potential proteins involved in cancer and suggest the potential role of DDX47 in angiogenesis associated with CC.

A32
THE NATURAL FLAVONOID APIGENIN IS ACTIVE AGAINST *Trypanosoma cruzi* EPIMASTIGOTES

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Trypanosoma cruzi is the causal etiologic agent of Chagas disease. In cultures, this parasite is mainly found in the epimastigote form and a low percentage in the infective form trypomastigote. The current chemotherapy against *T. cruzi* is insufficient because the available drugs, Nifurtimox and Benznidazole, have limited activity, and show toxic side effects in patients. Therefore, the "screening" of purified molecules from natural sources, mainly plant leaves has become an important tool for the fight against Chagas disease. Many natural compounds, extracted from native plants of Argentina, have been shown to be effective against the parasite. Among them, flavonoids are an important family of molecules that have been widely studied. In this work we analyze the effect of the natural flavonoid Apigenin (AGN) isolated from *Larrea divaricata*, on the growth of *T. cruzi* epimastigotes (strain *Dm28c*). AGN showed an antiproliferative effect on epimastigotes, even at low concentrations. This effect was irreversible even in the short term of exposure to the compound. AGN does not significantly affect the mitochondrial activity of the parasites, at all the concentrations tested (1, 5, and 10 µg/mL) but alteration in ROS levels were observed when 5 and 10 µg/mL of AGN were used. When we analyzed the ultrastructure of the parasites, we observed an increase in cytoplasmic vacuolization and the presence of structures that appear to be like "membrane blisters". From these results it is necessary to identify the molecular targets of the parasites for the action of this compound and to determine if AGN can affect the life cycle of *T cruzi*.

A33
BEHAVIOR OF RENAL HEK293 CELLS ON HYDROGELS BASED ON POLY-*N*-ISOPROPYL ACRYLAMIDE (PNIPAM)

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Synthetic or natural hydrogels have mechanical properties and physicochemical characteristics that simulate the properties of the extracellular matrix (EMC) of body tissues, providing an environment that mimics the native cellular milieus and allows cell growth and adhesion. Hydrogels based on poly-*N*-isopropylacrylamide (PNIPAM) and their co-polymers have attracted a great attention in the biomedical field due to their biocompatibility, smooth texture, and absence of cytotoxicity against several cell lines, characteristics that would allow tissue development. Based on this background, the aim of this study focused on evaluating the biocompatibility of PNIPAM and co-polymers surfaces with the HEK293 cell line, constituted by human embryonic kidney cells, under*in vitro* conditions. Cytotoxicity, genotoxicity, proliferation, adhesion, and cellular mitotic/fragmentation relation in contact with surfaces of PNIPAM and co-polymers with neutral or ionic characteristics in different proportions were carried out. The

viability and proliferation assays did not show cytotoxic or antiproliferative effects in the presence of any hydrogels studied. No DNA damage or cell cycle abnormalities were detected with PNIPAM. The adopted morphologies by cells on the surfaces, during cell growth and adhesion, varied with the physical-chemical properties of the materials and with the days of culture. Cytoplasmic and nuclear alterations were observed on hydrogels with the highest anionic and cationic charge. Similar results were observed in the mitotic/fragmentation relation. However, the cells seeded on the surfaces of PNIPAM and the co-polymers with a lower percentage of ionic monomer showed a very good compatibility. Therefore, these materials can be good renal cells scaffolds with clinical potential in the tissue engineering area.

A34
IMMOBILIZATION OF *Bradyrhizobium sp.* AND *Azospirillum brasilense* IN ALGINATE MATRIX DURING LONG PERIODS OF STORAGE IMPROVES THEIR VIABILITY AND CAPABILITY TO INTERACT WITH PEANUTS

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Immobilization of rhizobacteria offers advantages regarding the formulation of liquid inoculants, tending to stabilize the cells, minimizing the exposure to stress, improving the viability and stability during the inoculant production, storage, and handling. The objective of this work was to immobilize *Bradyrhizobium. sp* SEMIA6144 or *Azospirillum brasilense* Az39 cells in an alginate matrix, and evaluate cell viability and PGPR properties after 12 month-storage at 4°C. The microspheres were obtained using 2% sodium alginate by ionic gelling process and they were characterized by SEM and FTIR. The bacteria were extracted from the microspheres after storage of 1, 3, 6, and 12 months at 4°C, and the production of indole acetic acid (IAA), chemotaxis to radical exudates (RE), and bacterial adhesion to *A. hypogaea* (peanut) roots were determined. Bacterial immobilization in alginate reached a value of 10^7 CFU/microsphere. For Az39 the viability of the bacteria decreased after 4 months of storage at 4° C, however, for SEMIA6144 optimal cell viability values were reached after 12 months of storage (2.10^6 CFU/microsphere), showing low values of metabolic activity. Regarding the levels of IAA, a gradual decrease was observed in Az39 extracted from microspheres of 6 and 12 months of storage (11.5 to 9.21 µg IAA/mg dry biomass). Storage time affected the chemotaxis response to RE, after 6 months there was a significant decrease of 22% for SEMIA6144 and 36% for Az39 extracted from microspheres, compared to the chemotaxis of control cells. The adhesion to peanut root of SEMIA6144 and Az39 extracted from microspheres was 11.5% and 16%, respectively, higher than the control, except for the bacteria extracted from the microspheres after 12 months of storage; it was 11% and 27% lower than the control respectively. Likewise, peanut seeds were inoculated with microspheres-SEMIA6144, new (control) and 12 months of storage. Plant growth parameters and nodulation were evaluated after 30 days of growth under non-restrictive and restrictive water conditions (RWC). The inoculation of peanuts with microspheres-SEMIA6144 of 12 months of storage increased the length of the root, the dry biomass of peanuts; and in RWC increased the number of nodules in lateral roots. In addition, the total N content in the plant was 34% higher compared to liquid inoculation. Our results demonstrate that immobilization of SEMIA6144 and Az39 in a 2% alginate matrix is a potential alternative to improve peanut growth even under water restrictive conditions.

A35
USE OF A FLUOROMETER-BASED METHOD FOR REAL-TIME MONITORING OF CYTOSOL REDOX STATUS IN *Arabidopsis thaliana*

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Oxidative stress is a conserved defense mechanism that plants activate under biotic and abiotic stresses. Several assays enable the analysis of oxidative changes into plant tissues, including the use of proteins that function as redox sensors. roGFP-GRX, is a ratiometric probe that allows obtaining redox information of the glutathione redox couple (GSH:GSSG) throughout their excitation at different wavelengths. The GFP fluorescence depends on the oxidation of its Cys residues and its fusion to GRX (glutaredoxin) accelerates the equilibrium between Cys oxidation and glutathione redox state. Confocal fluorescence imaging allows monitoring redox changes on roGFP-GRX localized at different subcellular compartments. However, the heterogeneity of responses activated by different cells limits real-time monitoring of redox changes in leaf tissues. For this reason, we set up a fluorometric assay to study cytoplasmic redox changes in roGFP-GRX in Arabidopsis leaves exposed to biotic stress and were able to monitor a large number of samples. Several oxidant- and reducing-agent concentrations were tested to determine the full dynamic range between the oxidized and reduced protein configurations. A high H_2O_2 concentration was required to achieve full oxidation of the probe, possibly due to catalase, peroxidase, and superoxide dismutase activity. By contrast, full reduction requires fewer reducer levels. The elicitor peptide flg22 was used to trigger defense responses in leaf tissues and was found to alter the redox state of the sensor in a concentration-dependent manner. Furthermore, incubation with flg22 and a glucose-6-phosphate dehydrogenase (G6PD) inhibitor produced a lower oxidation of the sensor than flg22. As G6PD provides NADPH to cytosol, its activity could modify other NADPH-dependent enzymes, including the membrane NADPH oxidase acting as a main source of apoplastic ROS. Therefore, the accumulation of apoplastic ROS in response to flg22 would be associated with changes in the cytoplasmic redox homeostasis regulated by NADPH/NADP and GSH/GSSG.

A36
NEW EVIDENCE OF THE EFFECT OF OLIGODEOXYNUCLEOTIDE IMT504 ON MURINE BETA CELLS (MIN6B1)

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We have previously demonstrated that treatment with IMT504 promotes significant improvement in the diabetic condition in diverse animal models. We have also shown effects on gene expression in freshly isolated islets from diabetic, *in vivo* IMT504-treated animals and effects on enzyme activation and gene expression in *in vitro* IMT504-treated beta cells (MIN6B1). Based on these results, here we evaluated protective effects against beta cell apoptosis and PDX-1 expression as an indicator of beta cell functionality by IMT504 action. At the same time, we started to investigate possible binding and action sites of IMT504 on beta cells. A murine beta cell line (MIN6B1) was used. Cells were cultured in DMEM with 20 mM glucose, 15% FBS, 71 µM β-mercaptoethanol. Cell apoptosis was analyzed by ELISA, protein expression of PDX-1 by Western Blot and localization of IMT504 by immunocytochemistry. Cell apoptosis was induced by incubation with 0.75 mM H_2O_2 for 12 h. Then, cells were stimulated with IMT504 [2 (IMT2), 4 (IMT4) and 8 µg/mL (IMT8)] for 24 h. H_2O_2-induced cell death was reversed in a concentration dependent manner by treatment with IMT504, reaching similar levels to those of Control with IMT8 treatment [ANOVA with repeated measures: $P < 0.01$; N = 7]. To evaluate protein expression of PDX-1, cells were stimulated for 24 and 48 h with IMT504 doses as mentioned above. Protein expression of PDX-1 was significantly increased by 48 h stimulation with 4 µg/mL of IMT504 [ANOVA with repeated measures: PDX-1/actin (A.U.): $P < 0.03$; N = 4]. To evaluate IMT504 localization, cells were stimulated with 0.5 µg/mL of IMT504 linked to Texas Red for 15, 30, 60 min and 4 h. We found that beta cells were strongly positive for oligonucleotide IMT504 15 min after dosing, and it remained until 4 h. The label was detectable in cytosol. However, it was not detectable inside the nucleus. Taken together, our results demonstrate that IMT504 enters beta cells and prevents the decrease in cell viability triggered by H_2O_2. Moreover, we also demonstrate here that IMT504 increases expression of PDX-1, essential for adult beta cell functionality. Further analysis must be done to dilucidate the implications of these results on beta cell function recovery observed in diabetic animals.

A37
MYO1C PARTICIPATES IN CHLAMYDIAL INVASION AND DEVELOPMENT

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The obligate intracellular pathogen *Chlamydia trachomatis* cause sexually transmitted diseases. This bacterium, in women, can lead to several complications such as pelvic inflammatory disease, ectopic pregnancy, and infertility. *C. trachomatis* provoke an extensive remodeling in the host cell, including Golgi fragmentation, actin cytoskeleton reshaping, and cytokinesis inhibition. Additionally, *C. trachomatis* intercepts different intracellular trafficking pathways of the host cell to acquire essential nutrients for its survival and replication. We recently published that Myo1C stabilizes actin at the Golgi apparatus facilitating the arrival of incoming transport carriers at this organelle. Strikingly, *C. trachomatis* establishes a close relationship with the Golgi apparatus, receiving from this organelle a continuous supply of vesicles loaded with essential lipids. Our objective was to determine if *C. trachomatis* manipulated Myo1C as a strategy to ensure its development. We observed, by confocal microscopy, that endogenous and over-expressed Myo1C was recruited to the chlamydial inclusion. The knockdown of Myo1C impaired the *C. trachomatis* development and caused the destabilization of the actin belt that surrounds the inclusion. Moreover, we determined that Myo1C depletion provoked a decrease of *C. trachomatis* infection rate, assessed by flow cytometry and confocal microscopy. Our findings indicate that Myo1C is involved in both invasion and development of *C. trachomatis*.

A38
EFFECTS OF BENZOPHENONE 2 (BP2) AND 3 (BP3) ON AUTOPHAGY IN PANCREATIC BETA CELLS *IN VITRO*

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Benzophenones, frequently used in sunscreens and food packaging as UV blockers, are considered endocrine disruptors because they bind to estrogen receptors. The aim of the present study was to assess the effect of benzophenones 2 (BP2) and 3 (BP3) on pancreatic beta cell function, focusing on autophagy. Therefore, mouse pancreatic beta cell line MIN6B1 was treated with 10 µM BP2 or BP3 in the presence or absence of the autophagy-inhibitor Chloroquine (CQ, 10 µM) during 24 h. BP3 inhibited basal insulin secretion, and *Ulk1* transcription. But additional effects were uncovered when autophagy was modified by CQ. CQ decreased basal insulin secretion, without preventing BP3 insulin inhibition. Both, BP2 and BP3 counteracted CQ-induced *Lamp2* expression but did not compensate CQ-induced *Sqstm1/p62* gene transcription. Neither BP2 nor BP3 did alter the autophagic flux when analyzed by Immunofluorescence microscopy and Western blot. *In silico* analysis of the regulatory regions of the genes dysregulated by BP2 or BP3 showed the presence of estrogen receptor binding sites. In conclusion, benzophenones affect cellular adaptive responses related to autophagy, and lysosomal biogenesis, and hormone secretion in pancreatic beta cells. Therefore, BP2 and BP3 are able to alter beta cell homeostasis and could lead to beta cell dysfunction. *This work was supported by CONICET, ANPCyT, Fundación René Barón, and Fundación Williams.*

A39

RELATIVE EXPRESSION OF THE *lmb* AND *fbp* GENES BY *Streptococcus uberis* RC19 STRAIN

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Streptococcus uberis is one of the most prevalent environmental pathogens responsible for a significant proportion of clinical and subclinical bovine intramammary infections (IMI) in lactating and non-lactating cows in dairy herds worldwide. It is associated with clinical and subclinical IMI, both in the dry period and during lactation, and causes significant economic losses. The *S. uberis* adherence to the host's epithelial cells is an important initial and critical step in the colonization of the bovine mammary glands. Colonization requires the expression of potential virulence factors associated with the cell surface. To characterize the *S. uberis* RC19 strain in the early stages of colonization, we determined the adhesion and internalization ability of RC19 strain for 1, 2, and 3 h in co-culture with the cell line mammary MAC-T and evaluated the relative expression (R) of the *lmb* and *fbp* genes, encoding the laminin and fibronectin binding proteins, respectively. These genes were selected according to previous results. To evaluate the R of the genes, a MAC-T cell monolayer was co-cultured with the RC19 strain for 1, 2 and 3 h. The number of associated *S. uberis* bacteria at each time, expressed as CFU/mL, was determined. Total RNA extraction was performed with Trizol (Sigma-Aldrich) under three experimental conditions: *S. uberis* without contact with MAC-T cells, as a control, *S. uberis* in the supernatant of co-cultures with MAC-T epithelial cells (group 1), and *S. uberis* in the co-culture lysate (group 2). Genomic DNA was removed from RNA samples by using the DNase I, RNase-free (Thermo Scientific). The High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used as described by the manufacturer to obtain DNAc. Quantitative reverse transcriptase PCR (qRT-PCR) was performed in a MX3000 Multiplex Quantitative PCR system (Stratagene-Agilent) by using iTaq Universal SRYB Green 2x SuperMix kit (Bio-Rad) in duplicate in two independent experiments. To determine the R of the *lmb* and *fbp* genes, the CT value was used applying the delta-delta method. Differences in the expression levels of the *lmb* and *fbp* genes from *S.* uberis strain under different conditions were analyzed with respect to the control by unpaired *t*-test. A confidence level of ≥95% was considered statistically significant. We observed that the RC19 strain was able to adhere and internalize to MAC-T epithelial cells, exhibiting the highest adherence capacity after 2 and 3 h of co-culture. The expression of the *lmb* and *fbp* genes increased after the first hour of co-culture in bacteria that were in contact with the MAC-T cells, although not significantly. In bacteria with longer contact time with cells (2 h), the expression levels of the genes were significantly higher compared to the control group. Similar results to those previously obtained were observed after 3 h of co-culture. In conclusion, each gene showed an expression profile according to the condition and time of co-culture of the RC19 strain with the MAC-T cell line. These results report for the first time the expression of the *lmb* and *fbp* genes in one *S. uberis* strain, isolated from bovine subclinical mastitis from an Argentinean dairy herd, during co-culture with the MAC-T cell line at different times and conditions.

A40

MORPHOLOGICAL AND ULTRASTRUCTURAL CHARACTERIZATION OF FIFTH INSTAR NYMPHS HEMOCYTES OF THE HEMATOPHAGOUS INSECT *Dipetalogaster maxima* (HEMIPTERA: REDUVIIDAE)

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Hemocytes are cells present in the hemolymph of insects and other invertebrates. These hemolymphatic cells participate in different physiological processes including coagulation and immunity, as well as in the synthesis and transport of nutrients and hormones. The current hemocyte classification, mainly based in morphological characteristics, is a controversial topic particularly in the triatomines, the insect vectors of Chagas disease (Hemiptera: Reduviidae). Several authors have obtained contrasting results, partly because they used different experimental approaches. In this work, we have employed histological and cell biology techniques in order to characterize the hemocytes of fifth instar nymphs of the triatomine *Dipetalogaster maxima*. In addition, it was conducted for the first time an ultrastructural study by transmission electron microscopy. Using fresh preparations and contrast phase microscopy, the methodology with most consensus in the literature, were identified six populations of hemocytes: plasmatocytes, granulocytes, prohemocytes, oenocytes, adipocytes and giant cells, being the first two the most abundant. These cellular populations were also characterized by immunofluorescence by the employment of an anti-tubulin antibody and by transmission electron microscopy. All cell populations presented a high variation in morphology and size, explaining in part the scarce coincidence among the previously reported finding of diverse authors. This work provides novel information regarding a complex aspect of physiology and cell biology of triatomines and establishes the foundations for future studies directed to understand the specific roles of such hemocyte populations in the defense mechanism of insects.

A41

COVID-19'S PROTEASE 3CLpro STRUCTURAL STUDY REVEALS A QUINARY STRUCTURE

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At present, there are no specific therapies available for COVID-19 (SARS-CoV-2) patient treatment. The strategies that have been implemented are limited to preventive and supportive therapies, designed to prevent further complications and damage of affected organs. Chymotrypsin-like protease 3CLpro (main cysteine protease Mpro) and papain-like protease PLpro are involved in structural or non-structural protein processing for the replication and packaging of nascent viruses. Recently, it has been solved the 3CLpro three-dimensional structure which have been deposited in the Protein Data Bank (PDB). This protease represents a potential target for inhibitors of the virus replication. SARS-CoV-2 protease 3CLpro possesses

high sequence identity (96%) with its SARS-CoV counterpart. The aim of this work was to carry out the analysis of the known structural data in order to search for elements that might give us clues about their behavior *in vivo*. The study was made mainly with the PyMol, Chimera, Coot and CCP4MG programs. Some structural differences were observed between the two homologue proteases (SARS-CoV and SARS-CoV-2), principally in the catalytic region and in the protein surfaces. Both 3CLpro naturally dimerize and each monomer contains two regions, the N-terminal catalytic region and the C-terminal region. The 3IWM structure corresponding to the SARS-CoV 3Lpro protein was described as a tetramer formed by two dimers association. It was observed that the protein assembles to form a quinary oligomeric structure. This is a higher complexity association than quaternary structure (dimer or tetramer). This observation also applies to the SARS-CoV-2 homolog (6M2N and 6M2Q) due to the structural identity of the association regions. The quinary structure might be a way to storage large quantities of functional protease. This fact would allow enzime release by any change on the physiological environment. Enzyme release might cause non-specific proteolytic action on several proteins in humans, different from their natural targets. These observations would explain some of the clinical manifestations observed in patients with COVID-19.

A42
STRUCTURAL STUDY OF *Trypanosoma cruzi* NUCLEOSIDE DIPHOSPHATE KINASE 2 PROTEIN

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Chagas's disease is an anthropozoonosis caused by the flagellated protozoa *Trypanosoma cruzi*. This disease is transmitted by the insect vector *Triatoma infestans*. It has an overall prevalence estimated at 6–8 million cases and there are 65–100 million people being at risk of contracting the infection. No vaccines are available at present and the drugs used for treatment show undesirable side effects. The identification of new targets for chemotherapy is a major challenge in the control of the disease. Nucleoside diphosphate kinases play a key role in the maintenance of intracellular ratios of NTPs and dNTPs through the catalysis of the reversible phosphorylation of nucleoside diphosphates to nucleoside triphosphates. The X-ray crystallographic structure for canonical TcNDPK1 helical multi-hexameric oligomer was recently characterized in our laboratory. NDPKs can be divided in two groups according to the primary structure. Group I is composed of canonical NDPKs, which are broadly studied and found in prokaryotes and eukaryotes. Group II is formed by divergent NDPKs present in eukaryotes only. *T. cruzi* NDPK isoform 2 (TcNDPK2) is a 37kDa protein whose primary structure suggests an N-terminal of 88 residues DM10 domain and a catalytic C-terminal region. TcNDPK2 is a microtubule-associated enzyme mainly localized in the cytoskeleton and flagellum. The TcNDPK2 DM10 domain is sufficient and necessary for cytoskeleton delivery of this enzyme. In this work we have constructed a three-dimensional homology model for TcNDPK2. The structural model was visualized using Pymol and Chimera. It was validated using the Coot and PDBsum programs. The homology model of TcNDPK2 has an N-terminal domain corresponding to the DM10 domain, a linker region and a C-terminal NDPK domain. The proposed model is of great importance since the three-dimensional structure of TcNDPK2 has not been solved yet.

A43
DEHYDROLEUCODINE AND SOME CHEMICAL DERIVATES AFFECT PROLIFERATION OF *Trypanosoma cruzi* BY ALTERING DIFFERENT MOLECULAR TARGETS

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Chagas disease is endemic in Latin America and affects to millions of people worldwide. This pathology is caused by the trypanosomatid *Trypanosoma cruzi* (*T. cruzi*). The current chemotherapy is based on the nitroderivatives Benznidazole and Nifurtimox, but their use is restricted due to the severe side effects on the patients, justifying the continuous search for alternative drugs. The antioxidant defense system of the trypanosomatids is different from that of mammalian cells, having a particular set of reducing molecules and enzymes. Hence, the redox system of the parasite emerges as an attractive target for new antiparasitic therapies. The natural sesquiterpene lactone dehydroleucodine (DhL) is known to have an important antiparasitic activity. The α-methylene group of DhL could be responsible of its biological activities, possibly by blocking the thiols groups present in reducing molecules or enzymes. In this study we attempted to elucidate the mechanism of action of DhL and eleven derivatives (named DC-X1 to DC-X11) obtained by chemical substitutions on the methylene group. We confirmed the antiproliferative effect of this natural compounds, being the most active DhL, DC-X3, DC-X6 and DC-X11. Based on the background of DhL, we focused the study on the parasite antioxidative machinery. Thereby, *T. cruzi* epimastigotes were incubated with DhL and DC-X6 and intracellular ROS generation was evaluated. A significant increase of ROS was induced by DhL and at lesser extent by DC-X6. This effect was blocked by adding reduced glutathione. These results suggest that DhL and DC-X6 can induce oxidative stress in the parasites by inactivation of reducing enzymes, or by capture of reducing molecules (trypanotion or glutathione), affecting their redox homeostasis. On the other hand, we observed that DC-X11 alters the cell cycle of parasites synchronized with hydroxyurea (20 mM), which could lead to the apoptosis. Through the use of chemical derivatives, we confirmed the importance of the methylene group in the mechanism of action of DhL. Our study provides new insights about the possible targets for a potential drug against Chagas.

A44
TOLERANCE OF HIGH MOUNTAIN QUINOA ACHENE TO EXTRAPLANETARY CONDITIONS

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Chenopodium quinoa Wild (Chenopodiaceae) is an Andean traditional crop with a high nutritional value and distribution, including Colombia, Chile, and northwest Argentina. In the last years quinoa has received worldwide attention because it presents ecotypes able to adapt to different environmental conditions such as high altitudes (0–4000 MASL), contrasting temperatures, drought conditions, salinity, marginal soils with low nutrient concentrations, and it is also highly tolerant to UVB radiation and low atmospheric pressure. Due to its ductility against extreme environmental conditions, being a C3 species with high photosynthetic assimilation and presenting some ecotypes with a short life cycle, quinoa is considered an excellent candidate to be incorporated as an experimental crop in long-term space missions. These missions will require life support systems which provide nutritious and fresh food and regenerate resources such as oxygen. Currently, little is known about the survival, growth and development of plants exposed to extraplanetary conditions. In order to determine the quinoa tolerance for space travel, achenes (fruit-seed) from a high mountain ecotype (3800 MASL), adapted to high radiation, low pressure and sub-zero temperatures, were selected to be exposed to extra-planetary conditions (low pressure, P, 10^{-7} Tor, reached in a high vacuum chamber), laser simulated solar plasma radiation, Pl, and cryogenic temperatures, T, of −200°C, for 4, 8 and 16 h. The treated samples were later analyzed by scanning electron microscopy coupled to X-ray spectroscopy (SEM-EDS) to study the morphology and mobility of minerals. Seed viability was evaluated by germination and early growth of radicle and hypocotyl under normal atmospheric conditions for 14 days. Quinoa germination was not inhibited under any treatment. Final germination always reaches values of up to 90%. The rate and final germination subjected to low pressure (10^{-7} Tor) treatments during 4 h and 8 h were not different to control. The combined application of low pressure and cryogenic temperatures showed a delay in germination rates, nevertheless, final germination reaches a value of near 90% in both control and treatments. When plasma application was added, the germination rate was improved, reversing the delay observed in combined low P and T treatments. Early growth (radicle and hypocotyl length) was affected by different treatments being the radicle the most affected. The analysis of achenes by SEM-EDS indicated structural changes in the pericarp and in the K^+ content, which were reversed when adding low temperatures. Our result suggested that quinoa achene has a great tolerance to extraplanetary turning this high mountain ecotype into an excellent alternative for space missions.

A45
BENEFITS OF INOCULATING TOMATO WITH *Trichoderma harzianum* ITEM 3636

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Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetables worldwide. It is sensitive to low temperatures and is grown under cover (greenhouses) or in the field when the weather allows it. The fresh tomato market, both local and global, must be continuously supplied since world consumption has increased at the rate of 1 kg per inhabitant per year during the last 10 years. Tomato cultivation is intensive, a wide range of pesticides and other chemical inputs are used to ensure successful harvests. The use of ecological strategies to minimize the use of chemical inputs in traditional horticultural practices is the ultimate goal of sustainable horticulture. Several studies have shown that some rhizospheric strains of *Trichoderma* have direct effects on plants, increasing their potential for growth and nutrient absorption, as well as stimulating plant defenses against biotic and abiotic damage. In the present work, we analyzed the potential of *Trichoderma harzianum* ITEM 3636 to promote the yield of tomato plants in the field. Seeds belonging to the UCO 16 INTA variety were used, which were germinated in trays filled with a sterile mixture of soil:perlite (2:1) and placed into a growth chamber under controlled cycles of 16 h of light at 25°C and 8 h in the dark at 20°C, for 2 weeks. Then, elevated beds were mounted in the experimental field of the National University of Río Cuarto, Córdoba, for transplantation. The control without inoculation and inoculation with ITEM 3636 treatments were tested. At the time of transplantation, seedlings were inoculated by immersion of roots in a fungal suspension (1×10^5 conidia/mL). The furrow irrigation system was used. Chemical herbicides were not applied. Furthermore, neither chemical fungicides nor insecticides were used. Plants and their fruits were collected after 90 days of growth in the field. The evaluated parameters were number of fruits/plant, weight of fruits/plant, and yield/m^2. Pairwise comparisons were made with Student's *t*-test ($P < 0.05$). We observed that inoculation with ITEM 3636 caused an average of 33.6 fruits/plant higher than that of the control, although the difference was not statistically significant. However, this value represents an increase of 12%. In the case of mean fruit weight/plant and yield (kg/m^2), we observed that inoculation with ITEM 3636 caused significant increases of 14% and 15%, respectively, compared to control plants. Based on these results, we conclude that *T. harzianum* ITEM 3636 could have the potential to be formulated as a biofertilizer for application in horticulture.

A46
Trichoderma harzianum AS A GROWTH PROMOTING AGENT IN HYDROPONIC CULTURE OF TOMATO *(Solanum lycopersicum)*

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Tomato, due to its nutritional characteristics, is considered as a food of great commercial interest throughout the world. The importance of the crop has generated several studies that seek to increase its production through the application of biofertilizers based on beneficial microorganisms. Hydroponic cultivation is a method that allows plant production without the need to use the soil as a source of nutrients, or as a physical support.

Some advantages of hydroponic systems are: there is no competition for nutrients, the roots develop in better growing conditions, minimal loss of water, no issues with weeds, reduction in the application of agrochemicals and the system adjusts to non-traditional production areas. With this technique it is possible to obtain vegetables of excellent quality and health. The objective of this work was to evaluate the growth promoting capacity of *Trichoderma harzianum* ITEM 3636 in hydroponic tomato cultures. Seeds of the UCO 16 INTA variety were used, which were germinated in trays containing sterile substrate and placed in a growth chamber under controlled cycles of 16 h of light at 25°C and 8 h in the dark at 20°C. The seedlings were transferred 35 days after sowing to hydroponic culture containers containing 7 L of Hoagland's nutrient solution and kept in a chamber under the same growth conditions. Two treatments were carried out: A (uninoculated control) and B (treated with *Trichoderma harzianum*). Ten days after being transferred, treatment B was inoculated with 20 mL of a fungal solution of 1×10^5 conidia/mL. The parameters evaluated at 60 days post sowing were: root and shoot length and dry weight of roots and shoots from each treatment. Through the Analysis of Variance, it was observed that the treatment inoculated with *Trichoderma harzianum* (B) showed an increase in three of the parameters measured: root length, root dry weight and shoot dry weight, with statistically significant differences with respect to the control (A). These results allow us to infer that *Trichoderma harzianum* ITEM 3636 could be formulated as a biofertilizer and used as a growth promoter in hydroponic tomato cultures.

A47
FUSARIUM VERTICILLIOIDES MITOVIRUS 1: MOLECULAR CHARACTERIZATION OF THE FIRST MYCOVIRUS IDENTIFIED IN THE PHYTOPATHOGEN *Fusarium verticillioides*

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Fusarium verticillioides is a filamentous fungus with high incidence in corn crops and the major causal agent of stalk and ear rot in maize. In addition, *F. verticillioides* is a prolific producer of fumonosins, which can cause serious diseases in human and animal health when consumed. Synthetic fungicides are the most used strategy for *Fusarium* control. However, they do not show good results in the field, and they negatively affect the environment and people's health. In this context, biological control of plant pathogenic fungi by mycoviruses (fungal viruses) represents an environmentally friendly alternative. Mycoviruses lack of extracellular routes (intrinsic cytoplasmic elements) and their dispersion is through fungal spores and hyphal anastomosis. Some of them are capable of reducing host virulence (hypovirulence), which is an essential feature for its use as a biological controller. The objective of the present study was to determine the rate of viral infections and their molecular characterization from a survey of viral infections in *F. verticillioides* isolated maize from Argentina. The fungi were isolated from grains, grown on synthetic media (PDA), and nucleic acids were extracted with cellulose chromatography. We have detected a ~2.5 kb double-stranded RNA (dsRNA) in a single isolate, consistent with the mycoviral genomes. Total-RNA from the infected fungus was subjected to RNA-seq using Illumina NovaSeq platform PE-150 strategy (Novogene Inc.). The obtained readings were assembled de novo (Trinity) and the contigs were subjected to a local search with BLASTX against viral Ref-Seq deposited in NCBI. The viral genome sequence found was scanned with ORFfinder, which turned out to contain a single Open Reading Frame (ORF), which was then annotated structurally and functionally. The incidence of mycoviruses in *F. verticillioides* in maize from Argentina is 1.01%. The genome has 2,471 nt in length, with a 28.7% GC richness. The ORF has 2,184 nt, expanding from nt positions 219 (AUG) to 2,402 (UAA), flanked by 5'- and 3'-untranslated regions (UTRs) 218 nt and 69 nt in length, respectively. The mycovirus ORF encodes a 727 aa protein with a molecular mass of 84.85 kDa, with 100% of its Trp encoded by the codon UGA, as expected for a mitochondrial virus, and contained a conserved motif of the Mitovirus RNA dependent RNA polymerase Superfamily. The predicted aa sequence of the RdRp encoded by the virus exhibited the highest similarities to the RdRp aa sequence of Fusarium andiyazi mitovirus 2 and Plasmopara viticola-associated mitovirus 7 with identities of 83.63% and 75.10%, respectively, and *E*-value: 0 and Query Cover: 100% in both cases. According to ICTV rules we can conclude that this genome belongs to a novel mycovirus, a tentative member of a new species. We have named this novel mycovirus, the first reported in this fungal pathogen, *Fusarium verticillioides* mitovirus 1 (FvMV1). Future studies will be carried out to evaluate the effect of FvMV1 infection, with the objective of estimating the efficacy for biological control of *F. verticillioides*.

A48
POTENTIAL DISEASE-MODIFYING EFFECTS OF ANGIOTENSIN II TYPE 1 RECEPTOR (AT1) ANTAGONISTS FOR THE TREATMENT OF PARKINSON DISEASE

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Several studies in murine experimental models of Parkinson's disease (PD) suggest neuroprotective properties for Angiotensin II AT1 receptor antagonists (ARAs) and Angiotensin Converting Enzyme inhibitors (ACEis). We explored the potential disease-modifying effects of these drugs in the Parkinson's Progress Marker Initiative (PPMI) study database. The 423 treatment-naïve newly diagnosed PD patients from the PPMI were included in this study. In the first phase of this study, both the *t* and chi-square tests revealed no significant associations between exposure to ACEis and L-DOPA requirement (adjusted Odds Ratio, 95% confidence interval: 1.45, 0.58–3.59; $P = 0.4$). In the retrospective analysis of the first four years follow-up (the statistic used was the general estimation equations focused on changes averaged by subjects), MDS-UPDRS 2+3 scores in the "practically-defined OFF-condition" was significantly lower in patients exposed to ARAs (0.88, 0.79–0.98; $P = 0.03$) compared to unexposed patients. No associations were found with ACEis. These results show signals of a potential disease-modifying effect with ARAs in the PD. Further clinical trials are warranted.

A49
ESTROGENS MODULATE EXPRESSION OF CATHEPSIN D AND ACTIN IN A RAT MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons from substantia nigra pars compacta (SNc). A genetic study identified 24 loci that are associated with PD; 11 of them are involved in/or disrupt various functions of the autophagic-lysosomal pathway. Lysosomes participate in the degradation of macromolecules from endocytosis and autophagy processes. Epidemiological and clinical studies reveal a difference in the development of PD between genders, giving sex hormones a neuroprotective function and making them an interesting therapeutic proposal. The objective of this study is to analyze the effect of estrogens on the expression of lysosomal proteins in a rat model with the PD phenotype. Two-month-old male Sprague-Dawley rats were subjected to stereotaxic surgery to administer 6-hydroxydopamine (6-OHDA) or artificial cerebrospinal fluid (V) to 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 (V lesion); E (V + E injury); HP (6-OHDA injury) and HPE (6-OHDA + E injury). After the treatments, the animals were sacrificed, and the substantia nigra and prefrontal cortex were extracted and homogenized. Membranous and cytosolic fractions of the prefrontal cortex were obtained by differential centrifugation. The samples were processed for immunoblotting using antibodies against cathepsin D (CatD) and actin. Preliminary results show that chronic treatment with estrogens increases the expression of CatD and actin in the substantia nigra, and in the prefrontal cortex both proteins are increased in the cytosolic fraction. Since CatD reduces the α-synuclein concentration in PD, the results suggest that an increase of the lysosomal function would exert neuroprotective action on cells affected by the disease. Likewise, it is worth mentioning that estrogens could also modulate the organization of the cytoskeleton, as a stage in neuromodulation.

A50
EFFECTS OF *Prosopis strombulifera* (LAM.) BENTH AQUEOUS EXTRACT IN AN *IN VIVO* MODEL OF CUTANEOUS LEISHMANIASIS

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The leishmaniasis are a spectrum of diseases caused by infection with protozoal pathogens of the genus *Leishmania*, with an estimated 2 million new cases per year. *Leishmania* parasites are transmitted to a mammalian host through the bite of an infected sand fly. The clinical forms of the disease (cutaneous, mucocutaneous and visceral leishmaniasis) depend on the *Leishmania* species involved. In Argentina, it affects the northern region of the country with an incidence that has increased in the last two decades. Current treatments for leishmaniasis are unsatisfactory due to the associated high toxicity, cost, complex administration, and the emergence of resistant strains. Efforts have increased considerably in the last decade to identify new compounds with anti-leishmanial properties. Therefore, a strategy in the search for new compounds is the detection of purified molecules from plant sources. There are more than five hundred species of plants in the province of Mendoza, in the central west of Argentina, for which "folk medicine" has described various uses to preserve and help health. *Prosopis strombulifera* (Ps) has been used as an astringent, anti-inflammatory, and antidiarrheal agent. Recent studies have confirmed its biological activities against different microorganisms. The aqueous extract (AE) has been shown to be non-toxic in experimental animals. We evaluated the effect of PsAE in an in vivo model of cutaneous leishmaniasis. Male BALB/c mice were infected in the right hind paw pad with 1×10^5 *L. amazonensis* promastigotes and treated with PsAE 150 mg/animal/day administered orally in the drinking water, ad libitum. We observed that the treatment with the aqueous extract diminishes the swelling of the infection site compared to the mice treated with Glucantime, which was used as a positive treatment control. This is related to the significant decrease in parasite load, splenic index, and observed IgG levels. Although many more tests need to be done, PsAE may be effective in treating cutaneous leishmaniasis.

A51
SECONDARY STRUCTURE OF DOMAIN III OF THE NON-CODING RIBOSOMAL GENE 12S rRNA FROM SPECIES OF THE GENUSES *Heleobia* AND *Potamolithus* (GASTROPODA: TATEIDAE)

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The secondary structure of ribosomal markers is often useful for inferring phylogenetic relationships in mollusks and as supplementary information to overcome difficulties in the alignment of DNA sequences with high levels of insertion/deletion events. Alignment of non-coding DNA sequences poses particular problems since the conserved characteristics used to assume position homologies are not found in the nucleotide sequences themselves, but in the derived molecular structure. For this reason, the analysis of ribosomal genes requires structural information -in which the changes of nucleotide bases are relegated against the conserved structural characteristics-. This requires obtaining information on the variants in which the bases can be arranged throughout the sequences, such as covariation between sectors corresponding to stems formed by base complementarity in the secondary molecular structure of ribosomal RNA, or the more permissive variation in sectors of loops. The genetic material was obtained from the muscular tissue of the foot of specimens collected in localities of Cuyo (*Heleobia hatcheri*, (Pilsbry, 1911) and *Heleobia sp3*)

and from the Río de La Plata (*Potamolithus buschii*, (Frauenfeld, 1865) and *Potamolithus agapetus* (Pilsbry, 1911). For the analysis of the 12S rRNA gene, the folding was carried out based on the secondary structure of the third domain of this gene obtained for *Ischnochiton australis*. The fragments amplified by the L1091 and H1478 primers consisted of sequences between 366 and 369 base pairs long. These sequences corresponded to part of the third domain of the 12S rRNA gene (positions 62 to 369 in the alignment with *I. australis*.) The secondary structure obtained did not show important variations, since the mutations and even In-Del events (gaps) were scarce and located in particular sectors within the molecular structure. The results demonstrate a high conservation within the studied group and suggest little utility of this gene to explore phylogenetic relationships among the explored taxa.

A52
LARVAL MORPHOLOGY AND BIOLOGICAL DATA OF *Chrysoperla defreitasi* (NEUROPTERA: CHRYSOPIDAE), FIRST RECORD IN ARGENTINA

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Chrysopidae represent one of the groups of entomophagous insects of importance for the control of small phytophagous arthropods, especially aphids, whiteflies, mealybugs, thrips and mites, pests of crops of economic importance. Most of the species, in the larval stage, are active predators of the different biological states of these organisms. The biology of the neotropical species of *Chrysoperla*, and their larval morphology, are poorly known. In Argentina, three species of the genus *Chrysoperla* Steinmann have been described and cited: *Chrysoperla externa* (Hagen), *C. asoralis* (Banks) and *C. argentina* Gonzalez Olazo and Reguilón. In Argentina, three species of the genus *Chrysoperla* Steinmann have been described and cited: *Chrysoperla externa* (Hagen), *C. asoralis* (Banks) and *C. argentina* Gonzalez Olazo and Reguilón. *Chrysoperla defreitasi* Brooks, 1994 has been found for the first time in Argentina in agroecosystems of *Citrus* and other horticultural crops in Rosario de la Frontera, Salta. The knowledge of species immature stages is important in taxonomy and for the use in biological control programs. In this work the larval morphology and biological cycle of *Chrysoperla defreitasi* are described. In the laboratory, a breeding was established under control conditions starting of a 60-eggs cohort. Larvae were reared individually in plastic tubes and fed *ad libitum* with *Sitotroga cerealella* (Lepidoptera: Gelechiidae). First, second and third instars larvae were described after being fixed in Kaad solution and preserved in 65% glycerinated alcohol. Measurements were made with a micrometric ocular and express in millimeters. In this paper we record for the first time *Chrysoperla defreitasi* in Argentina. We also described for the first time the external morphology of the larval instars of *Chrysoperla defreitasi*, with illustrations and life cycle data. In addition, a key for the larval identification of the Chrysoperla' s species presents in Argentina is provided.

A53
STUDY OF THE METABOLIC IMPACT OF HYPERLIPEMIC DIETS IN PERIPHERAL BLOOD MONONUCLEAR CELLS: PRELIMINARY RESULTS

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Metabolic disorders associated with the diet are of great impact on health, due to their relation to chronic noncommunicable diseases. Peripheral blood mononuclear cells (PBMC) have an important role as early biomarkers in the study of the impact of fat-rich diets on lipid metabolism. These allow deepening the knowledge of the pathogenesis by non-invasive methods. Therefore, the objective is to study PBMC as a research tool for gene expression in alterations of lipid metabolism. Samples were obtained of ten New Zealand rabbits, divided in control group (N= 5) fed with balanced feed (C), and case group (N= 5) fed with the same feed supplemented with a 17% bovine fat (F). *Fat* groups do not receive fructose overload, keeping constant the concentration of carbohydrates and proteins, typical of the basic balanced food. Biochemical tests were performed to determine the levels of blood glucose (Gl), triglycerides (TG) and total cholesterol (CT). In PBMC, immunohistochemical tests (IHQ) were performed for SREBP1c and SERBP2 (*Protein binding to sterile regulatory elements*). Similar values of Gl (C: 140 ± 28.4 mg/dL *vs.* F: 118.3 ± 12.0 mg/dL) and TG (C: 144.1 ± 15.5 mg/dL *vs.* F: 135.6 ± 8.3 mg/dL) can be observed in preliminary biochemical studies of both groups, while group F shows an increase in CT (42.8 ± 21.6 mg/dL) compared to the group C (27.1 ± 4.5 mg/dL). However, some animals of group F have similar values to group C for CT (21.7 ± 2.4 mg/dL), *normocholesterolemia* group (NC). Thus, these animals do not show biochemical changes despite the intake of fat as occurs with others. In addition, liver tissue studies showed steatosis (oil red O stain), as well as the presence of SREBP1c (*perinuclear/nuclear* ratio*:* C: 2.0, F: 0.71, NG: 1.81) and SERBP2 (*perinuclear/nuclear* ratio*:* C: 3.29, F: 0.85, NG: 0.57) in PBMC. In conclusion, these results would indicate an activation of gene regulation without changes at the biochemical level. These results indicate that it is possible to study gene expression in PBMC, because it can observe the presence of specific molecules related to lipid metabolism.

A54
PROTEOME PROFILE PREVIOUS TO SOYBEAN EMBRYONIC AXES GERMINATION

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Seed germination implies restarting of embryonic axis growth, with expansion activity and without cell division defining radicle protrusion. Isolate soybean embryonic axes (A) [*Glycine max* (L. Merr)] start to enlargement from 10 h, completing germination at 12 h of incubation in water. In previous works, we identified 2677 proteins in A incubated 12 h, showing important metabolic activation and signalization, communication, and signal transduction, which would be affecting gene expression and triggering the development and structures establishment. The present work aimed to evaluate the proteome profile of A before visualizing germination. One hundred A were incubated 9 h in distilled water at 27 ± 1 ºC and in the dark. Samples of 1 g of A were powdered in liquid N_2, homogenized in 5 mM NaAc pH 4.6 buffers containing sucrose gradients (0.4–1 M), followed by sequential extraction in 0.2 M $CaCl_2$, 2 M LiCl and urea buffer (7 M urea, 2 M thiourea, 4% CHAPS and 1% DTT) and final precipitation in TCA 100%. Protein identification (CEQUIBIEM) was carried out by Masa Spectrophotometry (MS and MS–MS) using public databases (Uniprot and Phytozome) to assign the obtained peptides. For the successive extracts were identified, respectively, 40, 106, and 953 unique soybean proteins with, at least, two peptides of high confidence. The classification by gene ontology (AgriGO) for orthologue proteins of *Arabidopsis thaliana* showed enrichment in translation and gene expression processes, catabolism and energy, and embryo development ending in seed dormancy. These results evidenced a great cellular activity associated with early events defining soybean embryonic axes germination.

A55
INVOLVEMENT OF HIF-1A FACTOR IN CELLULAR FUNCTIONS RELATED TO OVARIAN TUMOR GROWTH AND DISSEMINATION

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Hypoxia is a characteristic feature of the tumor microenvironment, often associated with poor outcome of cancer patients. It is worth to mention that the advances in the treatment of ovarian cancer have been really scarce. The activation of HIF-1 (Hypoxia inducible factor-1) regulates several processes within cancer cells, such as metastasis, tumor angiogenesis, evasion of apoptosis and uncontrolled cell proliferation. We focus our studies in the effect of HIF-1α inhibition in a human ovarian cancer line, SKOV3. These cells were incubated with Acriflavine, a HIF-1α dimerization inhibitor, and cell proliferation, migration and expression of HIF-1α-related proteins were analyzed. Acriflavine 5 and 10 μM significantly decreased cell proliferation and Cyclin D1 protein expression ($P < 0.05$). Moreover, Acriflavine 5 μM decreased VEGFA protein levels and increased the Angiopoient 1/Angiopoietin 2 ratio in SKOV3 cells ($P < 0.05$). Whereas cellular migration remained unchanged, E-cadherin and N-cadherin protein expression decreased when the SKOV3 cells were incubated in the presence of Acriflavine 5 μM. With the present results, we show that Acriflavine decreases cell proliferation, alters the expression of angiogenic factors and cell migration-associated proteins in the SKOV3 cell line.

A56
RELATIONSHIP BETWEEN CARBONYL ACTIVITY AND SOD, ENSO AND INOS EXPRESSION IN THE LIVER OF FEMALE RATS WITH VITAMIN A DEFICIENCY WITH 6 MONTHS OF TREATMENT

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Vitamin A (VA) is crucial for the maintenance of tissues, growth, and cell differentiation. Oxidative stress can be the result of alterations in the antioxidant mechanism, such as decreased activity or expression of enzymes: catalase and superoxide dismutase (*SOD*). Antioxidant genes such as the *GPX* and *SOD* genes are activated by the action of retinoids. On the other hand, an increase in the levels of TBARs was reported in a deficient model of VA in the aorta, as well as an increase in the expression of endothelial and inducible nitric oxide synthase enzymes (*eNOS/iNOS*). This work aimed to determine the relationship between the activity of hepatic carbonyls (Carb) with molecular markers of the nitrosative and oxidative system: gene expression of *eNOS, iNOS* and *SOD* in a vitamin A deficient model of 6 months. Female Wistar rats were separated at weaning into three groups subjected to different diets: a control group with a sufficient diet in VA (8 mg of retinyl palmitate/kg of diet) for 6 months (c6m), a deficient group with a diet without VA for 6 months (d6m) and a third re-fed group that received a diet without VA for 5 months and then a sufficient diet on VA for 1 month (r6m). The liver was removed to determine Carb by ELISA and the expression of *eNOS*, *iNOS* and *SOD* by RT-PCR. Data were analyzed by linear regression and one-way ANOVA with GraphPad Prism, establishing a limit of $P < 0.05$. The results showed a decrease in the expression of *eNOS* ($P < 0.05$), *iNOS* ($P < 0.0001$) and *SOD* ($P < 0.005$), and an increase in carbonyls ($P < 0.01$), with feedback recovery only in *iNOS* ($P < 0.0001$). Regressions were significant between Carb and *eNOS* (R2 = 0.4892), and Carb and SOD (R2 = 0.6335), all with $P < 0.05$, with a negative slope. Conclusion: In VA deficiency there is an inverse relationship between the increase in Carb and the decrease in the expression of *SOD* and *eNOS*. We can suggest a breakdown of balance where a decrease in SOD, increases oxidative stress and protein oxidation. An attempt to compensate for oxidation would result in lower nitric oxide (NO) production through the lower expression of two enzymes that synthesize it (eNOS and iNOS), which have been shown to be regulated downwards in stress, where the concentration of nitric oxide is excessive.

A57

EFFECT OF CHLOROQUINE ON CATHEPSIN D DISTRIBUTION IN BREAST CANCER CELLS

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Breast cancer causes high mortality worldwide. Cells of some tumors have increased the lysosomal biogenesis as a response to its altered metabolism. These events affect lysosomal integrity and/or functionality, where increased levels of lysosomal proteases such as cathepsin D (CatD) are observed. In most cell types, lysosomal proteins are selectively transported from the trans Golgi network (TGN) to lysosomes by the mannose-6-phosphate receptors (CD-MPR and CI-MPR). In acidic compartments (e.g., late endosomes), the enzyme-MPR complexes are dissociated, and receptors can recycle back. Alterations in the lysosomal membrane release CatD into the cytoplasm, triggering apoptotic processes. Thus, lysosomes are considered as potential therapeutic targets for antitumor drugs. Usually, acidotropic amines accumulate in lysosomes and could induce an increase in lysosomal membrane permeability, leading to an escape of enzymes into the cytoplasm. Chloroquine (CQ) is an acidotropic amine known to affect lysosomal acidification and inhibits autophagy. The aim of this study was to evaluate the effect of CQ on the distribution of CatD and CD-MPR in human mammary tumor cells MCF-7. Cultures incubated with CQ at different times were processed for CatD and CD-MPR detection by indirect immunofluorescence. After 6 h of incubation with CQ, CD-MPR is mostly distributed in the cytoplasm, indicating that the increased lysosomal pH prevents the recycling of CD-MPR to the Golgi. In turn, the TGN becomes disorganized and co-localizes with CD-MPR. However, CatD exhibits an apparent increase in perinuclear staining compared to control cells. This effect could be due to a delay in the transport of the newly synthesized enzymes to lysosomes. Surprisingly, at 12 h of incubation, the perinuclear CatD signal is redistributed throughout the cytoplasm, indicating that it would have reached the lysosomes. At 18 h, CD-MPR and CatD fully recover their initial location, denoting that the CQ effect is lost over time. From these preliminary results, we conclude that CQ affects the endo-lysosomal system, altering the normal transport of CatD, although the mechanism by which the CQ effect is reversed should be elucidated.

A58

EVALUATION OF ROOT ANATOMY OF *Secale cereale* L. (RYE) AND *Medicago sativa* L. (ALFALFA) FERTILIZED WITH CHEMICAL TREATMENT AND CYANOBACTERIA

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Edaphic cyanobacteria are microorganisms that have the ability to fix atmospheric nitrogen, so their use in agriculture as biofertilizers has benefits. The objective of this work was to analyze the anatomy of *Secale cereale* L. (rye) and *Medicago sativa* L. (alfalfa) roots fertilized with different treatments. We worked with plants sown on plots located at the Department of Agricultural Sciences, FICA, UNSL, in a random plot design. Rye had four fertilization treatments: Control (T1), Cyanobacteria (T2), Cyanobacteria + Simple Superphosphate SPS (T3) and Urea + SPS (T4). Alfalfa was fertilized with three treatments: T1, T2 and T3. The plants were randomly extracted within each plot, on the same dates that cuts were made for evaluation of Dry Matter (four dates for rye, three dates for alfalfa); then, they were taken to the laboratory and were prepared to be analyzed. The anatomical cuts in both species were performed freehand in thicker roots, at 5 cm from the neck level. The sections obtained were observed under a light microscope, digital photos were taken, and permanent preparations were made with gelatin-glycerin. Alfalfa sections were stained with Lugol to detect the presence of starch granules. In rye, neither anatomical changes nor the presence of endophytic cyanobacterial cells or filaments were observed. In alfalfa obtained in the third date, in treatment T3, filaments and cells isolated from cyanobacteria were observed in cells of the radical crust. These initial results contribute to the study of cyanobacteria as biofertilizers in forage species.

A59

ROLE OF C-FOS AND LIPIN1B IN AUTOPHAGY REGULATION IN GLIOBLASTOMA

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c-Fos is a proto-oncoprotein belonging to the AP-1 family of transcription factors; in addition to its function as a transcription factor, it is associated with components of the endoplasmic reticulum (ER) and activates the synthesis of phospholipids and glycolipids by a mechanism independent of its genomic activity. c-Fos activates different enzymes involved in the synthesis of phospholipids and glycolipids, among them, the enzyme Phosphatidic Acid Phosphatase (Lipin1). AP-1 proteins have been involved in numerous pathologies and in the development of different types of cancer where their activity is often dysregulated, contributing to cell transformation, tumor progression, aggressiveness, and resistance to treatment. Autophagy is a process of degradation of cellular components and has currently been postulated as a therapeutic target in the treatment of tumors. Recent evidence proposes that this process plays both anti-tumor and pro-tumor roles. Previous results of our lab showed high levels of c-Fos expression in different tumors of the Central Nervous System (CNS), and a correlation between the degree of malignancy and the level of c-Fos expression as well, contrasting with the absent or almost undetectable expression in healthy tissues. This dichotomy highlights the potential of this protein as a therapeutic target. Our work aims to characterize the participation of c-Fos and Lipin1 in the regulation of autophagy, particularly in glioblastoma, the most aggressive CNS tumor. We studied the direct effect of c-Fos and Lipin1b overexpression in CHO K-1 cells as in a glioblastoma cell line, T98G. The overexpression of c-Fos, and the overexpression of Lipin1b, promoted the accumulation of the autophagy marker LC3II in CHO K-1 and T98G cells under starving conditions. Colocalization assays between LC3 and Lipin1b in CHO K-1 cells, and in a stable HeLa-LC3-GFP cell line, suggested that both proteins partially colocalize in punctuated structures under starving conditions. Results from an *in silico* analysis revealed that both c-Fos and Lipin1b have putative “LIR-motif”. Since we observed that c-Fos is capable of inducing autophagy and

that it presents a putative LC3II-interacting domain "similar to ATG3", we are interested in determining the cytoplasmic role of c-Fos in regulating autophagy and its importance in the biology of glioblastomas.

A60
EFFECT OF STEROID HORMONES ON THE MIGRATION OF BOVINE OVIDUCTAL EPITHELIAL CELLS

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Hormonal fluctuations throughout the estrous cycle influence gene expression and morphological changes associated with fertility. The oviduct, an organ the female reproductive system, responds to the action of sex hormones. The oviductal epithelium undergoes changes throughout the estrous cycle, which contribute to the generation of an optimal environment for fertilization and early embryo development. The epithelial cells of the oviduct are exposed to different factors that affect their integrity, such as components of the follicular fluid after ovulation or reactive oxygen species. Nevertheless, their regenerative capacity is important to maintain an adequate oviductal microenvironment. The steroid hormones, estrogen (E_2) and progesterone (P_4) regulate the activities of epithelial cells, such as proliferation, migration, apoptosis, and differentiation. The objective of this work was to evaluate the in vitro effect of E2 and P4 on the migration of bovine oviductal epithelial cells (BOEC) and to analyze the expression level of the focal adhesion kinase (*PTK2*) and paxillin (*PXN*) genes in BOEC cultures under different hormonal conditions. The cells were obtained by mechanical pressure from oviducts of heifers in the preovulatory stage. Monolayers and explants cultures were obtained and stimulated with different combinations of E_2 and P_4: (a) without hormones; (b) ethanol (vehicle); (c) E_2: 290 pg/mL; P_4: 6 ng/mL; (d) E_2: 86 pg/mL; P_4: 120 ng/mL; (e) E_2: 290 pg/mL; and (f) P_4: 120 ng/mL. By means of wound healing assays, the migratory capacity of BOEC was evaluated in the monolayer cultures at 6, 12, and 24 h. The results obtained indicated that high doses of P_4 inhibit the BOEC migration, while no effect on cell migration was observed in BOEC supplemented with a high E_2 concentration. The presence of *PTK2* and *PXN* transcripts was evaluated by semiquantitative RT-PCR in the explants BOEC cultures under the hormonal conditions previously mentioned during 24 h. High concentrations of E_2 did not affect the expression level of both *PTK2* and *PXN* genes, while P_4 inhibited their expression. In conclusion, P_4 affects the BOEC migration in vitro, possibly through the downregulation of *PTK2* and *PXN* genes involved in this biological process.

REPRODUCTIVE AND DEVELOPMENTAL BIOLOGY

A61
CHANGES IN N-ACETYLGLUCOSAMINE CONTENT ASSOCIATED WITH CAPACITATION AND CHEMOTAXIS IN PORCINE SPERMATOZOA

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In mammals, only a small number of ejaculated spermatozoa (SPZ) reach the region of the oviduct (ampulla) after the copula, where they encounter and fertilize the egg. It has been suggested that a sperm subpopulation is selected during their transit trough the female genital tract, so that only those with high fertilizing capability and the best skills for supporting embryo development can fertilize the egg. Fluids of the female genital tract, such as the follicular (FF), the oviductal fluid (OF), and the secretion of cumulus-oocyte complex (COCs), could promote the SPZ chemotaxis to the fertilization site. Progesterone (P4) is considered as an effective chemoattractant in most mammalian species, though other components of the fluids could attract SPZ even more efficiently. In the present study, we evaluated possible changes in carbohydrate composition of sperm surface after capacitation and chemotaxis. For this, we determined the content of N-acetyl-glucosamine (NAG) in porcine SPZ after the mentioned processes by using WGA-FITC lectin and flow cytometry. We observed that NAG content was significantly higher in the capacitated SPZ (30 min in capacitation media TALP, at 38.5ºC and with 5% CO_2) compared to fresh SPZ or SPZ stored in BTS (diluent media). For the chemotaxis assays, OF and FF collected from prepubertal gilts (OF0 and FF0) and periovulatory phase (OF2 and FF2) were used as chemoattractants. Six wells were filled with fresh spermatozoa (20×10^6/mL) from fertile boars (N = 3) selected in a discontinuous percoll gradient and immediately transferred to TALP, previously equilibrated at 38.5ºC and 5% CO_2. The opposite wells of the chemotaxis chamber (six) were filled with TALP (control group) or TALP supplemented with the chemoattractants as indicated: (1) TALP (control), (2) FF0 (1.25%), (3) FF2 (1.25%), (4) OF0 (1.25%), (5) OF2 (1.25%), (6) P4 (28.3 pM). After 20 min at 38.5ºC and 5% CO_2, the SPZ from the opposite wells were rescued, processed for NAG detection and analyzed by flow cytometry. We observed that NAG content was significantly lower in the SPZ obtained from the groups 3 and 6 compared to the control group or the original SPZ ($P < 0.05$). These preliminary results suggest that FF2 and P4 can selectively attract a SPZ subpopulation with low content of NAG in the plasma membrane under the *in-vitro* conditions.

A62
GLYCOSAMINOGLYCANS ISOLATED FROM FEMALE GENITAL SECRETIONS INCREASE THE EFFICIENCY OF IN VITRO FERTILIZATION IN PIGS

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Glycosaminoglycans (GAGs) are linear polysaccharides that contained repeating hexosamine disaccharides and are found in the oviductal fluid (OF), follicular fluid (FF) and in the cumulus oophorus secretion (COS) of several mammalian species. Based on their structure, two major classes of GAGs have been described: the sulfated GAGs, including heparan sulfate (HS), chondroitin sulfate (CS), keratan sulfate (KS) and the non-sulfated GAG like the hyaluronic acid (HA). Since the GAG hyaluronic acid appears to mediate the fertilization process, in this study we proposed to isolate the GAGs from pig FF (G-FF), OF (G-OF) and COS (G-COS) and evaluate their effect on the in vitro fertilization (IVF). GAGs were isolated by protease digestion, followed by lipid extraction and by sequenced steps of precipitations. The GAG composition was determined as previously described by Volpi *et al*. (2018)*. The IVF was performed in TALP medium containing either 120 µg/mL of G-COS, 120 µg/mL of G-FOP, 120 µg/mL of G-FF, 100 or 500 µg/mL of HA. Each group was co-cultured with 5×10^5 spermatozoa/mL and the fertilization parameters were determined. It was observed that G-FF showed lesser values for zona pelucida binding, while it showed the highest sperm penetration rate (%) and sperm efficiency to form pronucleous ($P < 0.05$, ANOVA followed by Tukey post hoc). However, no effect was observed after the incubation with HA or G-COS. This preliminary study suggests that isolated GAGs from FF can improve IVF outcomes. Further studies should be carried out on the embryo development and quality. **J Matern Fetal Neonatal Med* **22**: 1-61. 2018

A63
PIEZO-ICSI FERTILIZATION IN BOVINE OOCYTES

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ICSI is inefficient technique in bovine embryo production and chemical activation of oocytes is mandatory. The aims were to evaluate the bovine embryo production efficiency by Piezo-ICSI and to compare the PLC activity in bull and human sperm. In vitro matured bovine oocytes were fertilized by conventional ICSI and Piezo-ICSI. Number of pronuclei, embryo cleavage and sperm PLC enzymatic activity were determined by fluorometry. Oocyte survival (99 *vs*. 95 %), 2 pronuclei oocytes (22.3 *vs*. 5.9 %) and embryo cleavage rates (18.4 *vs*. 4.2 %) were higher when Piezo-ICSI was performed ($P < 0.05$). PLC activity was lower in bull than human sperm (5.2 ± 1.8 *vs*. 16.1 ± 4.4 mUI/10^6 cells, $P < 0.05$). Piezo pulse on sperm and oocyte membranes had positive effect on oocyte activation, increasing pronuclear formation and embryo cleavage. Membrane breakage by Piezo pulse led to lower oocyte lysis. However, fertilization and cleavage rates are still lower when compared to ICSI plus chemical activation. The low PLC activity in bull sperm may partially explain the oocyte activation failure after ICSI in this species.

A64
BLASTOCELE ASPIRATION IN LLAMA EMBRYOS. PRELIMINARY RESULTS

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Aspiration of blastocele fluid (embryo collapsing) is applied as a tool for optimizing cryopreservation protocols with the aim of improving the survival rate after vitrification or deep freezing of embryos beyond the expanded blastocist stage. Blastoceles contain liquid that could interfere with the process of cryopreservation as it potentially transforms to damaging ice during cooling. Hence, as expanded blastocysts dehydrate and shrink slower than earlier stage blastocysts, they are more likely to result in intracelullar ice formation. For this reason, reducing the blastocele decreases the potential danger of damage by ice crystals. The objective of this study was to aspirate blastocele fluid from llama embryos produced *in vivo* and evaluate their survival rate post collapsing, with the aim of evaluating whether the aspiration technique itself is harmful for the embyro. Follicular dynamics were monitored in embryo donor females by transrectal palpation and ultrasonography. Natural mating with a male of proven fertility was indicated in the presence of a dominant follicle in growth phase (≥ 7mm), together with an IV injection of 8 µg of buserelin (Receptal®). Transcervical uterine flushing for embryo recovery was carried out 8 days after mating. Embryos suitable for transfer (grades 1 and 2) were placed in Syngro® media, measured and then collapsed. Aspiration of the blastocele fluid was carried out with a Leica DMIL® inverted microscope equipped with Narishige® micromanipulators, using a holding pipette to immobilize the embryo and placing the inner cell mass at 12 or 6 o'clock to avoid damaging it during injection. An injection pipette was used to aspirate the blastocele fluid until the embryo collapsed. Then the collapsed embryos were cultured *in vitro* in Singro® media at 38°C, 5% CO_2 and 100% humidity for 2 h and their capacity to re-expand to their original size was evaluated. Embryo recovery rate was 44% (8/18) and 5 embryos were evaluated *in vitro*, all of which recoved their original size within the hour. The remaining 3 embryos were immediately vitrified after the blastocele was aspirated and are being maintained in liquid nitrogen until transfer to recipient females. The results obtained up to now would seem to indicate that the aspiration technique using micromanipulators does not affect llama embryo viability. Embryo re-expansion in *in vitro* culture demonstrates embryo viability as they are able to recuperate blastocele fluid. Also, contrary to the use of non-vital stains, this technique allows conservation of embryos at a stage that is transferable to the uterus of a female.

A65
CATHEPSIN D SORTING IN MAMMALIAN EPIDIDYMAL CELLS

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In mammals, sperm maturation occurs in the environment provided by the lumen of epididymal duct. The epididymal epithelium secretes high amounts of acid hydrolases, but the function of the enzymes and the mechanism of that secretion are still unclear. In the most cell types, the lysosomal enzyme cathepsin D (CatD) is transported from TGN to endo-lysosomal compartments through the mannose 6 phosphate receptors (CI- and CD-MPR), but alternative routes, mediated by Sortilin (Sor) or other receptors are known. Sor can transport CatD to lysosomes when the protease is complexed with the lysosomal protein, prosaposin. Previous results in the epididymal cell line RCE-1 confirmed that CatD forms complexes with prosaposin, suggesting that Sor participates in the CatD sorting. In our laboratory, a sortilin-knockdown epididymal cell line (RCE-1K) has been established, where CD-MPR expression is increased as a counterpart, suggesting that an interregulation between both receptors exists. Here, we correlated the distribution of CatD with that of CD-MPR in normal and RCE-1K cells by indirect immunofluorescence and quantitative co-localization analysis. Normal RCE-1 cells showed a perinuclear network-like Sor distribution. We also observed a high co-localization of CatD with Sor, but not with CD-MPR. In turn, CatD also co-localizes with endo-lysosomal markers. When RCE-1 cells are depleted of Sor (RCE1-KD), CatD still reaches lysosomes and its colocalization with CD-MPR through to the entire cytoplasm is increased. These results indicate that, Sor and CD-MPR could work cooperatively for CatD sorting in epididymal cells and provide new and important insights for the study of sperm maturation process.

A66
EVALUATING THE THERAPEUTIC POTENTIAL OF AMMONIUM TETRATHIOMOLYBDATE IN EXPERIMENTAL ENDOMETRIOSIS

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Endometriosis (EDT) is an estrogen-dependent chronic gynecological disease that affects 10–15% of women of reproductive age. It is characterized by the growth of endometriotic-like tissue outside the uterine cavity, causing microbleeds and producing a constant inflammatory-oxidative focus. This pathology still has no cure, and the most used current treatments (hormonal) can have side effects. Trace metals play an important role in the pathogenesis of EDT. Our research group reported a positive correlation between copper (Cu) levels in peritoneal fluid and endometriotic-like lesions volume induced in mice. There are also reports of elevated Cu in samples from patients with EDT by other authors. This metal is a key component in many essential enzymes, acting as a limiting factor in tumor progression, metastasis, and angiogenesis, as well as being able to enhance estrogenic action. Therefore, our objective was to investigate the therapeutic potential of Ammonium Tetrathiomolybdate (TM, Cu chelator) in EDT. For this purpose, we used an experimental model of EDT employing two-month-old female C57BL/6 mice. For surgical induction of endometriotic-like lesions, the right uterine horn was removed from the animal, divided longitudinally, cut into three square pieces of approximately 4 mm^2 each, which were then sutured in the intestinal mesentery. Three experimental groups were used: (1) Sham, placebo surgery; (2) EDT and (3) EDT + TM (N = 7 animals per experimental group). The EDT + TM group received 0.70 mg of TM/day/mouse in their drinking water for 3 weeks, starting on day 8 postoperatively. After one month of induced pathology, mice were sacrificed. The lesions were identified, counted, measured with caliper in two perpendicular diameters for volume evaluation, weighed, and stored at −80°C. Peritoneal fluid was also collected for the determination of estradiol by chemiluminescence. Data were statistically analyzed using Student's *t*-test and one-way ANOVA followed by Tukey's test (a P-value <0.05 was considered statistically significant). TM treatment did not affect the number of established lesions per mouse. However, treated animals showed lesions of lower weight and volume as compared to control animals ($P < 0.05$). Furthermore, the Cu chelator decreased estradiol levels in the peritoneal fluid, reaching levels similar to those of the Sham group ($P < 0.05$). In conclusion, our study shows promising results on the use of TM as a possible therapy for EDT.

A67
CHANGES IN SPERM MOTILITY AND ITS REGULATION BY PROTEIN KINASE C AND TYROSINE KINASE IN SPERM CAPACITATION WITH HYALURONIC ACID OR HEPARIN

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During capacitation, sperm motility increases to allow sperm to interact with the zona pellucida and trigger acrosomal exocytosis. Motility could be enhanced by the use of hyaluronic acid or heparin, so our aim was to evaluate it and to study the participation of protein kinase C and tyrosine kinase in its regulation in cryopreserved bovine spermatozoa. Hyaluronic acid and heparin are glycosaminoglycans present in the bovine female genital tract which induce capacitation in this species during *in vitro* conditions. GF-109203X and genistein were used as protein kinase C and tyrosine kinase inhibitors, respectively. Capacitation was evaluated by the chlorotetracycline epifluorescent technique and viability by trypan blue staining. Sperm motility was analyzed by Computer Assisted Sperm Analysis (ISAS). Data were analyzed by ANOVA and Tukey test ($P < 0.05$). Capacitation was significantly higher with hyaluronic acid (21.43 ± 4.58%) and heparin (32.54 ± 2.02%), while viability showed no significant differences between the inducers and their controls. Hyaluronic acid produced an increase in total motility (28.54 ± 7.88%), progressive motility (22.02 ± 7.95%) and in

the amplitude of lateral head displacement (3.57 ± 0.11 μm) compared with its control, while heparin treatment showed an opposite effect, reducing all these parameters respect to its control (32.82 ± 7.30%, 25.22 ± 3.25% and 3.87 ± 0.22 μm, respectively) ($P < 0.05$). Genistein produced a significant decrease in total motility (12.83 ± 6.95%) and progressive motility (10.63 ± 5.76%) in samples treated with heparin, while GF-109203X produced the same effect on both parameters in samples treated with hyaluronic acid (14.62 ± 6.12% and 11.86 ± 5.37%, respectively) ($P < 0.05$). On the other hand, these kinases inhibitors did not modify the amplitude of lateral head displacement in hyaluronic acid or heparin treatments ($P > 0.05$). The motility parameters indicated that hyaluronic acid potentiated total and progressive motility and also the amplitude of lateral head displacement. Both capacitation inducers produced a decrease in curvilinear velocity and average path velocity compared with their controls, indicating that they do not enhance them. In heparin capacitated spermatozoa motility would be mainly mediated by tyrosine kinase activity, while hyaluronic acid capacitation would involve protein kinase C activity in the signal transduction mechanisms which modulates changes in sperm motility.

A68
VARIATION OF SPERM MOTILITY AND MITOCONDRIAL POTENTIAL PARAMETERS DUE TO THE ANTIOXIDANT EFFECT OF TROLOX ON BOAR SEMEN REFRIGERATION

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Gamete cryopreservation is a fundamental biotechnological strategy in the productive field. However, in swine production, it is not regularly used due to the high concentration of unsaturated fatty acids makes boar sperm more susceptible to peroxidation produced by reactive oxygen species generated during their storage at low temperatures. Our aim was to study the effect of the addition of the synthetic compound derived from alpha-tocopherol called 'Trolox' to Modena modified diluent in porcine semen by means of functional sperm tests: evaluation of sperm motility, pre-capacitation status, sperm viability, membrane integrity, and mitochondrial membrane potential. We worked with a pool of semen samples that were separated into two equal aliquots, only one of them treated with Trolox, and both stored at 17°C. Both samples were evaluated on days 0 (fresh semen), 1, 2 and 5 of refrigeration. Subsequently, motility changes were studied using the Integrated Sperm Analysis System (software version 1.2, PROISER). Pre-capacitation was evaluated by the chlorotetracycline epifluorescent technique and viability by trypan blue staining. The functional integrity of the plasma membrane was evaluated through the Hypoosmotic Test, while the mitochondrial membrane potential was evaluated through the JC-1 fluorochrome. Data were analyzed by ANOVA/ Tukey test ($P < 0.05$). A significant decrease in progressive motility was observed in samples treated with or without Trolox during the refrigeration process, compared with fresh semen ($P < 0.05$). The progressive motility in fresh semen was 85.71 ± 1.74. No differences were observed in progresive motility between the samples refrigerated with and without Trolox. An increase in sperm vitality was observed on days 1 and 5 after refrigeration with Trolox compared with fresh semen ($P < 0.05$). The difference between the percentage of acrosomal and membrane integrity in treated and untreated samples was not significant ($P > 0.05$). Samples refrigerated with the addition of Trolox presented a significantly lower percentage of pre-capacitation on day 5 post-refrigeration compared with those which were not treated ($P < 0.05$). The mitochondrial membrane potential was 80.00 ± 4.69% prior to refrigeration and samples treated with Trolox (69.00 ± 7.75%, 58.75 ± 6.80% and 46.33 ± 12.66% at day 1, 2 and 5, respectively) presented higher values compared with the untreated ones ($P < 0.05$). The addition of Trolox to swine seminal samples produced an increase in their vitality as well as a decrease in the percentage of pre-capacitation and maintained the plasma membrane integrity while producing a decrease in sperm motility with a tendency to maintain a low intracellular redox state due to a low mitochondrial membrane potential.

A69
INVOLVEMENT OF SUPERIOR OVARIAN NERVE AND SPLEEN MACROPHAGES SECRETIONS ON NEUROIMMUNE-ENDOCRINE MODULATION IN UTERUS OF RATS WITH POLYCYSTIC OVARY

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Polycystic ovary syndrome (PCOS) is a public health important disease. The abnormal endocrine and metabolic characteristics of PCOS might be detrimental to endometrial function, manifesting as endometrial hyperplasia or cancer and receptivity reduction. Excessive sympathetic activity in the superior ovarian nerve (SON) plays an important role in inducing the PCOS symptoms in rats and humans. There is little evidence about the role of the innervation in endometrial PCO. We have shown in a PCO model that the ovarian steroidogenic response is differentially regulated by the macrophage secretions (MΦ-S) through the neural connection involving ovary-SON-coeliac ganglion-spleen in rat. The purpose of this work was to elucidate, in a PCO-induced rat model, whether *in vivo* bilateral SON transection (PCO-SONt) modifies MΦ-S effect on PCO uterus. For that, culture media of macrophages (MΦ) from PCO rat and PCO-SONt rats were used to stimulate *in vitro* uterus explants. Inflammatory, hormonal, and neural parameters were evaluated. PCO condition was induced at 60 days of age by i.m. injection of estradiol valerate (2 mg/rat). In a lot of PCO rats, the SON transection was performed 7 days before sacrifice. MΦ (1×10^6 cells) were cultured for 24 h in RPMI medium. Those MΦ culture supernatants were used to stimulate PCO and PCO-SONt uterus for 3 h. In uterine tissue we evaluated the expression of inducible nitric oxide synthase protein (iNOS) (by Western Blot), mRNA levels of nerve growth factor (NGF) and estrogen receptors alpha (ERα) (by RT-PCR) and nitric oxide (NO) release (by Griess reaction). The tumor necrosis factor alpha (TNFα) released in MΦ-S was measured by ELISA. In PCO group, MΦ-S from PCO-SONt rats decreased iNOS protein and NO release, compared to MΦ-S from PCO rats. Besides, the mRNA expression of NGF and ERα in PCO uterus incubated with MΦ-S from PCO-SONt rats were lower to that obtained with PCO MΦ-S ($P < 0.05$). MΦ from PCO-SONt rats released less TNFα ($P < 0.05$) when compared with PCO group. The results of the present study suggest the sympathetic innervation, through SON, is involved in uterus alterations observed in polycystic ovary syndrome. Considering that NGF is a mediator of the effects of steroids on the nervous

and immune systems in the uterus, the decrease of neurotrophin after SON transection could influence the hormone-mediated modulation of immune cells. We infer that the section of the SON, through its effect on MΦ, might has an ameliorative action against proinflammatory and proliferative PCO endometrial environment.

A70
SPLEEN MACROPHAGES SECRETIONS INDUCE A DUAL EFFECT OF INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSIONS AND PROSTAGLANDIN E2 CONTENT IN UTERUS OF RATS WITH POLYCYSTIC OVARY

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Polycystic ovary syndrome (PCOS) is the most common endocrine-reproductive-metabolic disorder in women, which can lead to infertility. The primary hormonal abnormalities in PCOS are characterized by higher androgen and estrogen but lower progesterone levels. PCOS negatively affects the endometrium, leading to implantation failure and proliferative aberrations. Although the precise etiology remains unclear, hormones and immune cells are reportedly a crosstalk in PCOS endometrial dysfunction. The aim of our study was to evaluate the effect of spleen macrophages secretions (MΦ-S) on the inducible nitric oxide synthase (iNOS) expression and prostaglandin E2 (PGE2) content in the uterus from PCO rats and the relationship with nitric oxide (NO) release and steroids receptors expression. PCO was induced in adult rats by estradiol valerate (2 mg/rat). After 2 months, control (C) and PCO rats were sacrificed. The PCO MΦ were isolated and cultured (1×10^6 cells) for 24 h in RPMI medium. Secretions were employed to stimulate C and PCO uterus (N = 6 per group), for 3 h in a metabolic bath. The uterine content of PGE2 (by RIA), iNOS protein (by Western Blot), androgen (AR) and estrogen (ERα) receptors gene expression (by RT-PCR) and nitrites release (by Griess reaction) was analyzed. In PCO uterus, under basal conditions, the NO release and INOS expression do not change, but PGE2 content increased ($P < 0.01$) in relation to basal C rat. Nonetheless, the PCO MΦ-S increased, in PCO uterus, the iNOS protein expression ($P < 0.05$), NO release ($P < 0.01$) and PGE2 content ever more ($P < 0.001$), compared to C group. In contrast, the PCO MΦ-S produced a decrease of AR mRNA ($P < 0.01$) without change in ERα mRNA expression in PCO uterus with respect to C. It is known that NO stimulates the activity of cyclooxygenase 2 (COX2), a limiting enzyme in prostaglandin synthesis. The increase in PGE2 content may be associated with the production of NO in the uterus SOP, induced by PCO MΦ-S. Greater amounts of tumor necrosis factor-alpha (TNFα) observed in PCO MΦ-S could explain in part the NO and PGE2 increase showed in PCO uterus. These results suggest that PCO MΦ-S can adversely affect the endometrial environment through the up-regulation of pro-inflammatory mediators. Besides, PCO MΦ-S could contribute to decreasing the uterine tissue sensitivity to androgens through the downregulation of AR mRNA levels. The above findings suggest that the interaction between iNOS, PGE2, and AR might be important in the regulation of physiopathologic events in PCO uterus, that compromise endometrial health long-term.

A71
SEMINAL TRANSFERRIN AND SPERMATIC PARAMETERS

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Seminal plasma (SP) is a complex fluid, produced by the male accessory glands of the reproductive system. It transports, protects, and nourishes the sperm from ejaculation to fertilization, and it also acts as a modulator of sperm function. For this reason, it is necessary to adequately characterize the molecular composition of SP, in order to understand how it is altered in male infertility. Transferrin (Tf) is the main iron transporter glycoprotein in the majority of organisms and therefore is an essential growth factor. Testicular Tf (TfT) is synthesized in Sértoli cells. These are the intratesticular cells responsible for the transport of micronutrients for the development of sperm cells through the blood-testicular barrier. The function of Tf in the testes (TfT) is still unknown, but it is believed that it could participate in the supply of iron for the development and differentiation of germ cells. The objective of this study was to compare the levels of TfT in PS with some quantitative clinical relevance variables of semen such us: sperm concentration, morphology, progressive sperm motility and total sperm count, to assess whether this protein is related to reproductive function. Sixty-six semen samples were studied from individuals aged 18 to 55 years old. Patients consulted for fertility alterations in the Urology Services of the Eva Perón School Hospital and Centenario Hospital, and healthy volunteers, during July 2018 to February 2019. Patients with the following pathologies, wich could interfere with Tf levels in blood plasma, were excluded such as acute or chronic liver disease, neoplasia, clinical/laboratory signs of infection/acute or chronic inflammation due to hepatitis virus (A, B or C) infections, leukocytosis, fever, hypoproteinemia, and iron metabolism diseases. Semen study was carried out according to WHO 2010 standards. TfT concentration was measured using the radial immunodiffusion technique adapted to low concentrations, developed in our laboratory. Statistical analysis was performed by the Spearman ordered rank correlation coefficient (r) for TfT versus each of the quantitative variables of the basic semen analysis. It was observed that TfT concentration has a direct relationship with the sperm concentration (r = 0.3872, *P*-value = 0.0070, $P < 0.01$, N = 66), the total sperm count (r = 0.515 ; *P*-value = 0.0008, $P < 0.01$, N = 66) and to a lesser extent with the percentage of progressive motile sperm (r = 0.3721; *P*-value = 0.0139, $P < 0.05$, N = 66); however, no correlation was detected with the variables associated with sperm morphology. Sperm abnormalities can be due to the action of multiple factors. The study of possible biomarkers, such as TfT, would contribute to the detection of alterations in sperm function, enabling an accurate diagnosis and proper treatment.

A72
IN VITRO MATURATION IN A DEFINED MEDIA WITHOUT OXIDATIVE SUBSTRATES

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Most metabolic studies carried out in cumulus oocytes-complexes (COCs) refer to carbohydrates as they are the main substrates in the culture media. There is limited information about the use of endogen lipids (EL) as oxidative substrates. The aim of this work was to study the endogen lipid consumption of bovine COCs during maturation in absence of other oxidative substrates. Maturation was performed in defined media, SOFm, (without pyruvate and lactate) supplemented with FSH, LH, EGF, gentamicin, insulin and PVA. Oocytes were randomly divided in 5 groups: (a) SOF + glucose 5.5 mM, (b) SOF + L-Carnitin (β-oxidation fatty acid stimulator), (c) SOF + glucose + L-Carnitin, (d) SOF + Etomoxir (β-oxidation fatty acid inhibitor), and (e) SOF (without any supplementation). The proportion of nuclear maturation was evaluated by the presence of metaphase II after Hoechst 33342 staining. The COCs endogen lipid content was determined by Nile Red staining and its fluorescence was quantified using digital photomicrographs taken with an epinofluorecence microscope. Significant increase in nuclear maturation rates were observed in presence of glucose, L-carnitin and the combination of both, respect to the group without any supplementation and the group supplemented with etomoxir. No difference was observed between these two last groups. For the endogen lipids content, it was observed that in media without supplementation, at the end of the maturation process, lipid content was lower than in the group supplemented with glucose ($P < 0,05$). However, no difference was observed between groups supplemented with β-oxidation fatty acid modulators (L-carnitin and etomoxir) and the media without any supplementation. In conclusion, endogen lipids can be used as oxidative substrates to hold, in part, nuclear maturation, parameter that was increased by glucose presence.

A73
MORIN'S (C15HI007; 2- (2,4-DIHIDROXIFENIL)- 3,5,7-TRIHIDROXI-4H-L-BENZOPIRAN-4-ONA) EFFECT ON PORCINE OOCYTE *IN VITRO* MATURATION

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Porcine *in vitro* maturation (IVM) efficiency is modulated by intra and extracellular redox balance. Morin is a flavonoid from the flavone family which eliminate free radicals and protect DNA damage caused by free radicals. Recent studies have shown that Morin's effect on cellular cultures is dose dependent. High doses of Morin stimulate oxygen reactive species (ROS) and promote apoptosis, whilst it has an antioxidant effect at low doses. The aim of this study was to evaluate the effect of adding different concentrations of Morin to the maturation medium on nuclear maturation and oocyte viability. We obtained pig cumulus oocyte complexes (COC) through follicular aspiration from slaughterhouse ovaries at 30–37°C. COCs were washed in PBS with 10% porcine follicular fluid (pFF). Groups of 50 COC per well were cultivated in 500 µL of medium at 39°C in a humidified atmosphere with 5% of CO_2. The control group was matured in base medium (medium 199 Sigma®, supplemented with 10% pFF, 2 mercaptoethanol, sodium pyruvate and antibiotics). For each treatment base medium was added with 100 µM of Morin (treatment 1), 50 µM (treatment 2), 10 µM (treatment 3) and 5 µM (treatment 4). The IVM procedure was done in 2 stages of 22 h. IVM medium was changed between stages and only the first one was supplemented with hMG and dAMPc. To evaluate oocyte viability, after IVM COC were denuded with hyaluronidase and were stained with Trypan Blue 0.16 % and observed under stereoscopic microscope. For nuclear maturation, oocytes were fixated with 4% paraformaldehyde and stained with Hoechst 33342, then mounted on slides to be observed under fluorescent microscope. Statistical analysis was performed with a logistic regression in R with GLM function from Stats package version 2.6.2. None of the treatments influenced oocyte viability and treatment 1 negatively affected nuclear maturation rate (P-value < 0.05). Thus, treatment 1 will be discarded for future determinations as it had a toxic effect. To conclude on the effects of adding Morin to IVM medium, oocyte redox balance must be analyzed.

A74
USE OF ENDOGENOUS LIPIDS AS UNIQUE OXIDATIVE SUBSTRATES DURING THE IN VITRO MATURATION OF PORCINE OOCYTES

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It has been postulated that endogenous lipids can be used as energy substrates during the *in vitro* maturation of porcine oocytes. However, those studies were conducted in complex culture media with various substrates, so the use of each metabolite could be overlapped. The main aim of this work was to analyze the implication of the metabolism of endogenous lipids as unique oxidative substrates during porcine oocyte *in vitro* maturation. Ovaries from slaughtered gilts were transported in an isothermal solution to the laboratory and subsequently prepared for follicle aspiration. Immature cumulus-oocyte complexes (COCs) were obtained by the aspiration of antral follicles (3–8 mm) and were morphologically classified by means of stereomicroscopes according to the characteristics of their cumulus. Only those oocytes surrounded by a dense and integral cumulus were selected and matured in the following culture media: (a) NCSU-37 (without pyruvate and glucose); (b) NCSU-37 + glucose (as positive control); (c) NCSU-37 + L-carnitine (to stimulate β-oxidation); (d) NCSU-37 + glucose + L-carnitine, and (e) NCSU-37 + etomoxir (to inhibit β-oxidation). The percentages of nuclear maturation were evaluated using an epifluorescence microscope by the presence of metaphase II after incubating the oocytes with Hoechst 33342 stain. The use of endogenous lipids was determined using Nile Red stain. Values with $P < 0.05$ were considered significantly different. The percentages of nuclear maturation in presence of glucose or L-carnitine were significantly higher than in the media without supplementation ($P < 0.05$). Moreover, their combination presented an additive effect (glucose + L-carnitine, $P < 0.05$). COCs incubated in presence of etomoxir did not present differences compared with the media without supplementation. Oocytes incubated in presence of L-carnitine showed an increase in lipid consumption compared with those incubated with glucose as their sole oxidative substrate ($P < 0.05$), whereas in the other study

groups no differences in lipid content were observed. These results indicate that endogenous lipids can be used as unique oxidative substrates and that the addition of Glucose, L-carnitine or their combination may contribute to improve *in vitro* maturation rates in porcine oocytes.

A75
EFFECT OF GLUTATHIONE ON BOVINE SPERM CAPACITATION

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Capacitation is a set of physiological changes that sperm undergoes to acquire the "ability" to fertilize the oocyte. *In vivo*, this process is positively associated with high levels of thiol groups in specific proteins present on the sperm surface, where these antioxidant molecules can trigger the release of fully capacitated cells from the oviductal epithelium. One of the most important molecules in the cellular antioxidant system is glutathione (GSH), a tripeptide that contains a thiol group in its structure. The aim of our work was to study the effect of GSH on the sperm capacitation process. One million of bovine spermatozoa obtained from straws of different animals were treated with 1 mM GSH for one hour. The percentage of motility, viability, vigor, acrosomal membrane integrity and cell capacitation were studied in control cells (PBS, GSH vehicle), cells treated with GSH, and cells treated with heparin (100 µg/mL, positive control). Capacitation was determined using chlortetracycline as a fluorescent probe. The results were statistically analyzed by one-way ANOVA and Bonferroni *post-hoc* test. Differences between groups were considered significant at $P < 0.05$. Sperm exposed to 1 mM GSH showed higher viability, motility, vigor, and percentage of capacitated cells than those incubated with heparin or control groups. The percentage of sperm with reacted acrosomal membranes was similar in all the conditions evaluated, while the percentage of capacitation of GSH treated cells was twice higher than the positive control. In conclusion, our results indicate that GSH improves bull sperm capacitation maintaining acceptable sperm viability and motility. Further studies are needed to confirm and extend these results.

A76
AMINO ACIDS METABOLISM DURING IN VITRO MATURATION OF THE BOVINE OOCYTE

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Glucose represents the main oxidative substrate present in the maturation media during the in vitro maturation process of the bovine cumulus-oocyte complex (COC). However, little is known about the role of amino acids during this process. The aim of this work was to study the bovine oocyte metabolism in a medium supplemented only with amino acids as oxidative substrates. COCs were obtained by aspiration of antral follicles from ovaries from slaughter cows. Those COCs with dense and compact cumulus were selected. Maturation was carried out in SOF medium (without pyruvate or lactate) supplemented with FSH, LH, EGF, gentamicin, insulin and PVA, under mineral oil at 39ºC and 5% CO_2 in humidified air for 22 hours. The COCs were randomly divided into 4 groups supplemented as follows: (a) SOF (C1−), (b) SOF + Amino Acids (T), (c) SOF + Glucose/Amino Acids (C+), and (d) SOF + Salicylate (Glutamate Dehydrogenase inhibitor) (C2−). Nuclear maturation was evaluated using Hoechst 33342 fluorescent staining, observing the presence of metaphase plate II. To study the metabolism of amino acids, the concentration of ammonia was determined at the end of maturation using a spectrophotometric technique based on the oxidation of NADPH by the enzyme Glutamate Dehydrogenase. A significant increase in the nuclear maturation percentage was observed in COCs matured in the group supplemented with amino acids (T) compared to both negative control groups (C1− and C2−) ($P < 0.05$). Maturation in the media supplemented with amino acids (T) was lower than the positive control (C +) ($P < 0.05$). In ammonia production a significant increase was observed in the medium supplemented with amino acids (T) ($P < 0.05$). From these results it turns that the amino acids during in vitro maturation of bovine oocytes are deaminated to obtain carbon skeletons as an energy source. The maturation is hold partly by the energy that comes out from the carbon skeletons of amino acids.

A77
EFFECT OF VITRIFICATION ON FUNCTIONAL PARAMETERS OF IN VITRO MATURED PORCINE OOCYTES

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Our aim was to evaluate the effect of the vitrification of porcine oocytes using a minimum volume system (Cryotech® method) on functional parameters throughout different recovery times after warming. Immature cumulus-oocyte complexes (COCs) were obtained by aspiration of antral ovarian follicles and were selected under a stereomicroscope. After 44 h of maturation, oocytes were partially denuded by gentle pipetting and then were vitrified using the Cryotech® vitrification method. Metaphase II plate recovery time analysis, *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) were carried out as functional studies to establish whether vitrification-warming affected oocyte nuclear and cytoplasmic competence. To study metaphase plate recovery time, vitrified-warmed oocytes were incubated for 2, 3, or 4 h in the same medium used for oocyte maturation. After incubation, oocytes were stained with Hoechst 33342 fluorochrome to establish the time point when most of the metaphase plates appear. IVF was performed using fresh semen from a Yorkshire boar of proven fertility and sperm head decondensation and/or pronuclear formation at 18 h post-insemination was evaluated. ICSI was carried out using an inverted Leica® DMIL microscope equipped with Narishige® micromanipulators. Briefly, the oocyte was fixed by the holding pipette and the injection pipette with an immobilized spermatozoon was pushed through the zona pellucida and subsequently through the oolemma into the cytoplasm. Presumed fertilized oocytes from IVF or ICSI were fixed on a glass slide with Carnoy's fixing solution for at least 24 h, incubated in a 10 mg/L Hoechst 33342 fluorochrome aqueous solution for 15 min at room temperature and observed under an epifluorescence microscope. Oocyte morphology was not affected by the vitrification-warming procedure. The

metaphase II configuration recovered 3 h after warming, but only 75% of the matured oocytes were able to recover the metaphase II plate configuration. No further improvement was found after 4 h, while 2 h of incubation post warming proved to be insufficient. Although IVF and ICSI fertilization rates did not differ from the controls, a significant decrease was found in the cleavage rate. Possibly vitrification-warming alters the cytoskeleton involved in the processes of metaphase II spindle formation and cleavage, thus reducing porcine oocyte competence. Therefore, improvements should be included in the vitrification protocols to minimize these alterations.

A78
OMEGA (n) 6 EXCEEDED DIETS MODIFY PLACENTAL VASCULARIZATION, REDUCING VITALITY AND FETAL GROWTH IN A MURINE MODEL

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The dietary n6/n3 ratio affects different reproductive parameters related to intragestational development of mammals. Objective: to evaluate the effect of diets exceeded in linoleic acid (LA)- n6 on placental vascularization, vitality, and fetal growth. Albino swiss mice fed from 0.5 gestation day (GD) with diets: control (C, commercial diet, LA = 1.6%, N = 25) or C with 10% of soy or sunflower oils (SOD, LA = 6.68%, N = 24 and SFOD, LA = 7.68%, N = 26). On 16.5 GD, we evaluated: placental and fetal weights, fetal vitality. In uterine horns, placental areas and placental necrosis areas were measured (H/E). Immunohistochemistry was performed for nitric oxide synthase III (eNOS) in placental labyrinth, quantifying marked area and intensity of staining, through reciprocal intensity, with FIJI software. Statistics: ANOVA, Kruskall-Wallis, Chi-square, Pearson's correlation, $P \leq 0.05$. SFOD placentas were significantly lighter than SOD and C (SFOD = 0.13 ± 0.02 g *vs.* SOD = 0.14 ± 0.02 g and C = 0.15 ± 0.02 g; $P \leq 0.005$). Fetuses from treated dams were significantly lighter than C (SFOD = 0.68 ± 0.01 g, SOD = 0.59 ± 0.01 g and C = 0.72 ± 0.02 g, $P \leq 0.005$). There was positive correlation between placental and fetal weights in the three groups (C = 0.75 g, SFOD = 0.59 g and SOD = 0.82 g, $r \leq 0.001$). Fetal vitality was lower in treated females than in controls: SFOD = 69.59% (N = 115) and SOD = 73.95% (N = 84) *vs*. C = 92.86% (N = 52), $P \leq 0.05$. There were no significant differences between the placental areas. SFOD placentas had a greater area of decidual necrosis than C and SOD (SFOD = 122.05 ± 32.38 μm^2 *vs.* SOD = 52.39 ± 28.59 μm^2 and C = 35.78 ± 11.91 μm^2, $P \leq 0.05$). The eNOS stained placental areas did not show significant differences; however, the reciprocal intensity of the stained area was lower in SFOD than in SOD and C (SFOD = 78.44 ± 3.80 μm^2 *vs*. SOD = 110.61 ± 9.07 μm^2 and C = 100.21 ± 4.76 μm^2, $P \leq 0.05$). The lower vascularization in SFOD would be due to a lower expression of eNOS because of the ω6 excess, with a low content of ω3; consequently, the nutrient supply to the fetus is reduced affecting fetal growth and vitality, as observed in this study.

A79
REPRODUCTIVE BEHAVIOR IN GABAB1KO ADULT MALE MICE

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Kiss1 neurons/cells co-express GABAB receptors (GABABR) and GABA is an important regulator of their physiology. *Kiss1* expression is a key factor in the control of reproduction, sexual behavior, and anxiety. Recently, we found *Kiss1* overexpression in male mice lacking GABABR (KO) in the medial amygdala (MeA) and the basal nucleus of the stria terminalis (BNST), areas associated with sexual behavior and anxiety. Therefore, we studied the sexual behavior of KO and WT male mice in the presence of a receptive female. KO males developed less mounts during the first half hour of the encounter (Chi-square test, $P < 0.05$), and showed less testosterone (*t*-test, $P < 0.05$), but no changes in serum luteinizing hormone were observed compared to WT. However, KO males copulated more than WT when they were left undisturbed during the night (Chi-square test, $P < 0.05$). Results show the importance of the GABABR in reproductive function exerted by *Kiss1* neurons/cells. *(CONICET, ANPCYT, ISN-CAEN, UBA, René Barón Foundation, Williams Foundation).*

A80
EFFECT OF THE COCULTURE OF PORCINE LUTEAL CELLS AND PORCINE *CUMULUS*-OOCYTE COMPLEXES ON CORTICAL REACTION, *IN VITRO* FERTILIZATION AND EMBRYO DEVELOPMENT

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In pigs, *in vitro* fertilization (IVF) is related to high rates of polyspermy and *in vitro* embryo production (IVP) is still an inefficient biotechnology. Coculture with somatic cells is an alternative to improve suboptimal *in vitro* culture conditions. The aim of this study was to evaluate the effect of coculture of porcine luteal cells (PLC) and cumulus-oocyte complexes (COC) during *in vitro* maturation (MIV) on the cortical reaction (CR), IVF and embryo development. Slaughterhouse ovaries were used to obtain PLC and COC. Selected COC were matured *in vitro* for 44 h in TCM-199 supplemented with human menopausal gonadotropin (control) or in coculture with PLC. The IVF was performed with refrigerated sperm in 100 μL drops of TCM-199 supplemented medium for 4 h. Following IVF, suspected zygotes were stained with Hoechst 33342, and sperm penetration, monospermic penetration (monospermic/penetrated), and IVF efficiency (monospermic/total oocytes) were assessed. Cortical reaction (cortical granule distribution) was evaluated with FITC-PNA staining after IVF. For embryo development, suspected zygotes were washed and cultured in PZM at 39°C, 7% O_2, 5% CO_2. Cleavage rates were determined on day 2 and blastocysts rates on day 7. More cortical granules were observed in the area

immediately below the oolemma, and the area outside the zona pellucida was more stained with PNA in the coculture group ($P < 0.0001$; Kruskal-Wallis and Dunn's multiple comparison test). The coculture system significantly increased monospermic fertilization rates ($P = 0.03$; Fisher's test), the IVF efficiency ($P = 0.02$; Fisher's test) and blastocysts rates ($P = 0.04$; Fisher's test). This simple coculture system could replace the conventional maturation medium with gonadotropins, with a more efficient CR, lower rates of polyspermy and greater embryo development.

CLINICAL MEDICINE AND ODONTOLOGY

A81
STANDARDIZATION IN THE MEASUREMENT OF POST EXTRACTION ALVEOLAR CRESTS WITH LOCALIZED VOLUMETRIC TOMOGRAPHY

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Cone-Beam Volumetric Tomography (CBVT) allows to analyze images of cranium facial complex in three dimensions, allowing in this case, to measure and compare alveolar dimensional changes altered by reabsorption processes after an extraction, which makes difficult, over time, a conventional or implant supported prosthetic rehabilitation. The objective of this work was to design a standardized technique using, us references, fixed anatomical repairs for the measurement of vestibular, palatal, or lingual alveolar ridges after an extraction. A total of 17 patients with precise indications were selected, preparing a removable surgical thermoformed plate, prior to surgery, us alveolar protector against masticatory impacts, placing it to the experimental group (11 patients), socket with collagen, and the control group (6 patients), socket without collagen, for one month. CBVT was indicated for both groups, one immediately after surgery and the other after three months, allowing to compare the dimensional changes through their measurements and, thus, obtaining the rates of reabsorption. The results of the treated patients, the statistical averages obtained, using fixed anatomical repairs us references through which the corresponding traces are made, showed that the control group obtained less bone resorption than the experimental group, both in height and width, demonstrating that the use of the plate in the socket without collagen would be more effective as a preventive of the dimensional bone resorption; concluding that the standardized technique turned out to be effective for the measurement of alveolar contours, since there is no scientific evidence of measurements made in 3D images with immovable fixed points.
Key words: Conebeam, Bone resorption, Thermoformed plate.

A82
STRESS AND DIETARY HABITS DURING QUARANTINE IN THE CONTEXT OF COVID-19 PANDEMIC

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The global Covid 19 pandemic, leads to quarantine, and is a stressor that would affect the subjects at different levels and intensity. An important aspect is the impact of the emotions on dietary habits health. The objective of this work was to investigate the stress and dietary habits in adult subjects during quarantine period forced to stay indoors due to COVID-19 pandemic. Surveys were made for its on-line answer and then they were analyzed. 85 total adult participants both sex, 87.9% women and 12.1% men. We determined that 90.3% spent with its relatives all this times, 9.7% was alone. Relation to changes in dietary habits, 60.2% consider that there is a relationship between current personal situation and their eating behavior. Respect to appetite during quarantine times: 70% refer to changes, 60% increased appetite and 7.1% decreased or absence of appetite. In addition, were reported changes in the organization, quality, and quantity of food 77.5% while 1.2% keeping same. About the increase in the consumption of certain foods or beverages, we observed that 71.8% increased the flour consumption, 34% refined sugars, 22.4% fats and oils, 12.9% soft drinks and 5.9% increased the consumption of alcohol. However, 45.9% increased the consumption of fruits and vegetables, 21.2% dairy products and 35.3% meat. The 61.2% changed feeding schedules. Relation to changes in body weight, 61.2% reported an increase and 45.2% do simple daily physical exercises. 80.7% report a relationship between their habits, daily routine, and diet during quarantine and 60% report sleep disturbances. Regarding to stress, 11.8% reported calmness, 83.9% expressed negative emotions and uncertainly about the future, and 68.8% have difficulties of organizing its life in these quarantine times. Our results indicated that COVID-19 pandemic led to stress conditions that could cause a dramatic change in the dietary habits, which could lead to an increase in body weight and sleep disturbances. In the future, this analysis should be carried out again to determine that the normality has returned, after the long period of quarantine and the different times flexibilities in our region.

A83
HISTOLOGICAL STUDY OF HUMAN DENTAL ALVEOLS TREATED WITH XENO-GRAFT IN IMPLANTOLOGY

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Dental implants are the treatment to replace missing teeth. The success of this therapy is achieved if the existing bone is sufficient to surround and stabilize the implant. While, when it is not, the application of a bone graft or substitute is essential. The most widely used is the autograft or autologous bone due to its biocompatibility. However, the market offers other variants, such as bovine bone xenografts that are used in daily practice.

The objective of this study was to evaluate 3 bovine xenografts (BIOSS, TIOSS and SYNERGY) applied to alveoli after human extraction during prosthetic rehabilitation with implants. Bone tissue samples were treated with: Group 1, TIOSS; Group 2, BIOSS; Group 3, Synergy; Group 4, without substitute (clot). Histopathological studies were carried out. The interpretation of bone biopsies was made following ISO Standards 10993-6 Year 2007: A- Biocompatibility: inflammation, foreign body reaction (FBR), abscesses, necrosis, fibrosis, macrophages. B- Bone neoformation: type of bone, presence/absence of particles and rate of resorption. Histometric and statistical studies. The histological results obtained at 4 months were: (A) Biocompatibility: Group 1 – TIOSS: moderate chronic inflammation. Presence of FBR, abundant lymphocytes. Group 2 and 3 – BIOSS and Synergy similar behavior with a few chronic inflammation and absence of FBR. Group 4 (clot) – absence of FBR. None of the cases presented necrosis and/or abscesses. (B Bone neoformation: Group 1 – TIOSS: amorphous particles surrounded by numerous congestive vessels and predominantly lax connective tissue and newly composed bone (38%). Group 2 and 3 – BIOSS and Synergy: particles surrounded by fibrovascularized tissue and newly composed bone (47% BIOSS and 49% Synergy) with abundant fatty bone marrow. Group 4 (clot) – laminar bone type (45%). New bone percentages did not show statistically significant differences ($P = 0.2$). The reabsorption rate of the particles was low in the 3 substitutes evaluated by their persistence at 4 months post placement in the alveolus. From the results obtained, we conclude that BIOSS and Synergy had better biological behavior due to the absence of FBR. All three stimulated bone neoformation. The low reabsorption rate prevented alveolar bone atrophy after extraction and maintained the dimensions of the receptor zone; beneficial events for the stability of the implant and its osseo integration.
Key words: Bovine xenografts, Alveoli, Bone regeneration.

A84
OPTICAL, POLARIZED, METALLOGRAPHIC AND ELECTRON SCANNING MICROSCOPIES FROM OSTEOFORMATION BY POLYLACTIC-POLYGLYCOLIC ACID

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Tooth extraction is one of the most common dental procedures. This situation is followed by irreversible alveolar bone resorption during the first three months of healing, although dimensional changes can be observed up to a year later. The clinical consequences limit the availability of bone for implant placement. Among the existing therapeutics, bone graft materials (BGM) are the choice, since in addition to providing structural support, they promote osteopromotion. In the present study, Polylactic-Polyglycolic Acid (PLA-PGA) particles were characterized and biological behavior as BGM in dental alveoli was evaluated. The in vivo model used was the post extraction alveolus in Wistar rats. Animals were divided into 2 groups: 1) Control Group (CG): without MRO; 2), Experimental Group (EG): with PLA-PGA. Bone samples were evaluated with soft X-rays (SXR), histological with light microscopy (LM) and polarized light (PL), histometric (H) and statistics studies at weeks 1, 2, and 3 after surgery. The characterization was carried out with a metallographic optical microscope (MOM) and a Scanning Electron (SEM). Results: MOM: particle conglomerates composed of small grains. Average size: 18 ± 6 µm. SEM: particle conglomerates with an ampfractuous and irregular surface, with wide interconnected channels limited by discontinuous and porous walls. In vivo model: SXR: CG: varied radiopacity areas, random, without reaching the apex of the alveolar ridges. EG: low radiopacity images (PLA-PGA particles) surrounded by more radiopaque areas (newly formed bone). LM: GC: alveolus covered by lamellar bone EG: alveolus covered by fibrous connective tissue, PLA-PGA particles surrounded by lamellar bone and primitive bone marrow. PL: in two thirds dense fibrous connective tissue birefringent reddish orange and in the apical third areas of reddish newly formed bone (type I collagen), with some yellowish green areas (type III collagen). GE: superficial third, birefringent dense fibrous connective tissue of intense reddish and orange colors (type I collagen), middle and apical third trabeculae of laminar neoformed bone, red orange and yellow birefringent (type III collagen), with persistence of PLA- particles PGA surrounded by a loose, reddish-orange fibrous area. EH: Bone volume in the GC was 75.12% and GE 78.26% at 3 weeks without statistically significant differences ($P = 0.35$). PLA-PGA was biocompatible and behaved as an osteoconductor and osseostimulator since it promoted the formation of type I and III collagen and bone neoformation around its particles. The alveolar space was preserved. Key words: bone regeneration, alveolus, PLA-PGA.

A85
HUMAN PREMOLARS: STUDY OF OCCLUSAL PITS INJECTED WITH DYE AND THEIR RELATIONSHIP WITH DENTINAL STRUCTURES

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The occlusal face of the premolars is formed by fusion of embryological lobes and where the union is missing, the occlusal pits appear. These are superficial excavations of the enamel that ecological niches to trap bacterial plaque and food debris. They cross enamel and its deep part ends close to the dentin, a permeable tissue. Enamel and dentin are separated by a limit crossed by dentinal structures: canaliculi, spindles and Linderer plumes. These teeth can get caries, a multifactorial disease whose anatomical factor could be enhanced if we verify that the dye injected into the occlusal pits passes into dentin and its structures. Our objectives: histologically classification of enamel located around terminal part of occlusal pits; form that the terminal takes; infiltration of dye into dentin and its structures. Descriptive-relational analysis, comparison between and within groups, Mann–Whitney test/5% bilateral test. We included healthy premolars, both sexes, extracted for orthodontic reasons at IUNIR, public and private dental centers. The roots were immobilized in molds and their crowns were left emerging, the dye was injected under pressure and allowed to dry. The crowns were devastated by their free faces until their proximity to the occlusal pits, the remnants were cut, and these were subjected to the technique of wear. The transparent sheets were examined at higher magnification with OM. Total, 30 teeth, 15 upper premolars (PMS) and 15 lower premolars (PMI). The PMS group was represented by 33% male and 67% female, mean age 15 ± 7.6; in PMI were 53% and 47%, average of 18 ± 9. The enamel surrounding the terminal of the occlusal pits there was classified in irregular: PMS 67%; in PMI 80% ($P = 0.35$), with fissures 40% and 33% respectively ($P = 0.99$), fissured-bonded to dentinal structures 13% in PMS and 53% in inferiors ($P = 0.0068$). The terminal shape on: narrow 34% in

PMS and PMI 20% ($P = 0.35$); rounded and larger 54% in PMS and 93% in PMI ($P = 0.0049$); fucsine infiltrated in dentin and its structures: 20% in PMS and 7% in PMI ($P < 0.0001$). In PMI group, there was majority of patients male of older average age, unlike the PMS group. The irregular enamel around terminal of occlusal pits is similar to the knotty at of the cusps and could have the function equal: resist the forces of chewing. Two characteristics in PMI group can predisposing to caries, the fissured enamel-attached to dentinal structures and the termination form of the rounded and larger occlusal pits that originates a greater contact surface. The dye step was scanty, we should try other means to spread it and deepen this topic.

A86
IMPLEMENTATION OF LASER AS A COMPLEMENT TO ENDODONTICS

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Introduction. Endodontics prevents and treats diseases of the endodontic, apical, and periapical region. This implies that the content of the interior of the root canals must be removed, including bacteria, toxins and the smear layer generated by the instrumentation itself. Success rates decrease significantly from 95% to 85% in teeth diagnosed with necrosis and therefore with a higher microbial presence. The smear layer has a penetration capacity in dentin of 40 µm, bacteria can colonize to a depth of 1,100 µm, while sodium hypochlorite, the most widely used irrigant due to its high disinfecting efficacy, only has a penetration power of 130 µm within the dentinal tubules. Over the years, various preparation techniques, irrigation protocols and intracanal medications have been developed with the aim of improving results, while one of the greatest difficulties still is the anatomical complexity. Currently, the implementation of the laser is suggested to achieve the disinfection of the canal system and the removal of the smear layer, since this type of therapy is effective even in areas of difficult access. However, we must emphasize that today it is considered a co-adjuvant to traditional endodontic treatment and seeks to potentiate and favor the action of all the elements involved in it, but so far it has not been able to replace it. The laser can be implemented with these objectives by means of two techniques, photoactivated disinfection (PAD) and Photon induced photoacoustic streaming (PIPS) as well as a biostimulant of soft tissues. Objectives**.** Taking into account the exposed information, this investigation project aims to evaluate the penetration power of the laser inside the dentinal tubules. Methodology. We will work in vitro on a sample consisting of 60 teeth with a diagnosis of necrosis and an indication for extraction. One group will be a witness, while another will receive an irrigation and instrumentation protocol and the last one, the same protocol plus the application of the Biolase Epic 10 Laser as a complement of the cleaning process. Results. Due to the impossibility of continuing the investigation this year due to SARS-CoV-2, we have only reached partial results during the second stage of the project. From the total of the collected sample, the pieces of the control group were selected to section them, observe them under the clinical microscope and prepare them for histological visualization**.** Discussion. Disinfection of the canal system is very difficult to achieve, especially in the apical part of the canal, isthmus, fins, and irregularities, despite the technological advances that have occurred in the field of Endodontics. Laser therapy has shown very promising results in achieving these goals.

A87
NON-HIGH-DENSITY LIPOPROTEINS-CHOLESTEROL AS CARDIOVASCULAR RISK PREDICTOR IN OBESE β LINE RATS

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Low-density lipoproteins cholesterol (LDL-C) is the most commonly accepted lipid cardiovascular risk predictor parameter, however lately its usefulness has been questioned. Instead, the value of cholesterol not bound to high-density lipoproteins (non-HDL-C) is proposed as the best predictive tool. By definition, non-HDL-C represents essentially the sum of all atherogenic particles, since it includes not only LDL-bound cholesterol but also that of medium (IDL) and very low (VLDL) density lipoproteins as well as particularly atherogenic remnants of VLDL. Non-HDL-C, as measuring the magnitude of the balance between atherogenic and antiatherogenic lipoproteins, becomes especially important in clinical situations such as diabetes mellitus, metabolic syndrome or visceral obesity, all of them characterized by an increase in plasma triacylglycerols (TAG). Rats of the β line show moderate obesity since peripuberty; obesity is more noticeable in males. They are normocholesterolemic, hypertriacylglycerolemic, glucid intolerant and finally diabetic at advanced age. The objective was to evaluate LDL-C and non-HDL-C cardiovascular risk predictive ability in rats of the obese β line. We worked with animals (n ~ 32) of both sexes and different ages (70, 120, and 250 days). After fasting, blood was extracted by puncture of the caudal vein and total cholesterol (COL), TAG and HDL-C were measured in plasma. Non-HDL-C was calculated as the difference (COL − HDL-C) and LDL-C with Friedwald formula (LDL-C = COL − (HDL-C + TAG / 5). Non-HDL-C < 130 mg/dL and LDL-C < 100 mg/dL were considered as normal values. Data were analyzed with the statistical program Prism 3.0; Student's *t*-test was applied to assess sexual dimorphism and a significant difference was considered when $P < 0.05$. Results are expressed as mean ± standard deviation. No HDL-C (mg/dL): 70 days: males: 43.75 ± 6.62 *vs*. females: 67.50 ± 4.25 ($P = 0.023$); 120 days: males: 87.75 ± 4.73 *vs.* females: 76.75 ± 5.15 ($P = 0.18$); 250 days: males: 157.00 ± 15.0 *vs.* females: 78.75 ± 4.19 ($P = 0.020$). LDL-C (mg / dL): 70 days: males: 31.25 ± 3.14 *vs.* females: 57.75 ± 3.47 ($P = 0.001$); 120 days: males: 57.70 ± 1.32 *vs.* females: 63.50 ± 5.31 ($P = 0.38$); 250 days: males: 64.25 ± 6.71 *vs.* females: 35.35 ± 6.50 ($P = 0.03$). No HDL-C increased with age; males at 250 days exceeded normal value and significantly differed from females. LDL-C remained normal in both sexes. In male β rats, No HDL-C would provide more accurate information on cardiovascular risk than LDL-C; the latter, since it does not reflect the true increase in atherogenic lipoproteins, would underestimate risk. These results should be considered when evaluating the efficacy of dietary and/or pharmacological interventions aimed at reducing cardiovascular risk. It is planned to study the correlation of these lipid parameters as indicators of atherosclerosis with the thickness of the carotid intima-media.

A88
PHENOTYPIC RE-CARACTERIZATION OF A MURINE MODEL OF OBESITY AFTER CHANGING HABITAT

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Laboratory animals constitute a biological reagent, and their "homogeneity" is essential. The metabolic and physiological alterations that can produce changes in the environment in which the animal is housed, could modify the response to treatments. That is why it was evaluated as imperative to re-characterize the IIMb /β rats when for its breeding and maintenance they were transfer from the bioterium of Cátedra de Biología–Facultad de Ciencias Médicas–Universidad Nacional de Rosario (CB) to the facilities of the Centro de Investigación y Producción de Reactivos Biológicos (CIPReB). Rats of the β line show moderate obesity since peripuberty; obesity is more noticeable in males. They are normocholesterolemic, hypertriacylglycerolemic, glucid intolerant and finally diabetic at advanced age. Biomass (BIOM), basal glycemia (GLU), triacylglycerolemia (TAG) and cholesterolemia (COL) values of a representative set of the last five years of male CB rats (N ~ 30) were compared with the corresponding values of animals from CIPReB (N ~ 32) at different ages: 70, 120, and 250 days old. Perigonadal (PAP) and retroperitoneal (PAR) adipose panicles of 250 days old animals were compared. Statistical analysis was performed with Student's *t*-test, significant difference $P < 0.05$. Results are expressed as mean ± standard deviation. BIOM (g): 70 days: CIPReB: 261.50 ± 7.31 *vs.* CB: 278.80 ± 4.48 ($P = 0.31$); 120 days: CIPReB: 382.30 ± 5.70 *vs.* CB: 367.50 ± 7.94 ($P = 0.15$); 250 days: CIPReB: 469.30 ± 11.66 *vs.* CB: 464.70 ± 9.55 ($P = 0.81$). GLU (mg/dL) 70 days: CIPReB: 144.3 ± 7.5 *vs.* CB: 114.1 ± 2.0 ($P = 0.06$); 120 days: CIPReB: 217.8 ± 8.3 *vs.* CB: 216.1 ± 9.0 ($P = 0.89$); 250 days: CIPReB: 245.5 ± 5.5 *vs.* CB: 201.8 ± 8.2 ($P = 0.01$). TAG (mg/dL): 70 days: CIPReB: 109.3 ± 16.7 *vs.* CB: 127.9 ± 3.5 ($P = 0.26$); 120 days: CIPReB: 169.0 ± 28.7 *vs.* CB: 251.9 ± 18.6 ($P = 0.22$); 250 days: CIPReB: 393.7 ± 82.0 *vs.* CB: 213.4 ± 21.8 ($P = 0.10$). Col (mg/dL): 70 days: CIPReB: 70.5 ± 10.0 *vs.* CB: 117.6 ± 1.4 ($P = 0.001$); 120 days: CIPReB: 133.5 ± 6.1 *vs.* CB: 209.6 ± 13.8 ($P = 0.11$); 250 days: CIPReB: 231.8 ± 21.0 *vs.* CB: 229.0 ± 15.0 ($P = 0.92$); PAP (g/100 g of biomass): CIPReB: 2.53 ± 0.25 *vs.* CB: 2.29 ± 0.10 ($P = 0.30$); PAR (g/100 g of biomass): CIPReB: 3.33 ± 0.42 *vs.* CB: 4.10 ± 0.26 ($P = 0.14$). Changing habitat would have affected nor biomass neither relative weight of abdominal adipose panicles, but it did modify plasma variables: in CIPReB, diabetes would be of earlier installation and in advanced maturity, plasma glucose and triacylglycerolemia would be significantly more altered than in CB.

A89
LIVER PRENEOPLASIA INDUCTION IN SPONTANEOUS DIABETIC RATS (eSS). STEREOLOGICAL ANALYSIS OF THE LIVER TISSUE AND THE ENDOCRINE PANCREAS

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Diabetes Mellitus (DM) and hepatocarcinoma (HC) share risk factors. To study whether there is a greater predisposition to developing HC due to diabetes and/or suffering alterations in the endocrine pancreas (EP), animal models are used. A line of spontaneously diabetic rats, eSS, produced at CIPREB was used to study the HC-DM association. Hepatic preneoplasia (HP) was induced with a biphasic model using diethylnitrosamine (inducer) and 2-acetylaminofluorene (promoter). The aim was to analyze hepatic preneoplastic foci (PF) development and the PE tissue of eSS rats with HP by stereological methods (SM). eSS and Wistar (W, controls) rats of 190 days (d) (N = 4) were induced for HP development. The liver (L) and pancreatic tissue were extracted and processed for histological studies. PFs were identified with anti GST-Pi antibody. Pancreatic tissue was stained with H&E and trichrome methods. A 121-point grid was used for SMs. In 20 microscopic fields, the volume density occupied by the PF (Vvpf) and the number of PF per unit area (N°.pf/a) in the L and the volume density of Langerhans islets (LI) (Vv.LI) and the number of LI per unit area (N°LI/A) in EP, were measured. Data for PF were analyzed with a mixed ANOVA model and the ones from EP, with Poisson regression (mean ± SD value, significant: $P < 0.05$). Vvpf and Nºpf/a, were significantly lower in eSS than in W: Vvpf, $P = 0.020$ and Nºpf/a, $P = 0.001$. In EP, no significant difference was observed between W and eSS, Vv. LI, $P = 0.800$ and N°LI/A, $P = 0.074$. We conclude that the induction of HP to eSS rats affected hepatic PF and no influenced the PE.

A90
COMPARATIVE STUDY OF POST EXTRACTION ALVEOLAR BONE REABSORPTION WITH COLLAGEN SPONGES AND ALVEOLAR PROTECTIVE PLATE

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After a tooth extraction, bone resorption and remodeling process inevitably occurs, which represents a problem for the rehabilitation, where size and morphology of the alveolar crest is modified. Dental extraction and bone resorption are the two main causes of alveolar bone deformities after healing. Clinical studies have documented an average of 4.0–4.5 mm of horizontal bone resorption after extraction procedures. Other studies have documented significant dimensional changes around the alveolar bone after extraction procedures. Clinical evaluation and tomographic comparison of bone repair process and alveolar ridge preservation of intact walls post extraction alveolus, with and without the use of bone substitute material based on intraalveolar collagen sponges as filler and physical barrier with an alveolar protective plate during healing process for three months. Patients of both sexes with normally implanted and clinically acceptable tooth with extraction indication were selected, applying the Exclusion criteria. Clinical and radiographic examination and Application of the Surgical Protocol. Impression of the field in order to build a 0.8 rigid thermoformed protective plate. Antibiotic treatment and antiseptic mouthwashes 48 h before the surgery. Surgical phase: Non traumatic Extraction

Technique, use of periostotomo for the luxation of the dental element to be extracted, a meticulous toilette of the wound, in experimental group placement of the collagen sponge inside the socket and closure with approximation suture. Placement of previously made in experimental and control group protective plate to be used during chewing for a period of 30 days. Post-operative hygiene care with the use of mouthwashes with 0.12% digluconate of chlorhexidine until suture removal. Immediate postoperative cone beam tomography and at 3 (three) months to measure and analyze the residual alveolar ridge. Records in the patient's clinical history of the results obtained and statistical analysis of the data. The results obtained showed that the control group obtained less bone resorption than the experimental group, both in height and width, demonstrating that the use of the plate in the alveolus without a collagen sponge would be more effective in minimizing bone resorption. *Key words: Bone regeneration, Collagen sponge, Dental extraction, Bone healing, Post-extraction alveolus, Alveolar protective plate.*

A91
CHANGES IN THE EXPRESSION OF THE 2-PORE DOMAIN POTASSIUM CHANNELS TASK1, TASK3 AND TRESK IN ORAL SQUAMOUS CELL CARCINOMAS

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Potassium channels have been proposed to promote cancer cell proliferation and metastases. Thus, we investigated the expression pattern of three 2-pore domain potassium channels (K2Ps) TASK1, TASK3 and TRESK in oral squamous cell carcinoma (OSCC), the commonest oral malignancy. The ultimate goal of the study is to identify new potential therapeutic targets to treat advanced OSCC. To achieve this goal, we studied the expression of TASK1, TASK3 and TRESK in human samples of SCC of oral origin. We carried out immunohistochemistry using validated antibodies to study the distribution and expression pattern of TASK1, TASK3 and TRESK in normal versus cancerous tissue. We also examined the expression of β-tubulin III (β-tub3), a marker associated with resistance to taxane-based chemotherapy and poor patient prognosis, and its correlation with the levels of expression of the aforementioned K2Ps. Immunohistochemistry on human SCC samples was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The retrospective study was limited to anonymous samples obtained from the Tissue Bank of the Faculty of Odontology (UN de Cuyo). Albeit the study was conducted anonymously we did obtain informed consent from the patients. The Bioethics Committee of the Faculty of Odontology approved this study (CUY 0040112/2018 and 102/2018 CD). We found that TASK3 was significantly up regulated whereas TASK1 and TRESK were both significantly downregulated in advanced, poorly differentiated OSCC. In a few samples of dysplastic tissue, we did not observe a significant change in the expression levels of these ion channels. In addition, human SCC showed a significant increase in the expression of β-tub3, whereas normal and dysplasic tissue exhibited very low levels of this protein. Interestingly, in OSCC the expression of β-tub3 correlated positively and significantly with TASK3 and TRESK, but not with TASK1. We conclude that the changes in expression and the co-localization with a marker of resistance to taxanes like β-tub3 turn TASK1, TASK3 and TRESK into potentially new prognostic tools and possibly new therapeutic targets for the treatment of advanced OSCC.

PHARMACOLOGY AND TOXICOLOGY

A92
PROTECTIVE ROL OF HEAT SHOCK PROTEINS (HSP) IN CADMIUM INTOXICATED RAT LUNGS. EFFECTS OF DIFFERENT PROTEIN SOURCES

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Heat shock proteins are a superfamily of highly conserved proteins that have an important role in folding/degradation of proteins, antigen presentation and immune response regulation. Their expression can be used as a sensitive biomarker when cells are under stress conditions, as microorganisms' infections, chemical substances, or temperature. We decided to study their expression in rat lungs with a subchronic intoxication with cadmium, analyzing the possible protective effects of a soy-based diet. 4 lots of adult Wistar rats were used: 2 lots received casein and 2 lots, soy as protein source. Within each group, 1 lot received regular tap water and the other 15 ppm of Cd (as $CdCl_2$) in drinking water for 60 days. Lungs were removed, fixed and paraffin embedded. Immunohistochemistry was realized using Hsp27 and Hsp70 antibodies. Some sections were hematoxylin-eosin stained for basic histologic assessment. Results showed that Hsp27 expression increased in both Cd intoxicated groups, being higher in soy fed groups. Hsp70 expression showed no differences between casein groups, but it revealed a significant increase in Soy–Cd *vs.* its control. Histological assessment revealed a loss of normal pulmonary histoarchitecture in Cas–Cd group, with a wide infiltration of connective tissue, fused alveoli, and capillary fragility. Injuries were less severe in Soy–Cd group, with infiltration zones just in lungs periphery. Cadmium intoxication generates injuries in lungs, which were less severe in soy fed animals. This could be related with higher Hsps levels found in those groups and their protective effects evidenced in the mostly conserved histoarchitecture in soy groups.

A93
STUDY OF NUCLEOLAR ALTERATIONS IN *Allium cepa* MERISTEMATIC CELLS USING SILVER STAINING (AgNOR)

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In a work carried out in our laboratory, in 2019, the genotoxicity of the consumption water of an educational entity in the city of San Luis, Province of San Luis was evaluated, through the *Allium cepa* test. Seven tap water samples were analyzed in seven school bathrooms. The results indicated irregularities in root growth, cell division, chromosomal and nuclear aberrations in all samples analyzed. However, sample number 7 warned a significantly higher percentage of nuclear aberrations, particularly an apparent increase in the number of nucleoli per cell was observed at the interface. Nucleolar alterations resulting from the action of chemical and physical agents can be important biomarkers of genotoxicity. The regions of the transcriptionally active nucleolar organizer (NORs), called Ag-NOR, are associated with nucleolar proteins, and are brown spots when dyed with silver. The objective of this work was to identify and confirm the presence of nucleolus in aberrant cells of *Allium cepa* using the silver staining technique (AgNOR). Apical meristems of *A. cepa* bulbs placed in distilled water (control treatment) and water sample number 7 (experimental treatment) were obtained and the classic methods for obtaining mitotic and silver staining preparations (AgNOR) according to Funaki *et al*. (1975) were performed. At least 1000 cells were tested per treatment. In control treatment, 100% of the cells in the interface stage showed one to three nucleolus per cell, which is typical in this species. In experimental treatment, 80% of cells had more than three nucleolus per cell at the interface. Silver staining revealed the presence of nucleolus in a higher proportion in sample preparations N°7 compared to the control samples. The increase in the number of nucleoli in aberrant cells, showed to be valid as a genotoxicity marker, and is an important parameter to use in environmental monitoring studies. These results strengthen what was concluded in the previous work regarding the presence of metal ions in the tested water sample causing aberrations and damage to the genetic material of *A. cepa*. From this work an investigation into the presence of these pollutants in the analyzed drinking water distribution network would then be desirable.

A94
MORPHOMETRIC ALTERATIONS OF CEREBELLUM INDUCED BY SUBCHRONIC CADMIUM INTOXICATION: THE PROTECTIVE ROLE OF A SOYBEAN DIET

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Cadmium (Cd) is an environmental contaminant. The aim of this study was to characterize its toxicity in cerebellum and the potential reversal by a vegetarian based diet. We determined the Cd levels in total blood, metals trace and Cd concentration in tissue, and also performed morphometric and stereological analysis. Female Wistar rats (12 animals/group) were fed with casein (Cas) and soybean diets (So) as protein source for 60 days. Simultaneously, half of the animals were administered either 15 ppm of Cd in water or water as control ad libitum. Morphometric analysis included quantifying the number of granule cell neurons (CGn) and Purkinje cells neurons (Pkn) in serial 20 µm-thick sections stained with cresyl violet along different lobules. We performed a three-dimensional volumetric reconstruction of the tissue and further quantification of the number of neurons through the use of a software Stereo Investigator. The thicknesses of the molecular and granular layers of the cerebellar cortex of lobules I-X were determined on digital images of 3 regions of each lobule and were analyzed with IMAGE J Software. Metal concentration was determined with an ICP-MS. Cd levels in total blood were incremented in CasCd *vs*. CasCo ($P < 0.01$) and in SoCd *vs*. SoCo ($P < 0.05$). Cd concentration in tissue was increased in CasCd *vs*. CasCo and *vs*. SoCd ($P < 0.0001$), with no significant differences between soy groups. Also, the trace elements were unbalanced along the Cd intoxication. Regarding selenium levels, a significant increase was observed in the SoCd group *vs*. CasCd. Likewise, manganese and zinc concentrations were significantly increased in CasCd *vs*. its control and *vs*. SoCd ($P < 0.0001$), while there were no differences between soy groups. In the case of copper, a significant increase was observed in the CasCd group *vs*. its control ($P < 0.01$) and *vs*. SoCd ($P < 0.05$). We found that sub chronic Cd exposure induces a decrease in the number of CGn in the CasCd groups *vs*. CasCo group ($P < 0.05$) and SoCd group ($P < 0.01$). On the contrary, the number of Pkn remained unchanged. In addition, Cd intoxication significantly reduced the internal granular layer of CasCd group *vs*. SoCd group in all the lobules tested, meanwhile no effect was observed in the thickness of the molecular layer. The thicknesses of the different regions of each folium from cerebellar lobules I-X did not show significant differences between the groups. Overall, these results unmask an irreversible toxic effect of low dose sub chronic Cd intoxication on cerebellum and identify a protective role by soy-enriched diet with potential as a therapeutic strategy for those individuals exposed to this dangerous environmental contaminant.

A95
HISTOLOGICAL ANALYSIS AND MORPHOMETRIC ALTERATIONS IN THE MAMMARY GLAND BY CADMIUM INTOXICATION. SOY-BASED DIET EFFECTS

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Cadmium (Cd) is a heavy metal and an important environmental contaminant, while Soy occupies an important place in the human diet and its benefits are widely reported. We studied the effects of Cd on the histoarchitecture of the rat mammary gland (MG) and also observed the effect of a soy-based diet. 4 batches of female Wistar rats were used: 2 batches received casein (Cas) and another 2 batches soy (Soy) as a protein source. Within each group, 1 lot received drinking water (Control-Co) and the other, 15 ppm Cd in water for 60 days (N = 6 per group). After the deadline, the animals were cycled and sacrificed in Diestro II. The MGs were extracted and immersed in Bouin solution, then they were stained with

hematoxylin-eosin for their subsequent analysis. First, the general morphology was visualized, and they were photographed with an optical microscope at different magnifications. In the CasCo group, a predominance of adipose tissue (AT) was observed with marginally located connective tissue (CT), while in CasCd the CT advanced over the AT. Regarding glandular development, it was higher in the CasCd group. The SoyCo group showed tree-like glandular development, and large diameter ducts with secretions were observed in SoyCd. The groups fed with Soybeans showed lower AT content compared to the Casein groups. Subsequently, an estimated percentage of mammary adipocytes was calculated by analyzing four micrographs per group using the ImageJ program. When comparing the percentages in the 4 experimental groups by OneWayANOVA, significant differences were found (**$P < 0.01$); a decrease in AT was observed in the SoyCo group with respect to CasCo (*t*-test, **$P < 0.01$). Finally, to determine the size of adipocytes in four experimental group, the areas of adipocytes were measured in 3 random fields (80–100 cells per field) from 3 individual micrographs using the Image Pro 10 program; Soy diet resulted in a smaller average size compared to the Casein diet. In conclusion, Cd impacts tissue development and histoarchitecture, which could lead to a loss of functionality. These effects are conditioned by nutrition: the estrogenic action of Soy is being perceived on breast tissue and the double treatment (SoyCd) causes notable histological damage and abnormal development in young adult virgin rats.

A96
CYTOTOXICITY AND CHARACTERIZATION OF SECONDARY METABOLITES OBTAINED FROM *Anagallis arvensis* L

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Anagallis arvensis L. is traditionally used with medicinal purposes as antifungal, antiviral, healing, anti-inflammatory, sedative, expectorant, and diuretic. In previous works we validated the antifungal activity of the ethanolic extract against *Candida albicans* strains, and the bioactive metabolites were partially characterized. The aims of this work were to optimize the obtaining of the complete triterpenic fraction, analyze the composition of present metabolites, and evaluate its cytotoxicity. Triterpenic fraction of *A. arvensis* (FTA) was obtained by precipitation with acetone, and purified by silica gel column chromatography, composition profiles were analyzed by TLC on silica gel revealed with sulfuric p-anisaldehyde. Ultra-high pressure liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) was used to analyze FTA. Cytotoxicity was evaluated on human lymphocytes isolated from peripheral blood (HLPBs), cultured in complete RPMI 1640 medium, at 37°C 5% CO_2 conditions, under activation or not by treatment with lipopolysaccharide (LPS). It was determined: cellular metabolic activity, measuring the reduction of 3- (4,5 dimethylthiazolyl-2) 2,5 diphenyl tetrazolium bromide (MTT 2.5 mg/mL) on ELISA reader at 550 nm; and membrane integrity, by exclusion of Trypan blue dye observed and counted on Neubauer chamber under optic microscope. FTA was tested for concentrations in the 0–100 µg/mL range. All experiments were performed in duplicate and statistically analyzed accepting $\alpha = 5\%$. FTA presented significant cytotoxicity at 100 µg/mL (less than 75% viability). However, at concentrations lower than 50 µg/mL, a viability ≥ 75% was reached, comparable to the viability control of the cell model tested. The UHPLC-MS/MS experiments allowed the identification of nine triterpenic glycosides, derived from oleanolic acid: Methylanagallosaponin I; Anagallisin A, B, C and D; Anagallosaponin II; Anagalloside A; monomethoxylated Anagalloside A and 3 Beta-[2-O-beta-D-Glucopyranosyl-4-O-[2-O-beta-D-xylopyranosyl-3-O-(3-O-beta-D-glucopyranosyl-beta-D-glucopyranosyl)-beta-D-glucopyranosyl]-alpha-L-arabinopyranosyloxy]-13,28-epoxyoleanano-16 alpha, 30-diol. These last two were informed for the first time for *A. arvensis*. These results show that FTA could be a viable alternative for medicinal use and the information obtained is being used in anti-inflammatory activity studies by our research group.

A97
STRUCTURAL BASES OF THE INTERACTION BETWEEN CYCLOOXYGENASE 1 AND FLAVONOIDS

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Inflammation is present in various pathologies, and it can be simulated using different animal models. Many substances are generated and are involved in the inflammatory process such as histamine, serotonin, prostaglandins, PAF, on the other. The enzyme cyclooxygenase (COX) is responsible for the generation of prostaglandins, and it is found in two isoforms COX 1 and 2. On the other hand, in previous works, flavonoids have shown gastric and intestinal anti-inflammatory and protective activity in animal models of paw edema induced by carrageenan, granuloma test, gastric ulcer induced by absolute ethanol and ulcerative colitis induced by 10% acetic acid. Among the flavonoids tested, it was observed that those that present in their chemical structure the presence of hydroxyls or oxymethyls in position 3'4' in ring B have a higher activity in the models tested. The aim of this work was to observe the possible interaction of the flavonoids 7-O-methyleriodictyl, nepetin, 7-O-methylsudachitin and quercetin with the enzyme COX 1 as responsible for the mechanism of action of these compounds in different animal models of inflammation and gastrointestinal protection tested. The Autodock and SwissDock programs were used to perform the *in-silico* binding (docking) of these compounds to the COX 1 enzyme. For this, the structural data of the COX 1 protein were used human (6Y3C) recently deposited in the Protein Data Bank (PDB). The binding of the compounds to the enzyme were observed using the Pymol and Chimera programs. The results suggest a strong correlation between bindings of compounds to COX 1 determined by *in silico* docking.

A98
SAFETY OF PEANUT (*Arachis hypogaea* L): CYTOGENOTOXIC STUDIES AND CHEMICAL COMPOSITION ANALYSIS

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Peanut (*Arachis hypogaea* L) is an economically important legume used for direct consumption as well as for manufacturing numerous food products. Argentina is one of the major peanut producers in the world and about 90% of its crop is produced in the province of Córdoba. This species also has numerous medicinal properties such as antioxidant, antiviral, antitumor. The objective was to determine the *in vivo* toxicity and genotoxicity of the peanut seed and to analyze its chemical composition. A peanut seed ethanolic extract (SEE) was obtained by a simple alcoholic extraction method. A chemical analysis was carried out: the content of total phenols was determined by Folin-Ciocalteu method, and the fatty acid composition was determined by gas chromatography; lipids were extracted with the Folch method and methylated with sodium methoxide. The separation, quantification, and identification of total FA methyl ester resultants were performed using a capillary column (20 m length × 250 mm id × 0.25 mm film tickness, SUPELCO) of polyethylene glycol in a Clarus500 GC and all fatty acids were identified using a commercial standard (Nu-check). All values were expressed as the total percentage area. On the other hand, *in vivo* cytogenotoxic studies were carried out: Balb/c mice (20 g) in groups of 6 animals (3 males and 3 females) were formed and inoculated with different concentrations of SEE (500, 1000, and 2000 mg/kg b.w.) diluted in physiological solution (PS) and dimethylsulphoxide (DMSO). A negative control group (25 µL of DMSO in 775 µL of PS) and a positive control (cyclophosphamide 20 mg/kg b.w.) were included. At 24 h, mice were sacrificed by decapitation and, bone marrow samples were taken from the femur for the micronucleus test and blood for comet assay. The chemical analysis of SEE indicated a content of total phenols of 15.05 ± 0.06 mg of GAE/g of dry extract. Linoleic acid (58.84%), oleic acid (11.31%), and palmitic acid (8.37%) were major compounds of SEE. *In vivo* cytogenotoxic studies showed that SEE was not genotoxic at the concentrations evaluated by the micronucleus and comet assays. In relation to the toxic capacity, the toxicity index values revealed non-significant differences ($P > 0.05$) between the negative control system and the treatments of SEE (500 and 1000 mg/kg), with the exception of the highest concentration of the extract (2000 mg/kg, $P = 0.0003$), which revealed some toxicity. In conclusion, peanut consumption is safe at concentrations recommended for healthy uses, such as nutrition, and phytomedicine.

A99
BIOGUIDED PURIFICATION AND CHARACTERIZATION OF AN ANTIBACTERIAL COMPONENT OF BARK TINCTURE FROM *Caesalpinia paraguariensis* BURK

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In previous works, antibacterial activity and toxicity of partially purified fractions of bark tincture from *Caesalpinia paraguariensis* Burkart. (Fabaceae) were determined. The fraction with the highest antibacterial activity (ethyl acetate), also exhibited high toxicity. Therefore, this work focused on purifying and separating the antibacterial component/s from the toxic/s present in the acetate-ethylic fraction (AEF), to characterize it/them chemically, determine their Minimum Inhibitory Concentration (MIC) and cytotoxicity. AEF was fractionated by CC-RP (C18) with water-methanol gradient (0–100%), sub-fractions obtained were analyzed by TLC silica gel and NP-PEG reagents under UV_{365nm} light, whose composition profiles allowed to separate eight different groups (G1-G8). Antibacterial activity of sub-fractions was assessed against *Staphilococcus aureus* ATCC 25923 (10^6 cfu/mL) by direct bioautography (3 mL ssMH inoculated medium, incubation at 37°C during 24 h), which was revealed by spray of MTT solution (2.5 mg/mL). MIC/MBC were determined against *S. aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, by microdilution in MH broth, and next subculture on agarized MH medium, according to CLSI protocols. The toxicity was tested determining viability of *A. salina* exposed to AEF, G6 or reference compounds between 1 and 1000 µg/mL (LC50) for 24 h (25°C). Survival percent human lymphocytes isolated from peripheral blood (HLPBs) cultured in RPMI 1640, exposed to G6 between 1–200 µg/mL and incubated (37°C, 5% CO_2, 24 h), was determined by metabolic activity assay measurable through MTT redox reagent, by ELISA multiplate reader at 550 nm. Chemical characterization of the most active sub-fraction was done by analyzing HPLC(DAD)-EM(ESI/Q-TOF), UV-Vis spectroscopy, TLC with specific revealers to phenolic compounds, searching in data base and specialized bibliography. Through bio-guided sub-fractionation a component was purified (G6) with bacteriostatic activity against assayed strains (MICs: 125-500 µg/mL), not toxic (CL_{50} > 1000 µg/mL), and not cytotoxic on HLPBs (cell survival > 75 percent at 200 µg/mL). TLC analysis revealed at *Rf* 0.7 a fluorescent yellow-orange spot, consistent with flavonoid-type phenolic compounds. A peak at *RT* 13.7 min was detected by HPLC-MS, compatible with the presence of flavonoid compound according to its UV-DAD spectrum [λmax (MeOH) 248, 366 nm], whose mass was 286 a.m.u., and $C_{15}H_{10}O_6$ its most probable molecular formula. The database and bibliography searching yielded on least six structures of same kind. The UV-Vis spectrum and characteristic color on TLC allowed to define the identity of the compound as fisetin. This flavonol was reported previously on species of Fabaceae and other families, in colorful fruits and their juices, but it is the first time that it has been identified for *C. paraguariensis*.

A100

EXPERIMENTAL STUDY OF BIOCHEMICAL AND STRUCTURAL EFFECTS OF METHYLPHENIDATE IN RENAL AND CARDIAC TISSUE IN RATS

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For treatment of attention deficit and hyperactivity, one of the psychostimulant drugs used is methylphenidate, pharmacologically similar to cocaine and amphetamine. There is insufficient evidence in long-term treated patients with this drug, in terms of modifications in biochemical and anatomopathological parameters. Objectives: To evaluate in experimental animals, biochemical and structural modifications in renal and cardiac parenchyma produced by methylphenidate. Materials and methods: adult white Wistar rats 4 groups (N = 2): (1) with ad-libitum water, (2) treated with methylphenidate, 20–30 mg/day (therapeutic) one month, (3) treatment group 2 2 months. (4) same treatment group 2, 3 months. Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and creatinine were determined. Renal and cardiac parenchymas were evaluated by optical microscopy with hematoxylin-eosin. Results: Group 2, no biochemical modifications or anatomophysiological alterations were found. In groups 3 and 4, significant differences were found in terms of total cholesterol, HDL cholesterol, LDL cholesterol and creatinine. There are no significant differences in triglycerides. All groups with methylphenidate showed weight gain *vs.* control group. Pathological anatomy: in groups 3 and 4, macroscopically weight gain was observed in the two organs studied. Microscopically: at the heart level they had bleeding in the right cavity and right atrial enlargement. At renal level, both showed macro glomeruli with slight increase in cellularity. Tubules exhibited intraluminal cell flaking with focal nuclear loss linking to tubular necrosis changes. Congestion was observed in the interstitium and vessels and only in group the presence of papillary proliferation and angioectasia was found. Conclusion: This study coincides with the literature on the need to expand research related to dosage, time and pathologies associated with the use of methylphenidate.

A101

IN VITRO CYTOTOXICITY OF *Condalia microphylla* AND *Schinus johnstonii* LEAF EXTRACTS

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In the last decades, the interest for studying the biological activities of natural products for food and/or therapeutic purposes has been increased. Ethnobotanical research reveals that *Condalia microphylla* (piquillín, Cm) and *Schinus johnstonii* (molle, Sj) shrubs, from Patagonian Monte, are used as food (alcoholic beverage) and medicine (Cm, fever-reducing and laxative; Sj, analgesic, anti-inflammatory, anesthetic, healing, balsamic, anti-catarrhal, antirheumatic, purgative and antimicrobial). However, determining natural extractsafety is an essential preliminary step indispensable for its use. The aim of this work was to determine the *in vitro* cytotoxic effect of two leaf extracts from *C. microphylla*: ethanolic extract (EE-Cm) and methanolic extract (EM-Cm), and methanolic extract from *S. johnstonii* (EM-Sj) leaves. To this end, the different extracts were tested in a wide range of concentrations (0.025–2 mg/mL) on the eukaryotic cell line Vero (*Cercopithecus aethiops*). The Neutral Red colorimetric method was used to determine cell viability. Vero cells in untreated culture medium were used as control. The concentration that reduced 50% of cell viability (CC_{50}) was determined from the cell viability *vs*. extract concentration curve. A dose-response relationship in the three extracts studied, with a relatively low toxicity, was observed. The CC_{50} for each extract was the following: EE-Cm = 0.775 mg/mL; EM-Cm = 0.480 mg/mL and EM-Sj = 0.170 mg/mL. Our results report for first time the *in vitro* cytotoxic concentrations from *C. microphylla* and *S. johnstonii* leaf extracts, reveal less cytotoxicity of EM from *S. johnstonii* than *C. microphylla*, and constitute the initial tests for subsequent cytotoxicity analysis of their fruits.

A102

ATRAZINE MONITORING IN GROUNDWATER AND BOVINE MILK FROM FARMS IN THE DAIRY BASIN OF VILLA MARIA, CORDOBA

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Atrazine (AT) is an *s*-triazine herbicide used for weed's pre- and post-emergence control in corn and sorghum crops. This compound is highly persistent in the environment due to its chemical stability, being able to bioconcentrate and bioaccumulate in the lipid tissue of higher organisms, such as mammals. Atrazine is an endocrine disruptor, and it is also able to produce mutagenic and teratogenic effects. Dairy cattle accumulate residues of herbicides from contaminated feed, water, and air. The aim of this work was to evaluate the presence of AT herbicide in groundwater and bovine milk of dairy farms of Villa María, Córdoba (Argentina). The dairy farms (N = 32) were located towards the south (N = 16) and north (N = 16) of the Ctalamochita river. Sampling was conducted during the herbicide application season (spring). Groundwater samples were obtained from the phreatic aquifer (8–30 m depth) and milk samples were taken from the milk tanks. Detection and quantification of AT in groundwater was performed by Capillary Electrophoresis (MEKC-UV), and in milk it was performed by the Atrazine ELISA Microtiter Plate commercial kit (Abraxis LLC, USA) with previous extraction of the herbicide. Of the total number of farms monitored, AT was detected in 28% of groundwater samples and 75% of milk samples. Atrazine concentrations in groundwater were 0.17 ± 0.27 µg/L in the north area and 0.12 ± 0.35 µg/L in the south area ($P < 0.20$). Atrazine concentrations detected in milk samples were higher in the north area (10.65 ± 9.03 µg/L) than in the south area (4.55 ± 6.48 µg/L) ($P < 0.05$). Correlation analyses between AT concentrations in groundwater and milk samples were significantly positive in the north area ($r = 0.68$; $P < 0.01$), showing that the quality of the water consumed by the dairy cattle influences the final composition of the milk. In the monitored dairy farms, 25% of the groundwater samples exceeded the AT maximum residue level (MRL) admitted by the European Union (EU) (0.1 µg/L). The

milk samples analyzed showed AT concentrations below the MRL established by the EU (50 µg/kg), whereas 12.5% of the samples exceeded the MRL allowed by the U. S. Environmental Protection Agency (USEPA) (20 µg/kg). The results obtained in this study show the arrival of the AT herbicide to groundwater sources and its potential to reach higher trophic levels. Bovine milk is a food of high biological value consumed frequently by the population, therefore monitoring and control of milk quality is necessary to protect the health of the exposed population.

A103
COMPARATIVE STUDY OF STRUCTURAL AND ELECTRONIC PROPERTIES AND REACTIVITIES OF A GUAIANOLIDES SERIE

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Sesquiterpene lactones (STL) constitute one of the largest biogenetically homogeneous groups of known secondary metabolites. The large number of these compounds, and their associated structural diversity, it is related to their broad spectrum of biological activities, which makes them of great importance for defense of the plants that synthesize. Most exercise biological activity through a common mechanism of action, based on the interference of the cellular macromolecule functions through covalent bonds formation between the partially electrophilic structures of STL and nucleophilic centers of biological targets, reason why they present diverse groups with a potentially reactive structure such as α-methylene-γ-lactone, α,β-unsaturated carbonyl, epoxide, etc., which make them more versatile with respect to their biological targets. Furthermore, lipophilicity and molecular geometry affect bioactivity of these compounds. From the pharmacological point of view, a wide spectrum of activities has been described: antitumor, antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory, antipyretic, antidiabetic, antiasthmatic, antioxidant, cell cycle blockers in meristematic cells and even meiosis in oocytes amphibian, hepatoprotective, nephroprotective, cytoprotective, analgesic, allergenic, of action on the central nervous system or on the cardiovascular system, etc. The aim of this work is present a theoretical study of structural and electronic properties and reactivities of a guaianolides group. Quantum-mechanical calculations were performed using Density Functional Theory with the B3LYP/6-31G (d) method using the Gaussian09W package. Geometric structures were optimized in both gas and liquid phases (self-consistent reaction field model, SCRF, solvent water); reactivity global descriptors were obtained from molecular border orbitals energies (HOMO and LUMO). Using UCA-Fukui software, local descriptors were obtained which account for the reactivity and site selectivity in a molecule. Taking into account the different descripting values, DhL is the compound that has the greatest reactivity of those analyzed. Considering local descriptors, the centers where these lactones are most likely to suffer a nucleophilic attack preferably correspond to α,β-unsaturated carbonyl when it is present, and if not, in the α-methylene-γ-lactone group. With some of these LSTs, biological tests were carried out on meiosis resumption in amphibian oocytes, observing that which causes the greatest effect is DhL (as predicted by computational calculations).

VETERINARY, ANATOMY, HISTOLOGY AND ANIMAL PHYSIOLOGY

A104
IN VIVO BIOCOMPATIBILITY OF HYDROGELS BASED ON POLY-N-ISOPROPYLACRYLAMIDE (PNIPAM)

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Over the last decades, biomaterials have been developed with great potential to be applied as scaffolds for cell growth, expanding the alternatives to organ and tissue transplants, within the field of tissue engineering. These scaffolds, in addition to allowing a correct *in vitro* development of the tissue of interest, must be accepted by the host avoiding any type of immunological rejection and must also be biostable in the time required for the desired tissue regeneration. Instead, its use *in vivo* introduces a relevant variable that must be studied: the interaction of the biomaterial with the cells belonging to the host's immune system. Hydrogels, especially those based on poly-N-isopropylacrylamide (PNIPAM), have been among the most studied in our group due to their similarity with the extracellular matrix (EMC). Based on this background, the aim of this study was to analyze the biocompatibility and immunological acceptance with three-dimensional (3D) hydrogels based on PNIPAM and its co-polymer, PNIPAM-co-3% APTA (3-acrylamidopropyl chloride) trimethyl- ammonium), in murine models of the Wistar strain. For this purpose, hydrogel discs were implanted in subcutaneous pockets, after sterilizing the materials. In the controls, the same procedure was performed without implanting the hydrogel. The healing process was monitored for 5 days, and the materials were recovered after 3 months. After this stage, the individuals were sacrificed, and blood samples were taken for hematological and biochemical analysis. No alterations in the healing process were observed regarding to controls in both treatments. Also, there were neither significant changes in the leukocyte formula, compatible with inflammatory processes, nor in the blood biochemistry that can be suggestive of kidney and liver dysfunction. Hence, these preliminary studies indicate that PNIPAM-based hydrogels do not generate immune rejection or significant alterations in the hematology and blood biochemistry of Wistar rats in a chronic period of 3 months.

A105
AFFECTING FACTORS ON DAILY PRODUCTION OF MOUNTAIN RANGE CREOLE GOATS IN NORTH-WEST ARGENTINA

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The parameters of the lactation curve fitting models and the estimates that are made from them, such as the yield peak, the peak days, persistence, and total milk production, can be affected by different environmental factors. Thus, the effects of the breed or genetic group, flock, year, year per biotype, delivery time, interaction year per season, number and type of delivery, production level, duration of lactation, feeding, handling and sanitary status are mentioned, among others. The aim of this study was to determine the effect of the environmental factors that affect the peak days, the production peak, and the estimated pick production value at 180 days of mountain range creole goats in North-West Argentina. The experimental batch was constituted by mountain range creole goats from the ANW. The flock was divided into two groups that were crossed in autumn and spring; each goat had only delivered once a year. To evaluate the milk production, weekly controls were carried out, until the females were dried. Data of 559 lactations were worked with, proceeding from the milk controls of 256 goats given birth over eight years (1998 to 2005). The period considered was the one between the birth and the day number 180 of lactation. The data of each of the lactations were individually adjusted, using the nls procedure of the R statistical package, with the Cappio model (Borlino *et al*., 1995). The model is as follows: $y(t) = atb \exp(-ct)$; where $y(t)$ is the average milk production in time t; a, b, and c are the parameters of the model, and e is the base of the Neperian logarithms. With the model parameters, the peak days, production peak and the production at 180 days of lactancy were estimated. The statistical software InfoStat was used to evaluate the effect of environmental factors. The model included as fixed effects year, season, season per year interaction, number and type of delivery as random components of the goats and errors. The comparisons of the averages were made with the DGC method. The peak days were affected by the year ($P < 0.001$) and year per season interaction ($P < 0.001$), being earlier in the goats that delivered during the spring with the exception of the years 2000 and 2002, in the which they were later. All the variation factors influenced the peak of production. Throughout the years 1998, 1999, 2001, 2004 and 2005, the peaks were higher in the goats that delivered in spring, in the rest of the years, they were similar. The peak of production was the lower in the goats that were delivering for the first time and the highest in those that were delivering for the fourth time. The peak was 0,253 higher in the goats of twin birth. Concerning the estimated production at 180 days, it was affected by the year ($P < 0.001$), the year per season interaction ($P < 0.01$), the birth type ($P < 0.001$) and the number of birth ($P < 0.001$). In the autumn goats, the accumulated milk productions were superior in the years 1998, 1999, 2000, and 2003. The productions estimated at 180 days were lower in the goats of the first delivery and in those of unknown delivery number, increasing in the following deliveries up to the maximum in the fourth delivery. The goats of single delivery produced 12 kilograms more than those of double delivery. It is concluded that all the environmental factors considered, systematically affected the milk production variables of mountain range Creole goats in North-West Argentina were systematically affected by the environmental factors.

A106
PERSISTENCE OF LACTATION OF MOUNTAIN RANGE CREOLE GOATS IN NORTH-WEST ARGENTINA

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The persistence of lactation is defined as the degree at which the peak of lactation or maximum average daily production can be sustained in time. The factors influencing persistence in goats include year, season, delivery, type of delivery, flock, and the duration of the lactation. The goal of this study was to determine the persistence of lactation on mountain range Creole goats in North-West Argentina and the effect of environmental factors that affecting it. The experimental batch consisted in creole goats of the ANW. The flock was divided into two groups that were crossed in autumn and spring; each goat delivering only once per year. To evaluate the milk production, weekly controls were carried out, until the females were dried. Data of 453 lactations was worked with, coming from the milk control of 256 goats, given birth over eight years (from 1998 to 2005). Only typical lactations were considered, defining as such the lactations that presented the peak from the second control. The period between calving and 180 days of lactation was studied. Persistence was calculated as a percentage, according to the following formula: $P = (p_{final} / p_{max}) \times 100$; where p is the persistence (%), p_{final}: production in the last control, and p_{max}: production in the peak. The effects of environmental factors were evaluated using the InFostat statistical software. The model included as fixed effects year, season, year per season interaction, delivery and delivery type, and error as a random component. Comparisons of averages were made under the DGC method. For all the evaluated data, the persistence was of 25%, a value that can be considered low, but which is reasonable, because it is dealt with a biotype that has not been selected for milk production. It was affected by the year ($P < 0.001$), season ($P < 0.001$), year per season interaction ($P < 0.001$) and delivery type ($P < 0.001$); no effect of the number of deliveries was observed. Except for the first year of the period of observations, in which the two calving seasons were similar, the goats that delivered during the autumn had a greater persistence of lactation, reaching the observed differences, with statistical significance. In the goats that delivered in autumn, the persistence ranged between 23% and 44%, while in the goats that delivered in spring it decreased by half (from 11% to 27%). Regarding the effect of the type of delivery, the goats with single deliveries were more persistent than those with double deliveries (27% *vs.* 25%). It is concluded that the mountain range Creole goats in North-West Argentina have a low lactation persistence, being systematically affected by different environmental factors.

A107
COMPARISON OF TWO METHODS OF FREQUENT USE TO DETERMINE THE ORGANIC CARBON CONTENT IN FORAGES OF THE UPPER DELTA OF PARANÁ

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Given the diversity and quantity of forage plants that this region presents, it was proposed to determine the energy potential (organic carbon content) of the same through two usual analytical techniques: Antrona method (non-structural carbohydrates, CNES) and oxidation with dichromate ($Cr_2O_7^{2-}$) (Oxidizable carbon, C_{oxid}). The plant species used in this work were the following: *Vigna luteola* (VL), *Polygonum acuminatum* (PA), *Panicum pernambuscence* (C1), *P. elephantipes* (C2), *Echinochloa polystachya* (C3), *Eichhornia azurea* (EA), *E. crassipes* (EC), *Baccharis salicifolia* (BS), *Lippia alba* (LA), *Salix humboldtiana* (SH), *Tessaria integrifolia* (TI), *Acacia caven* (AC), *Gleditsia triacanthos* (GT), and two *Medicago sativa* hays (HA16 and HA18). The samples were obtained monthly during the summer spring growth period, in a pre-flowering state by cutting with mechanical scissors, in the Islands that are in front of the city of Rosario. They were then dried at 60°C, ground and sieved with a 2 mm-screen. With the samples of each plant species, a composite sample was prepared. The determinations were made in duplicate. The average values, obtained with both techniques, presented a positive correlation of 0.596 ($P \leq 0.05$). C_{oxid} values (%) ranged from 7.5 to 15.4; being its general mean and standard deviation of 12.53 ± 1.97. CNES values (%) varied between 3.5 and 9.8; being its general mean and standard deviation of 6.94 ± 1.86. Regarding Coxid, the CNES represented only 55%, a predictable difference that indicates the proportion of saccharide derivatives over the total of reduced carbon compounds in the samples studied. The Coxid allowed to show and/or highlight existing differences in the carbon composition, which, without being associated with carbohydrates, influence the reducing power of forage (phenolic derivatives, for example). The results indicate that Coxid allows differentiating samples from the same plant species that presented similar CNES values both in the case of legumes (HA16, HA18 and AC) and in the case of grasses, which also presented similarities with aquatic or shrub species (EC, EA, C1, C2, C3, TI, or PA, LA). It is also concluded that between the studied species differences may occur at the level of the concentration of saccharide derivatives and that these are not reflected in the amount of Coxid that the forage presents (EA, PA, SH or C3, VL, LA), indicating the presence of saccharides with different reducing power. The study suggests the convenience of the complementary use of the two analytical techniques studied when it is required to characterize the energy potential of this type of forage as substrates in microbial processes such as methanogenesis and CO_2 production in the rumen of animals that are confined to the island for productive purposes.

A108
INCREASE OF ANTIOXIDANT COMPONENTS IN PLASMA OF HEIFERS FEED WITH SOYBEAN EXPELLER DURING NATURAL SERVICE

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Currently, there are numerous balanced diets formulated for beef cattle that contains soybean as an ingredient that supply a high-quality protein. Isoflavones belong to a group of flavonoids that are present in these type of food with an important protective role against cellular oxidative damage. The objective of this work was to evaluate the effect of the soybean expeller diet on the antioxidant state of heifer plasmas during the mating season, and its relationship with pregnancy and calving rates. For that, two groups of *Braford* heifers from the IIACS-INTA were fed for 3 months during the mating period differentially with a diet that contains soybean expeller at 0.6% of the body weight, and the other group was used as a control (diet without soybean). The research was carried out in 2 replicates in consecutive years, with 32 heifers per repetition, divided into two experimental groups. Blood samples from both groups were collected by puncture of jugular vein. Subsequently, hematocrits were determined for each sample. For the determinations of bioactive compounds and antioxidant activity, 10 plasma samples were selected and analyzed in duplicate from each group studied. The total content of phenolic compounds and flavonoid concentrations were determined by spectrophotometry. Animals that consumed soybean expeller during service had the highest levels of these bioactive components. Antioxidant activity was evaluated in plasma by measuring antiradical activity and the protective effect against enzymatic lipid peroxidation. An increase in antioxidant activity was determined for both measurements in the group that consumed soybean expeller. In addition, the level of malondialdehyde (MDA) was evaluated as a biomarker of oxidative stress by TBARS method. Plasma samples showed significantly lower MDA concentrations than in the control group. Finally, the hematocrits, the pregnancy and calving rates were not significantly different between groups. These results demonstrate, for the first time, that the inclusion of soy expeller in the diet of heifers during the mating season increased the antioxidant capacity of plasma maintaining adequate physiological conditions without affecting reproductive parameters.

A109
NEW TOOLS FOR *IN VITRO* HANDLING AND SELECTION OF CRYOPRESERVED SPERM SAMPLES IN EQUINES

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Cryopreserved sperm (SPZ) are used widely in domestic animals for assisted reproduction techniques (ART). However, cryopreservation procedures negatively affect SPZ quality, causing changes at the structural and molecular levels. Therefore, using cryopreserved SPZ for ART can decrease the efficiency of these techniques as well as the quality of the embryos obtained, being necessary to optimize the handling of this type of sample. Particularly in equines, ICSI is the most used technique for low-quality equine semen samples where SPZ is selected based on their motility and morphology. Physiologically, the oviduct epithelial cells (OEC) are involved in the selection of a population of SPZ suitable for oocyte fertilization,

which is released from the oviduct due to the capacitation process. This work aimed to evaluate different conditions for the *in vitro* manipulation and incubation of equine cryopreserved SPZ and to establish an *in vitro* OEC culture to select an SPZ population with fertilizing capacity suitable for its use in ART in this species. Regarding the handling and the effect on the motility of post-thawed equine cryopreserved SPZ samples, we used non-capacitating Whitten's medium, less effect on motility was observed through the time (120 min) when SPZ were incubated at concentrations of 30 mill/mL compared to lower concentrated samples (CASA, $P < 0.05$). Also, the motility decreased by half with centrifugations at 200g longer than 1 min ($P < 0.05$). On the other hand, we have established a culture model of OECs *in vitro*, in which we demonstrated the expression of epithelial markers (E-cadherin and cytokeratin) by both RT-PCR and immunofluorescence (IF). When we performed SPZ-OEC cocultures, we observed that the attached SPZs were motile and presented intact acrosome (PSA-FITC, IF), suggesting a selection by the oviductal model. Then, the co-cultures were incubated in capacitating conditions (capacitating Whitten´s medium) and the released SPZ population was recovered. Under these conditions, a greater number of live sperm (Hoechst258 assessed by IF), capacitated (activation of PKA and phosphorylation on tyrosine residues by IF), with progressive motility (CASA) and with the intact acrosome (PSA-FITC, IF) compared to the control was observed ($P < 0.05$). Moreover, the decrease in motility through the time was less in these SPZ than in the cryopreserved SPZ incubated in the absence of OECs. This sperm population could be recovered for its use in different ART. Improvements in handling and selection of cryopreserved SPZ not only generate tools to solve the problem that IVF presents in equines but would also improve efficiency in other ART such as ICSI, allowing the use of a population of higher quality gametes which it would positively impact the quality of the embryos obtained.

A110
TRACE MINERALS IN SERUM OF LACTATING SOWS

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Oligominerals such as iron (Fe), copper (Cu) and zinc (Zn) are essential for pig nutrition. Objective was to analyze the profile of oligominerals (iron, copper and zinc) in serum samples of sows lactation in two commercial farms of Argentina. The blood samples were extracted without anticoagulants, from 68 lactating sows of commercial genetics constituted by crosses of Yorkshine, Landrace and Pietrain breeds; randomly selected from establishments located in Santa Fe (A) and Entre Ríos (B). The diet was based on corn, soy expeler and enriched with mineral vitamin nucleus of inorganic origin for lactation category. Between both farms there is difference in management of the site of calving and lactation, in A they are carried out to field and in establishment B they are carried out in confinement. Samples for the determination of Fe, Cu and Zn in serum were analyzed by atomic absorption spectrophotometry (FAAS) method. Infostat program was applied, outliers were identified and eliminated prior to analysis, then Shapiro-Wilk test and Levene test were applied. All samples were found with normal distribution and showed homogeneity in their variances. T-test was performed assuming a significance of 0.05. Standard deviations and test values *t* were: Fe (µg/dL) 6.07, 19.19, t 0.00 – Cu (ppm) 0.35, 0.32, t 0.00 – Zn (ppm) 0.50, 0.42, t 0.01 for farms A and B, respectively. During investigation period were observed within normal, values average values of iron in both farms and copper of farm A. Establishment B, zinc was found slightly above the upper limit of reference range and same oligomineral, at farm A, showed averages above normal value. Means of three minerals were found to be significantly different ($P < 0.05$) for the lactation condition between the two farms. This behavior can be explained, if we consider that oligominerals Fe, Cu and Zn are normally added as correctors by commercial food companies at levels higher than recommended by most research centers.

A111
EVALUATION OF THE PROTEIN CONTENT IN SORGHUM FORAGE

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The use of marginal areas for livestock production and their edaphoclimatic limitations led to search adapted food for livestock, adapted to these conditions. Facing the development of sustainable systems, sorghum presents a very good potential for livestock feed. Sorghum cultivation resists conditions of extreme temperatures and drought, giving grain and forage in arid, sub-humid and even hot areas. It shows a great versatility for livestock feeding since it can be used as greening, deferred, silo, as grain etc. Geneticists, over the years, have sought to improve their biomass yields. Genetics improvement has achieved interesting increases in dry matter productivity per hectare. To be used as food for livestock, we are not only interested in its performance but also its nutritional value. The protein content is one of the parameters to consider when quantifying forage quality, due to the functions they perform in the animal's body. The objective of this work was to evaluate the crude protein content (CP) of commercial varieties of forage sorghum used as livestock food and to compare with data from varieties commercial sorghum used ten years ago. We worked with 20 samples of forage sorghum of 4 commercial varieties. The samples were dried in a drying oven at a temperature of 60°C until obtaining dry matter (DM). Then they were ground in a brand laboratory mill (Wiley®) with a 1 mm sieve and the flour obtained was used to determine the CP by the Kjeldahl method, according to the AOAC (1990), multiplying the value of N_2 obtained by the factor 6.25 to estimate the % of CP. The results obtained were the following MN°1: % CP = 9.32 ± 0.22; MN° 2: % CP = 8.97 ± 0.48; MN° 3% CP = 9.06 ± 0.29; MN° 4% CP = 9.32 ± 0.48. The data of commercial sorghum varieties used ten years ago, was obtained from the database of the Animal Nutrition Laboratory, FAZ, UNT. Whose medium value of % CP = 6.71 ± 0.51 *vs.* the mean value of the new varieties of % CP = 9.19 ± 0.15. Observing an improvement in the % of CP of 2.48. Concluding that in the new varieties, there was not only an improvement in the yields, but also in a nutritional parameter, as sensitive as the protein values.

A112

PROBIOTIC POTENTIAL OF *B. amyloliquefaciens* MEP$_2$18 AND ARP$_2$3 AS NEW ADDITIVES FOR CHICKEN FEEDING

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The genetic lines of broiler chickens are selected to express their maximum potential in the first weeks of life. This implies an increase in growth requirements, which is affected by nutrition, maturation of the immune and the digestive systems and exposure to pathogens. Probiotics based on strains of the *Bacillus subtilis* group are an efficient alternative to the replacement of Growth Promoting Antibiotics, currently excluded in animal feed. In our laboratory we have the native strains *Bacillus amyloliquefaciens* MEP$_2$18 and ARP$_2$3 that produce cyclic lipopeptides (CLP) with proven antibacterial and antifungal activities. The aims of this work were (1) to determine the effect of the addition of MEP$_2$18 and ARP$_2$3 to the diet on the productive variables of broiler chickens, (2) to identify bacterial species isolated from the intestinal tract, and (3) to perform antagonism tests in Petri dishes. In a preliminary trial, 24 male chicks (Cobb) were used, 2 of which were slaughtered at 12 h of life and 2 at 5 days of life, taking samples from the duodenum and cecum to know the basal cultivable bacterial flora. The remaining 20 chicks were divided into 4 groups of 5 chicks each receiving the following diets: TC, control (without *Bacillus*); T1, strain MEP$_2$18; T2, strain ARP$_2$3; and T3, mixture 1:1 MEP$_2$18:ARP$_2$3, all at 10^7 CFU/kg of feed. After 15 days of treatment, the productive variables were determined: Average consumption/bird (g), Average gain/bird (g) and Conversion Index. Then, the birds were slaughtered to extract tissue samples. The samples were diluted with buffered peptone and cultivated in selective culture media. Isolated colonies with different morphologies were replicated and biotypified by MALDI-TOF. Weight gain was observed in T1 and T2 treatments (686 g and 675 g, respectively) compared to TC and T3 (670 g and 652 g). This result was reflected in improved feed conversion index in T1 and T2 (1.30 and 1.35) compared to TC and T3 (1.38 and 1.38). Most of the bacterial isolates that could be cultivable in all samples were *Escherichia coli*, followed by *Enterococcus faecalis* and *Proteus mirabilis*. *Escherichia fergusonii* was also found. Preliminary antagonism trials showed that the CLPs produced by MEP$_2$18 inhibited *E. coli* and *P. mirabilis*. The presence of *Lactobacillus* sp. was also detected in all samples indicating early colonization of the intestinal tract. Conclusion: The addition of MEP$_2$18 and ARP$_2$3 in the feed for broiler chicks for 15 days generated a better Conversion Index, observing weight increase, balancing the bacterial microbiota present in the intestinal tract and influencing towards a beneficial flora. These were evidenced by the presence of *Lactobacillus* sp, which enhances the maturity of the intestinal immune system. However, no differences were observed with respect to the control group when the 1:1 MEP$_2$18:ARP$_2$3 mixture was applied, preliminarily indicating that the combination of strains would not exert a synergistic effect.

A113

DIFFERENCIAL INVESTMENT IN CELL IMMUNE RESPONSE ON RATS INFECTED WITH *Trypanosoma cruzi* AND/OR *Trichinella spiralis* EXPOSED TO FOOD RESTRICTION

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One of the most studied resistance mechanisms is the immune response. The variability of investment in such a mechanism has been shown to be modulated by the context faced by animals in the wild. The aim of this study is to evaluate the leucocyte profile as an indicator of the investment in cellular immunity in a model of infection by *Trichinella spiralis* and/or *Trypanosoma cruzi* in laboratory rats exposed to food restriction. Both parasites are etiological agents of zoonotic diseases of relevance in Argentina, and rodents can participate as reservoir and transmission sources. After four weeks of exposure to food restriction or no challenge (control), the rats were inoculated with one of the parasites or both. Two weeks after infection, the absolute differential counts were evaluated. Lymphocyte count raised in animals exposed to *T. cruzi* alone and in co-infection, more markedly in food restriction, while in those infected with *T. spiralis* the count tended to decrease. The count of eosinophils increased in food-restricted animals in presence of mono-infection with *T. spiralis*, while in control animals the increase was less pronounced. Contrary to what we expected, no significant changes in neutrophil counts were observed. Finally, the monocyte count decreased in rats infected with *Tri. spiralis* and increased in the presence of *T. cruzi* in both treatments, although more markedly in food restriction. The increase of eosinophils in the presence of *T. spiralis* is expected in helminth infections. However, such trend was not maintained in co-infection. This would indicate a modulation of the response to nematodes in presence of another infection and a greater investment during scarcity of resources. The greater increase in monocytes and lymphocytes in food restriction compared to the control group in the presence of the protozoan could be demonstrating a modulation of the strategy in scarcity of resources. In the other hand, the decrease in the number of lymphocytes is expectable in the presence of *T. spiralis*. These results are relevant for the understanding of the variability of the investment in resistance and its consequences in the modulation of the defense strategy. Rodents play a key role in the transmission of innumerable diseases of economic and health importance, so understanding the determinants that modulate their capacity as reservoirs is essential in veterinary medicine and public health.

A114
AMPHIBIAN SPERM QUALITY: EVALUATION OF FUNCTIONAL PARAMETERS

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Amphibians, having a biphasic life (water-land), are excellent bioindicators of environmental quality, mainly in the aquatic environments where larval and middle development takes place to which they return, when they are adults, to reproduce. The demographic increase in the Argentine Northwest threatens the development of autochthonous amphibian species such as *Leptodactylus chaquensis*. For this reason, we seek new parameters in the determination of sperm quality that can be applied in *in vitro* fertilization techniques, contemplated in species conservation programs. In previous studies we determined physio-morphological parameters that can be used as quality standards. However, we consider important the study of physiological parameters, until now not reported for this species. The aim of this work was to evaluate the functional integrity of the plasma membrane and to determine the presence and activity of hyaluronidase in *L. chaquensis* spermatozoa. Samples of spermatozoa from animals captured in the breeding period (November–February), obtained by testicular dilaceration, were selected and fractionated in Mother Ringer (MR). For cytological tests, samples with a percentage of vitality greater than 80% were used. Sperm suspensions were exposed to a hypoosmotic solution with different osmolalities (9.4, 18.8, 37.5, 75, 150 mOs) incubated at 25 ± 3°C for 5 min. As controls, MR and distilled water were used. Swelling of the plasma membrane, visible as a drop on the end piece of the flagellum, indicates a positive response. To test the hyaluronidase activity, different sperm extracts (3, 5, 10, 20 $\times 10^6$ sp/mL) were incubated on agar plates (0.03, 0.05, 0.10% agar) at 25 ± 3°C for 24 and 48 h. 1× hyaluronidase and MR were used as controls. The formation of a hydrolysis halo around the well where the sample is deposited indicates a positive response. The acrosomal status was analyzed to correlate the samples with previously established morphological parameters of normality. Spermatozoa in hypoosmotic means 9.4 mOs show a maximum positive response (86.33 ± 2.03%). The tests carried out with different concentrations of sperm extracts did not show positive results in any tested concentration of agar. All the samples used exhibited 92.67 ± 0.49% intact acrosome. These data show that: –The gametes obtained by testicular dilaceration maintain high percentages of functionality and integrity of the plasma membrane. –An apparent absence of hyaluronidase. However, another methodology is required to evaluate the acrosomal functionality of this species. These preliminary data would contribute to the establishment of sperm quality standards in anurans.

A115
CARCASS AND MEAT QUALITY IN HEAVY LAMBS

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Lamb meat is low consumption in Argentina, contrasting with meat from other species, this is primarily due to the seasonality in his offer (from November to March) and the absence of cuts in retail commerce. Heavy lamb is a strategy that has allowed in other countries to increase consumption and open export markets. The objective of this work was to evaluate the quality of carcass and meat in heavy lambs in our region. Twenty male lambs, 10 Corriedale breed and 10 Hampshire Down, with weaning at 19–20 kg, were studied. Lambs were grazing on grain oats and alfalfa hay, supplemented with corn grain (220 g/animal/day). The sacrifice was made between 31–36 kg of live weight, after rest and fasting. The objective measurements in carcass included hot carcass (HCW) and cold carcass weight (CCW), after 24 h of cold. Carcass yields were calculated, measurements and indices were made in the carcasses, the performance was determined at cuts and the percentage of each tissue in the back. For the meat evaluation were measured pH and temperature in the left *Longissimus dorsi* (LD) muscle (5th–10th rib) at 0 h, 45 min, and 24 h post slaughter. Samples were taken from the left LD muscle (5th–13th rib), to determine color using a colorimeter, cooking losses (CL%), water holding capacity (%WHC) and tenderness with Warner Bratzler's shearforce. Statistical analysis was performed using ANOVA. The average results for HCW were 14.00 ± 1.12 kg and CCW 13.70 ± 1.09 kg. The average carcass yields observed were: abattoir yield 44.5%, commercial 40.3%, biological 56% and true 57%. The measurements in the carcass showed variability in carcass compactness influenced by the breed, meat biotypes showed greater compactness than double purposes breeds. At cuts yields we find the following distribution of pieces: 10% Neck, 5% Front ribs, 16 to 17% Rib, 33.5 to 34.2% Leg, 20% Shoulder and 12.3 to 13.6% Breast and Flank. The tissue composition of the shoulder showed a high percentage of muscle (61.6% to 64%), variable amount of fat between 9% and 11.5%, bone 22% and waste 5%. The physical chemical quality determinations of meat showed in pH at 24 h values above the optimus (5.9), therefore pre and post slauther factors should be evaluated. The tenderness results were below the acceptable, with averages of 1.09. These meats showed lower WHC (32.8%) and lower CL (25.3%) than those observed in other studies and with different weights. The heavy lambs showed good results in the objective carcass evaluation and meat quality parameters evaluated show these meats have acceptable quality according to standardized calues al national and international level.

A116
IMPORTANCE OF THE BLOOD HEMOGRAM IN THE DIAGNOSIS OF *Hepatozoon canis* IN CANINE PATIENTS IN THE VETERINARY HOSPITAL OF THE NATIONAL UNIVERSITY OF TUCUMÁN

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The hemoparasites are one of the most frequent pathologies in dogs. The development of this diseases is directly related to its transmission vector, the tick. The general signs include fever, anemia, thrombocytopenia, hepatosplenomegaly, and eventually pancytopenia in chronic cases. Faced with clinical suspicion, the diagnosis is confirmed by evaluating the presence of parasites in capillary blood smears or smears from the phlogistic layer of the hematocrit. However, occasionally it is possible the diagnostic through routine peripheral blood smears (especially in high-level parasitemias). During the second semester of 2019, through the service provided by the Veterinary Hospital of the National University of Tucumán, 10 patients

were identified with haemoparasites, in 6 of them, *Hepatozoon canis* was identified through routine peripheral blood hemogram. The main objective of this work consisted in evaluating in what form some parameters of the hemogram are presented, in the patients in whom *H. canis* was identified. Regarding the methodology, all the data were collected from patients that attended the Veterinary Hospital during the second semester of 2019. The results show that the hematocrit level decreases significantly compared to the reference range, unlike the total protein levels. The total number of leukocytes falls into the reference range in most cases, although there are remarkable alterations in blood differential, like an increased number of neutrophils (neutrophilia) and a decreased number of lymphocytes. Conclusions: some alterations in the hemogram like the low hematocrit and neutrophilia seems to correlate well with the identification of *H. canis* gametocytes in the cytoplasm of monocytes and neutrophils through routine Romanowsky-stained blood smears. Although the probability of diagnosis in peripheral blood smears is low, the routine blood count can be very useful for detecting parasite forms, especially in cases where there are not strong clinical sings.

A117

PREVALENCE OF RISK FACTORS IN SMALL PIG FARMS IN THE CENTRAL VALLEY OF CATAMARCA

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Food of animal origin constitutes a vehicle for the transmission of zoonosis. The important role played by these products in the chain of transmission of foodborne illness does not escape the interest of the health team; they are frequently contaminated from their origin, as a consequence of the presence of *Trichinella spiralis* in animals. Trichinellosis is a cause of morbidity in Catamarca and its surveillance constitutes a significant element to alert about its evolution, as it is a reportable pathology. The objective was to describe the prevalence of risk factors for this disease in subsistence pig farms after the last record of human cases. An observation of phenomena in their natural environment was carried out with a non-experimental, cross-sectional design study, corresponding to the first farms measured in an integral way (N = 103) in the population of small producers in the central valley, based on surveys of variables associated with the event and focused on behavioral, demographic, and environmental risk factors. Out of the total of those surveyed, 20 (19.40%), elementary facilities 14 (13.6%), hygienic-sanitary conditions 3 (2.91%), veterinary care 1 (0.97%) were found to have an elementary level of knowledge, enumeration 50 (48.54%), balanced diet 35 (33.98%), health management 10 (9.70%), drinking water58 (56,030), effluents 3 (2.91%), prevention 9 (8.75%), biosecurity (0%), home slaugther103 (100%). All pig farms have conditions for the nematode to lodge and allow its transmission. The risk factors evaluated show that the characterization of the situation recognizes those points on which to implement population-based prevention strategies and counseling and control interventions that effectively and affordably reduce the precarious situation and thus avoid infection in man.

A118

URETEROCELE IN *Felis catus*, ULTRASOUND DIAGNOSIS. CASE PRESENTATION

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Ureterocele is a condition of the urinary system at the mouth of the ureter in the bladder, causing a cystic dilation in the submucosa region due to the bulging of the dilated segment in the bladder. They are classified according to their location as orthotopic or ectopic and can be unilateral or bilateral. In addition to this, it can be classified on the basis of clinical signs as Grade 1 (no evidence of upper urinary tract disease), Grade 2 (ipsilateral, with hydroureter, hydronephrosis, or chronic kidney disease), or Grade 3 (bilateral, with hydroureter, hydronephrosis, or chronic kidney disease). They have been described in small animals, both in dogs and cats, mainly in female dogs, the ectopic type being the most common, causing urinary incontinence. Intravesical ureterocele in dogs is usually asymptomatic unless it is large enough to cause flow obstruction and hydronephrosis, among other types of complications, such as recurrent urinary tract infections and lithiasis formation. Animals generally develop signs at a young age. The following case came to the clinical consultation: Nazarena. Female Feline, 4 years old, breed: Common European. The Medical History: Anamnesis: she goes to the consultation because she urinates with blood, she vomits, she manifests pain when they grab her. The pet lives with other cats and dogs and eats standard food. A clinical examination was performed showing normal mucous membranes, normal hydration, chest auscultation without particularities, moderate overweight, pain on abdominal palpation, particularly in the bladder, non-plethoric bladder, and a temperature of 40°C. Presumptive diagnosis: lower urinary tract disease, which is why abdominal ultrasound with a urinary focus is indicated. The report indicated: Bladder: (Length 2.86 cm, Width: 2.13 cm, Height: 1.74 cm, Volume: 5.52 ml), small in size, irregular walls with hyperechoic mucosa, compatible with chronic cystitis, abundant hyperechogenic sediment is identified and images with net acoustic shadow of 0.29–0.37–0.93 cm in diameter compatible with lithiasis are visualized. At the mouth of the right ureter, a circular, thin-walled and hyperechoic image is observed, with dilation of the right ureter and mild right kidney pylectasis, compatible with ureterocele. Treatment: the patient is referred to surgery. Evolution: the patient dies before surgery. In the present case, a Grade 2 orthotopic ureterocele was diagnosed by ultrasound, complicated with lithiasis and microlithiasis associated with chronic cystitis; detected after a conventional urinary tract treatment, to which it was refractory. The essentials of a complementary diagnostic method with high sensitivity are exposed to more efficiently and ideally face a treatment, in this case surgery to solve the bad formation and later correct the associated pathologies.

A119
ASPERGILLOSIS AS A CAUSE OF DEATH IN CAPTIVE BIRDS OF PREY

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Aspergillosis is a fungal infection caused by fungi of the genus *Aspergillus spp*. This is a ubiquitous organism that grows easily in decomposing organic matter and in states of immunosuppression it produces opportunistic infections. The spores of the fungus are very resistant and spread easily through the environment. It is described as the major cause of morbidity and mortality in birds. Predisposing factors are diverse, unpredictable, and tend to suppress the host's immune response. The objective was to determine the percentage of deaths due to aspergillosis in captive raptors, since it is reported as a common cause of deaths in these birds. The study was carried out in the Mendoza Ecopark (32°53' south latitude and 68°56' west longitude), located on the eastern slope of Cerro de La Gloria within the General San Martín Park property. A retrospective study was carried out on the causes of death of birds of prey housed in said institution and those that entered the quarantine sector. The period considered was from January 2016 to January 2020. In total, there were 26 deaths of birds of prey (18 from the campus and 8 that entered quarantine). The necropsy protocols corresponded to: 5 chimangos (*Milvago chimango*), 1 peregrine falcon (*Falco peregrinus*), 4 belfry owls (*Tyto alba*), 1 crowned eagle (*Harpyhaliaetus coronatus*), 4 mixed hawks (*Parabuteo unicinctus*), 1 long-eared owl (*Bubo virginianus*), 2 common harriers (*Geranoaetus polyosoma*), 5 black eagles (*Geranoaetus melanoleucus*), 1 royal jote (*Sarcoramphus papa*), 1 black-headed jote (*Coragyps atratus*) and 1 caracara (*Caracara plancus*). Two deaths due to aspergillosis were detected in the black eagle, with a difference of 2 years between one and the other; It should be noted that both birds belonged to the establishment. The diagnosis was made based on the macroscopic lesions of the necropsy and the imprints stained with Stain 15 where the presence of conidiospores and conidia characteristic of *Aspergillus spp*. were present. In both necropsies, the presence of granulomatous material was observed in the larynx and trachea, one of the birds also presented it in the tracheal bifurcation. The lungs and air sacs showed white-yellowish nodulations, umbilicated, 1-5 mm in diameter. The diagnoses of deaths due to aspergillosis were 13%. It has been reported that certain species of birds of prey present greater susceptibility to the disease, however, these species are not found in the country. In this study, in the span of four years, the affected species have been black eagles. Further studies will be required to define whether there was a coincidence or if said species has a certain predisposition. Regarding the percentage of cause of death due to this disease, it is low, being a little lower than the range reported in zoos, which is 15–30%.

A120
Cryptococcus neoformans SURVEY IN BIRDS OF PREY

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Cryptococcosis is a fungal disease caused by species belonging to the *Cryptococcus neoformans* complex, formed by *C. neoformans* and *C. gattii*. The main route of transmission is through the inhalation of basidiospores or yeasts, directly from environments contaminated with excreta from pigeons and other birds. Birds of prey have a high potential to spread zoonotic agents due to the wide distances they travel and because of their keeping in zoological institutions for conservation and rehabilitation purposes. Studies show that they can act as carriers and disseminators of *Cryptococcus neoformans* and other zoonotic yeasts. The objective was to determine the presence of *Cryptococcus neoformans* in the excreta of birds of prey from the Mendoza Ecopark. The study was carried out at the facilities of the Mendoza Ecopark (32°53' south latitude and 68°56' west longitude) located on the eastern slope of Cerro de la Gloria within the Parque General San Martín property. The sampling was carried out in pool in 13 enclosures that are located in the institution's walk sector, and individually to the birds that were and entered the quarantine sector, during the period from July 2016 to December 2019. A total of 50 raptors were sampled corresponding to: enclosure 35, royal jote (*Sarcoramphus papa*) (N = 1); enclosure 36, common harrier (*Geranoaetus polyosoma*) (N = 3); enclosure 37, crowned eagle (*Harpyhaliaetus coronatus*) (N = 1); enclosure 38, carancho (*Caracara plancus*) (N = 6); enclosure 39, common harrier (*Geranoaetus polyosoma*) (N = 2); enclosure 40, moorish eagle (*Geranoaetus melanoleucus*) (N = 3); enclosure 41, moorish eagle (*Geranoaetus melanoleucus*) (N = 2); enclosure 44, caracara (*Caracara plancus*) (N = 2); enclosure 45, black eagle (*Geranoaetus melanoleucus*) (N = 2); enclosure 46, mixed hawk (*Parabuteo unicinctus*) (N = 3); enclosure 47, chimango (*Milvago chimango*) (N = 8); enclosure 48, moorish eagle (*Geranoaetus melanoleucus*) (N = 3); high enclosure, owl (*Bubo virginianus*) (N = 1); quarantine sector, mixed hawk (*Parabuteo unicinctus*) (N = 7), carancho (*Caracara plancus*) (N = 1), chimango (*Milvago chimango*) (N = 2), red-headed jote (*Cathartes aura*) (N = 1) , black-headed jote (*Coragyps atratus*) (N = 1), common harrier (*Geranoaetus polyosoma*) (N = 1). The excreta were collected from the enclosures in clean jars and transferred to the Ecopark laboratory, where a small amount was taken with a swab and placed on a slide. Negative staining or India ink was used, placing a drop, and then placing a cover slip on it to observe it in a Nikon Model YS 100 brand optical microscope. The result was negative for all the samples observed. Cryptococcosis is a zoonosis that affects more than one million people worldwide. Its survey in birds of prey kept in captivity, as well as those that enter institutions to be rehabilitated, is an important preventive measure in public health.

EDUCATION AND EXTENSION

A121
VIRUSES, NEWS AND MICROBIOLOGISTS IN TODAY'S SOCIETY

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With the premise that scientific disclosure allows the link between science and society, providing knowledge to an audience that is clearly an essential actor in health prevention, our objective is to improve the skills of future professionals so that not only have knowledge of microbiology, particularly, virology but also that they can mobilize, transfer and make decisions in the field of scientific research, diagnosis, and characterization of viral agents, considering in all cases the social impact of this action. The present work proposal was mandatory for the subject Virology and Diagnostic Virology, of the fourth and fifth years of Microbiology (UNRC). In the first instance, we worked on scientific disclosure productions (2016–2018), a totally innovative experience for the subject, being outstanding, from the disciplinary and social aspects, the productions obtained, as well as during the development process of the same, the student bonding, engagement, and connection beyond the limits of the classroom. In the year 2019, we presented the PIIMEG work project *Viruses are in the news,* the pandemic generated by SARS CoV-2 in the present year, accelerated the objectives of work for the course Diagnostic Virology. Virological knowledge became vertiginous worldwide, manifesting itself in the news of all kinds and scientific publications, which led us to raise and debate about the content of the subject and recently published research, as proved to be the design of new diagnostic equipment to determine the circulation of the pandemic virus in the same experience of implementation. The results achieved in this context were extremely productive, the uncertainty of real-life was shared and the emergence of new virological knowledge to be treated in the subject. This allowed discussion of the subject, its validation, implementation of new diagnostic equipment, strengthening capacities through the design and implementation of solutions to the various specific problems triggered by the SARS CoV-2. Capacities that are expected of a microbiologist, especially in the exercise of their profession and that must be stimulated. In addition, the social and ethical commitment that should be manifested in their professional practice was strengthened and highlighted, through the evaluation of the various voices of international virologists. The evaluation focused on the processes carried out and the achievements of the activity, planned or not.

A122
SECOND-YEAR MEDICINE STUDENTS VALUATIONS REGARDING THE USE OF A VIRTUAL EDUCATIONAL PLATFORM IN 2019 AND 2020

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The virtual platform Schoology is a tool that provides students a collaborative workspace, which is currently used in Biochemistry, a 2nd-year Medicine subject. Schoology was implemented in 2019, and in the current year, its use was prioritized in the context of Covid-19. The objective of the work was to compare the assessment of Biochemistry students in 2019 and 2020 regarding the use of the educational platform. 64 students participated, out of a total of 76, who took the course in 2019; and 62 students, out of a total of 100, who attended it in 2020. They previously signed informed consent. A survey was used, composed of 7 proposals that addressed whether the platform: facilitates access to study material and communication between peers and with teachers, if it improves academic performance and subject organization and whether it was considered easy to use, and if it should be used in other subjects. The propositions were evaluated using a Likert scale: strongly disagree (MDA in its Spanish acronym), disagree (DA); agree (A); strongly agree (MA). The application of the instrument was voluntary and anonymous. The Chi-square homogeneity test was used, with a significance level of 5%, for each proposition an observed value -VO- and a theoretical value -VT- were obtained. Access to material VO = 0.6080; VT = 5.99. Communication with teachers VO = 7,063; VT = 7.815. Communication with partner VO = 1.488; VT 5.99. Application in another subject VO = 10.57; VT = 7.815. Improved academic performance VO = 10.73; VT = 5.99. Ease of use VO = 6.31; VT5.99. Organization of the subject VO = 7.88; VT = 5.99. In the first three propositions, a similar statistical result was obtained, so it is concluded that there are no significant differences between both years. In the rest of the propositions, differences are observed. This result may be due to the fact that, in recent times, there has been a greater deployment of virtual education within the framework of compulsory social isolation. This allows students to have a wide range of choices, and comparisons between educational platforms. The assessment of these results is relevant to establish the degree of conformity in specific and general aspects of Schoology, and in which of them there is less approval.

MICROBIOLOGY AND IMMUNOLOGY

A123
COMMERCIAL ESSENTIAL OILS WITH ANTIFUNGAL EFFECT AGAINST THE MAIZE PHYTOPATHOGEN FUNGUS *Fusarium verticillioides*

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Fusarium verticillioides (Sacc.) is a fungal pathogen of maize and the major causal agent of stalk and ear rot worldwide. The application of synthetic fungicides is the most commonly used strategy to control food deterioration by fungi. However, these chemical substances proved to be toxic for the environment and organisms. In this context, there is an increasing public demand for the development of natural and safer antifungal agents, such as plant essential oils (EOs). Essential oils are hydrophobic substances of complex mixtures of volatile organic compounds. The aims of the present work were to study the chemical composition, antifungal, and anti-conidiation activities of *Curcuma longa*, *Pimenta dioica*, *Rosmarinus officinalis*, and *Syzygium aromaticum* EOs using a multivariate approach (Principal Component Analysis; PCA). The chemical composition of the EOs was analyzed by GC/MS. The antifungal and anti-conidiation activities were evaluated by the agar diffusion method. Different aliquots of each EO were diluted in Czapek Dox Agar (CDA) culture medium to achieve the following concentrations: 1000 ppm, 500 ppm, 250 ppm and 125 ppm. Data were analyzed by one-way analysis of variance (ANOVA) followed by a Multiple Comparison test. *Syzygium aromaticum* EO reported the highest antifungal effect, followed by *P. dioica* and to a lesser extent *C. longa*. The major compounds of these EOs were eugenol (88.7% in *S. aromaticum* and 16.7% in *P. dioica*), methyleugenol (53.09% in *P. dioica*), α-turmerone (44.7%), β-turmerone (20.67%), and Ar-turmerone (17.27%) in *C. longa*. The bioactivity of eugenol is attributable to the free –OH group that act as the hydrophilic portion increasing its solubility in the plasma membrane and forming hydrogen bonds with the active sites of different enzymes. On the other hand, turmerones are ketones with an extra double bond between the alpha and beta carbons. These α,β-unsaturations increase the polarizability of the molecule, allowing them to bind with amino acids and nucleic acids and affecting different fungal metabolic pathways. *Rosmarinus officinalis* poorly inhibited fungal growth, but was the only EO that inhibited conidial production, being its major components 1,8-cineole (53.48%), α-pinene (15.65%), and (–)-camphor (9.57%). Our results showed that some compounds are capable of decreasing mycelial growth without affecting sporulation, and vice versa. However, not all the compounds of an EO are responsible for its bioactivity. In the present work, we were able to identify different major compounds or mix of major compounds that were responsible of antifungal and anti-sporulation effects. Further experiments combining these pure components are necessary in order to achieve a highly bioactive plant-based formulation against the phytopathogen fungus *F. verticillioides.*

A124
POLYEXTREMOPHILE ACTINOBACTERIA: STUDY OF RESISTANCE TO COPPER BY SCANNING ELECTRON MICROSCOPY

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Actinobacteria are a group of Gram-positive bacteria with a high content of G and C in their DNA and physiologically very diverse, they produce a great number of metabolites which are important in biotechnology. Due to this, emphasis is being placed on the isolation of these microorganisms from promising sources, such as the High-altitude Andean Lakes (HAAL), located in the region of the South American Central Andes, at a height of more than 3500 MASL. This place presents a microbiodiversity with unique mechanisms to adapt to extreme environmental factors, such as high salinity, dryness, UV radiation and heavy metals. In this piece of work, the morphological characteristics of polyextremophile bacteria were determined by Scanning Electron Microscopy after being subjected to copper stress. To this end, *Nesterenkonia sp.* Act20, isolated from the soil in Lake Socompa (3750 MASL), was used. In addition, this was later compared to *Nesterenkonia halotolerans* DSMZ 15474, isolated from high saline soils in China. Resistance profiles were studied after being subjected to 3 mM $CuSO_4$ in different times (24, 48, 72, 96 h). For each of the times, were taken samples for scanning electron microscopy, they were fixed with Karnovsky fixative (p-formaldehyde 8% v/v, glutaraldehyde 25% v/v and phosphate buffer, pH = 7). The samples were dehydrated successively with increasing alcohol concentrations (30, 50, 70, 90, and 100 %) and acetone. The final dehydration was carried out with the critical point technique. Samples were mounted on scanning electron microscopy sample stubs and gold coated. Specimens were observed under vacuum using a Zeiss Supra 55VP (Carl Zeiss NTS GmbH, Germany) scanning electron microscope. The results demonstrate that the presence of heavy metals generate bacterial aggregation, the production of extracellular material and some morphological modifications such as increased surface roughness and apparent turgor. Furthermore, *Nesterenkonia* Act 20 presents a higher growth than *Nesterenkonia halotolerans*. These results contribute to reaffirm the biotechnological potentiality of the microorganisms isolated in LAPAs.

A125
PRELIMINARY STUDIES OF LACTOBACILLI STRAINS FOR USE AS BIOPRESERVATIVE AGENTS IN BREWER'S GRAINS

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Brewer's grains (BG) are used by pig producers as feedstuffs. The high content of moisture makes this byproduct susceptible to contamination by deteriorating and/or pathogenic microorganisms. On the other hand, lactobacilli strains are promising candidates to be used as biopreservative agents in animal feed; they are safe and produce a wide range of antimicrobial metabolites. The aims of the present study were to study the kinetics of inhibition of *E. coli* F4-K88 (producer of diarrhea in pigs) by lactobacilli in co-cultures and evaluate the growth parameters of each lactobacilli strain in BG medium. Firstly, mixed cultures were prepared in MRS broth of *L. brevis* L52, *L. plantarum* L54 and *L. cellobiosus* L56 with *E. coli* in a 1:1 ratio, at different times plate counts of each microorganism were performed and growth parameters were calculated. In the second experience, a BG-based liquid medium (BGM) was performed to assess the growth of each lactobacilli strain separately. L52, L54 and L56 strains inhibited the development of *E. coli* F4 when both strains were co-cultivated. In the pathogen control culture, the maximum count value was 9 log cfu/mL at 16 h of incubation. While in co-culture with the different strains of lactobacilli, it was observed that approximately 8 h after incubation, *E. coli* reached a maximum microbial population with values that ranged from 4×10^6 to 3×10^7 cfu/mL, representing an inhibition percentage in the range of 19–29% respect to the control. Furthermore, the lactobacilli strains eliminated *E. coli* from the culture medium after 24–36 h of incubation. In general, lactobacilli decreased the growth rate values of *E. coli* and increased the generation time of the pathogenic microorganism. In the second assay, lactobacilli strains were added to the BGM in order to evaluate their adaptability to the substrate. Analysis of variance did not show significant differences in the growth rate between lactobacilli strains. The maximum counts were 8 log cfu/mL at 12 h of incubation. Finally, it was observed that the addition of these microorganisms to the BG extract produced a decrease in pH values that ranged from 3.6 to 5.7. In conclusion, *L. brevis* L52, *L. plantarum* L54 and *L. cellobiosus* L56 isolated from BG inhibited the growth of the main bacterium responsible for post-weaning diarrhea in pigs. Also, they demonstrated adequate growth adaptability in a similar medium to the substrate where they will be applied in the future.

A126
ASSESSMENT OF NEW OVULES CONTAINING FREEZE-DRIED PROBIOTIC *Lactobacillus fermentum* L23 (GENBANK GQ455406.1): CONTROL OF VIABILITY AND ANTIBIOFILM ACTIVITY DURING STORAGE TIME

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The growing interest in the development of bio-products containing safe microorganisms as a strategy to restore the vaginal ecosystem and prevent or combat pathogenic microorganisms in this niche has re-emerged in recent years. However, in most Latin American countries, including Argentina, there is low availability of these vaginal products in the market. In the development of new ovules containing probiotic strains, the control of viability and probiotic properties in storage is relevant. The aims of this work were (i) to select a vaginal formulation that ensures the best level of *L. fermentum* L23 viability over time and, (ii) to evaluate the maintenance of antibiofilm activity after recovering from the ovules through time. To obtain a high biomass production, successive cultures of L23 strain in MRS broth (pH 5.5) were made. Bacterial cells were concentrated 20-fold in 10% skimmed milk solution and subsequently, lyophilized. Three vaginal formulations (F1–F3), combining lyophilized powder of L23 with different proportions of glycerol, gelatin, skim milk, lactose or Tween80 were tested. Then, the ovules by fusion method were obtained. To determine bacterial viability recovered from the ovules over storage time, samples every 30 days for 270 days were taken. Bacterial counts on MRS agar plates were done, and their average (log CFU/mL) was used to calculate the survival rates (%). As an important antagonistic property from L23 strain, the anti-biofilm activity over time was tested. Thus, the effect of bioactive metabolites produced by L23 after recovering from ovules was evaluated on pathogenic biofilm formation every 90 days until 270 days. Pure cell-free supernatants (CFS) and neutralized CFS containing (organic acid + bacteriocin) and (bacteriocin), respectively, were tested on two biofilm-producing pathogens, *Streptococcus agalactiae* (SGB) and *Staphylococcus aureus*. Then, the antibiofilm activity (%) was calculated by spectrophotometer measures. Formulations F1 (base components) and F2 (skimmed milk + lactose) allowed to maintain the viability of L23 strain for 180 days, whereas in F3 (skimmed milk, lactose + Tween80) only remained for 150 days. The survival achieved with F1 and F2 was meaningful in comparison to F3 until the end of the experience ($P < 0.05$). For F1, F2, and F3, the average (%) reduction on the SGB biofilm over time produced by the CFSs and NCFS of L23 ranged between 70–73% and between 57.3–66.33%, respectively. Similarly, on the *S. aureus* biofilm, the percent reduction ranged between 62–70% and 55–63% for CFS and NCFS, respectively. In conclusion, F1 and F2 formulations were the better options for reaching optimal viability levels of this strain over time. Likewise, the strong anti-biofilm activity maintained over time confirms its technological potential.

A127

STUDY OF THE SYNERGIC INTERACTION EFFECT BETWEEN NISIN AND THE *Shigella flexneri* 2'S ANTIMICROBIAL PEPTIDE, ON FOODBORNE BACTERIAL PATHOGENS

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Bacteriocins are antimicrobial peptides that have antagonistic effects against the development of other organisms. Nisin is the most studied bacteriocin able to inhibit a broad spectrum of food spoilage. We demonstrated that the *Shigella flexneri* 2 AC172 strain produces a peptide, which has similar characteristics to that of bacteriocins that display antimicrobial activity against the *Escherichia coli* AB1133 strain. In this work, we evaluated the synergistic effect produced by the combination of nisin and *S. flexneri* 2 AC172's cell-free supernatant on the growth of different pathogenic strains, which cause foodborne illnesses. The antimicrobial activity of these peptides, alone or in combination, against foodborne bacterial pathogens, was determined by the minimal inhibitory concentration (MIC) method, followed by the determination of the optical density at 600 nm in a microplate reader. For this purpose, a 96-well microplate containing serial double dilutions of nisin, *S. flexneri* 2 AC172's cell-free supernatant, or different nisin/cell-free supernatant amount combination, were inoculated with a suspension of testing bacterial containing 104–105 CFU/mL. These microplates were then incubated for 24 h at 37°C. The MIC values were used to determine the Fractional Inhibitory Concentration (FIC) and the FIC index (FICI), which finally defined the synergistic effect exerted by both antibiotic compounds. In this work, we observed a synergistic effect between both bacteriocins, capable of increasing the antibiotic sensitivity of pathogenic strains. These natural antimicrobial combinations represent a biotechnological strategy applicable to the preservation of food, in order to combat foodborne pathogens that can affect human health.

A128

MICROBIOMES STUDY IN MEDICAL-ASSISTENTIAL ENVIRONMENTS BY ELECTRON MICROSCOPY SWEPT TECHNIQUES

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The identification of microbiomes present in the health field is extremely important given the close relationship with human diseases. The aim of this study was to investigate the composition of microorganisms on the laboratory surfaces of the Central Blood Bank "Dr. César Guerra", (PRIS-SI.PRO.SA). Duplicate samples from the countertops, air conditioners and the equipment on the Production, Distribution and Molecular Biology services were taken using paper tape and swabs. Plating was carried out in LB pH7 culture medium containing Cycloheximide (CH) and Cyclohehimide/Nalidixic Acid (CH/NA) antibiotics. Both the paper tape and the colonies obtained from the cultures were subjected to Scanning Electron Microscopy (SEM). Sampling using paper tape allowed the presence of microbial biofilms to be detected in the internal part of the Production service centrifuge, in the Distribution service platelet shaker and in the Molecular Biology service countertops. They presented a complex three-dimensional organization characterized by microorganisms of different morphology arranged in layers immersed in abundant extracellular material. The tape analysis also revealed the presence of isolated bacteria (cocci and bacilli) or the formation of small groups of them at the different sampling sites. The cultures allowed the isolation of predominant microorganisms from countertops, air conditioners and equipment. A total of 45 colonies (Gram+ and Gram−) that exhibited various morphotypes (cocci, bacilli, and coccobacilli) were isolated. Using SEM, it was possible to analyze in detail the structure, organization, and morphology of the bacteria in culture. Furthermore, it was observed that many colonies established close contacts. The SEM study revealed a wide spectrum of associations among them. It was possible to analyze the contact points among interacting colonies, revealing morphological changes in the bacteria as well as a large amount of extracellular material at the interaction sites. In addition, the topographic analysis of the colonies showed differences in the conformation of the different sectors in some of them. This work, aimed at analyzing the microbiological communities developed *in situ* in healthcare settings, proposes high-resolution microscopy techniques as key tools for the study in situ of biofilms on a surface, which study is lacking in our country.

A129

COMBINED TREATMENT WITH AMPHOTERICIN B AND CLOMIPRAMINE AGAINST MACROPHAGES J774.A1 INFECTED WITH *Leishmania amazonensis*

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Leishmaniases are important neglected tropical diseases caused by parasites of the genus *Leishmania* (Trypanosomatidae) with broad geographical dispersion. Current antileishmanial available drugs have shown to be limited by toxicity, high cost, and long-term treatment, resulting in low patient compliance with the treatment. Combined therapies based on available drugs for Leishmaniasis treatment and novel uses for old drugs, have been proposed as promising therapeutic alternatives. On the one hand, Amphotericin B (Amph-B) is a drug that exerts its leishmanicidal action by increasing permeability, leading to cell death due to the leakage of cellular content. However, it has shown serious toxic effects. On the other hand, Clomipramine (Clo), a tricyclic antidepressant, is a competitive inhibitor of the enzyme trypanothione reductase (TR), which is only found in *Leishmania* and *Trypanosoma cruzi*. Inhibition of TR produces an increase of non-reduced intracellular peroxides, leading to toxic effects on the parasite. The aim of this work was to study the combined effect of Amph-B and Clo against macrophages from J774.A1 line infected with *L. amazonensis*. Macrophages (2×10^5 cells/mL) were infected with promastigotes in a ratio of 5 parasites per mammalian cell. Then, the infected

macrophages were incubated with concentrations of Clo (0.15 and 0.30 µg/mL) and Amph-B (0.5 and 1.0 µg/mL) alone or in combination (N = 3). Both infected control without treatment (IC) and control without infection were included. After 72 h, Giemsa staining was performed, and cells were randomly counted to determine the infection index (Inf I). The Inf I was calculated as the percentage of the infected macrophages and the average number of amastigotes per macrophage. Results have shown that with respect to IC, the treatment with Clo at 0.30 µg/mL reduced the Inf I by approximately 24% whereas the treatment with Amph-B at 1.0 µg/mL reduced the Inf I by approximately 40% ($P < 0.0001$). Combination of Amph-B 0.5–Clo 0.30 µg/mL and Amph-B 1.0–Clo 0.30 µg/mL reduced 75% of Inf I with respect to IC ($P < 0.0001$), without statistically differences between these two groups ($P > 0.05$). It is also important to point out that these results were achieved by keeping the cellular viability of macrophages between 76 and 92%. In conclusion, complementary mechanisms of action of Clo and Amph-B enhance leishmanicidal activity avoiding lethal effects on host cells.

A130
EFFECT OF CULTURE MEDIA AND CHEMICAL AGENTS ON THE ANTIMICROBIAL ACTIVITY OF *Bacillus velezensis* SL-6

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Antimicrobial metabolites produced by members of the genus *Bacillus* constitute a great source of antibiotics with potential application in veterinary and medical sciences. Evaluation of culture media and the stability of these metabolites against different agents are important for their optimal production. In the present work, the influence of culture media and the effect of different chemical treatments on the antimicrobial activity (AA) of *Bacillus velezensis* SL-6 were studied. Batch cultures of the SL-6 strain were performed in Tryptone Soy Broth, Brain-Heart Infusion, Mueller Hinton Broth, Glucose-Peptone Broth and Synthetic Mineral Broth (SMB). After 24 h, the biomass (g/L) was determined by the dry weight technique, and the AA (AU/mL) of the cell-free supernatants (CFS) against *Candida albicans*, *Staphylococcus aureus*, and *Yersinia enterocolitica* was measured by the agar well diffusion method. In addition, the residual AA of the SLCE after treatment with divalent salts of Ca^{2+}, Mg^{2+}, Cu^{2+} and Mn^{2+}, detergents (Tween 20 and Tween 80) and buffer solutions with pH between 1 and 11 were determined. Bioactive compounds of SL-6 strain were produced only in the SMB with antimicrobial activity titers of 1600, 400, and 800 UA/mL for *C. albicans*, *S. aureus*, and *Y. enterocolitica*, respectively. The residual AA against *C. albicans* and *S. aureus* remained stable after each treatment, showing values between 76 and 100%. On the other hand, the activity against *Y. enterocolitica* was totally inhibited by both detergents, reduced by 32% when treated with Ca^{2+} and affected by extreme pH values, remaining stable at pH range 5.0–8.0. Characterization of the antagonistic activity of *B. velezensis* SL-6 suggests that the secretion of antimicrobial metabolites against bacteria and yeasts was highly dependent on the culture media used. Additionally, the stability of these metabolites was differentially modified by the chemical agents tested, the biocompounds involved in anti-*Yersinia* activity being highly sensitive.

A131
Nesterenkonia sp. Act20 UV-RESISTOME: GENOMICS AND ULTRASTRUCTURE

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Nesterenkonia sp. Act20 is an actinobacterium isolated from arid soil surrounding Lake Socompa, located at 3750 m, in the province of Salta, Argentina. It is characterized by its multi-resistance to extreme conditions, highlighting its ability to survive high levels of UV irradiation has been linked to its efficient DNA repair mechanisms and the production of primary and secondary metabolic products. Our objectives are to analyze the behavior of Act20 after exposure to artificial UV-B radiation at different times. The strain was grown in both liquid and agar media and visualize using the Scanning Electron Microscope (SEM). Zeiss SUPRA 55VP (Carl Zeiss NTS GmbH, Germany) belonging to the Electron Microscopy Core Facility (CIME), Bio-imaging data was likewise correlated with the strain genomics. Genomic survey indicated the presence of coding genes for flagellum biogenesis: flagella biosynthesis proteins, flagella motor proteins, and the flagella hook, among others, whose structure was evidenced by observing plated colonies using SEM. Likewise morphological and ultrastructural changes were observed due to the arrest of the cell division cycle caused by the different doses of UV-B exposure, in both culture conditions. Despite this, there were a low number of cell deaths, which shows the resistance of the strain to this stress factor. This work adds up evidence towards the definition of the UV-resistome of extremophilic strains from Andean Lakes.

A132
MICROENCAPSULATION OF PROBIOTIC LACTOBACILLI IN A MATRIX OF MILK PROTEINS: INCREASING VIABILITY DURING DIFFERENT HEAT TREATMENTS

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Microencapsulation of probiotic microorganisms is an important strategy to increase bacterial viability against different stressful technological and physiological conditions. Previous studies demonstrated that microencapsulation of *Lactobacillus fermentum* L23 and *L. rhamnosus* L60 protected them during long refrigerated storage and under simulated gastrointestinal conditions. Exposure to high temperatures is one of the most important technological conditions that decrease beneficial bacteria viability during processing of certain foods. The objective of this work was to evaluate the resistance to high temperatures of *L. fermentum* L23 and *L. rhamnosus* L60 microencapsulated in a matrix of milk proteins. Both lactobacilli strains

were microencapsulated by the process of emulsification and rennet catalyzed gelation of milk proteins. Microcapsules containing *L. fermentum* L23 and *L. rhamnosus* L60 were added into MRS broth and exposed to 50°C and 65°C in a water bath during 30 min. Lactobacilli were recovered from the microcapsules and bacterial counts were determined at time 0 and after each heat treatment. The same procedure was performed with free lactobacilli (controls). The results of this work showed that the bacterial viability of both free lactobacilli strains significantly decreased when they were subjected to the temperatures tested. From an initial mean count of 8.62 log CFU/mL of free L23 strain, the bacterial viability after exposure to 50°C and 65°C for 30 min decreased to 7.74 and 3.87 log CFU/mL, respectively. For free L60 strain, lactobacilli counts were reduced from an initial population of 8.29 to 6.43 and 3.96 log CFU/mL after the heat treatment at 50°C and 65°C, respectively. When lactobacilli strains were microencapsulated, they showed higher stability to high temperatures. Microencapsulation of L23 allowed maintaining mean log bacterial counts of 8.56 and 6.26 log CFU/mL after exposure to 50°C and 65°C, respectively. While microencapsulated L60 strain showed mean log counts of 7.87 and 6.17 log CFU/mL for both temperatures tested. Both microencapsulated lactobacilli showed very high survival rates, which were in the range of 95–99.3% at 50°C, and 72.5–74.4% at 65°C. In conclusion, the microencapsulation process used in this study effectively protected these *Lactobacillus* strains against the different heat treatments tested. These findings suggest that the microencapsulation of lactobacilli in a matrix of milk proteins has biotechnological potential to be applied in the development of new functional foods.

A133
ECOPHYSIOLOGICAL CHARACTERIZATION OF FUNGAL STRAINS WITH NEMATOPHAGE CAPACITY ON *Nacobbus aberrans*

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Nacobbus aberrans is a recurrent phytoparasite in the crops of the Río Cuarto horticultural belt. A low environmental impact practice for nematode management is the use of biocontrol agents. The objectives of this study were: (a) to evaluate the effect of different environmental factors on the growth of 5 fungal strains with a nematophage capacity on *N. aberrans*; (b) to evaluate the capacity of these strains to produce extracellular enzymes. The effect of temperature (t) (20, 25, and 30 °C), water activity (aw) (0.99, 0.98, 0.95, and 0.93) and matric potential (Ψ_m) (−0.7, −3, −7, and −10 MPa) on the growth rate (GR) of *Purpureocillium lilacinum* SR7, SR14, SR38, *Metarhizium robertsii* SR51 and *Plectosphaerella plurivora* SRA14 was determined. Furthermore, the production of extracellular enzymes (proteases, chitinases, amylases and lipases) of the 5 fungal strains was evaluated by the plate assay method, and finally the chitinolytic activity was quantified. At 25°C the GR of the 5 fungal strains was similar (0.36–0.60 cm/ day), while at 30°C this parameter increased significantly (12%) ($P < 0.05$) for *P. lilacinum* SR14 and *M. robertsii* SR51. The optimal aw for the development of the five fungal strains was 0.99, while GR decreased to lower aw (0.95: 71%; 0.93: 96%). The optimal Ψ_m for fungal development (0.38–0.60 cm/ day) was −0.7 MPa, while the GR was reduced at higher values (−3 MPa: 047; −7.0 MPa: 82; −10 MPa: 100%) ($P < 0.05$). The enzymatic studies showed the production of chitinases by the 5 nematophagous fungi. The quantitative test showed that *P. lilacinum* SR7 was the strain with the highest enzymatic activity ($P < 0.05$) (0.18 U/h mL), while *P. lilacinum* SR14 and SR38 and *M. robertsii* SR51 produced levels of chitinases in the order of 0.9–0.14 U/h. This study revealed that modifications in aqueous availability can significantly affect the GR of these fungi. However, the 5 fungal strains developed under the ranges of environmental conditions evaluated, which represents an advantage in the competition with other soil organisms. In addition, the *P. lilacinum* SR7, SR14 and SR38 and *M. robertsii* SR51 strains produced chitinases, enzymes involved in the infection process of plant parasitic nematodes such as *N. aberrans*.

A134
IN VITRO EVALUATION OF THE NEMATICIDAL ACTIVITY OF BRASSICACEAE EXTRACTS ON *Nacobbus aberrans* AND ITS COMPATIBILITY WITH NEMATOPHAGOUS FUNGI

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Nacobbus aberrans is one of the recurring biotic adversities in undercover crops in the Río Cuarto horticultural belt. Its handling through the application of methyl bromide has been prohibited in Argentina since 2006. Consequently, biotic adversities that were "silenced" became relevant. The combined use of botanical extracts and nematophagous fungi could be a promising alternative for controlling *N. aberrans*. The aims of this work were (a) to evaluate the nematicidal activity of the aqueous extracts (AEs) of broccoli (*Brassica. oleoracea* var. *italica*) and cabbage (*Brassica oleoracea* var. *capitata*) on the infective stage J2 of *N. aberrans* and (b) to determine the *in vitro* compatibility of the AEs with 5 fungal nematophagous strains (*Purpureocillium lilacinum* SR7, SR14, SR38, *Metarhizium robertsii* SR51 and *Plectosphaerella plurivora* SRA14). The nematicidal activity of 7 concentrations (100, 50, 25, 20, 17.5, 12.5, and 6.25 %) of broccoli and cabbage AEs on the larvae was evaluated. For this, 980 µL of the AEs solutions were placed in vials containing 20 J2s and incubated at room temperature. The estimation of the immobile J2s was performed after 2, 4, and 24 h of incubation. Eight replicates were made, and the test was repeated in time. To determine compatibility, 0.1 mL of each spore suspensions (10^1 and 10^2 spores/mL) of the 5 fungal strains was placed in Soil Extract Agar supplemented with the corresponding doses of each AE (90; 50; 25, 20, 17.5, 12, 6, and 3 % for broccoli and 90, 50, 25, 12, 6, and 3 % for cabbage). The effect of AEs on the viability of the fungal propagules was determined by comparing the counts (CFU/mL) with the respective control. Both AEs showed high nematicidal activity with LD_{50} of 12.7% for broccoli and 10.96% for cabbage, at 24 h of exposure. The nematicidal action increased with the exposure time. Broccoli AE was proved to be compatible with *P. lilacinum* SR14 and SR7 at the lowest doses tested (3, 6, and 12.5 %), while it completely inhibited fungal development at concentrations higher than 25%. The results obtained in the present study demonstrate the possibility of using broccoli AE at the nematicidal concentration of 12.5% in combination with *P. lilacinum* SR14 and SR7 for the control of the phytoparasitic nematode, *N. aberrans*.

A135
INFLUENCE OF IMMATURE STAGES ON THE POSITIVE PREDICTIVE VALUE OF DEMODICOSIS DIAGNOSIS

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Demodex spp. is a habitual resident mite of human skin, however it has a function as an etiological agent or adjuvant in certain dermatological diseases. Its diagnosis consists of finding the parasite in superficial skin lesions by microscopic observation of superficial biopsy samples or of those obtained with adhesive tape. The present work studies the influence of the mite infective stages on the positive predictive value (PPV) of the diagnosis of demodicosis when adhesive tape is used as a sampling method. Three hundred and sixty-six positive samples from patients with and without compatible symptoms including healthy volunteers were analysed. They were separated into subgroups according to the presence / absence of the different stages and their combinations, and the PPV and confidence intervals (95% CI) were calculated for the total and each data subgroup, taking the dermatological clinical characteristics as a reference value. The global PPV was 0.88 (95% CI 0.85–0.92) (N = 366) and the combinations of the different stages of this parasite gave different PPV according to the morphologies found. When only adults were found in the samples, the PPV was 0.81 (95% CI 0.75–0.88) (N = 129), while values of 0.87 (95% CI 0.73–1.01) (N = 23) were reached when considering only larval stages and 0.85 (95% CI 0.73–0.96) (N = 39) with only eggs. When combinations of infecting stages were considered: 1.00 (95% CI NA) (N = 12) for eggs and larval stages, 0.94 (95% CI 0.87–1.01) (N = 48) for eggs and adults and 0.9 (95% CI 0.82–0.98) (N = 50) for larval and adult stages. The combination of the three morphologies simultaneously gave a PPV 0.97 (95% CI 0.93–1.01) (N = 65), while the set of all samples in which immature forms were found either accompanied or not by adults yielded a PPV of 0.92 (95% CI 0.89–0.95) (N = 237). From the above, it can be deduced that the presence of adults was the most frequently found morphology. On the other hand, although several of the confidence intervals obtained overlap each other, the simultaneous combination of the three morphologies significantly improved the PPV with respect to the global parameter, while the samples that showed the presence of eggs and/or larval stages (with or without the presence of adults), had a significantly higher PPV than those in which only adult forms were found.

A136
ANTIMICROBIAL EFFECT IN A BASE CREAM OF FREE CELL SUPERNATANTS FROM *Lactobacillus paracasei ssp paracasei 1. sl57*. SAN LUIS. ARGENTINA.

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Enterococci are part of the normal intestinal flora. Taxonomically they were classified as group D streptococci, actually are considered a new genus. In humans, they commonly cause urinary tract infections, bacteremia, and endocarditis, as well as intra-abdominal and pelvic infections, skin, soft tissue, and wound infections. Up to 90% of enterococcal infections in humans are caused by *Enterococcus faecalis*. The strains resistant to currently available antibiotics pose real therapeutic difficulties. The probiotics are presented here, as an alternative for the treatment of enterococcal skin infections. The protective role of lactic acid bacteria (LAB) lies in their capability to decrease pH and synthesize of bacteriostatic and bactericidal substances. These substances include hydrogen peroxide, lactic acid, carbon dioxide and bacteriocins which are defined as peptides produced by bacteria that inhibit other related and unrelated microorganisms. There are few studies on the use of lactic bacteria in creams. Strain selected for this study was isolated and named as *sl57,* from samples of goat milk samples collected from a regional dairy. The *LAB* strain was biochemically typified as *Lactobacillus paracasei ssp paracasei 1*. The purposes of this study were to evaluate the spectrum of antimicrobial activity of a *LAB* strain and study its antimicrobial effect on a base cream. The antimicrobial activity of cell-free supernatant (CFS) and neutralized cell-free supernatant (NCFS) of a culture of the strain under study was evaluated against *E. faecalis* (indicator *strain*) for liquid medium method. The inhibition percentage (%I) was calculated according to the formula I = 1 − As / Ac, considering As and Ac as sample absorbance and control absorbance respectively. In control assay, the CFS was replaced by MRS broth. Aliquots of 8 mL of the dilution of base cream were treated with 1 mL of CFS or 1 mL MRS broth (controls) and 1 mL of indicator strain suspension (10^8 CFU/mL). Aliquots were incubated at 35°C for 12 h, 1 mL of 10-fold serial dilution of each treatment was poured on a Petri dish using Tripticase Soy Agar medium. The plates were incubated at 37°C for 24 h. *E. faecalis* counts in samples were determined according to National Standard Test Methods for Food Microbiology. CFS and NCFS from *L. paracasei spp paracasei I sl57* showed inhibitory activity of 74.5% and 67%, respectively, against *E. faecalis*. On the other hand, significant differences in counts were observed, therefore, CFS inhibits indicator growth in the treated base cream (count values expressed as CFU/mL), compared to growth of indicator strains in controls (aliquots of samples treated with MRS broth). These results give evidence of the possibility of the use of probiotics for the topical treatment of infections, this is important due to the scarcity of studies in this regard and the high resistance to antibiotics presented by this bacterial genus.

A137
EVALUATION OF A DIAGNOSTIC SYSTEM FOR THE DETECTION OF IGM AND IGG ANTI-SARS-CoV-2 ANTIBODIES IN COMPARISON WITH MOLECULAR DIAGNOSTICS

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As of April 7, 2020, the World Health Organization (WHO) reported a total of 417.416 confirmed cases of SARS-CoV-2 virus infection (COVID-19) in the Americas and 12.597 deaths. Of these 1715 infected and 60 dead corresponded to Argentina, while for our province of San Luis there were 11 infected and no dead. The diagnosis of SARS-CoV-2 virus infection is made by means of the real-time reverse transcriptase-polymerase chain

reaction (RT-PCR) test, which detects the presence of viral RNA. This molecular test is useful in the first three weeks of infection and is currently the standard reference method recommended by the WHO. Immunological tests can be a complementary diagnostic aid and important support in epidemiological surveillance. These tests are based on the detection of IgM and IgG immunoglobulins against SARS-CoV-2, which appear from the second week of infection. In San Luis, until the end of June 2020, the diagnosis of COVID-19 was only made through molecular tests. In a scenario of an increasing number of cases, it could generate an under-recording, so in this context, the Ministry of Health decided to perform rapid serological tests. However, before their application on a large scale, it was necessary to evaluate their usefulness by comparing them with the molecular test. The objective of the study was to evaluate the functional and operational characteristics, as well as to determine the diagnostic performance of two commercial rapid serological test systems for the detection of IgM and IgG antibodies, by calculating their additional diagnostic performance compared to RT-PCR for the detection of SARS-CoV-2 infection. A cross-sectional study was carried out, through a universe constituted by 340 blood samples, all of them from people who voluntarily decided to undergo the test and through the corresponding informed consent and belonging to the localities of Quines, Candelaria, Luján, Los Cajones, and Lafinur in the Department of Ayacucho in the Province of San Luis. These samples came from asymptomatic patients, with no epidemiological link in the area of our province, but with contacts from the neighboring town of Villa Dolores which was experiencing an outbreak of more than 130 cases in two weeks. All samples were processed by the SD Biosensor Standard Q COVID-19 IgM/IgG Duo system. In turn, samples positive to serological tests were tested by means of nasopharyngeal/oropharyngeal swab for molecular diagnosis (RT-PCR), and 14 days after this first sample were re-tested for serological status by the same techniques. The additional diagnostic performance of the rapid serological test was evaluated in relation to the molecular one. The sensitivity and specificity of these tests were also estimated. The rapid serological test yielded 28 positive samples (27 IgM-positives and one IgG-positive) with 8.2% positive results compared to 0% for the molecular test. The rapid serological test detected 312 cases that were also negative by the initial RT-PCR and the additional diagnostic yield was 91.7% compared to RT-PCR. Re-testing 14 days after this first result gave a value of 9 positive samples (7 positives for IgM and 2 positives for IgG) out of these 28 positives samples. The specificity value and negative predictive value (NPV) were calculated for IgG (99.7% and 100%, respectively) and IgM (92% and 100%, respectively). Sensitivity and positive predictive value cannot be calculated because all samples were negative using the technique *gold standard* (RT-PCR). Disagreement was found between the values found and those expressed by the commercial kit in its insert. In conclusion, rapid serological testing, while simple, quick, and easy to perform, with no additional equipment requirements, provides additional diagnostic performance to the molecular tests for the pathology of COVID-19, but they have a lot of interferences, as well as a high number of positives false.

A138
ANNUAL VARIATION OF ATMOSPHERIC BIOPARTICLES IN GENERAL ALVEAR, MENDOZA

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Among the application lines of Aerobiology, those related to agriculture are very important, through studies about pollination phenology, the control of fungal weed pests and the harvest forecast. In addition, numerous works relate concentrations of spores in the atmosphere with plant diseases caused by fungi. On the other hand, Aerobiology has relevance in the Medicine area, in topics related mainly to pollen allergies. The objective was to evaluate the aerobiological spectrum of General Alvear from the pollen and fungal types present in the atmosphere and to prepare annual pollen and sporal calendars from daily records. This work was carried out in General Alvear department, located 300 km from Mendoza city, in a period of one year (July 2019–June 2020). The biological particles in suspension were sampled with a Hirst-type volumetric sensor, Lanzoni brand. For the analysis and particle count from the samples, were identified and counted the number of each of the types of aeroparticles that were deposited in 4 prefixed horizontal stripes, homogeneously distributed on the surface of the slide. The analyses were made using a Celestron binocular optical microscope at 40×10 magnification. During the period studied, an average concentration of 274.54 bioparticles/m^3 of air has been detected, the maximum concentration was observed in February (461.66 bioparticles/m^3 of air) and the minimum in June (136.4 bioparticles/m^3 of air). Regarding the pollen spectrum, it showed an annual average of 36.9 grains/m^3 of air, expressing the highest concentration in September (158.55 grains/m^3 of air) and the lowest in April and May (0.54 grains/m^3 of air). The fungal spores showed an annual average of 237.65 spores/m^3 of air, being the maximum in February (449.1 spores/m^3 of air) and the minimum values in August (119.07 spores/m^3 of air). 17 pollen types were identified, the most representatives belong to Cupressaceae, Oleaceae, Ulmaceae, Moraceae, Chenopodiaceae-Amaranthaceae, and Poaceae, among others. Likewise, 30 spore types were identified belong to *Alternaria*, *Aspergillus-Penicillium*, *Leptosphaeria*, *Cladosporium*, *Torula*, *Didymosphaeria*, and *Drechslera*, among others. The contributions of this work could help to substantially improve the production of fruit and vegetables in the region in addition to making forecasts on the incidence of pollinosis in the region.

A139
ANTIVIRAL ACTIVITY OF NORDIHYDROGUAIARETIC ACID ON LOCAL ISOLATIONS OF FORT SHERMAN VIRUS (ORTHOBUNYAVIRUS)

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The genus Orthobunyavirus (arbovirus) is a group of viruses, which can cause infection in humans and is characterized by its lack of therapeutic treatment or preventive vaccine. We proposed to study the *in vitro* antiviral activity of nordihydroguaiaretic acid (NDGA), the most important active compound of *Larrea divaricata* Cav. (*Zygophyllaceae*), against two local isolates of the Fort Sherman virus (FSV) as a model for the genus Orthobunyavirus and to evaluate the effect of NDGA as a lipolytic agent in the cell cycle of these viral isolates. The method of reducing plaque forming units in LLC-MK2 cells was used to evaluate the action of NDGA on FSV isolates, CbaAr426 and SFCrEq231, under different conditions. Furthermore, the ability of the NDGA to be incorporated into these cells was quantified by HPLC. NDGA showed antiviral activity with a similar

dose-dependent inhibition in both isolates (> 90 I%). The NDGA selectivity index was 4.8 and 4.6 for CbaAr426 and SFCrEq231, respectively. It was established that NDGA has a better inhibition (> 90 I%) 1 h post-infection (p.i.), showing a different behavior on each viral isolate. The effect of NDGA was equally important at 8 hrs p.i. on the CbaAr426 isolation. However, on SFCrEq231, it was active during the first 2 h p.i. The antiviral effect of NDGA has previously been related to its ability to alter lipid metabolism by interfering with the sterol regulatory element-binding protein (SREBP) and 5-lipoxygenase (5-LOX) pathway. Using caffeic acid, an inhibitor of 5-LOX, we determined that the inhibition of this enzyme negatively affected replication in both isolates; and through the use of resveratrol, an inhibitor of SREBP1, it was demonstrated that the negative regulation of this pathway only had an action on the reduction of SFCrEq231. We estimate that NDGA acts intracellularly since it showed the ability to incorporate in LLC-MK2 cells (15% enters between 0–15 min). The information provided in this work makes NDGA a potential antiviral candidate for Orthobunyavirus infections, especially on FSV isolates circulating in Argentina. On the other hand, the results obtained make new contributions to the biochemical study of FSV, a poorly studied and potentially dangerous infection.

A140
CHEMICAL AND MICROBIOLOGICAL PARAMETERS IN THE EVALUATION OF THE TECHNOLOGICAL PROCESS OF PAPRIKA PRODUCTION

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The determination of color, volatile organic compounds (VOCs), and microbiological aspects are parameters related not only to the quality of the product but also to make it possible to evaluate comprehensively the technological process of manufacturing the paprika that comes from the fruit of *Capsicum annuum L*. The objective of this work is to determine the ASTA color, the profile of the majority of volatile compounds, and the microbiological aspect that characterize the paprika from 3 drying systems, grown in the department of Santa María, Catamarca. The samples were taken at the Diaguita Cooperative and other producing establishments. Around 15 samples of 2 varieties were studied: *Elephant trunk* and *Black* with different drying processes, microtunnel drying, with solar panels, and traditional drying. VOCs were analyzed by gas chromatography (HS-CG) and (CG-MS). Microbiological analyzes were performed according to the methods of the American Public Health Association (APHA). In the determination of the ASTA color or total carotenoid content, the ASTA 20.1 method of (American Spice Trade Association. The following VOCs were identified; myristic acid, palmitic acid, linoleic acid, and α-tocopherol in 100% of the samples, acid, methyl ester in 95%, palmitic acid methyl ester in 70%, ethyl acid ethyl ester in 75%. Color values between 60 and 240 ASTA were obtained. The microbiological analyzes included total coliforms, *E. coli*, *Salmonella spp*., *Bacillus cereus*, *Clostridium perfringens*, molds, and yeasts; they complied with the SENASA regulations by 90%. In relation to the results obtained, it is concluded that color and the presence of some VOCs are visibly related to the drying system and variety. The VOCs profile obtained reveals that the temperature and drying time conditions are adequate in non-traditional dryers, avoiding the loss of volatiles. The microbiological aspect in the processes is acceptable, which implies that the water activity of the product is low, favoring an appropriate state. It is recommended to take extreme care in the post-harvest stage with reference to storage conditions, to avoid deterioration, applying good manufacturing practices (BPM) in all phases.

A141
BIOLOGICAL ACTIVITY OF NORBELADINE ANALOGS AGAINST METHICILLIN-RESISTANT *Staphylococcus aureus*

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Staphylococcus aureus is a pathogenic microorganism and a frequent colonizer of human mucosa and skin. Nasal carriers are important reservoirs at risk of transmitting or spreading this bacterium to susceptible people or developing endogenous infections. Mice are a good model for the study of bacterial and host factors that influence nasal colonization by *S. aureus*. Medicinal plants that present active metabolites with antistaphylococcal activity offer an important alternative for the treatment of infections caused by methicillin-resistant *S. aureus* (MRSA). 4'-O-methylnorbelladin is a protoalkaloid which can undergo three different types of phenol oxidative coupling reactions to give *Amaryllidaceae* alkaloids such as hemanthamine, licorin, and galantamine. The latter is primarily isolated from daffodil (*Narcissus* spp.), snowdrop (*Galanthus* spp.), and summer snowflake (*Leucojum aestivum*) and is currently used in the palliative treatment of Alzheimer's disease in the early stage. In this study, the *in vitro* and *in vivo* activity of two halogenated analogs of 4'-O-methylnorbelladin were evaluated: 2'-chloro-MN **(1)** and 2'-bromo-MN **(2)** against *S. aureus* ATCC 43300, a methicillin-resistant strain (MRSA). Compounds **1** and **2** as hydrochlorides, were synthesized by condensation of the corresponding substituted aromatic aldehydes and tyramine and further reduction with sodium borohydride. For both compounds, the minimum inhibitory concentration (MIC) was determined by the microdilution method in broth and the minimum bactericidal concentration (MBC) by subcultures in tryptic soy agar. The *in vivo* study was implemented during three consecutive days. The first day, eight BALB/c mice were infected by instilling 10 µL of a bacterial suspension with 1×10^8 CFU/mL in each nostril; on the second day, the compound **1** suspension was administered intranasally at a concentration of 250 µg/ml in half of these mice, using the other half of mice as control (without compounds). The third day, all mice were sacrificed. The nostrils and internal organs (spleen and lung) were extracted and homogenized for bacterial quantification. The same experience was carried out with compound **2**. MIC and MBC values against MRSA for both compounds were 250 µg/mL each. Compounds **1** and **2** showed significant *in vivo* antistaphylococcal activity reflected in a significant decrease in the bacterial count of MRSA in nasal homogenates compared to the control group ($P = 0.0001$, $P = 0.0017$, respectively). Bacteria were not isolated from internal organ homogenates. There are a limited number of studies on the

bioactivities of precursors or analogues of *Amaryllidaceae* alkaloids. This study contributes to the discovery of new compounds with antibacterial properties, demonstrating an antistaphylococcal effect in an *in vivo* mouse model.

A142
EFFECT OF *Zinnia peruviana* ROOT EXTRACT ON THE PRODUCTION OF MICROBIAL BIOFILMS

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Accelerating the increase and spread of antimicrobial resistance is a growing global public health concern affecting the control of clinical infections. One mechanism by which bacteria acquire resistance to antibiotics and evade the immune system is the biofilms. Approximately 80% of chronic and recurrent microbial infections are due to this resistance strategy produced by a diverse group of organisms including Gram-positive and Gram-negative bacteria, as well as fungal species. This emerging context has motivated the search for new antimicrobial substances that inhibit or impede microbial growth or interfere with the production or eradication of biofilms. The extensive and varied flora of the Central West Region of Argentina offers an important resource for the study of natural products in the search for antimicrobials with potential therapeutic use in clinical infections. Within these species, *Zinnia peruviana*, is an herb with antimalarial and antimicrobial properties. In its phytochemical characterization it presents sesquiterpene lactones with antifungal activity, of the elemanolid type with γ-lactone-α,β-unsaturated groups and formyl-α,β-unsaturated groups. Previous work of our research group motivates the advancement and deepening of the study of this plant species as a potential source of new natural antimicrobial agents. The objective of this work was to evaluate the effect of the addition of acetonic extract from the roots of *Z. peruviana* on the biofilm production of *L. monocytogenes*, *E. coli* and *C. albicans*. Biofilm production was evaluated by determining adherence to 96-well microplates U-bottom. To each well was added: 50 µL of culture medium (supplemented with 1% glucose), 50 µL of bacterial inoculum (10^8 CFU/mL) and 100 µL of extract at different concentrations. In parallel, wells with medium and inocula without the addition of extract were used for their comparative analysis. Incubated at 37°C for 48 h. The culture was discarded, and the adhered content was washed with sterile physiological solution, fixed with methanol, and stained with crystal violet. The optical density reading was performed at 550 nm. The experience was carried out in quadruplicate and was repeated twice. The tested extract inhibited 29% and 50% of the biofilm production of *L. monocytogenes* with 0.625 mg/mL and 5 mg/mL respectively. For *E. coli* the reduction was 33% (0.625 mg/mL) and 51% (0.078 mg/mL) while for *C. albicans* a significant reduction in biofilm formation was observed with 3 concentrations of extract: 59% (5 mg/mL), 42% (0.625 mg/mL) and 44% (0.078 mg/mL). All the biofilm reduction values showed significant differences ($P < 0.05$). The high biological activity of the roots of *Z. peruviana* represents an alternative potential for the treatment and control of microbial infections.

A143
ELABORATION OF A FUNCTIONAL FOOD WITH THE ADDITION OF GRATED COCONUT

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Yogurt is understood according to the Argentine Food Code (CAA), the product whose fermentation is carried out with protosymbiotic cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, which in a complementary way can accompany other lactic acid bacteria. Functional Foods have the characteristic of acting on the body promoting a positive physiological effect, beyond its traditional nutritional value. The objective of the work was to make yogurts with the addition of grated coconut as an optional non-dairy ingredient (Chapter VIII, Art. 576 3b of the CAA). Materials and methods: two different types of yogurts were made five times. The formula used to make them was: 1L of partially skimmed liquid milk, 190 g of firm yogurt, 20 g of partially skimmed milk powder, 15 g of common sugar (formula F1 yogurt control) and with the addition of 25 g of grated coconut (formula F2); were carried out 21 days after elaborate bacteriological analyzes were carried out Total Coliforms at 30°C and 45°C (Wilson's method), *Escherichia coli* (Petri film *E. coli*), molds and yeasts (glucose potato agar) and determination of total lipids (by alkaline hydrolysis, AOAC-1995- method 905.02) and fibers (AOAC-1995- method 985.29). Results: in bacteriological determinations, the count of total coliforms at 30°C and 45°C, *Escherichia coli*, molds and yeasts was <10 CFU/g in both formulas. Control yogurt (F1) provides 1.71 (g%) of total lipids, while yogurt with grated coconut (F2) 2.91 (g%); the fiber value in formula F2 was 0.395 (g%), while formula F1 lacks it. All yogurt formulations presented a good state of conservation, and their organoleptic characteristics were satisfactory until the end of the study. Conclusions: according to the results obtained, the formula F2, provides 1.20 g% more of total lipids than F1 and also 0.395 g% of fiber, which means an improvement in the nutritional value of yogurt F2 by the contribution of grated coconut. This functional food would benefit the health of any type of consumer.

A144
PROPERTIES OF THE DICHLOROMETHANE SUBEXTRACT OF *Flourensia blakeana* ON PATHOGENIC MICROORGANISMS

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The antimicrobial activity of plant extracts is correlated with the various metabolites present. These extracts with active molecules are promising in the search of antimicrobials. The present work deals with the study of possible antibacterial properties of *F. blakeana* dichloromethane subextract

(DMC) against pathogenic bacteria that causes acute diarrhea, food poisoning and other diseases together with the identification of some compounds present in the subextract. The test was carried out with 5 bacterial strains: *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Bacillus subtillis* BSGC 168 and *Salmonella enterica ser.* Enteritidis ACC PA03. Activity was determined by the plate diffusion method. The plant material was collected in Tafí del Valle department, Tucumán. The DCM sub-extract was obtained by partitioning the ethanolic extract of leaves and flowers. In the phytochemical study, a combination of TLC, CC, CCV chromatographic techniques was used and flavonoids, derivatives of costic acid, monoterpenes and sesquiterpenes were purified. The structural elucidation of the compounds was determined by NMR spectroscopy in one and two dimensions (^{1}H, ^{13}C, HSQC, HMBC). The DMC subextract showed antimicrobial activity against *B. subtillis* 168 and *S. enterica ser*. Enteritidis AC PA03. The inhibitory concentration was 250 µg/mL against *B. subtilis* and 100 µg/mL, against *S. entérica*. These results indicate that the *F. blakeana* DMC subextract has selective antibacterial properties against some of the bacteria studied. possibly related to the identified metabolites, for which reason it is interesting to search for active molecules and work continues to isolate the active compounds.

A145
Salmonella BIOFILMS: PARTICIPATION OF THE RCSCDB REGULATORY SYSTEM

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Bacterial biofilms are complex communities consisting of microorganisms embedded in a self-produced extracellular matrix. *Salmonella* is able to form biofilm on the surface of the gallstones that produce the persistence of the bacterial colonization in the carrier patients. The RcsCDB phosphorelay system has an important role in the bacterial physiology, mainly in the response to extracytoplasmic stress signaling. It was shown that the factors affecting the cell envelope leads to the activation of the system and consequently the modulation of capsule synthesis, motility behavior and biofilm formation. Previously, in our laboratory we characterize the *rcsC11* mutant, in which RcsCDB constitutive activation happens, as a not virulent strain that can be used as an attenuated vaccine. We here investigated whether the RcsCDB system activation conditions have the ability to affect the red dry/rough (RDAR) morphotype and the levels of biofilm formation on polystyrene plates, distinctive of *Salmonella*. For this purpose, we used the 14028s wild type strain harboring the p*rcsB* plasmid, and the *tolB* and *rcsC11* mutants as RcsCDB system activation conditions. In addition, we compared the ability of biofilm formation of the attenuated mutant respect to the wild-type strain on uniform gallstones mainly composed of cholesterol, removed from a single lithiasic patient. To this end, gallstones were incubated in LB medium without salt, supplemented or not with bile salts, previously inoculated with wild type and *rcsC11 Salmonella* strains. After 7 days, the biofilm formed was evaluated by scanning electron microscopy. Our results demonstrated that the RcsCDB system activation negatively affects the *Salmonella* biofilm development. In addition, our findings on the inability of the *rcsC11* strain to form biofilms supports that this mutant is an excellent candidate for the development of attenuated vaccines.

A146
USE OF BACTERIAL SIDEROPHORES COMBINED WITH BENEFICIAL BACTERIA TO IMPROVE THE GROWTH OF CORN CROP (*Zea mays* L.)

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Siderophores are low weight molecular compounds, secreted by microorganisms in lack iron situations to grab this metal from its environment. Numerous bacteria are capable of internalizing iron by the use of their own and heterologous siderophores, namely produced by other bacteria, which gives them an adaptive advantage over other microorganisms when they compete for this micronutrient in different environments, as for example, in the rhizosphere. The objective of this work was to study the effect of the eteroquelin siderophore, produced by *Escherichia coli*, over the growth, biofilm formation and the stimulating action of the vegetable growth of the beneficial bacterium *Bacillus velezensis* FZB42. It has been used for these studies an enterobactin-enriched extract obtained from the supernatant of the *E. coli* strain BW25113. The effect on growth and biofilm formation capacity of the bacterial strain was evaluated *in vitro* by using growth curves and by quantifying the ability of cells to adhere to the walls of multiwell plaques, respectively, in the presence and absence of the siderophore. To study the effect under the bio stimulant action, plant growth tests in controlled conditions were carried out for 30 days, by inoculating corn seeds with an enterobactin-enriched extract, with a spore suspension of *B.* velezensis FZB42, with a combination of both and with distilled water (control). It was observed that the addition of an enterobactin-enriched extract to the culture medium produced acceleration in the initial growth of *B. velezensis* spores. Regarding to the biofilm formation, no differences were observed in the presence of low concentrations of the siderophore with respect to the control, while the addition of high concentrations caused an inhibition in the biofilm formation by the bacterial strain. Finally, it was determined that the inoculation of *B. velezensis* combined with enterobactin extracts enhanced the aerial growth of maize plants, with respect to the control and the single treatments. On the other hand, all the applied treatments showed better results in the root length and weight compared to the control, but they did not differ significantly from each other. The results obtained show that the enterobactin siderophore can enhance the in vitro growth of spores and the beneficial effect of the bacterium *B. velezensis* FZB42 on maize plants. This would be due to the ability of the bacterium to use the siderophore, which would allow a better colonization of this PGPR in plant roots. Additionally, it was observed that the application of siderophore on maize seeds alone has a positive effect on plant growth, indicating that this bacterial metabolite can be used as a biostimulant to improve the growth of this crop.

A147
ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS OF *Cuminum cyminum* L. FROM CATAMARCA, ARGENTINA, AGAINST *Fusarium verticillioides*

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Cumin (*Cuminum cyminum*), an aromatic herb Apiaceae native to the Mediterranean basin, is cultivated in northern Argentina, particularly in Catamarca. Its seed have a strong odor due to volatile compounds that make up its essential oil (EO) and that have proven antimicrobial activity. *Fusarium verticillioides* is the main phytopathogenic fungus that infects the corn plant, it produces toxins, mainly fumonisin B1 (FB1), which affect the productivity of the plant and the quality of the grain. The objective of the work was to evaluate the antifungal activity against *F. verticillioides* and antimicotoxicogenic against FB1, of EOs extracted from cumin seeds of different producing departments of Catamarca, and of cuminaldehyde, one of the main components of EO. It was tested with standard cuminaldehyde and with twenty-three samples of pure AEs obtained by hydrodistillation of dried and ripe cumin fruits from the departments of Belén, Capayán, Pomán, Santa María, and Tinogasta. Antifungal activity was evaluated *in vitro* by the dilution method on Czapek-dox agar. The following parameters were determined: percentage of growth inhibition, growth rate, and lag phase of the fungus. To study the effect against FB1 production, we worked with EOs at 500 ppm and the toxin was determined by reverse phase fluorescence/HPLC. Bioassays were performed in quadruplicate and the results were considered significant at $P < 0.05$. In all cases, the pure cuminaldehyde showed greater antifungal activity against *F. verticillioides* than the AEs. At the maximum concentration tested (1000 ppm) of AE, the average inhibition of fungal growth was 79.99 ± 12.16%; the growth rate decreased by 25% and the lag phase lasted from 57.41 ± 16.11 h (control) to 117.38 ± 25.29 h (treatment). The AE of cumin from Capayán was the most active against *F. verticillioides*. The average activity of 1000 ppm of AE was equivalent to that observed for 500 ppm of pure cuminaldehyde; therefore, the observed activity is mainly attributed to this compound. At 500 ppm EO, no inhibition in FB1 production was observed, rather a slight stimulation. EOs from cumin from Catamarca and pure cuminaldehyde could be an alternative for the control of this maize pest.

A148
IDENTIFICATION OF COLICINS PRODUCED BY CLINICAL ISOLATES FROM PATIENTS SUFFERING GASTROENTERIC DISEASES

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Bacteriocins are small molecules secreted by enterobacteria species to compete against the microbiota for the ecological niche or for survival. In this work, we identified and analyzed antimicrobial compounds produced by clinical isolates (CI) of the *Shigella* genus, which were obtained from patients suffering gastrointestinal infections during the 2013–2018 period in the Northwestern region of Argentina (Catamarca, Santiago del Estero, and Tucumán). In previous studies, we had identified 54 CIs able to produce antimicrobial agents that inhibit the growth of the *Escherichia coli* AB1133 strain. Based on the above results, 11 CIs were analyzed to select those capable of producing and secreting into the supernatant the compounds with higher antimicrobial activity. The serological classification of these 11 CIs was confirmed by multiple PCR using specific primers. The antimicrobial activity of the cell-free supernatants was determined against the pathogenic *E. coli* O157:H7 strain and other pathogenic *Salmonella* genus CIs obtained in the laboratory. In addition, we studied the resistance pattern of the 11 producer CIs against different antibiotics and their plasmid content in order to identify a relationship between both patterns. Three antimicrobial compounds were selected from the 11 CIs analyzed, since they presented the highest antimicrobial activity and wider spectrum of action. Then, a partial purification of selected compounds was performed by ammonium sulfate precipitation and the antimicrobial activity was determined. Finally, the molecular mass of the antimicrobial compounds was studied by polyacrylamide gel electrophoresis. Our results demonstrated that these 3 compounds were different but had similar characteristics to colicins, mainly because of their high molecular mass, suggesting that they could be used for new antibiotics or food preservatives development.

A149
SUSCEPTIBILITY ASSAY TO TRICHOSTATIN A IN *Tritrichomonas foetus* ISOLATES

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Tritrichomonas foetus is a flagellated protozoan parasite and the etiologic agent of the Bovine Trichomonosis (TB), a venereal disease of cattle. In countries like Argentina, where the natural service is used as a reproductive method, the pathology is endemic and causes great economic losses due to the decrease in the pregnancy rate in herds, as a consequence of transitory infertility and occasional abortions caused by the parasite. Infected animals are frequently slaughtered because there is still no effective drug therapy. The most widely used drug is Metronidazole (Mz) and its derivatives, with a range of IC50 (half –50%– of the minimum inhibitory concentration) around µM. Currently, strains resistant to this drug have been reported, which has led to a decrease in its use. For these reasons, the search for new effective drugs at low concentration, with few side effects is highly relevant. Recently, it has been used several drugs with effects on epigenetic mechanisms that regulates cellular adaptation process of microorganisms. Trichostatin A (TSA) is a deacetylase enzymes inhibitor that modifies gene expression and affects cell proliferation and growth of related protozoan parasites. In this regard, the objective of this work was to evaluate the susceptibility to Trichostatin A (TSA) in *T. foetus* isolates from different regions of the country, through the calculation of IC_{50} and MLC (minimum lethal concentration). For this purpose, 6 isolates of *T. foetus* were incubated with different concentrations of TSA under anaerobic conditions for 24 and 48 h. Live parasites were stained with FDA

(fluorescein diacetate), then they were counted by flow cytometry to estimate IC_{50} and LMC was determined by recovering live parasites in a drug-free medium. The IC_{50} values were in the range of nM (2.2 to 13.2 nM at 24 h and from 2.6 to 7.1 nM at 48 h) and the LMC were higher than 50 nM and 100 nM. The parasites exhibited a biological variability in the response between isolates and a high susceptibility to low concentrations of TSA. Based on these results, very low doses on TSA or analogues drugs could be used as new treatment strategies for this infection that produce economic losses in our country.

A150
DETECTION AND TYPIFICATION OF NATIVE CASES OF DENGUE VIRUS INFECTION (DENV 1-4) IN SAN LUIS

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Dengue is an infection caused by an RNA virus from the *Flaviviridae* family. There are four serotypes among which there is no cross immunity but there is homotypic immunity for life. It affects large areas in the world including Central and South America, within which Argentina is one of the countries with thousands of cases per year. As a consequence of the dengue epidemic that affected and affects several provinces of northern Argentina, especially our neighboring Province of La Rioja, which exceeded 5000 confirmed cases; the first local cases were registered in San Luis, there being no previous health records of native cases. The objective of this work is to communicate the cases of dengue diagnosed by the Public Health Laboratory of San Luis, the geographical area of residence, the temporal and spatial relationship, and the local conditions of transmission of this disease in this city from January to May 2020. Dengue cases were defined in: (a) suspect case: patient with an acute onset fever with no apparent focus; (b) confirmed case: patient with acute febrile syndrome and detection in serum of dengue virus IgM by enzyme immunoassay (ELISA) and/or detection in the same sample of viral antigen NS1 also by the same technique, typified by molecular biology within the first five days of evolution by a positive real-time polymerase chain reaction (RT-PCR); (c) a native case (local transmission of the virus): patients who had remained in the study area in the ten days prior to the onset of symptoms. Clinical and biochemical data are analyzed. For the analysis of the geographical distribution and the temporal and spatial relationship of the occurrence of cases, they were geo-referenced according to their address. In imported cases, the origin, date of onset of symptoms and days of viremia upon entry to the region were analyzed. In native cases, the origin and date of onset of symptoms were analyzed. During January and May 2020, 115 cases of acute febrile syndrome suspected of dengue were received, fulfilling the definition of a confirmed case of 28 patients (24.3%). According to the place of transmission, 16 were imported (57.1%) and 12 native (42.8%). The total of confirmed, imported and native cases corresponded to Dengue virus Type 1 (DENV-1). The possibility of introducing dengue as a locally transmitted disease is related to the epidemiological situation on the border with Provinces where they have already had native cases such as Córdoba and La Rioja. In the first epidemiological weeks, there were imported cases from both provinces that were quickly detected by the health system. In local transmission, it is necessary to consider the time of the extrinsic incubation period in the vector and the time of the incubation period of the disease, this time being around 15 days, which explains the appearance of the first native cases in early April. Another striking fact has been the maintenance of local transmission until mid-May. The persistence of high temperatures in autumn made local transmission possible on these considered dates. The presence of the vector and the virus are necessary conditions for local viral transmission, but a sensitized system in the search and quick action in blocking and other strategies are essential to truncate any possibility of an outbreak in any new area.

A151
SEARCH FOR ANTIFUNGAL AGENTS IN EXTRACTS OF *Prosopis nigra* USEFUL IN THE CONTROL OF *Cercospora kikuchii* AND *Septoria glycines*

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Soybean is affected by late season diseases produced by *Cercospora kikuchii* and *Septoria glycines* that lead to defoliation and yield losses due to a bad seed filling. These fungi are controlled with azole fungicides alone or in mixtures with strobilurins. The appearance of fungal resistance conducted to the increase in the doses and frequency of application of the fungicides. New antifungals are needed in order to overcome this problem. The aims of this work were: (1) The evaluation of the antifungal activity of extracts from *Prosopis nigra* against *C. kikuchii* and *S. glycines*. (2) The isolation and identification of the main metabolite responsible of the antifungal activity in the most bioactive extract. Leaves, bark and hearthwood of *P. nigra* collected in Paraná (Entre Ríos) were separated in portions that were extracted with methanol, ethyl acetate or dichloromethane. The extracts were evaporated to dryness and the residues tested on *C. kikuchii* and *S. glycines* by the disc diffusion method. Minimum inhibitory dose (MID) and diameter of inhibition at the MID were measured. The most bioactive extract was subjected to a bioassay guided isolation involving TLC bioautography, column chromatography (silica gel and Sephadex LH20) and preparative TLC. The identity of the bioactive metabolite was estblished by NMR. The anticercosporin activity was also evaluated. Difenoconazol was used as positive control. The leaf methanolic extract showed MID = 0.2 mg/disc on both fungi (DI = 18.5 mm, *C. kikuchii*; 20.5 mm, *S. glycines*). The remaining extracts were inactive or had higher MID values. Tryptamine was identified as the main antifungal constituent and had anticercosporin activity. It is promisory for control of *C. kikuchii* and *S. glycines*.

A152
ANTIFUNGAL ACTIVITY OF *Euphorbia* SPECIES NATIVE FROM LA PAMPA

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Fusarium verticillioides and *F. graminearum* cause cereal ear rots. They reduce grain yield and contaminate the grains with mycotoxins noxious for humans and animals. New antifungals are required in order to control the ear rot diseases. The aims of this work were: (1) To evaluate the antifungal activity of extracts from *Euphorbia* species native from La Pampa against *F. verticillioides* (NRRL 25457 and LABI7) and *F. graminearum* (NRRL 28063 and LABI11). (2) To isolate, identify and characterize the antifungal activity of the main antifungal metabolite of the most bioactive extract. Aerial parts of *Euphorbia collina*, *E. serpens*, and *E. schickendantzii* from La Pampa were sequentially extracted with hexane, ethyl acetate and methanol. Extracts were evaporated to dryness and their residues were tested on strains of *Fusarium* by the microdilution method. The concentration of 50% of fungal growth (IC_{50}) was calculated. The antifungal constituents of the most bioactive extract were separated by a gradient chromatography in silica gel and their identities were established by GC-MS. The hexane leaf extract of *E. collina* had the lowest IC_{50} (814–824 µg/mL, *F. verticillioides*; 360–392 µg/mL, *F. graminearum*). A mixture of cycloartenol and 24-methylen cycloartanol was isolated. The hexane leaf extract of *C. collina* had the highest antifungal activity which was due to a mixture of two pentacyclic triterpenes.

BIOCHEMISTRY, PHYSIOLOGY, PATHOLOGY AND PLANT PRODUCTION

A153
NEW ARGENTINE VARIETY OF BUCKWHEAT (ALMNO13): *IN VITRO* ANTIOXIDANT ACTIVITY

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The consumption of grains has been associated with the prevention of diseases that depend on oxidative stress (atherosclerosis, cancer diabetes, Alzheimer, etc.). Natural antioxidants play an important role in inhibiting free radicals. Buckwheat (*Fagopyrumesculentum* Moench) is a pseudocereal, which has gained increasing interest in recent years based on its nutritional values. The objective of this work was to evaluate bioactive compounds and the antioxidant properties of the flour of an experimental variety of Argentine Buckwheat, produced in the central region of our country, where work has been done to obtain new experimental lines of pseudocereals, including buckwheat, called ALMNO13, in order to improve its adaptability, uniformity and performance to growing conditions and promote its use in food. The seeds (ALMNO13) were dried in a forced air oven, at 45ºC for 48 h. The dried product was ground in an electric coffee grinder (CG-8 Stylo, 220 V, 50 Hz, 90 W, China) and sieved (200 mm). A slightly whitish powder (buckwheat flour) was obtained. The ALMNO13 flour was degreased with n-hexane. Bioactive compounds and antioxidant activity were evaluated by the following methods: (i) Total Polyphenols, by Folin-Ciocalteau, (ii) Total flavonoid content, by the colorimetric method, using AlCl3, 510 nm; (iii) Anthocyanins were determined by a differential pH method; (iv) DPPH test, to determine free radical scavenging activity, and (v) NO test, to determine scavenging activity against nitric oxide. The results of buckwheat flour (ALMNO13) were: Total Polyphenols, 323.5 ± 12.1 mg gallic acid, Flavonoids (mg/g) 189.80 ± 15.0, Anthocyanins (mg/g) 167.06 ± 11.0; IC50 DPPH (mg/mL) 0.473; DPPH % inhibition: 686.30 ± 1.1 and NO % inhibition, 53.8 ± 2.5.

A154
PLANT VIGOR VARIABILITY IN TWO SPONTANEOUS POPULATIONS OF *Pappophorum vaginatum* BUCKLEY FROM LA PAMPA DEPRIMIDA

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Pappophorum vaginatum is an America native warm-season forage perennial grass present in regions of Argentina where grasslands are the based for livestock production. For instance, *P. vaginatum* is part of the halophyte steppes of the Pampa Deprimida where the reincorporation of selected native germplasm would be important not only to increase livestock production but to maintain biodiversity. Thus, the study of characteristics related to implantation control such as plant vigor, is crucial. The objective was to evaluate plant vigor variability and other associated characteristics and their correlations in two spontaneous populations of *P. vaginatum* from halophyte steppes of the Pampa Deprimida grown in substrate with no limitations. Caryopsis with their covers (lemma and palea) of two spontaneous populations of *P. vaginatum* (P1, P2) were collected in Magdalena and Punta Indio municipalities (Buenos Aires province), respectively. Then, the caryopses were individually weight (PC) and sown (10 October 2019) in plastic trays (with cells of 180 cm^3) filled with typical Argiudol soil as substrate in a greenhouse. Fifty-two days after sowing 80 plants of each population were retired and washed in a stream of water on a sieve. It was determined: aerial length (LA), radical length (LR), total length (LT), longest adventitious root length (Ladv), number of adventitious roots longer than 3 cm (n° adv), number of green leaves totally unfold (n° hoj) and tiller number (n° mac). Then, each plant was dissected at the root neck height, were put in a stove at 60°C and aerial (PSA) and radical dry weight (PSR) were determined, and the total dry weight (PST) was calculated. Ratios PSA/PSR and LA/LR were calculated. Variability within populations was analyzed by means the following parameters: average, standard deviation, range, and coefficient of variation (%). Variation between populations

was analyzed by means the *t*-test. Besides, phenotypic correlations (Pearson's coefficient) between PST and the other studied characteristics were analyzed. There were no significant differences ($P > 0.05$) between populations for any of the studied characteristics, except for the PSA/PSR ratio for which P2 resulted superior ($P \leq 0.05$) to P1. Both populations showed significant ($P \leq 0.05$) and positives correlations between PST and LA, LR, LT, LA/LR, Ladv, n° adv, n° mac, and n° hoj. The variability found within *P. vaginatum* populations for all the studied characteristics and between them for dry biomass partition (PSA/PSR) would be promissory for the genetic improvement of the implantation. Although populations did not differ in plant vigor (PST) in the studied period, the associations found between characteristics linked to it would be useful for its possible application in indirect selection.

A155
INTERACTION OF FORAGE AVAILABILITY OF DEFERRED *Digitaria eriantha* PASTURE, WITH DISTANCE TO WATER POINT

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In the south-central area of San Luis, the availability of forage was evaluated previously to a grazing period, on a 450-ha paddock with only water point, without presence of trees or natural watering places, implanted from 25 years ago, *Digitaria eriantha* pasture "digitaria". The production of the entire growth cycle was used in winter as deferred for more than 10 years, for 40–45 days depending on the amount of forage. The stocking rate remained constant over the period and was adjusted to dry matter (DM) available. Measurements were made before animals grazing period and after that (remaining forage), approximately at 250 (C), 1000 (M) and 2300 (L) meters from the watering place. Through regression analysis and the statistical software InfoStat, relationships between these variables were established (pre- and post-grazing DM availability and distance to watering point); the degree of significance of the coefficients and parameters ($P < 0.10$) and the adjustment of the models (R^2: Determination Coefficient) allowed the selection of the most representative. Initial forage accumulation varied with distance from watering and was significantly higher ($P < 0.10$) in L than in the rest of the paddock (C: 860, M: 1123, L: 2246 kgMS/ha), and the estimated grazing efficiency with respect to initial availability was 70–97 %. Forage accumulation had a closely relationship with the distance from the watering point; with a high and positive exponential modeling adjustment (α and β parameters were very significant). The post-grazing remnant had a positive correlation with the availability of forage before the animals grazing, adjusting the relationship to both a linear ($R^2 = 0.75$) and logarithmic ($R^2 = 0.84$) models. As for the parameters, the linear model and the independent variable were very significant ($P < 0.01$), while the constant was significant at 10%. In the case of the logarithmic model, α and β parameters were highly significant ($P < 0.01$). The relationships determined show the influence of the distance to the water, as a determinant of the effect of the animal on the productive memory of the pasture, since despite being used outside its growing season (deferred), the action of grazing affects more in the area with the highest animal concentration (C) and not in remote areas with less grazing pressure. The initial forage availability of deferred digitaria, shows a close relationship with the distance to water point, and will determine changes in the remnant after grazing, so it is possible to model and predict pasture behavior.

A156
STRUCTURAL VARIABLES AND RESERVATION STORAGE SITES IN *Digitaria eriantha* PASTURE UNDER GRAZING

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Plant reserves provide the energy for the regrowth of forage plants and, in the case of perennials, to tolerate the dormant period. They are mainly stored in roots, rhizomes, stolons, crowns, leaf sheaths and lower parts of the stem, so these organs should not be damaged by grazing. High levels of reserves invigorate the growth of leaves and roots, while if they are low, they reduce it or can cause death. *Digitaria eriantha* is a perennial mega-thermal that have a well adaptation to semi-arid environments, whose deferred forage satisfies the winter requirements of grazing cows. Previously of a grazing period, individual plant structure and production were evaluated at distances of 250–1000–2300 m from the water point (C–M–L, respectively), in 450 ha-paddock of the center of the province of San Luis, historically deferred grazing by breeding cows. The basal surface of individual clamps did not differ statistically between sectors, although it tends to increase in the nearby sector (C: 36, M: 29, L: 29 cm^2). The proportion of leaf sheaths over the total accumulated material does not have a great variation between extreme sectors (C: 6.6 and L: 8.1%), while the proportionover foliar material increases in sector C, subject to higher grazing pressure. The proportion of reproductive structures in C increased by more than a third, compared to the other sectors (44% and 32%, respectively), with significantly lowers ($P < 0.01$) reproductive and vegetative heights. In sector M with respect to C, the proportion of leaf sheaths decrease (7.5% of the total and 10.8% over the foliar material), but not the production (488 *vs.* 395 kgDM/ha of availability). Sector L is similar to M, but with a higher DM accumulation (994 kg/ha), probably due to lower grazing pressure and the possibility of accumulating a greater amount of reserves. Despite the "effort" in the morphological modification of the plant to persist in the face of high grazing pressure, the production of reserve structures is higher in individuals subjected to a lower grazing pressure (C = 57, L = 98 kgDM/ha). Although there are only statistically significant differences ($P < 0.05$) in production of DM per hectare, and the relative values are averages for each sector, these results seem to indicate that digitaria, when faced with a moderate grazing pressure, decreases its production, but without significant changes in the structure of the plant, compared to the situation with less intensity of grazing. By increasing the grazing pressure -C-, digitaria tends to modify its foliar structure, possibly trying to encourage the accumulation of reserves (mainly in leaf sheaths) and produce more reproductive structures as a response to resilience to bovine herbivory.

A157
IMPACT OF WATER STRESS ON THE ENDOGENOUS PHYTOHORMONES CONTENT IN SUNFLOWER PLANTS DURING LATE VEGETATIVE GROWTH (V8)

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It has been well documented that water stress produces morpho-physiological, biochemical, and molecular alterations. A physiological change typically observed under water stress is the modification in endogenous levels of phytohormones. The aim of this study was to evaluate the impact of water stress on the endogenous hormonal levels of Abscisic acid (ABA), Jasmonic acid (JA), Salicylic acid (SA), Brassinosteroids (BRs) and Strigolactones (SLs) in shoot and roots of sunflower plants. The experiment was conducted on plants of four inbred lines of sunflower, previously characterized as tolerant to water stress (B71), sensitive to water stress (B59), moderate tolerant to water stress (C803) and moderate sensitive to water stress (R461-4), subjected to moderate water stress by watering suppression for 15 days, under controlled environmental conditions, during the late vegetative growth (V8). The extraction and purification of endogenous phytohormones was carried out from 200 mg of dry weight of shoot and roots; the identification and quantification were performed by LC-ESI/MS-MS. Our results showed that in the four inbred lines the ABA level increased in shoot and roots of water-stressed plants, whereas SA significantly increased its level only in the shoot. Regarding BRs, an increase of Epibrassinolide was detected in shoot of stressed plants of all inbred lines, and in shoot of B71 line there was an increment of Catasterona. In shoot, the endogenous level of both SLs (Dimetilsorgolactone and Deoxistrigol) did not show modifications in response to water stress, whereas in roots a reduction in their levels was quantified. Finally, no evident changes in JA endogenous level were observed. These findings suggest that in the sensitive B59 line the accumulation of various phytohormones detected as response to water stress treatment could be probably related to the demand of high adjustment capactity in signaling and regulation of its hormonal response. The tolerant line (B71) did not present significant changes in hormonal levels, which would confirm its tolerance to water deficit in late stages of vegetative growth. The R461-4 line triggered its endogenous hormonal levels as a consequence of imposed water stress in a similar fashion to the sensitive B59 line, therefore was characterized as moderately sensitive to water stress. The C803 line had a similar hormonal profile to B59 line although its morphological response was comparable to B71, accordingly it was identified as moderately tolerant to water stress.

A158
MORPHOLOGICAL CHARACTERIZATION OF ARBUSCULAR MYCORRHIZAL FUNGI AND THEIR COLONIZATION IN TRAPS AND OLIVE SEEDLINGS FROM A MIXED INOCULUM OF OLIVE TREES

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Arbuscular mycorrhizal fungi (AMF, Phylum Glomeromycota) are worldwide distributed and form symbiotic associations with almost 80% of the vascular plants of the earth. In this symbiosis the fungus transfers nutrients to the plant in exchange for carbon compounds, but also improves the tolerance of plants to various biotic and abiotic stress (such as pathogens, salinity, drought, and others). Spores isolated from field-collected rhizospheric soil mixtures indicate the spore abundance and diversity sporulation in the soil. Therefore, the importance of completing these studies with those of trap plants that allow multiplying species those do not sporulation in such conditions or do so by season. In this work, rhizospheric olive soil (cv. 'Arauco') samples were extracted in Chilecito, province of La Rioja. The multiplication and obtaining of the mixed inoculum were done in trap plants of white clover (*Trifolium repens*) and the use thereof for the inoculation of olive cuttings (*Olea europaea*). At 30 and 60 days, respectively, samples of roots were extracted in order to obtain the mycorrhization percentages. Root colonization percentages were high (around 60%) for both cases. On the other hand, spores of AMF present in the samples collected from the olive rhizosphere, trap plants and olive cuttings were isolated and identified at the morphospecies level. 20 different morphospecies were identified distributed in 4 families Acaulosporaceae, Claroideoglomeraceae, Ambisporaceae, and Glomeraceae of which only 3 were found in olive and the remaining 17 presents in trap plants, 7 were common in both crops. This would be demonstrating of selection of olive cultivation and an affinity for certain morphospecies.

A159
CONTENT OF TOTAL POLYPHENOLS, FLAVONOIDS AND TANNINS CONDENSED IN PLANTS OF *Mentha spicata* L. FERTILIZED WITH UREA

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Many aromatic species have antioxidant compounds, which can be used in food industry, cosmetic, or nutritional supplements. Natural antioxidants are highly demanded in the international market because they are healthy and do not present the adverse effects of their synthetic substitutes. Among the antioxidants present in plants, polyphenols stand out, and their concentration is affected by environmental factors, such as the chemical composition of the soil. The objective of this work was to determine the effect of urea fertilization on the content of total polyphenols, flavonoids, and condensed tannins, in *Mentha spicata* plants. The tests were carried out in the Experimental Field of the Facultad de Agronomía y Agroindustrias, UNSE, located in El Zanjón, province of Santiago del Estero. The following urea doses were used: 0, 100, and 150 kg/ha. The crop was harvested in full bloom, and the plants were air-dried at room temperature to constant weight. The plant material was ground in a Wiley mill, and the total polyphenols were extracted in 80% (v/v) methanol. In this extract, the concentrations of total polyphenols, flavonoids, and condensed

tannins were quantified spectrophotometrically. A completely randomized experimental design with 5 replications was used, and the data were analyzed with ANOVA and Tukey's test. The 100 kg urea/ha dose increased the concentrations of total polyphenols, flavonoids, and condensed tannins by 106, 60, and 133%, relative to the control, respectively. In the 150 kg urea/ha-dose, the concentrations of these compounds increased by 186, 220, and 200%, relative to the control. It is concluded that the urea fertilization increases the concentrations of total polyphenols, flavonoids, and condensed tannins, in *M. spicata.*

A160
VALUE-ADDED SAN LUIS NATIVE FRUITS

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The native fruits of San Luis range have great potential for economic exploitation, constituting a new alternative for the food industry and insertion of new native species of our region in Argentine production systems. The addition of native fruits to food as a way of enriching it with bioactives, could give added value to new regional-based products. Three fruits were selected, Tuna (*Opuntia prasina*), passionaria (*Passiflora caerulea*) and peje (*Jodina rhombifolia*) due to their color, aroma and high content of vitamin C. Tuna has a unique aroma and flavor that have been described as reminiscent of the kiwi and plum and semisweet; Passionaria has a mild sweetness, an astringency similar to berries and a dark red color. Peje is an intense red drupe, with a mild flavor and a particular aroma reminiscent of walnut due to its fatty acid content. The addition of dried fruits is proposed as carriers of their natural bioactives and flavor, color, and aroma ingredients to commercial black tea (*Camellia sinensis*), although black tea is a source of antioxidants, its bitter taste and the sensation of astringency induced by high concentrations of polyphenolic compounds cause a negative sensory reaction in the consumer. Dried peje, tuna and passionaria are added to Black Tea in order to increase the functionality of the product and provide an improvement in its organoleptic characteristics (aroma, flavor and color). Two formulations were made: F1 and F2, with 5%, 10%; 15% and 10%, 15% and 20% of peje, tuna and passionaria, respectively. Total polyphenols, total anthocyanins, and ascorbic acid were analyzed. The sensory evaluation was performed using a hedonic scale by a panel of 30 untrained evaluators who scored from 1 (unpleasant) to 4 (very pleasant), the characteristics evaluated were: color, texture, flavor, aroma of the three treatments obtained. The content of total polyphenols, such as gallic acid, was 81.50 mg/g; total tannins was 1013 mg/L; Total anthocyanins from 6.5 to 8.4 mg/100 g and Vitamin C was from 1.07 to 2.16 g. The results showed that the F2 treatment had the highest acceptance by part of the panel, the aroma and flavor of the fruits being two of the main sensory attributes of acceptance. The results indicate that the addition of dried native fruits to commercial black tea constitutes an adequate nutritional and organoleptic strategy, as a source of vitamin C and contributors of color, odor (anthocyanin pigments and volatile compounds), relevant sensory attributes in infusions, since the consumer it judges them mainly by their appearance and relates them directly to their quality.

A161
VOLATILE ORGANIC ALCOHOLS AS A NATURAL ALTERNATIVE FOR THE CONTROL OF *Sitophilus zeamais*

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storage in Argentina. Most of the synthetic insecticides currently used cause environmental damage and their incorrect application generates pest resistance. Consequently, natural volatile organic compounds (VOCs) are an alternative to combat pests. However, they loss efficiency in their insecticidal capacity when they are applied directly in storage systems. Due to this situation, the incorporation of VOCs into a paint binder provides them a long and effective lifespan. The objective of this work was to evaluate the insecticidal capacity of different volatile alcohols (VAs) against *S. zeamais* and to evaluate their effects on a matrix of polyvinyl acetate paint. The insecticidal fumigation technique was used, thus, the insects remained separated by a microgrid from the compound absorbed on the filter paper. Volatile alcohols with triple bond and structured by 7 and 8 carbons, 1-heptyn-3-ol and 1-octyn-3-ol showed the higher toxicities reflected in their lethal concentrations (LC50) of 0.74 and 1.13 µL/L, respectively. In addition, the chronic insecticidal activity was evaluated by fumigation assays with the presence of food (corn) in the paint matrix with 1-heptyn-3-ol incorporated (compound with higher insecticidal activity) with a dose of 14.7 µL/L corresponding to the LC99. The VAs-free paint was not toxic to *S. zeamais*. However, with the additive treatment, a 20-fold decrease in the rate of corn consumption by insects and total mortality was observed. The results show that the toxicity of the molecules does not depend on the length of the carbon chain, but it is influenced by it, at least in homologous molecules of up to 8 carbons. The compounds with unsaturated triple bond showed an increase in the toxicity in comparison with saturated and unsaturated double bonded compounds.

A162
EFFECT OF NITRIC OXIDE AND ABSCISIC ACID ON TOMATO PLANTS CV. MICRO-TOM METABOLISM SUBJECTED TO WATER STRESS

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The purpose of this work was to evaluate the regulatory effects of nitric oxide (NO) and abscisic acid (ABA) on the profile of first and secondary metabolism, as well as the antioxidant enzymes activity in tomato plants (*Solanum lycopersicum*) cv. Micro-Tom subjected to different water stress conditions. For this, since flowering to harvest period, Micro-Tom and *Notabilis*, its ABA-mutant, were exposed to the following treatments: water

stress (WS, irrigation treatment, 50% field capacity), 100 µM SNP (NO donor), 100 µM ABA, and 500 µM LNNA (NO synthase inhibitor). Leaves from both genotypes, were sampled to determine enzymatic activities. In parallel, protein total content (PT) and, chlorophyll, polyphenols and anthocyanins were measured in the treated leaves. The interaction between irrigation and chemical treatments (SNP, ABA, and LNNA) was significant for PT and pigments such as chlorophylls (a, b, and total), carotenoids, and total polyphenols. Under irrigation, NO and ABA treated leaves increased the activity of APX and the content of total polyphenol while CAT activity did only by NO. Under drought, NO and ABA stimulated APX activity, chlorophyll content (a, by totals), but NO on total polyphenols contents. Also, differences in the biochemical parameters were observed between genotypes due to the treatments. For example, under irrigation both, NO and ABA, increased APX, CAT and POX the activities in *Notabilis,* while the total anthocyanin content was not stimulated by NO. This in contrast to previous studies, where ABA and NO can act differentially against water stress.

A163
POTENTIAL CONTAMINATION BIOMARKERS IN *Salvinia rotundifolia* PLANTS, DURING THE SIMULTANEOUS REMOVAL OF Cr (III)/(VI) PRESENT IN TANNERY EFFLUENTS

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The integrated management of water resources, including the restoration of polluted aquatic environments, has driven the demand for cleaner, cheaper, and more sustainable technologies for the removal of pollutants from industrial effluents. In this way, natural treatment systems that involve the use of plant species (Phytoremediation) have raised growing interest as an alternative method to traditional technologies for the treatment of contaminated water. The exposure of plants to heavy metals induces the appearance of various metabolic and physiological responses with more or less pronounced changes in certain parameters, which some authors call biomarkers. Thus, the objective of this work was to analyze the accumulation patterns of metabolites that can be used as biomarkers in the accumulation of Cr(III)/(VI) in *Salvinia rotundifolia* plants growing in tannery effluents. Three sampling points were selected from where effluent samples were taken in an area where a tannery is located in the town of Nonogasta, La Rioja. Subsequently, a known weight of plants was placed in plastic containers containing, on one side, the same volume of the Cr(III)/(VI) mixture in dilutions of the effluent under study and samples of the effluent without the exogenous addition of metal were used in any their oxidative states and their effects on the content of MDA, H_2O_2, protein and non-protein thiolic compounds and soluble and insoluble phenols were evaluated. The results showed in all cases that the parameters analyzed presented alterations in the accumulation patterns and that these in turn depend on the organ analyzed (fronde or lacinia) and the species of Cr analyzed. The simple exposure to the effluent was enough for the plants to show considerable alterations, mainly in the values of peroxide and thiolic compounds. Although more studies are necessary, the results show that the selected parameters would be reliable indicators of contamination in *S. rotundifolia* plants.

A164
STRIGOLACTONES AND BRASINOESTEROIDS AND THEIR INTERACTION WITH ABSCISIC ACID IN THE BREAKING OF SEED DORMANCY OF SUNFLOWER (*Helianthus annuus* L.)

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In agriculture, the successful and uniform seed germination is important to avoid problems in the establishment of seedlings that ultimately impact on the crop yield. Thus, dormancy is an undesirable trait, for this reason different treatments have been implemented to overcome this problem. Germination and dormancy are processes modulated mainly by the abscisic acid (ABA)/gibberellin (GAs) balance, but other hormonal groups such as strigolactones (SLs) and brassinosteroids (BRs), also participate in its regulation. The objective of this investigation was to elucidate the hormonal changes that occur in the embryonic axis in response to exogenous treatments with growth regulators that also impact on the breaking of seed dormancy in sunflower seeds. Cypsels of Xi3 line, characterized as dormant at harvest, were supplied by the Argentine Cooperatives Association (ACA) and used in this research study. Treatments with GA_3 and Ethephon, both effective in the sunflower dormancy release, were applied alone or in combination. Endogenous hormonal determinations were performed in the embryonic axis of control and treated seeds at 0 (dry seed), 3, 6, and 12 h of imbibition. ABA, BRs (Castasterone –CS–) and SLs (deoxystrigol –DS–) were extracted twice with diethyl ether and identified and quantified by Liquid Chromatography-Tandem Mass Spectrometry (LC–ESI/MS–MS). Regarding ABA, during the imbibition all treatments reduced their endogenous level respect to 0 h, but no differences were observed between treatments and control condition. It is widely accepted that not only endogenous levels of ABA and GAs but also the ABA/GAs ratio constitutes a central mechanism in the regulation of seed germination and dormancy. In this sense, the ABA/GA_1 ratio showed a significant decrease at 12 h for all treatments in comparison to the control. Respect to DS, at 12 h of imbibition reached relatively lower endogenous levels in the embryonic axis treated with respect to the control condition, which suggests that DS should not exceed a threshold level of approximately 40,000 pmol/g PS to trigger germination. In relation to CS, a notable increase was registered at 12 h imbibition, which would indicate its participation in germination. In conclusion, it is necessary that endogenous levels of ABA and DS remain low and DS level increase in the embryonic axis during imbibition for the germination takes place successfully.

A165
PHYTOCHEMICAL ANALYSIS OF PLANT SPECIES IN THE PAMPEANA REGION

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In recent years the search for natural sources of compounds with antioxidant activity has become very important, due to its high content of antioxidants; however, not all vegetables produce them in the same quantity and type. This is due to the secondary metabolites possessed by the plants, and which have been a starting point for the discovery of new bioactive substances. In this work, phytochemical analysis was performed with the aim of evaluating the antioxidant capacity of the fruits of *Schinus molle* L (false pepper) and *Condalia microphylla* (piquillin). To this end, phytochemical screening was performed using test tube reactions and the determination of antioxidant capacity by a quantitative assay using the DPPH method, where absorbance was read at 517 nm. The total phenol content was determined by the Folin–Ciocalteu method and the absorbance was measured at 760 nm. The fruits were collected and subsequently extracts were made by maceration mixture water/ethyl alcohol (1:1) at room temperature. Phytochemical sifting identified the presence of flavonoids, tannins, phenols, reducing sugars, saponins, triterpenes, and steroids. The anti-radical activity expressed as a percentage of free radical trapping capacity (% CARL) of *S. molle* extracts was (94.20%) and (46.03%) for *C. microphylla*, values that are within acceptable parameters. The polyphenol content was 0.689 mg gallic acid/L (AG) for *S. molle* and 5.50 mg gallic acid/mL (AG) for *C. microphylla*. The results obtained conclude that the fruits of *S. molle* and *C. microphylla* have high antioxidant capacity values, with a direct correlation between the values of total phenols being observed with the values of radical capacity.

A166
PHYSICOCHEMICAL CHARACTERIZATION AND ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES OBTAINED THROUGH NATURAL CHEMICAL REDUCERS

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Aqueous plant extracts are a promising option for the synthesis of nanoparticles (Nps) since they contain reducing agents among their components. Due to the wide field of application, Nps have acquired great importance as a potential agent with antifungal activity to counteract the negative effect of some phytopathogenic microorganisms. The objectives of this work were to synthesize AgNps, their characterization using techniques such as transmission electron microscopy (TEM) and UV-Vis spectrophotometry, and evaluation of the antifungal activity against *Fusarium sp*. The synthesis of AgNps was carried out using aqueous extracts of aromatic plants such as rosemary (*Rosmarinus officinalis*); pichana (*Baccharis spartioides*) and false sunflower (*Heliantus petiolaris*), replacing chemical reducers, using ascorbic/citric acid as a control. For the synthesis of NPs, 10^{-2} M $AgNO_3$ silver nitrate was used, mixing the plant extracts in the presence of 0.13 mM polyvinylpyrrolidone (PVP) with constant stirring, at a temperature of 80ºC. The evaluation of the antifungal activity of the AgNPs against the fungus was carried out by means of the inhibition of the mycelial growth of *Fusarium* sp. Potato Dextrose Agar (PDA) was prepared, sterilized in an autoclave and then 50% Potato Dextrose Agar and 50% of the different concentrations of the extract were placed in Petri dishes, allowed to solidify, and refrigerated at 4°C until later use. Slices of 3 mm diameter of *Fusarium* sp. colonies were plated onto the center of the Petri dish and later sealed and incubated at 26°C. The results obtained were evaluated considering active ingredients that present a percentage of inhibition greater or equal than 20%. The percentage inhibition values found for the control AgNPs and the AgNPs obtained from the plant species false sunflower, pichana, and rosemary were 75, 70, and 30, and without inhibition, respectively. Through TEM it was observed that the predominant morphology of the AgNPs was spherical with diameters in the range of 20–40 nm. The absorbance of the NPs was measured with UV-visible spectroscopy, whose absorption bands were manifested at wavelengths in the 400–450 nm range, values attributed to the excitation of surface plasmons by AgNps. From the analysis of the results, it is concluded that the AgNPs obtained with false sunflower and pichana extracts are potential antifungal agents against *Fusarium* sp.

A167
GENOTYPIC VARIATION OF ROOT GROWTH OF PEANUT SEEDLINGS, ACCORDING TO TEMPERATURE DURING GERMINATION

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The high germination percentage of a seed lot does not ensure the success of the establishment of the crop. There are a set of factors that limit the germination process, among them the characteristics of the growth of the roots of the genotype, the water, and the temperature. The roots of peanut seedlings (*Arachis hypogaea* L.) must have the capacity to grow rapidly to absorb the water necessary for the growth of the aerial part and thus emerge. The absorption process is limited by the low temperatures during planting and the low water-holding capacity of the soils where this cultivation is carried out – frank sandy textures. The objective of this work was to evaluate the length of the roots during the germination of four peanut genotypes at different temperatures. The test consisted of exposing four peanut genotypes: "Valencia", ASEM 400, Granoleico and EC98, to eleven (11) temperature levels: 14, 18, 22, 26, 28, 30, 32, 34, 36, 38, and 40 °C. Seeds of the same size were used (retained in the 8 mm-sieve) with similar quality (germination percentage –PG– and vigor). In the experience, the seeds were conditioned between paper with 2.5 times the weight in water and placed in growth chambers at constant temperature, according to the treatment, during the period seeds were germinating. A daily evaluation was performed to identify the normal seedlings to which root length was recorded (LR). With the obtained data, the PG, the mean germination time (TMG) and the development rate (TD) were calculated. The statistical program InfoStat ver. Professional was used to perform the correlation analysis between variables and Analysis of Variance (ANOVA), with a significance level of $P < 0.05$. The length that the root reached at

the end of growth of normal seedlings was modified by temperature with differences between genotypes; at low temperatures (low PG) they were short comparatively with those that grew in the range of temperatures in which the PG did not vary (from 18 to 34 °C). In this temperature range, a lengthening of the main root is observed when the temperature of the germination environment is far from the optimum (18, 20, 32, and 34 °C), even in one of the genotypes (EC98) a minimum value can be recorded that coincides with the optimal temperature for germination (highest TD). In genotypes related to the Virginia botanical type, LR is negatively correlated with temperature (ASEM 400: −0.61; EC98: −0.70; Granoleico: −0.75) and TD (EC98: −0.64; Granoleico: −0.68) and positively with the TMG (ASEM 400: 0.64; EC98: 0.78; Granoleico: 0.76) and the PG (EC 98: 0.39). The results show that there are differences between genotypes in the LR in the temperature range where germination is high and similar, which allows identifying genotypes that produce longer roots at low temperatures and can be used as one of the strategies to increase the sowing period of this crop.

A168
CHARACTERISTICS OF PEANUT SEEDLINGS ACCORDING TO THE SIZE OF THE SEEDS AND THEIR RELATION TO QUALITY

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International standards for seed analysis establish parameters to identify normal seedlings based on the characteristics of the aerial part and the root, determining the result of the germination standard test. Peanut seeds (*Arachis hypogaea* L.) have different sizes, so it is necessary to elucidate whether there are morphological differences between them that prevent them from being jointly evaluated. Although, given the growing conditions of the crop they may present different qualities, even being formed in the same mother plant. The objective of the work was to evaluate the characteristics of the seedlings according to the size of the seeds and their relationship with quality. The treatment was the size of the seeds with 6 levels: 9 mm (Z9: 77.75 gr), 8 mm (Z8: 69.37 gr), 7.5 mm (Z7.5: 57.81 gr), 7 mm (Z7: 46.52 g), 6.5 mm (Z6.5: 39.56 g) and 6 mm (Z6: 29.81 g). The mother plants grew in the field of the FAV-UNRC, of the 2018/2019 cycle, and given the conditions to which they were exposed, seeds of different qualities were available (germination percentage (PG): 18 to 100%). When seeds reached the equilibrium humidity, they were conditioned between moistened paper (water: 2.5 times the weight of the paper) and put to germinate in a growth chamber at 25ºC for 10 days. At the end of this period, normal seedlings were evaluated to record the diameter of the region near the hypocotyl and the length of the root, the number of secondary roots and leaves, and the PG was estimated. The statistical program InfoStat was used to perform correlation and variance analysis ($P < 0.05$). The results show that the length of the root –between 1.7 and 34 cm– does not depend on the size of the seed. Instead, the other characteristics of the seedlings are influenced by size; the largest seeds (Z9 and Z8) had a lower number of leaves per plant and a greater diameter of the root and number of secondary roots per plant. Given these characteristics, the size of the seeds influenced the PG; it is higher in Z9, Z8 and Z7.5, intermediate in Z7 and Z6.5 and lower in Z6. This behavior is explained by the positive correlation between the PG with the size of the seeds (0.91), the diameter of the root (0.92) and the number of secondary roots (0.87). On the other hand, the length of the root was one of the parameters that defined the normal seedlings, consequently the PG; the seed lots of lower quality had lower length and diameter of the root and number of secondary roots and leaves per plant. Therefore, these characteristics allowed the identification of batches of different qualities. Regarding the evaluation of seeds of different sizes, the results show that it is possible to use the length of the root to analyze them since it does not vary between them when they have the same quality.

A169
EFFECT OF ENDOGENOUS COMPETITION BETWEEN CADMIUM AND ZINC ON OXIDATIVE STRESS AND ANTIOXIDANT ENZYMES IN *Glycine max* PLANTS

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Heavy metal contamination in soils and the contact of these with plants generates a competition between the contaminating metals and the essential ones. Zinc (Zn) is an essential element for both plants and animals, in excess it causes alterations in important processes for the development of the organism. Cadmium (Cd) is a heavy metal without biological functions, in low concentrations it causes damage at cellular and molecular level. Because it has the same charge than essential metals, the plant absorbs Cd and Zn through the same transporters (IRT1-ZIP), generating interferences in the entry, transport and use of Zn. There is also competition with other cations such as Calcium (Ca) and Magnesium (Mg). The symptoms generated in plants by the presence of Cd and/or excess Zn, are similar: reduction of biomass, decrease in elongation and darkening of the main roots, chlorosis and necrosis of leaves, and changes in oxidative stress parameters. In our model, Glycine max leaves and roots were used, which after 10 days of development and adaptation to hydroponic conditions in Hoagland's nutrient solution, were subjected to contamination with Cd and Zn, for 6 days. Zn was used at the concentrations of 0, 1.2, and 6.1 mM, with a constant concentration of 40 µM CdCl. Endogenous studies of the competitive capacity between Cd, Zn, Ca, and Mg were carried out to determine them when absorbed through the roots. Root and stem length and leaf area were determined, MDA was measured as an oxidative stress parameter and catalase (CAT) as an antioxidant enzyme. The results of the endogenous content in both leaves and roots showed that by increasing the concentration of Zn, the absorption of Cd decreased significantly ($P < 0.001$) on the contrary, the absorption of Zn did not show significant changes in the presence or absence of Cd in root, but there was a decrease in the endogenous content of Zn in the leaf as its concentration increases ($P < 0.001$). Ca and Mg showed a significant decrease with respect to the control ($P < 0.01$). The morphophysiological parameters showed a significant decrease in both root and stem length in all treatments respect to the control ($P < 0.001$), a significant increase was observed in MDA content in the last two treatments with Cd + Zn ($P < 0.001$), both in leaf and root. The activity of CAT decreased in roots with the last treatment compared to the control ($P < 0.05$), in leaf a significant increase of the enzyme was observed in all the treatments respect to the control ($P < 0.01$). The objective of this study was to establish the relationship of the endogenous

content of the aforementioned ions with respect to the morphophysiological and biochemical changes generated in soybean plants. We can conclude that the presence of Cd and Zn modifies the content of essential ions, which generates changes in both antioxidant and pro-oxidant activity, with a greater response in leaves than in roots, mainly with high concentrations of Zn, which could be attributed to a plant response to Cd and Zn toxicity.

A170
COMPOSITION AND SACCHARIFICATION OF THE SUGARCANE LIGNOCELLULOSE

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The full utilization of the sugarcane (*Saccharum spp.*) stalk is a promising alternative to satisfy the increasing demand of biofuels and the reduction of GGE. Understanding the interaction between lignocellulose components and its digestibility results essential for second generation ethanol production as well as other bioenergetic uses. In the present work, lignin (LIG), crystalline cellulose (CC), matrix of polysaccharides (MP) and saccharification rate (SAC) were evaluated in internodes of the cultivar LCP 85-384 (LCP) and the energy cane biotype INTA 05-3116 (B1) at ratooning (RT), grand growing (GG), early ripening (ER) and late ripening (LR) stages. Optical microscopy was used to observe LIG distribution in internodes. Linear mixed models and correlations were used to assess relationship between traits. LIG did not differ between genotypes but increased as the cycle progressed (18% in RT, 26% in GG, 29% in ER, and 31% in LR). At RT, LIG was detected in vascular bundles and cortex, and thereafter in parenchyma, especially in B1. CC did not depend on genotype but was higher at the onset of the cycle (41% in RT, 27% in GG, 30% in ER, and 29% in LR). Maximum SAC was observed in RT for B1 and in GC for LCP; then it decreased towards the end of the cycle. SAC was statistically correlated with LIG ($r = -0.34^{**}$) and with MP monosaccharides: arabinose ($r = -0.34^{**}$), xylose ($r = -0.34^{**}$), galacturonic acid ($r = 0.25^{*}$), glucuronic acid ($r = 0.33^{**}$), and glucose ($r = 0.48^{**}$). This study revealed effects of genotype and stage in the SAC, as well as a complex interaction with lignocellulose components.

A171
SUSTANAIBLE APPLICATIONS OF BIOCHARS FROM LIGNOCELLULOSIC WASTES

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In Argentina, recycling abundant lignocellulosic wastes in order to produce high value-added products for technological applications is a sustainable alternative to minimize environmental insults. Biochars produced from pyrolysis of sunflower seed hulls, sugar cane bagasse and cob corn were studied to be applied as soil amendment, pollution remediation, and as energetic materials. In pursuing this, a wide variety of biochars properties were measured by N2 sortometry, FTIR, CEC measurements, fixed and volatile C determination, heating capacity, particle size, among others. Pyrolysis was performed at bench scale, at 470°C, under nitrogen flux, at different contact times using raw biomasses as well as acid washed ones. Technical-economic and environmental feasibility of biochars production was assessed for different applications. As a result, biochars with different properties were obtained. Materials with large specific surface area and microporosity are suitable for pollution remediation, specifically for water pollutant absorption. Biochars possessing macroporosity and functional groups are appropriate for soil amendment, whereas materials with large particle size, low ashes loading, and high calorific power are suitable for energetic applications. It is worth noting that the both co-products of pyrolysis, biogas, and bio-oil, show properties with technological application. As for biogas, its high calorific power matches with energy production, while bio-oil is studied as grain biocide.

A172
IN VITRO MICROPROPAGATION OF PINOT NOIR (*Vitis vinifera*) AND OBTAINING WHOLE VIRUS FREE PLANTS

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Plant biotechnology, especially plant micropropagation, makes it possible to produce virus-free plants with genetic uniformity. The objective of this study was to obtain mother plants of the Pinot Noir strain through *in vitro* propagation. The cultivation was started from uninodal cuttings of Pinot Noir plants, which were disinfected with 70% alcohol for 3 s and 15% sodium hypochlorite for 20 min with sterile water rinses. They were seeded in 50% Murashige Skoog (MS) nutrient medium with the addition of 0.01% indole acetic acid. For eight weeks, data on establishment, rooting and sprouting of leaves were recorded and statistically analyzed. The *in vitro* plants obtained were transplanted to a sterilized mixture 1:1 of perlite and fertile soil, under adequate conditions of moisture and temperature, for acclimatization *ex vitro*. For the descriptive analysis of the number of leaves in the different weeks, the average central tendency: median and mode were calculated, along with measures of dispersion as standard deviation and variance. With the establishment and rooting data, frequency tables were constructed. The tests of normality (Kolmogorov-Smirnov, $P < 0.05$) and homocedasticity (Levene test, $P < 0.05$) were also performed. Because the tested assumptions were not met, the Mann–Whitney and Kruskal–Wallis *U*-tests were used for the correlation analysis. A total of 83% of the explants were established, there was 57% rooting and 1.2 average leaves per plant. There was a correlation between week of establishment and leaf production, being more productive the plants that established in the first and second week. The percentage of acclimatized mother plants was 100%. Pinot Noir proved to be a genetic material with good yield and excellent performance in vitro.

A173
PROTOCOLS FOR *IN VITRO* MICROPROPAGATION OF CABERNET FRANC AND PINOT NOIR (*Vitis vinifera*) VINE

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In view of the need to provide the food industry in Saint Louis with virus-free plants from grapevines of great vitivinicultural value, the objective was to find economic protocols to carry out the *in vitro* cultivation of Cabernet Franc and Pinot Noir grapevines. Previously, mother plants of both strains had been obtained, from which we started to carry out two experiments. The culture medium was: Murashigue Skoog (MS) salts at 50%; Vitamins (Thiamin 0.04 g/L, Nicotinic Acid 0.04 g/L, Pyridoxine 0.05 g/L) 1 mL; Adenine sulphate 0.8 g/L, 10 mL; EDTA Fe (FeSO47H2O) 2.78 g/L + (Na 2 EDTA) 3.73 g/L 5 mL; Sucrose as carbon source 30/L; Agar-agar 8 g/L; pH 5.7–5.8 to which was added in Experiment I: without auxins and Experiment II with auxins: AIA (Acetic Indole Acid) 0.01 g/L 10 mL. The culture media were transferred to glass flasks and covered with aluminum paper and sterilized 1 atm for 15 min at 120ºC in autoclave. Uninodal cuttings of both strains were disinfected with 70% alcohol for 3 s and 15% sodium hypochlorite for 15 min, with three sterile water washings between each step and sowed in laminar flow chamber. After 8 weeks of growth in culture chamber with photoperiod of 16:8 light/dark, the following results were observed. In the experiments I/II in the stage of Establishment of explants Cabernet Franc had 60%/100% and Pinot Noir 90%/100%; the average of leaves Cabernet Franc 7/66 and Pinot Noir 18/92, both with significant differences between experiments; the percentage of plants that produced roots *in vitro* was in Cabernet Franc 0%/80% and Pinot Noir 20%/90%, both with significant differences between experiments. Micropropagation of cuttings obtained from *in vitro* micropropagated mother plants, produced viable virus-free plants in Cabernet Franc and Pinot Noir strains, being more optimal the use of MS 50% medium with addition of AIA.

A174
PRIMING IN WHEAT (*Triticum aestivum* L) SEEDS WITH SPERMINE AS A TOLERANCE STRATEGY TO CADMIUM STRESS

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Previous exposure of seeds/plants to certain chemicals compounds (H_2O_2, NO, etc.) could induce homeostasis alteration that allows them to tolerate a future abiotic stress, a phenomenon known as pre-conditioning or priming. Cadmium (Cd) is a pollutant that produces oxidative stress and interferes with the entry, transport and use of nutrients. The current work is part of a project whose principal aim is to evaluate if polyamines (PAs) applied to wheat seeds could induce a priming event and improve the response to abiotic stress produced by Cd. In order to test this, wheat seeds (*Triticum aestivum L*) were primed with distilled water (C) or 25 µM spermine (Spm) for 3 h in a shaker (120 rpm), 24 ± 2 °C, and darkness. Then seeds were germinated (30 h, 24 ± 2 °C, darkness) and, afterwards, 3 seedlings were transferred to each pot with vermiculite. They were carried to a growing chamber (14:10 h light/darkness, 24 ± 2 °C, 50% relative humidity) and were watered with Hoagland ¼'s solution (C and Spm) with or without the addition of 200 µM $CdCl_2$ (Cd and Spm-Cd). After 7 days from the beginning of the experiment, they were harvested, the roots were washed with distilled water and separated from the aerial part. Results showed a significant increase in root length in Spm-Cd *vs*. Cd. Cd content in root and leaf was similar for Cd and Spm-Cd, while the contents of Mg, Ca, Fe, and Zn, were modified. The homeostasis of the free and conjugated PAs was altered in both organs by Spm and Cd. For example, Spm (with and without Cd) increased the level of cadaverine and diaminopropane (DAP) regarding C and Cd. The Spm-Cd treatment increased the activity of superoxide dismutase (SOD), guaiacol peroxidase (GPOX), the content of indol acetic acid (IAA), and decreased abscisic acid (ABA) in roots. In leaves, ABA increased in Spm-Cd regarding to the other treatments. In conclusion, priming with Spm improved tolerance in presence of Cd, proved an improvement in growth, redox, and hormonal balance in wheat plants. The use of priming with Spm could help a better establishment of the plant in soil, providing a better acclimatization to Cd stress.

A175
EFFECT OF PYRROLIZIDINE ALKALOIDS OBTAINED FROM *Senecio rudbeckiaefolius* ON THE MIDGUT OF PEST-INSECTS OF STORED NUTS

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Currently, research and development of vegetal based biopesticides are intended to mitigate environmental pollution caused by chemical pesticide residues and promote agricultural sustainable development. One of the problems of great economic implication is the control of insects that attack agricultural products that, due to their seasonal nature, must be stored. Such is the case of walnut production in the provinces of La Rioja and Catamarca. Productivity depends on many factors; particularly, damage caused by insects during storage is a key point and it mainly affects small producers. Among insect species that frequently attack walnuts, we found *Oryzaephilus surinamensis* (Coleoptera) and *Plodia interpunctella* (Lepidoptera). Plant secondary metabolites are an interesting alternative due to their rapid biodegradability and low impact on ecosystems. Harmful effects of botanicals against insects can be manifested as toxicity, growth inhibition, and reproductive disorders. Despite the insecticidal potential of many secondary metabolites, target organs or tissues and their action mechanisms are frequently unknown. The digestive membranes, composed of

the intestinal epithelium and a peritrophic membrane, have been postulated as some of the targets of insecticides. In previous studies, we demonstrated lethal and sublethal effects of a pyrrolizidine alkaloid (PA) fraction obtained from a methanolic extract of *Senecio rudbeckiaefolius* (Asteraceae) on *O. surinamensis* and *P. interpunctella* larvae. The aim of this work was to histologically analyze *S. rudbeckiaefolius* PA effects on the larval midgut of both species fed with walnuts impregnated with 50–250 mg/L PA hydroalcoholic solutions. For this, larvae of both species were processed using the histological technique for arthropods and histological sections (3–5 µm) were stained with Hematoxylin-Eosin. The microscopic study of the *O. surinamensis* larvae revealed irreversible damage on intestinal lining and signs of melanization in surrounding tissues, compatible with previously detected toxic effects. By contrast, the *P. interpunctella* larvae preserved the integrity of their digestive mucosa and showed cytological changes associated with the cellular immune response of insects. The identification of potential biopesticides' target tissues or organs provides a scientific basis for their incorporation in insect-pest control programs within a sustainable agriculture framework.

A176
CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS OF *Mentha spicata* FERTILIZED WITH UREA

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Secondary metabolites present in aromatic species constitute a promising alternative in the control of phytopathogens, since they produce less environmental impact than synthetic fungicides. This work aimed to evaluate the effect of urea fertilization on the chemical composition of *Mentha spicata* essential oils, and their antifungal activity. The essential oils were extracted using distillation by drag of vapor, from leaves of unfertilized plants (control), or fertilized with 150 kg/ha of urea. Its chemical composition was determined by gas chromatography and mass spectrometry. Its fungicidal activity was determined, calculating the percentage of inhibition of colonies of *Alternaria solani* and *Fusarium oxysporum*. The results were analyzed with ANOVA and Tukey's Test. The most abundant component in the essential oil was carvone and limonene in both treatments, control and nitrogen fertilization. Fertilization with urea increased the content of carvone in essential oils by 47%, compared to the control. In the control, 10 and 15 µL doses of Petri^{-1} box essential oils were required, to inhibit 100% of the development of *A. solani* y *F. oxysporum,* respectively. In the treatment fertilized with 150 kg^{-1} urea, only doses of 6 and 9 µL Petri^{-1} box essential oils were required, respectively. In conclusion, the essential oils of *M. spicata* possess antifungal activity on *A. solani* and *F. oxysporum*. Fertilization with urea increases this property, making it possible to use it as a biological fungicide.

A177
EFFECT OF NITROGEN FERTILIZATION ON THE CHEMICAL COMPOSITION AND ANTIDIABETIC PROPERTIES OF ESSENTIAL OILS OF *Mentha spicata* L.

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Among the homeopathic uses of essential oils, the control of type 2 diabetes stands out, due to the ability to inhibit the activity of the enzymes α-amylase and α-glucosidase. The therapeutic properties of essential oils depend on their chemical composition, which is affected by environmental conditions (mainly concentration of nutrients in the soil and photoperiod). Nitrogen fertilization can increase the yield of essential oils in *Mentha spicata*, but its effect on their chemical properties is unknown. This work aimed to evaluate the effect of nitrogen fertilization on the concentration of carvone, and the antidiabetic properties of essential oils of *M. spicata*. The tests were carried out in the town of El Zanjón (Santiago del Estero) under irrigation, applying the following urea doses: 0, 100, and 150 kg/ha. The crop was harvested in full bloom, and the essential oils were extracted by steam entrainment distillation. The chemical composition of essential oils was determined by gas chromatography and mass spectrometry. The inhibitory capacity of essential oils and ascarbose (a standard antidiabetic) on the activities of the α-amylase and α-glucosidase enzymes was determined, which was expressed as IC_{50} values (concentration of the sample that produces 50% inhibition in enzyme activity). A completely randomized experimental design with 5 replications was used, and the data was analyzed with ANOVA and Tukey's test. The nitrogen increased the content of carvone in essential oils with respect to the control, although no significant differences were obtained between the 100 and 150 kg urea/ha doses (the yields were 51, 60, and 71 % of carvone in the treatments of 0, 100, and 150 kg urea/ha, respectively). The essential oils of *M. spicata* had a greater inhibitory capacity of the α-amylase and α-glucosidase enzymes than ascarbose. The increase in carvone content was accompanied by an increase in the inhibitory capacity of the enzymes α-amylase and α-glucosidase. IC_{50} values for α-amylase inhibition were 135, 93, and 92 µg/mL in treatments of 0, 100, and 150 kg urea/ha. For the inhibition of α-glucosidase, IC_{50} values of 98, 74, and 76 µg/mL were obtained in the treatments of 0, 100, and 150 kg urea/ha. In ascarbose, IC_{50} values of 372 and 195 µg/mL were obtained for the inhibition of the α-amylase and α-glucosidase enzymes, respectively. It is concluded that nitrogen fertilization increases the concentration of carvone, and the antidiabetic properties in essential oils of *M. spicata*.

A178

Pseudomonas fluorescens SF4c PRODUCES TWO TYPES OF RETRACTABLE TAILOCINS

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Microorganisms in the rhizosphere produce metabolites, including bacteriocins, which allow them to compete for a specific niche. Bacteriocins antagonize bacteria which are phylogenetically related to the producing strain. Bacteriocins showing structural similarities with bacteriophage tails are known as tailocins. The native *Pseudomonas fluorescens* SF4c strain produces two tailocins (type R and type F). R tailocin has a common ancestor with the tail of *Enterobacteriophage* P2 of the *Myoviridae* family, while the same is true for type F with phage lambda of the S*iphoviridae* family. A F tailocin mutant derived from *P. fluorescens* SF4c was previously constructed in our laboratory. The present work aimed to construct a mutant in R tailocin and to compare them to each other. Primers were designed to amplify two fragments corresponding to the beginning and the end of the region that comprises the structural genes of R tailocin. Thus, 12000 bp of the cluster were deleted and replaced by a kanamycin gene. The antimicrobial activity of the R and F mutants was analyzed against different strains of the genera *Pseudomonas* and *Xanthomonas*. F tailocin inhibited 7 of the 8 strains tested, including *Xanthomonas vesicatoria* Xcv Bv5-4a which causes tomato and pepper spot disease, while R tailocin showed antimicrobial activity only against *P. fluorescens* CTR212. Thus, F tailocin had a wider killing spectrum. Therefore, it could be used in the future as a tool to control spot disease. A phylogenetic study of *P. fluorescens* SF4c bacteriocins was also performed. The structural genes of each SF4c tailocin were aligned using MEGA software with those of tailocins produced by other plant-associated *Pseudomonas*. Furthermore, EDGAR software was used to analyze the average amino-acid identity matrix (AAI) and to generate a Venn diagram of the orthologous gene cluster. Phage prediction was carried out on PHASTER software with the structural gene sequences for R and F tailocins. These bioinformatic analyses showed that F tailocin was grouped with R-type tailocins from others plant-associated *Pseudomonas*. Likewise, the prediction of related phages indicated identity with bacteriophages of the *Myoviridae* family, which have long retractable tails. In conclusion, the results suggest that *P. fluorescens* SF4c produces two types of retractable tailocins, called R1 and R2, with different spectra of action. An orthologous gene analysis could help to understand the phylogenetic evolution of these elements and their distribution in nature.

A179

POTENTIAL OF CARDOON LIGNOCELLULOSIC BIOMASS TO GENERATE THERMAL ENERGY

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In last year, the use of biomass as fuel has been increasing worldwide. Replacing fossil energy input with renewable energy within the energy consumption matrix is not only an environmental responsibility, but also a benefit in production costs. Cardoon (*Cynara cardunculus var. altilis*) is a perennial species belonging to the Asteraceas family, it has an annual crop cycle, low maintenance requirements and produces between 15 to 20 tons/ha/year of lignocellulosic biomass. The aim of this work was to evaluate the potential of cardoon lignocellulosic biomass for thermal energy production. A triturate sample of lignocellulosic biomass (5 kg) was used to determine: apparent density (DA) and percentage of moisture (on wet basis) and ash content (% Cz), volatile matter (% MV), fixed carbon content (% CF) and heating value (on dry basis). Analyses were performed according to standardized protocols reported in UNE 164001: 2005 EX. The energetic potential was calculated considering that the main demand of the energy generated by combustion is for thermal energy production (hot air, hot water, or steam). The biomass presented a DA = 139.88 kg/m^3, slightly lower than recommended value (150 kg/m^3). The moisture percentage was 16.08% m/m, the % Cz was 7.60% m/m, MV = 74.98%, and CF = 7.41% m/m. The higher heating value was 4123.56 kcal/kg while the lower heating value was 3117.42 kcal/kg. This last value indicates that the heat energy yield after combustion is similar to other solid fuels, such as firewood (3000–3700 kcal/kg). The energy density presented a value of 442.81 Mcal/m^3. These results were used to determine the energy potential by direct combustion of cardoon biomass. It was found that to heat a 100 m^2 room, a thermal energy power of 11000 kcal/h is required, which is reached with 4.9 kg of biomass/h; while to obtaining hot water in a horizontal humotubular boiler (required thermal energy power 3000 kcal/h), 1.5 kg/h are needed and for stream production (thermal energy power 480000 kcal/h) the requirement amounts to 275 kg/h. According to these results, it is concluded that the crushed cardoon is suitable as an energy source, mainly in short logistics schemes and also it can be used for multiple thermal energy applications. In future studies, the residue generated by combustion will be characterized to determine whether an additional benefit is obtained from its use as a by-product.

A180

ROLE OF THE CHROMATIN REMODELER MOM1 IN THE PRIMING AGAINST PATHOGENS IN *Arabidopsis thaliana* (L.) HEYNH

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After recognition of a pathogen, plants induce local and distal defenses. Systemic resistance programs are usually associated with an immune alertness, immunological "memory" or priming. Despite not showing constitutive defenses, a primed plant responds faster and/or strongly to recurrent infections. Priming may involve an increase in immune receptors, accumulation of inactive signaling proteins and/or epigenetic modifications of chromatin that predispose to transcriptional activation of defense genes. The chromatin remodeler "Morpheus Molecule" (MOM1) was recently proposed as a priming factor associated with the activation of defenses during aging. Here, we analyzed the role of MOM1 in the immunological "memory" of *Arabidopsis*. We studied the susceptibility of mom1 mutant plants to chemical and biological inducers of priming against pathogens. We also determined whether the increased pathogen resistance observed in plants lacking MOM1 is caused by aging and/or growth conditions. We found that, independently of the development stage, under optimal conditions of growth and sterility mom1 mutant plants

show increased levels of immune receptors without the induction of defenses. In addition, mom1 is more sensitive to the priming inducers azelaic acid (AZA) and aminobutyric acid (BABA). Consistently, transgenic plants that express a minimal but functional version of MOM1 (mini-MOM1) do not respond to these inductors. Moreover, treatments with AZA or BABA reduced the transcript levels of MOM1 in wild-type plants. Together, our results position the chromatin remodeler MOM1 as a negative regulator of the priming against pathogens in *Arabidopsis*.

A181

IMPACT OF *Azospirillum brasilense* Az39 INOCULATION ON THE MAIZE MICROBIOME

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Azospirillum is one of the most used genera for agriculture worldwide. Inoculation with many plant growth-promoting rhizobacteria (PGPR) modifies the microbial communities associated with plants, but few reports are available for *Azospirillum*. In order to understand if the *A. brasilense* Az39 inoculation modifies the bacterial populations associated with maize rhizosphere, we performed a metagenomics analysis under experimental controlled conditions. For this purpose, maize seeds were pre-germinated for 7 days and transplanted to pots containing soil samples obtained from a georeferenced agricultural region of the Córdoba Province (Argentina). The seedlings were inoculated at root level with 100 ⌈L of 1×10^8 cfu/mL of *A. brasilense* Az39 (inoculated rhizosphere). An equivalent volume of sterile distilled water was added to non-inoculated seedlings (non-inoculated rhizosphere) and non-inoculated soil treated with an equivalent volume of sterile water (bulk soil) were used as control treatments. After 14 days-incubation, the whole DNA was extracted from the rhizosphere, and the V4 region of the bacterial 16S rRNA gene was amplified and sequenced using the Illumina MiSeq platform. The genus relative abundances were evaluated and both alpha and beta diversity were assessed. The microbial differences were explored using LEfSe and IndicSpecies algorithms and a co-occurrence network analysis was performed. Results showed that *Azospirillum* was the most abundant genus in inoculated samples which confirms its ability to colonize the maize rhizosphere. A significant difference was observed for alpha diversity according to the Pielou index. No differences in the beta diversity between Az39 inoculated and non-inoculated rhizospheres were observed. However, these communities maintained a differential structure in comparison with the bulk soil. According to LEfSe index, the four most abundant genera associated with Az39 were *Burkholderia, Massilia, Sphingobium* and *Cupriavidus*, while *Azospirillum* and *Pseudomonas* were the most abundant genera according to IndicSpecies. An increase in relative abundance of some members of the Rhizobales order was observed by inoculation of Az39 in the maize rhizosphere. Finally, the co-occurrence network showed a positive interaction between *Azospirillum* and *Pseudomonas* genus. The ability of *Azospirillum* to colonize the maize rhizosphere induced changes in relative abundance of some bacterial genera in the rhizosphere and such changes could involve new interactions between these recruited microorganisms in the microbiome and together with some differential effects on plants.

A182

Azospirillum brasilense CHANGES THE ROOT ARCHITECTURE OF *Arabidopsis thaliana* BY DEPENDENT AND INDEPENDENT INDOLEACETIC ACID MECHANISMS

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Azospirillum brasilense is a plant growth-promoting rhizobacteria (PGPR) with the ability to produce several phytohormones such as indole-3-acetic acid (IAA). The positive interaction of *Azospirillum* with plants has been simplified and explained through the bacterial capacity to produce IAA. Typical changes on root architecture by promoting the number of lateral roots and hair formation and reducing the primary root length were established in inoculated plants. These changes increase the root surface and organ volume improving the water and nutrients acquisition by inoculated plants. The mechanism by which *Azospirillum* induces such changes fails to be explained only by the bacterial capacity to produce IAA. The main objective of this work was to evaluate the role of the IAA produced by *A. brasilense* Az39 as co-responsible for the changes observed in root architecture of *Arabidopsis thaliana* and to evaluate the nature of the IAA-independent mechanism. In our experiments *A. thaliana* root architecture was evaluated after inoculation with *A. brasilense* Az39 or with its IAA deficient mutant named Az39Δ*ipdC*. Disinfected and stratified seeds of *A. thaliana* ecotype Col-0 and its mutant *tir 1.1* deficient for lateral root formation were germinated vertically in Petri dishes containing Murashige and Skoog (MS) medium for 7 days with 16/8 h photoperiod at 22ºC. Then, seedlings were aseptically transferred to MS plates containing 10^8 cfu/mL of the wild-type strain (Az39); its mutant (Az39 *ipdC*$^-$); an equivalent number of heat-inactivated cells of Az39 (Az39φ) or equivalent title of *E. coli* DH5α used as negative control. After 5 days post-transference, the root architecture was evaluated by the use of an image analysis system. Our results demonstrate the ability of *A. brasilense* Az39 to modify the primary root development of *A. thaliana* through IAA biosynthesis, while other IAA-independent mechanisms were related to an increase in the development of lateral roots and the number of root hairs. The physical presence of the bacteria, even metabolically inactive (Az39φ) in the culture medium seems to mediate the development of root hairs in inoculated seedlings and this mechanism being common to other bacteria as the non PGPR strain *Escherichia coli* DH5α. Our results suggest that inoculation with *A. brasilense* induces morphological changes in root architecture of *A. thaliana* through IAA-dependent and IAA-independent mechanisms. The biosynthesis of IAA by *A. brasilense* Az39 reduces the length of the main root of the inoculated seedlings, while the contact of the bacterial cells with the plant at root level (even inactivated cells) increases the root hair production. Finally, the production of some active compounds by the metabolically active cells of Az39 increases the growth and development of lateral roots. This is the first report presenting some preliminary evidence about this novel interaction for the *Azospirillum* genus.

A183
BROMATOLOGICAL CHARACTERIZATION OF *Passiflora caerulea* L.

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Passiflora caerulea L. of the family Passifloraceae is a native species to Argentina. It has been used in traditional medicine for the treatment of nervous disorders (anxiety, insomnia) but its nutritional proprieties need to be explored. It is known by the names of "Biricuyá", "Burucuyá", "Flor de Cristo", "Flor de Pasión", "Flor de la Pasión", "Granadilla". *P. caerulea* has alternate, palmate, penta-lobed leaves like an open hand, 10–18 cm long and wide. The fruit is a yellowish orange oval berry 6 cm long by 4 cm in diameter, containing numerous seeds. In this work, the fruit of *Passiflora caerulea* was studied in terms of its bromatological characteristic for use in the food industry. Official methods of food analysis were used and previously optimized and validated techniques (AOAC 942.15/90 AOAC, 925.10; 945.39, 978.04–968.01, 962.09, 945.46, (AOAC 942.15/90; AOAC 10.041/84; AOAC 932.12/90). Proximal analysis of *P. caerulea* showed a moisture, ash, fiber, fat, protein, and carbohydrate content of 52.43%, 0.75%, 3.5%, 0.18%, 1.78%, 41.36%, respectively, and its caloric value of 81.04 kcal/100g (calculated using Atwater factors of 4 kcal/g for proteins and carbohydrates and 9 kcal/g for fats). Also, the fruit was evaluated in its pH, acidity, total solids, and total sugars showing pH = 5.07, acid index of 0.83 g citric acid/100 g, 5.09° Brix, and 9.67 g total sugars/100 g. The fruit of *P. caerulea* presented a high percentage of moisture, fiber, and protein but low fat content, values close to the FAO (Food and Agriculture Organization) benchmarks. The energy value of the Passionflower fruit seems to be influenced mainly by carbohydrates, which were its main macronutrients. The acid value was within the accepted range for consumption and the pH value was of medium acidity, as proposed by the Food and Drug Administration (FDA, 2016). The values of total solids of the fruit of the Passion are related to the sweetness of it that proposes it as a fruit of good acceptance. The results obtained allow us to infer that the native fruit of *P. caerulea* has an outstanding chemical and nutritional composition to be used in the food industry and in the development of new food products. This will allow the preservation and revaluation of this regional fruit and consider it as an alternative in the diet of the population of the province of San Luis, allowing its inclusion in the Composition Tables of Argentine Foods.

A184
THE AUXIN CATABOLISM BY *Bradyrhizobium japonicum* E109 AND ITS IMPORTANCE FOR THE SOYBEAN-*Bradyrhizobium* SYMBIOSIS

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Bradyrhizobium japonicum E109 is one of the most widely used strains for the soybean inoculant formulation in Argentina. This strain is considered a real plant growth-promoting rhizobacteria (PGPR) due to its capacity to fix biological nitrogen and to biosynthesize several phytohormones as the indole-3-acetic acid (IAA). The IAA metabolism comprises several mechanisms, such as the biosynthesis, catabolism, conjugation, and hydrolysis of auxins conjugates, which globally regulate the IAA status. The IAA catabolism has been partly elucidated in *B. japonicum* E109 under saprophytic conditions but its importance for the soybean-*Bradyrhizobium* symbiosis still remains unclear. The aim of this work was to evaluate the catabolism of IAA and its impact on the saprophytic and symbiotic behavior of *B. japonicum* E109 and *B. diazoefficiens* USDA110 and its mutants, which are incapable of degrading the hormone. To this end, aliquots of 40 µg/mL of IAA were added to pure cultures of *B. japonicum* E109, *B. diazoefficiens* USDA110 and USDA110 Δ*iac*A, Δ*iac*C and E109 Δ*iac*C mutants during exponential growth phase (1×10^9 cfu/mL). After 24 h-incubation, both the saprophytic and symbiotic behavior was evaluated for each strain. The saprophytic performance was evaluated through the biomass production (OD_{595}), cell viability (cfu/mL) and IAA concentration in the culture medium (µg/mL); while the symbiotic performance was assessed by the evaluation of root nodulation through the Burton's test. For each treatment, 30 seedlings were maintained under growth chamber conditions for 21 days with 16/8 h photoperiod at 28ºC and were irrigated with nitrogen-deficient sterile N-free Hoagland's solution [25% (v/v)]. Our results demonstrated that the exogenous addition of IAA caused significant changes in the saprophytic behavior of all the evaluated strains. In the case of biomass production (OD_{595}) a 32.1% increase was determined in E109 and USDA110 wild type strains associated with 3.16% increase in the number of viable cells (cfu/mL). In the case of the Δ*iac*C and Δ*iac*A mutants, they increased around 29.7% the biomass production and 3.3% the number of viable cells (cfu/mL). In the case of the soybean seed inoculation, the exogenous treatment with IAA increased the number of *Bradyrhizobium* viable cells recovered from inoculated seeds after 4 h-inoculation (RF%) around 27.4% in comparison with control treatment without addition of IAA. In relation with the symbiotic performance, treatment with IAA increased the percentage of root nodulation of the Δ*iac*A mutant by 30% and decreased by 55% in the case of USDA110. E109 and Δ*iac*C maintained a percentage of nodulation around 100%. Finally, the number of total nodules increased significantly in the Δ*iac*C and Δ*iac*A mutants by an average 30% by exogenous addition of IAA. These results suggest that the exogenous addition of IAA to E109 and USDA110 or their mutant cultures causes a positive effect on both saprophytic and symbiotic behavior of these bacteria, independently of their ability to degrade the hormone in the culture medium.

A185
CHARACTERIZATION OF THE ACIL-HOMOSERINE LACTONE DEGRADATION BY *Azospirillum brasilense* Az39

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Azospirillum brasilense Az39 is a PGPR strain with the ability to promote the plant growth and crop yield under agronomic conditions mainly through its capacity to fix atmospheric nitrogen and to produce several phytohormones. Although the mechanisms related to growth promotion have been studied in detail in this bacterial genus, we know little about the mechanisms that govern the bacteria-bacteria interaction and particularly those related with the *quorum* phenomenon. In our laboratory, we have confirmed that *A. brasilense* Az39 does not produce acyl-homoserine lactones (AHL) (*quorum sensing*) but instead degrades these molecules (*quorum quenching*) due to an enzymatic activity. The objective of this work was to determine the type of enzyme involved in the degradation of AHLs in Az39 and to verify its cellular location. Initially, we analysed whether the inactivation of AHLs was due to the presence of lactonase-type enzymes and for do it, we cultured the bacteria in 5 ml of MMAB medium supplemented with 10 µM of AHLs (C_6-HSL and C_{10}-HSL) for 16 h at 240 rpm, 30 °C and pH 6.5-7.0. After the incubation time, 1 mL of the culture was taken and 1 M HCl was added until achieving a pH of 2.0, finally, 5 µL samples were taken from each reaction mixture at different time intervals (5, 30, and 60 min) to be analyzed by bioassays with reporter strains. To determine the location of the enzyme, some pre-incubated treatments with different AHLs were performed. These treatments were: (T1) LB + 10 µM of each AHL; (T2) Az39 filtered supernatant; (T3) Az39 filtered and denatured supernatant; (T4) Az39 denatured (100°C–30 min) and (T5) Az39. All treatments were analyzed using reporter strains. Finally, for the identification of the gene responsible for the degradation activity of AHLs in Az39, an insertional mutant strain was obtained for a putative gene. Our results did not show re-lactonization of AHL after acid treatment, therefore the participation of lactonases in the inactivation of these intercellular signaling molecules by Az39 was ruled out. In contrast, denaturation tests allowed us to suggest that the enzyme responsible for the acylase activity could be bound to the plasma membrane or released to the periplasm. The mutant strain was developed in a gene annotated by sequence homology as a putative *penicillin acylase* (EC. 3.5.1.11), and it was unable to degrade synthetic AHLs *in vitro*. These results confirm that *A. brasilense* Az39 does not produce AHLs but degrades them through the expression of a gene that codes for a putative acylase-type enzyme with a cellular location.

A186
DORMANT RUPTURE AND HORMONES LEVELS IN *Jatropha curcas* L. AND *Jatropha macrocarpa* GRISEB SEED

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Jatropha curcas L. and *Jatropha macrocarpa* Griseb are perennial shrub with the greatest importance mainly from its biofuel potential. Seeds of both species present a hard seminal covering that encloses the endosperm and the embryo. Several authors consider that this tegument is one of the factors that induce dormancy. The aim of the work was to study the role of tegument and abscisic acid (ABA) y jasmonic acid (JA) in dormancy and germination in these species. *J. macrocarpa* present dormancy since it does not germinate by traditional methods. The seeds of *J. macrocarpa* were subjected to different treatments to break seed dormancy: (T1) Control; (T2) Scarification with sandpaper; (T3) Total elimination of the tegument; (T4) Immersion in boiling water for 1 min and then immersed in cold water for 24 h; (T5) Alternating hot and cold water for 5 min, each one; (T6) Immersion in concentrated H_2SO_4 for 15 min; (T7) Immersion in concentrated H_2SO_4 for 30 min; (T8) Stratification in wet and cold paper (4°C) for 90 days; (T9) Stratification in moist sand and cold (4°C) for 90 days. After each treatment the seeds were placed in Petri dishes containing 3 mL of distilled water at 30°C temperature. The test was conducted under dark condition. Germination percentages (GP) were determined for 30 days. We used 20 seeds by treatment, with three replications each one. The seeds of *J. curcas* don't were subjected to different treatments scarification and stratification because they have no dormancy. ABA and JA were extracted and purified from both *Jatropha* species tegument. These hormones were identified and quantified from tissue using reverse-phase high-performance liquid chromatography (HPLC)-mass spectrometry (MS). Analysis of variance (ANOVA) was applied, and data were subjected to the Multiple Range Duncan Test, using the software INFOSTAT-UNC. The total removal of tegument showed a 50% increase in germination percentage, with the other treatments achieved between 0–10%. ABA and JAs were detected in tegument of *J. macrocarpa* and *J. curcas* seeds. JAs were the most abundant compound. ABA level was higher in *J. curcas* (628%) than in *J. macrocarpa*. This would indicate that the tegument ABA level is not directly linked to germination and/or dormancy of these *Jatropha* species. In contrast, level of JAs was higher in *J. macrocarpa* (101%) than in *J. curcas.* In effects JA could have a roll in inhibition of germination of *J. macrocarpa* seeds.

A187
TOXIC AND REPELLENT EFECTS OF NATURAL PRODUCTS OBTAINED FROM *Lippia turbinata* FOR THE CONTROL OF *Oryzaephilus surinamensis*

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The walnut cultivation (*Juglans regia*) and its subsequent commercialization as dried fruit, represent important economic sources in La Rioja province, Argentina. Production performance and quality depend on several factors, from crop handling practices to postharvest control. Stored nuts

are frequently attacked by pest-insects, which decrease the quality and therefore the profitability of the product. Pest control in storage systems depends mainly on the use of fumigants, but small walnut producers do not apply these inputs in storage sheds. Plant secondary metabolites represent an interesting alternative for the control of pest-insects, while minimizing the negative effects on the environment and human health. The objective of this work was to evaluate the toxic and repellent effects of ethanolic extract (EE) and essential oil (EO), obtained from aerial parts of *Lippia turbinata* (Verbenaceae) on larvae and adults of *Oryzaepilus surinamensis* (Coleoptera), one of the main regional pests of stored nuts. For the toxicity bioassays, batches of 15 larval/adult individuals were topicated with *L. turbinata* EE at concentrations of 1000, 3000, and 5000 mg/L and EO at 300, 400, and 500 mg/L and 3 repetitions were carried out. The insects were placed in Petri dishes under controlled conditions and fed with nuts. Survival was recorded at 72 h and the mortality rate was calculated for both treatments. To evaluate repellency, an ambient olfactometer was used, in which a filter paper impregnated with the natural product to be tested and another with the solvent as a control were placed; 48 individuals per product were evaluated. The behavior was recorded according to the area of choice and the data was analyzed with the R-Medic test. The *L. turbinata* EE exerted a concentration-dependent toxic effect, reaching values of up to 72% mortality in larvae and 47% in adults. On the other hand, the *L. turbinata* EO caused a repellent effect at 300 mg/L on adults and at 500mg/L on *O. surinamensis* larvae. These results allow us to infer that the natural products obtained from *L. turbinata* are promising as potential phytosanitary products for the control of this pest of economic relevance in the region.

A188
EFFECT OF NODULATION OF *Sinorhizobium meliloti* ON THE GROWTH OF *Medicago sativa* EXPOSED TO SALINITY AND CADMIUM

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Alfalfa is a species of great plasticity, morphologically and physiologically adapted to tolerate abiotic stresses. The aim of this work is to evaluate the behavior of two varieties of alfalfa, inoculated with tolerant rhizobia, by determining physiological and antioxidant parameters. For the trial, seeds of var. Trinidad 87 and CW 660 were previously germinated and then planted in a terrine system with sterilized vermiculite. A first replicate was watered with Hoagland nutrient solution (SN) and the second replicate contains this same nitrogen limiting solution. The plants are inoculated with *S. meliloti* capable of nodulating the roots. The control plants were watered with nitrogen and nitrogen-limiting SN, and the treatments consisted of SN with nitrogen + Cd 75 µM + 100 mM NaCl; nitrogen-limiting SN + Cd 75 µM + 100 mM NaCl. After 5 weeks of cultivation, morphological parameters were measured: air length, fresh air weight and fresh root weight. Oxidative damage was determined by lipid peroxidation (MDA), and enzymatic activity: superoxide dismutase (SOD) and catalase (CAT). The statistical analysis performed was a multifactorial ANOVA (SPSS Statistics for $P \leq 0.05$). In CW 660, the morphological parameters showed significant differences between the inoculated and uninoculated plants, as well as by the treatment applied, but there was no significant effect of the inoculation on the treatment. The same was observed in the determination of SOD. A significant effect of inoculation on treatment was observed for catalase activity. In var. Trinidad, inoculation was beneficial for all morphological parameters, although no difference in SOD and CAT was observed. MDA, in both varieties, increased with inoculation and with stress treatments independent of inoculation.

A189
CHARACTERIZATION OF THE *BNT1* IMMUNE RECEPTOR IN *Arabidopsis* TARGETED TO PLASTIDS

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Plants depend on a non-adaptive immune system triggered by receptors after the detection of pathogens. Among them, nucleotide-binding leucine-rich repeat receptors (NLRs) family are key immune sensors that recognize specific effectors used by pathogens to promote their virulence. NLR proteins have been found in various subcellular compartments and they can be relocated after activation, indicating that trafficking is involved in their function. However, NLRs acting from endosymbiont organelles such as plastids or mitochondria have not been described. NLRs consist of a N-terminal region that is similar to the "Toll/Interleukin-1 receptor" (TIR) or forms a "coiled coil" (CC), a nucleotide binding domain (NB) and a leucine rich repeat (LRR) (TIR/CC-NB-LRR). Recently, we have found a novel N-terminal localization signal for plastid or mitochondria targeting. Notably, among the *Arabidopsis* predicted proteins that possess this signature one is the NLR receptor BNT1. In this work, we have characterized the changes in transcripts and localization of BNT1. We carried out an *in-silico* analysis of the transcriptional variations of *BNT1* isoforms generated by alternative splicing (AS). By RT-PCR we validated and examined the *BNT1* isoforms level in basal and biotic/abiotic stress conditions. We also generated BNT1-GFP fusions for transient expression in *Nicotiana benthamiana* to determine its localization using confocal microscopy and subcellular fractionations. Several BNT1 N-terminal mutated versions were also analyzed. Together, our results suggest that *BNT1* isoforms generated by AS specifically participate in different stress responses. Additionally, the differential localization of BNT1 mutated versions suggest a key role of its N-terminal region during defenses against pathogens. The elucidation of BNT1 biological role could represent a paradigm to understand the function of NLR immune receptors localized in plastids and different subcellular spaces.

A190

SURVEY OF PATHOGENS ASSOCIATED WITH SPECIES OF *Cucurbita* IN EARLY STAGES OF DEVELOPMENT AND THEIR RELATIONSHIP WITH DISEASES IN RIPE FRUIT

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In the horticultural belt of Rosario, various vegetables are grown intensively or semi-extensively, being the *Cucurbita* genus of great importance for the farmer. Over the years there has been a reduction in the quality of the fruits, seeds and flowers that are demanded and consumed by the public dew to their nutritional value. The diseases that affect the ripe fruit can affect all the phenological stages and tissues of the plant, therefore, their study is relevant. The objective of this work was to detect the pathogens associated with the cultivation of squash (*Cucurbita moschata* Duch.) in early developmental and reproductive stages. As experimental material, various *Cucurbita* inputs implanted in the Horticultural Production lot of the Faculty of Agrarian Sciences (33º01'SL and 60º53'WL) were used, including groups of defined cultivars, from different origins and populations of the wild form. Three evaluations were carried out during the flowering and fruit formation stage: 01/02/20 (R1), 01/08/20 (R2) and 01/13/20 (R3). Koch's postulates were applied to isolate the pathogens from the various symptomatic plant organs: (a) in immature fruit, the pathogen was isolated from fructifications (if any), and/or epidermal tissue and pulp disinfected with 2% sodium hypochlorite for 30 s, adding a portion of the pulp without sanitizing; (b) the symptomatic leave sections were placed in a humid chamber for 4–7 days until the appearance of signs; and (c) pollen grains from closed flowers prior to anthesis, were evaluated without surface treatment. In all cases, the plant material was seeded in Petri dishes with 2% APGA culture medium (2% glucose potato agar, acidified) and incubated at 27 ± 2 °C for 7 days. Each fungal genus was determined on the basis of the macro and micro-morphological characters using various taxonomic keys. The incidence (%I) of each fungal genus was calculated as number of colonies / total colonies × 100. Evaluated plant samples were: 24 immature fruits (11 (R1) + 12 (R2) + 1 (R3)), 17 symptomatic leaves (2 (R1) + 15 (R2) + 0 (R3)) and 20 closed flowers were evaluated for pollen extraction (0 (R1) + 14 (R2) + 6 (R3)). Results were: the 75% of the immature fruits showed interactions with fungal pathogens: *Fusarium* (66.7%), *Aspergillus* (22.2%), *Pythium* (22.2%), *Phyllosticta* (11.1%), *Rhizopus* (11.1%), *Myrothecium* (5.6%), *Phialophora* (5.6%), *Epicoccum* (5.6%), and *Alternaria* (5.6%). The 11.8% of the symptomatic leaves only showed associations with *Fusarium* genus, while the 70% of the pollen samples (from closed flowers) showed associations with *Fusarium* (42.9%), *Phyllosticta* (28.6%), *Pythium* (21.4%), *Aspergillus* (7.1%), *Cladosporium* (7.1%), *Nigrospora* (7.1%), *Drechslera* (7.1%), *Stemphylium* (7.1%), and *Phomopsis* (7.1%). The identification of the fungal species associated with the varieties of zucchini produced in our region, will promote to advance in the production strategies of quality and safe horticultural foods, and boost regional economies.

A191

PLANT VIGOR VARIABILITY IN TWO SPONTANEOUS POPULATIONS OF *Stapfochloa berroi* (ARECHAV.) P.M. PETERSON FROM LA PAMPA DEPRIMIDA

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Stapfochloa berroi is a perennial grass of spring-summer growth that in Argentina is native and is found in grasslands important for animal production in several provinces. In the halophyte steppes of the Pampa Deprimida, *S. berroi* is an important resource due to its good forage value and its adaptation to these restrictive environments (sodium soils, low organic matter content, waterlogging, drought) for other forage species. Reincorporation of selected native germplasm would be important both to increase livestock productivity and to maintain biodiversity. Thus, it is key to study characters related to implantation control such as plant vigor. The objective was to analyze variability in plant vigor and associated characteristics and their correlations in two spontaneous populations of Sb from steppes of halophytes of the Depressed Pampa growing in substrate without limitations. Caryopsis with their covers (lemma and palea) of two spontaneous populations of *S. berroi* (P1, P2) were collected in Magdalena and Punta Indio municipalities (Buenos Aires prov.), respectively. Then, the caryopsis were individually weight (PC) and sown (10 October 2019) in plastic trays (with cells of 180 cm^3) filled with typical Argiudol soil as substrate in a greenhouse. Fifty-two days after sowing 80 plants of each population were retired and washed softly in a stream of tap water on a sieve. It was determined: aerial length (LA), radical length (LR), total length (LT), longest adventitious root length (Ladv), number of adventitious roots longer than 3 cm (n° adv), number of green leaves totally unfold (n° hoj) and tiller number (n° mac). Then, each plant was dissected at the root neck height, were put in a stove at 60°C and aerial (PSA) and radical dry weight (PSR) were determined, and the total dry weight (PST) was calculated. PSA/PSR and LA/LR ratios were calculated. Variability within populations was analyzed by means the following parameters: average, standard deviation, range, and coefficient of variation (%). Variability between populations was analyzed by means the t test. Besides, phenotypic correlations (Pearson's coefficient) between PST and the other studied characteristics were analyzed. Significant differences ($P \leq 0.05$) were observed between populations for all the evaluated characteristics, except for Ladv and PSA/PSR. In both populations there were significant ($P \leq 0.05$) and positive correlations between PST and LA, LT, LA/LR, n° adv, n° mac, and n° hoj. The variability found within and between *S. berroi* populations for the studied traits would be promising for genetic improvement of implantation. Furthermore, the associations found between characteristics linked to plant vigor would be useful for its possible application in indirect selection.

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RELATIONSHIP BETWEEN THE FOLIAR EXTENSION OF *Digitaria eriantha* (DIGITARIA) AND VARIABLES CLIMATIC OF VILLA MERCEDES, SAN LUIS, ARGENTINA

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Digitaria eriantha (cv Irene) is a perennial, summer-growing, drought-resistant grass that is complemented by *Eragrostis curvula* and semi-arid natural grasslands, making it promising for San Luis' livestock systems. It was proposed to find predictive models of foliar growth of digitaria subjected or not to cuts or N and water subsidies, according to climatic variables (thermal sum "ST" in ºCd, with base temperature of 7–10 ºC, and accumulated precipitation "PP" in mm), during four cycles. With fertilization and water, potted plants were subjected to the combination of two levels of N and irrigation: 200 kg N/Ha (N200, potential) and control (N0), eventual irrigation to avoid withering (Re) and frequent irrigation to maintain high soil moisture (Rf). The length of leaf blades of the tiller (LL), distal leaf length of the tiller (LFd) or length of accumulated leaf blades per tiller (LLac), was periodically evaluated from the regrowth from a cleaning cut at the end of winter. The adjustment models between these response variables and the climatic variables were obtained by regression analysis ($P < 0.10$). During net spring growth, LL was related only to ST by linear adjustment (R^2: 0.63) and reached a maximum of 190 mm at 319°Cd in November. During the active growth seasons, spring-summer, both ST and PP explained the LLac variations with a moderate determination coefficient (R^2: 0.60 for ST and 0.58 for PP). The maximum LLac was 722 mm (February), with 10 leaves, requiring approximately 1700°Cd and 600 mm of PP. When the species was subjected to intense defoliation (cuts at 10 cm from the ground, at the beginning of the senescence of the tiller), it gave 3 shoots, corresponding to spring, late spring-summer, and autumn. Leaf size (LFd) was stimulated by ST in spring (R^2: 0.85) and by PP in spring-summer and autumn shoots (R^2: 0.80 in both cases). In this experience, the average length of the tillers (LFd) without cuts reached 600 mm when accumulating 1400°Cd in the cane (January), then decreased towards the end of the cycle (April). When considering the fertilization and irrigation treatments, it was found that, RfN200 and ReN200 presented the highest LLac per tiller (1000 to 1200 mm) around 500 to 600°Cd. From the relationship between LLac and ST, polynomial regression models were derived with good fit values (R^2: 0.94 and 0.88, respectively). On the other hand, RfN0 and ReN0 reached their maximums of 900 mm around 1400°Cd, much later than the fertilized ones. In this case, they also fit polynomial models with lower levels of fit (R^2: 0.28 for RfN0 and R^2: 0.41 for ReN0). The cuts and fertilization had an impact as important growth factors by increasing or limiting leaf length and the speed with which it reaches its maximum or accumulates. The extension of the leaves of *D. eriantha* responds to climatic factors considered whether or not it is subjected to cuts, throughout the cycle; but ST behaves as a better predictor of leaf growth, mainly in spring shoots or with subsidized N without water restriction.

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RELATIONSHIPS BETWEEN STRUCTURAL VARIABLES OF *Eragrostis curvula* (WEEPING GRASS) AND CLIMATICS OF VILLA MERCEDES, SAN LUIS, ARGENTINA

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Eragrostis curvula is a perennial Poaceae, type C4 and resistant to drought, which makes it a suitable fodder resource for the semi-arid. It was proposed to find predictive-representative models of structural changes of weeping grass canopy, fertilized or not with N, according to climatic variables (thermal sum and rainfall). For this purpose, it was evaluated, during four cycles, the extension and foliar density before the climatic conditions of each cycle. It was calculated the thermal sum "ST" in °Cd, with a base temperature of 7–11 ºC, and the accumulated precipitations "PP" in mm. The structure of the canopy was measured periodically after a cleaning cut at the end of winter and represented with the length of leaf blades of the tiller (LL), length of accumulated leaf blades per tiller (LLac) or maximum leaf length (LFm), and the number of leaves generated by the plants (N°H), located in plots or pots. When fertilizing and eventually watering plants in pots, two levels of N were considered: potential, equivalent to 200 kg N/ha, and a control without N. The models found ($P < 0.10$) met the assumptions of normality, homocedasticity and independence from residues ($P > 0.05$). In the spring growth of weeping in plots (from regrowth, September, until the beginning of leaf senescence, October), LL was related to ST and PP by multiple regression (R^2: 0.79) and reached a maximum of 240 mm with 140°Cd in October. In the complete cycle of plants in plots (from September until the end of growth in March), LLac was related to ST and PP by polynomial adjustments (R^2: 0.96 and 0.83, respectively), reaching maximum values of 1200 mm, with 1500°Cd and 450 mm of PP. It presented a maximum N°H (10) with 1600°Cd, being directly related to ST (R^2: 0.95). In the complete cycle of potted plants, it was adjusted to polynomial models by relating LLac and N°H with the climatic variables. ST was a better predictor of growth and leaf density (R^2: 0.96 and 0.98, respectively) than PP (R^2: 0.90 and 0.95, respectively). LLac reached adjusted maximum values (1400 mm) with 1300°Cd and 325 mm of PP. The maximum N°H (11) was obtained with higher ST and PP (1900°Cd, 475 mm). In spring–summer, when fertilizing with N, LFm was conditioned to PP, and when it was not fertilized to ST (R^2: 0.79 and 0.96, respectively). Leaf density, with or without N application, was closely linked to ST ($R^2 > 0.91$). In this case, the relationships between dependent and independent variables were linear and direct. Without fertilization, it reached its maximum N°H (11) between 1700-2000°Cd; when fertilized, the maximum N°H (12) occurred between 1900–2000 °Cd, and in any situation with 660 mm of PP. Without N subsidy, ST acquires importance in the structural behavior of the canopy of *E. curvula*, while with N subsidy the growth is subordinated to water. The extension and density of leaves of this species responds to the climatic factors considered, but ST is a better predictor of leaf elongation under N-restrictive conditions.

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ANTI-INSECT ACTIVITY OF ESSENTIAL OILS OF *Cuminum cyminum* L. FROM CATAMARCA, ARGENTINA, AGAINST *Sitophilus zeamais* M.

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C. cyminum is an aromatic herb Apiaceae rich in essential oil (EO) that is cultivated in northern Argentina. The EOs present various biological activities that can be used for medical, veterinary or pest control purposes. The *Sitophilus zeamais* weevil, a pest that attacks the corn grain, produces significant losses to the corn production sector. The objective of the work was to evaluate the anti-insect activity of cumin EOs from different producing departments of Catamarca, against *S. zeamais* and to compare it with the activity of cuminaldehyde, the main component of EO. We worked with twenty-three samples of pure EOs obtained by hydrodistillation of dried and ripe cumin fruits from the departments of Belén, Capayán, Pomán, Santa María, and Tinogasta. Insecticidal activity was determined by fumigant toxicity against adult *S. zeamais* individuals at concentrations of 300; 150; 75; 37.5 and 18.75 $\mu L_{EO}/L_{air}$, and the lethal concentrations 50 and 95 (LC_{50} and LC_{95}) were calculated by probit regression analysis. Attraction-repellency was evaluated at concentrations of 4, 0.4, and 0.05 $\mu L_{EO}/L_{air}$ using a two-way olfactometer and response indices (RI), and inhibition of acetylcholinesterase (AChE) activity, were calculated by a colorimetric method at concentrations of 9.2 and 2.3 mg/L. Under the same conditions, standard pure cuminaldehyde was experimented. Bioassays were performed in five times and the results were considered significant at $P < 0.05$. All AEs showed high insecticidal activity and were generally more active than cuminaldehyde. The provincial mean values of LC_{50} and LC_{95} were, respectively, 74.68 and 214.55 $\mu L_{EO}/L_{air}$, while those of cuminaldehyde were 59.31 and 267.19 $\mu L_{EO}/L_{air}$. The EOs of cumin from Pomán and Santa María were the most active with mean values of LC_{50} (26.33 and 32.01 $\mu L_{EO}/L_{air}$) and LC_{95}, (89.06 and 79.03 $\mu L_{EO}/L_{air}$), respectively, although an EO from Bethlehem showed the lowest LC_{50} (14.93 $\mu L_{EO}/L_{air}$). AChE inhibition was 88.39 ± 15.45% at 9.2 mg/L. The behavioral responses of the insect to the experimental stimulus were compared by paired samples *t*-test, showing that the AE repel at the three concentrations tested, $P \leq 0.002$. The AEs of cumin from Catamarca present important insecticidal and repellent activity against *S. zeamais* and could be used for the integrated control of this maize pest.

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MODIFICATIONS IN THE LIPID METABOLISM OF *F. graminearum* AND BARLEY ROOTS IN RESPONSE TO *B. subtilis* ATCC 6633

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Fusarium graminearum (Fg) is a plant pathogen that produces significant economic losses in the agricultural sector of the province of Córdoba. The establishment of the pathogen is given by its asexual propagation structure, "the macroconidia", when they come into contact with the different plant organs. Current agricultural practices and consumer demands for agrochemical-free food drive the development of microbial formulations capable of controlling pests as well as improving food quality. The use of biological control agents is an important strategy for the integrated management of fungal diseases and strains of *Bacillus* sp. They could suppress soil diseases due to their antagonistic properties regarding competitiveness with pathogens in the rhizosphere and the production of toxic metabolites. The objective of the work was to analyze the inhibitory action of *Bacillus subtilis* ATCC 6633 (Bs) as a biocontrol agent on Fg, its effect at the lipid and structural level, and its implication in the lipid signaling mechanism for this pathogen in barley. Thus, plaque inhibition and recovery assays, lipid profile analysis by TLC, measurement of phospholipase A2 (PLA2) and phospholipase D (PLD) activity, EROS production and free proline content were carried out. The results showed that the inhibition of Bs on Fg given by compounds released to the medium was independent of the concentration of Bs used in the inhibition tests. While the recovery of Fg after the inhibition was dependent on the concentration of Bs. The macroconidia obtained from inhibited colonies showed alterations in their length, foot cell angle and number of septa. Likewise, these structures presented alterations in the lipid pattern with a decrease in the content of triacylglycerides, free fatty acids, ergosterol, phosphatidylcholine and phosphatidylglycerol and an increase in phosphatidylethanolamine. In the presence of Bs, the PLD activity and the EROS production previously described for the barley-Fg pathosystem showed no changes. However, the activity of PLA2 was different between Bs and Fg, but similar between Bs and Bs/Fg. This latter behavior was also observed in the accumulation of free proline. Thus, the antagonistic action of Bs on Fg affects its optimal development, preventing its establishment. Concomitantly, Bs + Fg would induce mobilization or metabolism of proline in the plant by modulating, by a mechanism not yet elucidated, the recognition of the pathogen. Thus, the barley seedling in the presence of Bs does not recognize the pathogen, avoiding activating various immunity mechanisms.

A196

FOLIAR ANATOMY, GLANDULAR TRICHOMES, HISTOCHEMISTRY AND BIOLOGYCAL ACTIVITY OF *Argyrochosma flava* HOOK.

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Argyrochosma flava Hook. (Pteridaceae) is a rupestral fern that grows from the Andes in Colombia to central Argentina where is frequently seen in the chaqueños forest. The sporophyte measures between 15–18 cm, the fronds are bipinnate and the petioles are brown to dark brown and lustrous. The abaxial surface of the fronds is covered by a characteristic yellow farinose exudate, secreted by glandular trichomes that produce and store bioactive secondary metabolites. The farina is mainly constituted by the chalcone 2'6'-dihydroxy-4'-methoxyhalcone, along with other flavonoids and

terpenes. Chalcones usually have antifungal activity against different fungi. The aims of this work were to study the morphology and histochemistry of glandular trichomes of fronds and to evaluate the antifungal activity against phytopathogenic fungi of farinose exudate. For anatomical studies, young and mature fronds were diaphanized and the density of trichomes in both epidermises was evaluated using conventional techniques. Fresh frond material was hand-picked and stained with relevant histochemical assays using the following reagents: Toluidine Blue (polysaccharides), Ruthenium Red (pectin), NADI reagent (terpenes), Sudan Black B, Sudan IV, Nile Blue, and Neutral Red (lipids), Ferric Chloride and Vainillin/H_2SO_4 (phenols), Vainillin/HCl, Aluminium trichloride and Benedict reagent for flavonoids. The air-dried sterile fronds were rinsed with ethyl acetate to dissolve the farinose exudate and its antifungal activity was evaluated by using broth microdilution technique against *Penicillium digitatum* CCC-102, *P. italicum* CCC-101, *Botrytis cinerea* CCC-100 and *Molinilia fructicola* INTA-SP345. The anatomical results showed that the fronds are dorsiventral and the palisade and spongy parenchyma have a similar thickness. Both epidermises are unistrate, the upper one has larger cells than the lower one and the stomata are at the same level as the abaxial epidermal cells. Glandular trichomes in this species have a basal epidermal cell, a 1–2 celled basal stalk, and a spherical secreting head, in both epidermis and rachis in young fronds, and only in the abaxial epidermis in mature or senescent fronds. The histochemical test showed the lipophilic and hydrophilic nature of the secretion that contained polysaccharides, lipids, terpenes, phenols, and flavonoids. The farina was active against the four pathogenic fungi evaluated with MICs between 31.25 and 125 µg/mL showing the highest inhibition for *M. fructicola*, *P. digitatum*, and *P. italicum* (31.25 and 62.5 µg/mL, respectively). This is a promising result since the diseases caused by *Penicillium* are two of the most difficult to eradicate in citrus.

A197
IN VITRO ANTI-GIARDIAL ACTIVITY OF PLANT EXTRACTS FROM THE TRIBE VERNONIEAE (ASTERACEAE)

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Giardiasis, caused by *Giardia lamblia*, constitutes a parasitosis of great epidemiological and clinical importance due to its high prevalence and pathogenicity, mainly among children. World Health Organization considers it among neglected diseases. The first-line drug to treat this parasitosis is metronidazole, which has considerable adverse effects, has teratogenic and embryotoxic potential and it is considered a possible carcinogen in humans. Due to the side effects of conventional drugs and the increased resistance of parasites to treatment, it is necessary to identify new, more effective, and safer giardicidal agents. Historically, natural products, particularly plant derivatives, have been the most successful source for the discovery of new drugs. The concept that medicinal products derived from plants depend on the action of a single active ingredient was modified in light of the discovery that there are, in many cases, adjuvant substances in plants that increase the activity of the components responsible for the effect. The aim of the present work was to evaluate the activity of 16 vegetable extracts obtained from three species of the tribe Vernonieae (Asteraceae) against the intestinal parasite *G. lamblia*. The selected species were: *Vernonanthura nebularum*, *Centratherum punctatum*, and *Elephantopus mollis*, which stand out for being rich in sesquiterpene lactones, metabolites with a wide spectrum of biological activities. To obtain the extracts, solvents of different polarities were used, extracts of surface washing and maceration were obtained, and they were concentrated in a rotary evaporator. The *in vitro* antiparasitic activity of the extracts was evaluated against *G. lamblia* trophozoites, the IC_{50} measurement was performed using the resazurin (2 mM) fluorometric method, working at an excitation and emission length of 540–590 nm, respectively, with a parasitic population of 7.5×10^5 trophozoites/mL, incubated at 37°C for 48 h. *V. nebularum* leaf and flower rinse extracts were the most active with IC_{50} values ≤ 15.5 µg/mL. The remaining extracts had a moderate effect with IC_{50} values between 79.9 and 35.9 µg/ mL, with exception of the methanolic extracts, which were inactive. The results obtained show that the selected species are promising candidates for further studies as giardicidal agents and to evaluate the synergism between sesquiterpene lactones and other constituents present in the extracts against the parasite.

A198
INVOLVEMENT OF THE ARABIDOPSIS DNA GLYCOSYLASE MBD4L IN OXIDATIVE STRESS RESPONSES

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Plants accumulate reactive oxygen species (ROS) in response to biotic and abiotic stresses. These molecules can have either toxic effects on lipids, proteins, and DNA, or beneficial functions acting as signals that modulate plant growth, development or defense pathways. Paraquat (PQ) is a ROS-inducing agent that inhibits photosystem I, favoring the transference of electrons to molecular oxygen for generation of superoxide anion ($O_2^{\cdot-}$). An important DNA lesion generated by ROS is 7, 8-dihydro-8-oxoguanine (8-oxo-G). In *Arabidopsis*, AtOGG1 is the main DNA glycosylase of the base excision repair system that recognizes and eliminates 8-oxo-G from DNA. Overexpression of the *AtOGG1* gene increases tolerance to PQ and mannitol. Interestingly, overexpression of the DNA glycosylase *AtMBD4L* gene also enhances oxidative stress tolerance, although this enzyme does not recognize 8-oxo-G as substrate in vitro. We wondered if AtMBD4L deficiency reduces the oxidative stress tolerance. To evaluate this, we used comet assay to detect DNA strand breaks in nuclei isolated and treated with exogenous ROS from wild type and AtMBD4L mutant (*mbd4l*) seedlings. DNA damage levels were similar in both plants. Using plate growth assays, we performed a time course analysis of germination and root length parameters. The *mbd4l* showed a faster germination and a longer root length than wild type plants. In addition, chlorosis at aerial tissues was lower in the mutant. Using NBT histochemical staining we evaluated the $O_2^{\cdot-}$ content and detected similar levels in mutant and control plants. Finally, we analyzed the *AtOGG1* gene expression by RT-PCR and observed its significant induction in *mbd4l*, but not in wild type plants. Taken together, our results suggest that lack of AtMBD4L does not prevent the repair of nuclear DNA lesions derived from exogenous ROS treatment, but the highest tolerance to oxidative stress observed in *mbd4l* seedlings would be related with the exacerbate *AtOGG1* activity.

A199
Aloe maculata GEL SERS ON SILVER NANOPARTICLES

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Aloe maculata ("maculata" = with spots) originating from South Africa, has a stellate shape with a short stem and 4 or 5 leaves per level, mottled with white, with brown teeth on the edge and at the upper end of the leaf, flowers of orange colour. It is cultivated for cosmetic use (due to its large amount of gel) and also as an ornamental. Raman spectroscopy is a non-destructive analytical technique based on the inelastic scattering of monochromatic light (generally provided by a laser source) by the vibrational movement of molecules and gives qualitative and quantitative information about the structural chemical groups in a given sample: a Raman spectrum is "molecular fingerprint", and its intensity is proportional to the analyte concentration. Unfortunately, the applicability of Raman spectroscopy is limited by its poor sensitivity. To obtain an increase in Raman intensity, the analytes can be adsorbed on a nanostructured metal surface with adequate characteristics, producing an improved surface Raman scattering spectrum (SERS). In recent years, researchers in the field of nanotechnology have discovered that metallic nanoparticles have all kinds of benefits. The objective of this work is to characterize spectroscopically the *A. maculata* of this region. Leaves of the *A. maculata* plants from Raco and San Miguel de Tucumán were collected, they were separated into two groups: leaf senescence (LS) and young leaves (Y). Spectroscopic measurements (SERS, Raman, FTIR, UV) of the extracted gel were carried out. A very effective and simple way of producing silver colloids for surface enhanced Raman scattering (SERS) was used to perform SERS measurements. The reduction of silver nitrate was carried out with hydroxylamine hydrochloride at alkaline pH and at room temperature obtaining, in a short time, highly sensitive SERS colloids. The mentioned colloids can be used for SERS spectroscopy immediately after their preparation. The general procedure is quick, simple, and characterized by a high preparation success rate. The lyophilized Aloe gel was resuspended, and it was incorporated into the silver nanoparticle solution in the same volumetric proportion. The results of the SERS showed the presence of a significant amount of polysaccharides and in lesser amounts of amino acids. If we compare the Raman spectra with the SERS, shifts in the bands corresponding to the C=O group of approximately 20 cm^{-1} are observed, thus demonstrating the strong interaction of this group with the nanostructured silver surface. These first SERS measurements reveal us the presence of polysaccharides, already reported in previous works, but also the stretch band of the C=O group at 1745 cm^{-1}, and allow us to conclude that in this sample of *A. maculata* from Tucumán, there is a considerable amount of amino acids derived from cysteine. This is a first step in the characterization of the Aloe from the northern region of Argentina.

A200
NITROGEN FERTILIZATION IN SOYBEAN INOCULATED WITH DENITRIFYING STRAINS

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Soybean (*Glycine max*) is one of the most important economic legume crops in Argentina and with a larger planting area coverage in Córdoba province. Soybean–rhizobia symbiosis results in nitrogen biological fixation (NBF) and plays an important role in plant cultivation and fertilizer application. However, there are many problems in agricultural application of NBF; one of them is the high concentration of nitrate in soil. The experiment was conducted to evaluate response to nitrogen fertilization with 180 kg/ha urea (fert) and inoculation with different denitrifying strains of *Bradyrhizobium* spp. in yield of *Glycine max*. The experiment was developed in Río Cuarto, Córdoba, with a fully randomized design with 7 treatments: (1) uninoculated and unfertilized control; (2) control fertilized with urea (180 kg/ha); (3) soybean inoculated with *Bradyrhizobium diazoefficiens* USDA110 and fertilized with urea (180 kg/ha); (4) soybean inoculated with *Bradyrhizobium* Per 3.64 and fertilized with urea (180 kg/ha); (5) soybean inoculated with *Bradyrhizobium japonicum* E109 and fertilized with urea (180 kg/ha); (6) soybean inoculated with *Bradyrhizobium* Per 3.61 and fertilized with urea (180 kg/ha); and (7) soybean inoculated with *Bradyrhizobium* Per 1.12 and fertilized with urea (180 kg/ha). Results show that NBF was unaffected by nitrogen fertilization. Inoculation treatment with the strain Per 1.12. presented higher values in all the variables analyzed (number of nodules at the root and secondary roots; dry weight of nodules (mg/plant) and grain yield (kg/ha), except dry weight of aerial biomass (g/plant). Seed production for *G. max* grain yield was higher in Per 1.12 compared with the other treatments. In conclusion, Per 1.12 strain represents a promising output as *G. max* inoculant, improving yield in soil with high content of nitrate and in water deficit condition. Results show empirical evidence of NBF contribution to the nutrition economy of soybean cultivation.

A201
pH, DRY MATTER AND °BRIX OF SORGHUM SILAGE INFECTED WITH ERGOT

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Sorghum is an excellent crop for producing fodder reserves such as silage, constituting a key component in the feeding of cattle in the province of San Luis Argentina. Sorghum silage provides wet fodder of similar nutritional value to the original one, giving certainty to the production process in summertime due to its high potential for quality dry matter (DM) production. The stabilization of the forage is done by lactic fermentation and is an indicator of a good silage pH value, between 3.3 to 4.2, it is also important that the crop to be silage has 30 to 40% of DM. Sorghum ergot is a fungal disease generally caused by *Claviceps africana* and *Sphacelia sorghi* that infects the crop during anthesis. Environmental conditions such as days with high relative humidity and low temperatures favour infection. Some characteristics of the silage of a sorghum crop (BMR 135, Gentos) severely affected by ergot were evaluated in Villa Mercedes, San Luis. The material to be silage was harvested 7 times between March 11 and July 3, in 4 plots distributed at random within the crop. The trial crop was found at the beginning of anthesis during the first cutting dates and reached the end of its growth cycle in the last ones. The sorghum was chopped with a MAINERO 4771 precision grinder and placed in PVC tubes of 110 mm in

diameter and 450 mm long. The material was manually compacted until it reached a density of 500 kg of green material/m^3, then each tube was closed with hermetic caps, ensuring the process of anaerobic fermentation. Four microsilos were made for each cutting date. Once they were stabilized, after 30 days, the percent (%) of DM was determined at a constant temperature of 60°C, °Brix (°B) with a manual refractometer (0–32 °B) and pH by means of a digital pH meter. ANOVA was performed ($P < 0.05$), considering as treatments the cut-off dates. It was specified between which moments there were or were not significant differences for each variable by means of the Fisher LSD test ($P < 0.05$ and $P > 0.05$, respectively). The pH values varied between 3.93 (April 24) and 4.25 (May 27), although they showed significant differences between dates April 24 and May 27, they are considered appropriate at the biological level for this type of silage. There were significant differences in DM between the cuts of March and April, the values ranged from 20% (March 11) to 25–26% (April 24). Although DM increased as the crop cycle progressed, it was always below the recommended optimal range and in coincidence with the ergot attack. Regarding °B in the last cut date (May 14) the lowest value was determined (8.75), showing significant differences with the rest of the evaluation moments that behaved homogeneously (10.25–11.7). According to the evaluation of ergot-infected sorghum silage, the variables pH, DM, and °B behaved similarly to those obtained with healthy materials.

A202
THE EFFECT OF OZONATED WATER ON *Botrytis sp*.

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Postharvest rots are a common problem for fruit and vegetable production. Unfortunately, to prevent this issue, disinfectants that may have polluting effects are used. In order to replace this practice with more environmentally friendly alternatives, our research team has been working on different lines aimed at evaluating the effectiveness of the use of ozone. The objective of this study was to evaluate the *in vitro* effect of ozonated water on fungi contaminating fruits belonging to the genus *Botrytis*. The fungus was isolated from an infected fruit and further placed in Petri dishes with a growth medium for its multiplication. A solution of the pathogen was prepared with distilled water and the number of conidia was recorded, which resulted in an initial concentration of 7.8×10^6/mL. To introduce the ozone, 500 mL of distilled water at a controlled temperature of 3ºC were placed in a 1000 mL-cylinder, which was bubbled with ozone to a concentration of 1.35 ppm. An aliquot containing *Botrytis* conidia was added and samples were taken at four exposure times (0, 1, 3, and 5 min). Serial dilutions were made of each sample and further seeded in triplicate. After incubating them at 26°C for 3 days, the colony count was performed. This test was repeated twice. The results of the first test showed outstanding differences ($P < 0.10$) at the exposure times of 0 and 5 min. The average colony counting was of 33.5, 28.6; 21, and 16.6 during the increasing incubation times. Considering colonies belonging to 0 time as 100%, the counting of the three remaining times were 85%, 62%, and 49% for 1, 3, and 5 min. Significant differences were found in the second trial ($P < 0.05$) at 0 and 1 exposure times with respect to times 3 and 5. The average colony counting was of 22.8, 22.5, 11, and 6.2 for 0 and 5 exposure times, respectively. Considering colonies belonging to 0 time as 100%, the counting of the three remaining times were 98%, 48%, and 27% for 1, 3, and 5 min. The average reduction of colonies was 45% and 62% with respect to the initial colonies at 3 and 5 min. We conclude that ozonated water has a positive effect on the *in vitro* control of *Botrytis sp*.

A203
OZONE EFFECT ON LEAFY VEGETABLES

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Postharvest treatment with ozone in fruit and leafy vegetables are diverse in terms of effectiveness and efficiency. The washing and disinfection processes is one of the alternatives of using ozonated water. The aim of this work is to evaluate the effect of ozonated water in the postharvest conservation of leafy vegetables. The treatments were carried out with leaves of two species, chicory (*Cichorium intybus*) and rocket (*Eruca sativa*), immersed in water with an ozone concentration of 0.2 ppm. The immersion times were 0, 1, 3, and 5 min and observations were made initially at the end of each dive time in each treatment and at 7 days, individually preserved in closed trays at 4°C. For control treatments the same method was applied, but with distilled water without ozone. The observations were based on color intensity, compared with pattern charts, evaluated with the naked eye, turgor, and presence of necrosis and/or symptoms of diseases, calculating the affected area. In the observations made at the initiation of the trial it was observed in general that leaves, treated with ozonated water have greater turgidity than the control, without differentiating between species and immersion times. For the second observation, at 7 days, it was observed in the 2 species, a more intense green color and greater turgor in control treatments than in the ones treated with ozonated water. In the case of the rocket a difference in color or turgidity is not observed, but it is clear the presence of necrotic surfaces and soft rots in the ones which have an immersion time of 3 and 5 min. In the case of chicory, damage is also visible, but with greater intensity in leaves with a time of immersion of 5 min, with soft rot, but without necrotic surfaces. These results show that the treatments with ozonated water for the conservation of leafy vegetables are not convenient with the parameters set in this trial.

A204
IN VITRO MICROPROPAGATION OF HOP IN ORDER TO PRODUCE HOMOGENEOUS SEEDLINGS

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Hop (*Humulus lupulus L.*) is a crop originating in northern Europe and western Asia, with incipient production in Argentina. The female inflorescence, also called the “cone”, is made up of multiple leaf scales known as bracts and/or ‘bractéolas’, in which the lupulin gland is generated, an organ of

interest for the beer industry, due to its high content of substances such as acids bitters, essential oils, and/or various polyphenols. The objective of the work was to micropropagate hop plants in vitro, to obtain new healthy and genetically homogeneous individuals, in a short period of time. Starting from rhizomes of plants grown in soil and in hydroponics, a 50% Murashige Skoog (MS) (1962) medium was used with Indole Acetic Acid (AIA) and Adenine Sulfate 30 g/L of sucrose and solidified with 7.5 g/L of agar-agar. The pH of the media was 5.8 and they were sterilized for 15 min at 121°C in an autoclave. Buds emerged from rhyzomes were used which were washed with water and detergent. Subsequently, they were disinfected with 70% alcohol 3 min and sodium hypochlorite 15–20% 20 min with rinses of sterile distilled water. They were seeded in sterile culture medium and grown in a culture chamber with a 16:8 light/dark photoperiod, with an intensity of 100 μE/m^2s and 25 ± 1°C temperature. The explants from rhizomes grown in soil were not established *in vitro*. The explants that came from rhizomes grown in hydroponic cultures were established *in vitro* by 50% and they formed mother plants with leaves and roots. The next step will be to micropropagate mother plants in order to obtain a large number of seedlings with genetic homogeneity.

A205
EDITING AND REMODELING OF PHOSPHATIDIC ACID DURING TEMPERATURE STRESS RECOVERY IN BARLEY ROOTS

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Low temperature exposure affects the composition and biophysical properties of plant membranes, interfering with their proper functions and triggering signaling pathways. Plants respond to temperature stress by altering lipid class composition and lipid unsaturation to maintain membrane integrity. Cold tolerance acquisition involves activation of several stress cellular mechanisms while adverse condition is persisting. However, the correct timing and rate of recovery resulting in loss of cold tolerance, repairing structural damage, and redirecting the energy resources to resume growth and development is equally relevant for plant fitness and survival. Despite the critical role of the membrane when conditions are restored, limited lipidomic studies have been reported during stress recovery. The objective of this study was to describe lipid rearrangements of barley roots (*Hordeum vulgare*) during chilling and short and long stress recovery. Glycerolipidome was performed in roots of barley seedlings exposed to suboptimal temperatures 4°C (36 h) and recovered at 25°C during short (2 h) and long (24h) periods. Lipids were obtained by a modified version of multi-extraction method based in a single-extraction method with a polar solvent mixture and were identified by mass spectrometry (ESI–MS/MS). Then, the unsaturation index was determined. The data showed a significant increase in total lipid composition in response to cold stress as a consequence of an increase in phospholipids, with no significant differences in galactolipids and lysophospholipids. Total lipid content decreased to control values during recovery as a result of the reduction of the following lipid classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS) and phosphatidylinositol (PI). Contrasting, phosphatidic acid (PA) showed an opposite behavior and it increased significantly after 24 h of recovery. Analyses at the molecular species level revealed the main contribution of PA 34:2 and 36:4. Similarly, PA species that increased were the same PC species that decreased in recovery and showed a negative correlation according to the correlation analysis (based on Pearson's coefficient). These results suggest that PA recovery-formed is derived primarily from PC. Post-stress PA edition might establish a new membrane configuration modifying membrane curvature, lipids-binding to proteins and consequently, signaling functions. Further analyses on model membranes are required to unravel how polyunsaturated species of PA modify its biophysical properties during recovery. Moreover, biochemical analyses of PA formation will describe recovery signaling pathways as a key determinant in the acquisition of tolerance to low temperatures.

A206
PROLINE EFFECTS ON REDOX METABOLISM OF PEANUT MICROSYMBIONTS EXPOSED TO DROUGHT STRESS

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Water deficit is one of the environmental stresses that most affects the symbiotic legume-rhizobia association. In particular, the exposure of microorganisms, in their natural environment, to adverse environmental conditions can directly impact the possibility of establishing interaction with plants, so it is of interest to evaluate the oxidative and antioxidant response of peanut micro-symbionts growing under drought stress. As a biotechnological alternative, proline, an osmoprotective amino acid and determinant of cellular redox balance, could increase tolerance to drought stress. Thus, in order to improve peanut production in regions susceptible to the occurrence of abiotic stresses through the application of seed inoculants prepared in culture media supplemented with proline, the effect of this molecule on growth and cellular redox balance of peanut microsymbionts under drought stress conditions was analyzed. For this purpose, the rhizobia recommended as peanut inoculants *Bradyrhizobium* sp. C-145 and *Bradyrhizobium* sp. SEMIA 6144, were used. The treatments were: (I) control; (II) 50 mM proline; (III) 30 mM polyethylene glycol (PEG 6000), as an inducer of drought stress; (IV) 30 mM PEG 6000 + 50 mM proline. Bacteria growth was determined through viability (CFU/mL), intracellular proline content and in the culture medium, production of a reactive oxygen species (ROS) (hydrogen peroxide, H_2O_2), oxidative damage to lipids by quantification of the content of thiobarbituric acid reactive substances (TBARs) and specific activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). The results showed that the addition of 50 mM proline increased the viability and growth rate of *Bradyrhizobium* sp. C-145 with respect to the control, while under stress (PEG 6000, 30 mM) these variables were decreased. The growth of this strain in the presence of PEG 6000 and proline reached similar values to the control at 48 and 72 h. In the presence of stress, the cells showed an increase in intracellular proline content and activity of the antioxidant enzymes SOD and CAT, while in response to exogenous proline addition, the cells exposed to stress had these indicator values similar to the control. In *Bradyrhizobium* sp. SEMIA 6144, the amino acid did not modify the viability or the growth rate with respect to the control, but the addition of PEG 6000 reduced the viability. Furthermore, the presence of the amino

acid did not reverse the effect of the addition of PEG 6000. The cells showed oxidative burst, due to an increase in the content of H_2O_2, oxidative damage to lipids and activation of the antioxidant system (SOD and CAT) in the stress condition. While, in response to proline addition, the H_2O_2 content and the specific activity of the enzymes still remained high. In conclusion, the addition of proline to the culture medium had a protective effect on the growth of *Bradyrhizobium* sp. C-145 in the presence of drought stress that can be associated with the maintenance of cellular redox balance. Thus, the addition of the amino acid to peanut inoculants of said strain would constitute an agrosustainable strategy to mitigate the adverse effects of drought stress in crops.

ECOLOGY, ETHOLOGY AND BIODIVERSITY

A207
THE VEGETATION OF THE EDGE OF *Chrysanthemum* CROPS AS A RESERVOIR OF ENTOMOPHAGOUS ARTHROPODS IN THE PREPUNA ECORREGION OF JUJUY, ARGENTINA

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The importance of natural vegetation around crop fields is to be recognized as a reserve of entomophagous arthropod diversity. The objective is to know the composition of potential biological controllers of pests present in the edge vegetation of the *Chrysanthemum* crop in the Prepuna jujeña. The study was carried out on three fields, between October and December/17 and February/18. The arthropods were collected with Moericke traps, four in spontaneous vegetation (VE) and four in wind curtains (CV). A total of 1126 entomophagous arthropods were collected, 472 in VE and 654 in CV, distributed in 40 families. 897 parasitoids and 229 predators were identified. The most abundant and rich order was Hymenoptera (967/26). The most abundant parasitoid was Encyrtidae (28.6%) and among the predators, Anyphaenidae (5.5%). The results show the necessity to continue with studies on the importance of selecting edge plant species for the support of the beneficial entomofauna.

A208
SOIL MORPHOLOGY OF THE SAN LUIS PROVINCE SOUTHWEST AFFECTED BY VOLCANIC ASH

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This work studies the soil morphology affected by volcanic ash in soils in the southwest of the San Luis province, coming from the eruption of Quizapú that occurred in April 1932. This volcanic ash is found in Entisols and in most cases was low thickness, so it affected very few agronomic activities that developed in the sector, such as land and grasslands management and conservation; apparently with low influence on soil genesis. In this sector, San Luis province has a cold continental climate of dry to semi-arid characteristic with low humidity index and marked water deficit; the study area include isohyets from 450 mm in the east to 350 mm in the west. Soils affected distribution is presented in the province where continuous or discontinuous accumulations of volcanic ash are currently found by direct observation. These situations occurs when the disarm for cultivation, removal by trampling of livestock, action of the mesofauna and wind remobilization was carried out. The ash is found in two geomorphological environments: (1) the medanous plain with grasslands, and (2) the loessic plain with scrub and grasslands. This sector comprises the series Batavia (Torripsament ústic), Nahuel Mapa (Torripsament typic), Río Salado (Torripsament typic) and Alto Negro (Torriortent typic). Almost 90 years after the fall of volcanic ash, due to climatic conditions of aridity and semiaridity in the area, in some places the tephra remains almost intact. Because of the apatite content (in small amounts), in conditions of favorable humidity can enhance these soils fertility. Soil profiles analysis, classification and mapping are carried out for productive purposes, and three possibilities are proposed: (1) when volcanic ash is less than 5 cm-thick, it would be incorporated into the morphological description of the Ap horizon and sampled as a composite sample. The horizon sequence would be: Ap (which includes tephra), 2A, 2AC, 2C. (2) When the thickness of the tephra is greater than 5 cm and keeps a certain catenary regularity in the landscape would be described separately as a layer of sediment, placing the Arabic number 2 in the profile sequence, but indicating that it is the same series of soils as in the previous case; the horizon sequence: Ap, 2C, 3A, 3AC and 3C. (3) When the ash layer is mixed with the upper sediment, it is advisable to identify and mention its position in the morphological description; the sequence of horizons: Ap (with mixed volcanic ash), A2, AC.

A209
SURVEY OF SCORPIONES, PSEUDOSCORPIONES, SOLIFUGEA AND OPILIONES (ARTHROPODA: ARACHNIDA) IN AMBATO DEPARTMENT, CATAMARCA

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The Arachnida class includes chelicerated arthropods. It includes Scorpiones, Pseudoscorpiones, Solifugae and Opiliones, among other orders. For Catamarca, there is little work related to the study of these animals, so little is known about their diversity, abundance, and distribution. The objectives of this investigation were to survey organisms of the orders: Scorpiones, Pseudoscorpiones, Solifugae and Opiliones, in four localities of

the Ambato department, province of Catamarca and determine fauna richness (RF), and abundance (A), as measures of diversity. The biological material was collected in La Puerta, Las Juntas, Los Varela, and El Rodeo, in the Ambato department. In each locality, an environment corresponding to the Chaco Serrano ecoregion was selected, in which two transects were traced, one with a north-south orientation, and the other with an east-west orientation, 50 m long × 2 m wide, on which, 10 pitfall traps were placed, with ethylene glycol solution (1:9). The traps remained active for 28 days, renewed every seven days. The collected organisms were determined down to the lowest possible taxon to discern. 227 specimens from four orders and 10 families were collected. Order Scorpiones: Buthidae (A = 1), Bothriuridae (A = 97); Order Pseudoscorpiones: Atemnidae (A = 2), Cheliferidae (A = 49), Chernetidae (A = 15), Lechithiidae (A = 8), Withidae (A = 2); Order Solifugae: Mummuciidae (A = 9); Order Opiliones: Cosmetidae (A = 20), Gonyleptidae (A = 24). The information generated contributes to the knowledge of this important group of arthropods for the province of Catamarca.

A210
FIRST RESULTS ON THE DIVERSITY AND DISTRIBUTION OF SPIDERS (ARTHROPODA: ARANEAE) IN THE DEPARTMENT AMBATO, CATAMARCA

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Spiders are very diverse and abundant chelicerate arthropods. They occupy different ecosystems and they play an important role as controllers of insect populations. Few species in Argentina, can cause accidents to man. The arachnofauna of the province of Catamarca is still little known. The objectives of this research were: know the diversity and distribution of spiders in four locations in the Ambato department, Catamarca province and list the species of sanitary importance. The biological material was collected in La Puerta, Las Juntas, Los Varela, and El Rodeo, in the Ambato department. In each locality, an environment corresponding to the Chaco Serrano ecoregion was selected, in which two transects were plotted with north-south orientation; East West, 50m long × 2m wide, on which 10 pitfall traps were placed, with ethylene glycol solution (1:9). The traps remained active for 28 days, renewed every seven days. The collected organisms were determined up to the family taxonand and genus for spiders of sanitary importance. Morotypes were recognized in each family. 630 individuals from 33 families were collected. More abundant were: Lycosidae (16.03%), Salticidae (13.97%), Thomisidae (12.22 %), Araneidae (11.11%), and Lyniphidae (6.83%). The most diverse families were: Salticidae (12 morfotypes), Araneidae (8 morfotypes), Theridiidae (5 morfotypes), and Lycosidae (5 morfotypes). The genera of sanitary importance were *Latrodectus* sp. and *Loxosceles* sp. The data obtained is the first for the Ambato department, thus contributing to the knowledge of the rich spider fauna of Catamarca.

A211
SELECTION AND PRODUCTIVITY OF *Aedes aegypti* BREEDING SITES

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Aedes aegypti is the main vector of dengue, Zika and chikungunyas, and studying their breeding sites is important for their control. In Diamante (April 2015) 100 houses were studied at random, the containers that could contain water were counted and those with larvae and pupae. *A. aegypti* abundance was estimated with Index of: Container (CI), containers prevalence; houses (HI), houses prevalence; Breteau (BI), n° of breeding sites/100 houses, and density of Pupae/ha (PD). The availability (ABS) and use (UBS) of the breeding site resource were studied. The containers were categorized according to their function in: related to Construction (C), Domestic (D), Gardening (G), Useless (U), Pet (P), Water reserve (WR), Returnable (RT), and Others (O). For each one, the CI, adult productivity, and Ivlev's electivity index were calculated (IEV = 0, no choice; IEV = 1, maximum choice). The categories with the highest ABS were P and WR. For the city as a whole, it was observed: HI = 12, CI = 6.06, BI = 18, and PD = 4.03. A total of 187 immature were counted, the most frequent UBS being categories U and G, and in the P, we never found immature. The highest value of the CI was for C and then U. The highest productivity of adults resulted in categories U and G, following in importance C. High IEV was observed in C, and minimal in G, great evasion in P, and slight in D and RT. The main containers (P, RT, WR, D and G) that contributed to the ABS would be related with their functions of contain water per se, at least at some point. The values of the indices and density of pupae/ha (average of 2 female adults/ha) were high. The electivity of C and U and evasion in P would be influenced by the anthropic intervention and not so much a reflection of the mosquito's choice. However, U and G were the most productive, and this could be a characteristic of a more favorable environment and a consequence of a choice for vector development.

A212
FLOWERING CALENDAR OF THE CITY OF SAN LUIS (CAPITAL), ARGENTINA

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In plants, flowering is a process that is perpetuated over time with almost constant regularity. In urban spaces, knowing the plants that bloom at different times of the year, allows reaching diagnoses of pollinosis or allergy to pollen. The objective of this work is to present a flowering calendar in the city of San Luis, which indicates a possible user who suffers from pollinosis or hypersensitivity to plants or a local allergist, to identify the plant genera that cause this condition. This calendar was built by surveying the plants of the city center of San Luis for three consecutive years (2017–2019). The period when the highest flowering peak occurs is before and during spring. Some of the genera or species that flourish during this period, in order of appearance are: in August: *Cupressus* spp. *Morus* spp., *Ulmus* spp., *Schinus areira*, *Schinus molle*, *Acacia dealbata*, *Fraxinus*

exelsior, *Fraxinus pensilvanica*, *Vachellia caven*, *Acer* spp. *Populus* spp.; in September: *Melia acedarach*, *Handroanthus impetiginosus*, *Tecoma stans*, *Broussonetia* spp. *Callistemon* spp., *Geofroea decorticans*, *Prunus* spp. *Tamarix ramosissima*, *Robinia psudoacasia*, *Ricinus communis*, *Salix* spp., *Citrus* spp.; in October: *Jacaranda mimosifolia*, *Liquidambar* spp., *Eucalyptus* spp. *Erythrina crista-galli*, *Senegalia visco*, *Ligustrum* spp., *Olea Europaea*, *Catalpa bignonioides*, *Quercus* spp., *Eleagnus angustifolia*, *Lithraea molleoides*, *Brachychiton* spp. *Grevillea robusta*, *Nerium oleander*, *Gleditsia triacanthos*, *Cinnamomum glanduliferum*, *Dracaena* spp.; in November: *Albizia julibrissin*, *Tipuana tipu*, *Lagerstroemia indica*, *Prosopis* spp., *Tilia* spp.; in December: *Hibiscus* spp., *Bahuinia* spp., *Manihot grahamii*, *Styphnolobium japonicum*. Some pollinosis-producing plants are in flower in the months less close to spring, they belong to the Amaranthaceae/Chenopodiaceae families, which have a late flowering period in summer. In addition, in the month of May, *Parthenium hysterophorus* blooms, an herbaceous plant that produces allergic symptoms. Another group of plants that belong to the genus *Parietaria* spp., flower during spring and summer, produce copious allergies in hypersensitive people. The use of a flowering calendar allows assigning the origin to seasonal allergic processes by local allergists and people who inhabit the city of San Luis, to reach the diagnosis of pollinosis with greater specificity. Another report is that this species flowering calendar contributes at an educational level, not only to contribute to the knowledge of the plants themselves but also as ornamentals in houses, buildings, and public spaces.

A213
CYANOBACTERIA FROM BIOLOGICAL SOIL CRUST (BSCs) FROM FIRE AFFECTED MOUNTAINS OF SAN LUIS AND CÓRDOBA

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BSCs are present in most degraded ecosystems, for example, by forest fires. Cyanobacteria are initial BSC formers. The objective of this work was to determine the relationship between fire and cyanobacterial species present in BSCs from three sites affected by fires of different magnitudes (high, medium, and low) in San Luis and Córdoba mountains. Samples were sown in specific Watanabe medium and were grown under controlled conditions of 12 h of light and a temperature of 25°C. They were observed weekly for 90 consecutive days under a light microscope and the percentage classification of non-fixing (NF) and fixing (F) species was carried out; non-fixing (NF) and fixing (F) species were, in turn, classified into heterocystized (FH) and non-heterocystized (FNH). Taxonomic determination was made using specific bibliography. It was observed that, in the three analyzed sites, *Nostoc*, *Nodularia*, *Scytonema*, and *Cylindrospermum* genera represent F, and *Phormidium* and *Oscillatoria* genera represent NF. The fixing species (FH and FNH) suffer the greatest disappearance effect after the occurrence of fires, being *Cylindrospermum* the most susceptible. *Scytonema* and *Nostoc* have greater resistance to high fire intensities and show good reappearance capacity in the short term after fire, and *Nodularia* presents intermediate conditions. For the NF, *Phormidium* percentage presented little decrease, while *Oscillatoria* percentage decreased to intermediate and high fire intensities. These preliminary results allow us to conclude that both *Sytonema* and *Nostoc* as F species, and *Phormidium* as NF species are the most resistant to high intensity fires.

A214
THE WINTER MIXED-SPECIES FLOCKS IN SUBTROPICAL MOUNTAIN FORESTS OF THE SAN JAVIER PARK, TUCUMAN, ARGENTINA

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Mixed-species flocks (BM) are associations of different bird species that are formed as a strategy to increase foraging efficiency and reduce the risk of predation. In the subtropical mountain forest of the protected area, Parque San Javier (Tucumán, Argentina), BMs are formed mainly during the autumn/winter season, when the climatic conditions are unfavorable, and the food resource is scarce. We evaluate how the structure and composition of the BM varies between 2006 and 2016. The samplings were carried out from May to September (2006 and 2016), in 10 transects of 300 m long, respecting the same design and sampling effort in both years. Richness and abundance were specified by BM, stratum and substrate use of observed individuals. The number of participating species was similar in both years, 26 species for 2006 and 28 for 2016.The average number of species per BM was similar for both years, as was the average number of individuals per BM. The range of participating species was equal, from 2 to 13 species/BM and the range of individuals was similar, from 3 to 21 individuals/BM. The frequency of occurrence of the species varied between the years, however, the use of stratum and substrate was similar. The results would indicate that participating in a BM would be beneficial as a strategy by the bird community of Parque San Javier during the non-reproductive season (autumn-winter). Although, in general, the structure and composition did not vary, the appearance of two new species (*Chlorostilbon lucidus* and *Piranga flava*), between the years evaluated, on the one hand, would reinforce the role of the protected area (site in good state of conservation for birds) and, on the other hand, as a response to the loss and/or modification of the environment around the Park. BM could be considered bioindicators of environmental conditions in future research.

A215
POST-FIRE RECOVERY OF NATIVE WOODY PLANTS IN LOMA BLANCA (SAN LUIS)

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Most woody species that grow in the Chaco Serrano region have survival strategies that allow them to persist as adults, re-emerging in the face of disturbances such as the incidence of fire. The study area is located in Loma Blanca, a location near San José del Morro, province of San Luis, it is an area of grassland and mountain forest, with stony soil and rainfall that fluctuates between 500 and 600 mm annually. Recurring fires occur in this

area, generally of anthropogenic origin, one of them occurred in August 2019, burning approximately 1500 hectares of grassland and native forest. It was an intense fire, of great dimensions, spreading rapidly in the rural area due to the prevailing atmospheric conditions, strong winds of changing direction. In the months following the fire, periodic observations were made to determine the post-fire behavior of woody species. The species that up to the present have sprouted in the burned area belong to the following families Anacardiaceae, Asteraceae, Cannabaceae, Cervantesiaceae, Fabaceae, Rhamnaceae, and Solanaceae. Among those that re-emerged after fire are trees and shrubs. Regrowth was evident in the following tree species: *Celtis tala*, *Geoffroea decorticans*, *Jodina rhombifolia*, *Prosopis caldenia*, *Prosopis torquata*, *Schinus fasciculatus*, *Vachellia caven*, and in bushes like *Baccharis aliena*, *Cestrum parqui*, *Colletia spinosissima*, *Condalia microphylla*, *Erythrostemon gilliesii*, *Prosopis campestris*, and *Senna aphylla*. Two months later, and despite strong winds having reappeared, strong winds caused the fall of more than 20% off the individuals off *Prosopis caldenia* and *Vachellia caven* most affected by the fire. Among those that re-sprouted, despite the fact that some specimens did not survive after a year after the fire occurred, there are *Baccharis aliena* (especially 50% off the largest individuals) and *Prosopis campestris* (by 20 %) possibly due to the subsequent drought they endured after the fire. Finally, two genera germinated in the most affected areas *Geoffroea* and *Vachellia*. It is concluded that the aforementioned woody species have the ability to sprout after fire, but not all of them survive 100%.

A216
REGENERATION AFTER A FIRE IN NATIVE HERBACEOUS OF SAN LUIS

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Fire is one of the most important disturbances to which terrestrial ecosystems are subjected and is a factor of recognized incidence in the province of San Luis, especially in the mountain area of forest and in bush and grassland areas. Various species of plants in arid and semi-arid areas have the ability to adapt to fire due to the generation of sprouts and/or post-fire germination ability. The study area is located in Loma Blanca, a location near San José del Morro, province of San Luis. In August 2019, the rural area was affected, for two days, by a fire that disturbed approximately 1,500 hectares of grassland and native forest. Since 2007, this research team has been carrying out studies of the native vegetation in the area and to date 90 herbaceous species have been identified, without considering those belonging to the Poaceae family. Starting in September 2019, periodic observations were made to determine the post-fire behavior of the herbaceous plants and analyze their regeneration. The regeneration of thirteen species corresponding to nine families was observed: Acantaceae, Asteraceae, Cleomaceae, Convolvulaceae, Fabaceae, Ranunculaceae, Solanaceae, Turneraceae y Verbenaceae. The genres observed were *Evolvulus*, *Chaptalia*, *Clematis*, *Dichondra*, *Galactia*, *Glandularia*, *Hysterionica*, *Nierembergia*, *Rhynchosia*, *Stenandrium*, *Tarenaya*, *Trichocline*, and *Turnera.* In the regenerated species, the presence of regrowth from gemiferous roots was evidenced in 7%, basal buds in 23% and the presence of rhizomes in 69%. Specifically, in *Tarenaya*, plants originated from seeds were also observed. These species resisted the effects of fire, presenting the ability to sprout from the aforementioned morphological structures.

A217
ENVIRONMENTAL DETERMINANTS IN THE CYCLE OF *Fasciola hepatica* AND TRANSMISSION PATTERNS

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Fasciola hepatica transmission dynamics has strong environmental determinants that model and generate particular transmission patterns in each region that depend on a wide variety of abiotic, topographic, biological, and livestock management factors. The analysis of the natural habitat and its environment together with the climatic conditions are the factors that become crucial. The objective of the study was to characterize habitats and relationships with climatic factors to understand the cycle transmission pattern in human endemic areas. Work was carried out in two areas, Fiambalá (27°29'52.62"S, 67°35'53.51"W; altitude: 1630 m), and the Ipizca dam (28°49'08.83"S, 65°32'20.00"W; altitude: 944 m). Annual variation of climatic factors was obtained. The data were obtained from the agroclimatic base of the FAO Agromet Group (FAOCLIM). The interpolation method applied to evaluate the climatic characteristics used the New LocClim 1.10 FAO software. The factors analyzed included maximum, average and minimum temperatures, precipitation, potential evapotranspiration, wet period (vegetation season) and dry period (aridity conditions). The climatic classifications used were obtained from the Koeppen indexes (vegetation as an expression of the climate); Budyko (evaporation of precipitation); From Martonne (aridity/humidity) and Gorczynski (oceanic influence). The results highlight the desert-arid environmental characteristics of the town of Fiambalá and the less extreme ones of Ipizca; as well as seasonal averages with peaks in January and July, with higher values in Ipizca. Rain availability is very low in both areas. The analysis of the annual variation of precipitation and potential evapotranspiration shows a small peak in January-February (slightly higher in Ipizca) and almost nonexistent rainfall during the period April-May to October-November. The extreme, desert, and arid environmental characteristics around Fiambalá and the less extreme conditions of semi-aridity and aridity in Ipizca, as well as the very low annual precipitation in both locations, are surprising and very different from the typical environmental characteristics surrounding the transmission foci of fascioliasis.

A218
MICROPLASTIC AEROBIOLOGY: A PRELIMINARY STUDY OF INDOOR AIR QUALITY

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The atmospheric air inside buildings, as the outdoor air, has a large number of suspended particles whose origin can be biological or non-biological. These particles can affect the quality of the air, which influences both the health of the people who inhabit the place, and the ecosystem in general.

Microplastics are among the aerovagant inorganic particles of interest, these are structures of less than 5 mm diameter that can be easily inhaled and cause respiratory conditions. The objective of this work was to identify inorganic particles, comparable, according to the bibliography, with microplastics in a continuous aerobiological sampling of 5 months (November 2012–March 2013). To get samples, it was used a volumetric aerobiological collector, Hist type, Lanzoni brand, model VPPS 2000, located in the basement of the UNSL "Facultades" building. Those samples were observed with a Binocular microscope at 1000× magnification. Microplastic compatible particles were quantified as fibers/m^3 of air. The averages in the months of November and December 2012 were 304.31 fibers/m^3 of air and 140.28 fibers/m^3 of air, respectively, while in January, February, and March of the year 2013, the respective values were: 107, 61, 254.03, and 275.78 fibers/m^3 of air. The conclusion derived from this analysis is that in the months with more anthropic activity inside the building, the number of microfibers in the air increases significantly, while in the periods in which the number of people decreases (December and January) the number of these particles is lower. It is planned to continue with this and other more specific studies like spectrphotometry in order to arrive to concrete results regarding indoor air pollution, in this and other buildings.

A219

Asio stygius, A RAPACIOUS BIRD TYPICAL OF URBAN AREAS IN TUCUMÁN PROVINCE, ARGENTINA

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Barn owls and owls are part of the Strigiformes group and the well-known group of prey´s birds, which are at the top level of food webs and are therefore important in preserving the stability of the environment. In this very particular group of birds *Asio stygius* ('black owl'), is rare and difficult to observe species that inhabits mainly in jungles areas, forests, and savannas. This owl has a discontinuous distribution throughout the American continent, from northern Mexico to Paraguay, southeastern Brazil, and northern Argentina. It is a solitary, nocturnal bird, and feeds mainly birds and bats; during the day it rests on leafy trees. Their hunting strategy consists of moving from one perch to another, seeking to locate their prey. The objective of this work was to compare the feeding of two Stygian Owl specimens and see the variability of their food items. In 2016, during the months of August and September, a specimen of *A. stygius* was observed for the first time in the Botanical Garden of the Miguel Lillo Foundation located in San Miguel de Tucumán, capital of the province. Twenty pellets (food boluses) were collected and analyzed to identify the food items. The taxonomic determination was carried out up to the maximum possible level and it was determined that 100% of their prey were birds. In 2019, between the months of April and September, another individual was detected in the West Cemetery, also located in the capital of Tucumán. In this case, 21 pellets were collected, which were analyzed with the same techniques as in the previous case. The results were again that 100% of the items ingested were birds. The results obtained so far in these two specimens examined would indicate that the preferred food for this species, in urban environments, birds. In turn, the presence of the Stygian Owl in urban areas corroborates the importance of green spaces as reservoirs of biodiversity, within urbanizations. Therefore, it is essential generate new sites within cities with native vegetation as much as possible, which maintains the original structure and composition of the area, preserving the existing ones. These actions would not only improve the quality of environment, but also that of the inhabitants who live in the area.

A220

MALVOIDEAE BURNETT (MALVACEAE JUSS.) SUBFAMILY IN SAN LUIS (ARGENTINA)

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The family Malvaceae s.l. includes 243 genera, and about 4225 species and is largely tropical to temperate. Of these 129 genera and 1900–2200 species are native to the Neotropics. Subfamily Malvoideae is almost cosmopolitan with 111–115 genera and 1800–2000 species, throughout the warm temperate and temperate zones worldwide, but mostly in the New World where 78 genera and about 1200–1400 species are present. In Argentina includes 35 genera, 198 species. 48 species and 1 variety are considered endemisms for Argentina. The aim of this work is to develop a catalog of the Malvoideae Burnett. of San Luis, thus the botanical entities of the Malvaceae family, Subfamily Malvoideae and their distribution in the province surveyed to date are reported. The specimens were collected in the 2014–2019 period in agroecosystems, urban areas, mountain and plains in different phenological states, were georeferenced and identified using traditional botanical methods through specialized bibliography and iconography and were deposited in the Herbarium of Agricultural Sciences of the UNSL (VMA), the nomenclature of the specimens deposited in the Herbaria of the EEA INTA Villa Mercedes (VMSL) and of Ciencias Agropecuarias-UNSL (VMA) were updated. As a result, 41 species were identified, grouped into 18 genera and 1 variety: *Abutilon*, *Anoda*, *Callianthe*, *Gaya*, *Herisanthia*, *Krapovickasia*, *Lecanophora*, *Malva*, *Malvastrum*, *Modiola*, *Modiolastrum*, *Pavonia*, *Pseudoabutilon*, *Rhyncosida*, *Sida*, *Sphaeralcea*, *Tarasa*, *Wissadula*. An illustrated key of genera based on simple systematic characters to facilitate their use both in the field and in the laboratory is given, as well as distribution area of taxa was established. Three new records for the province are reported: *Pavonia sepium*, *Krapovickasia flavescens*, and *Tarasa trisecta.*

A221
PRELIMINARY STUDY OF THE INCIDENCE OF EFFLUENTS FROM THE RIO SALI BASIN IN REPRODUCTION EVENTS IN *Rhinella arenarum*

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Previous investigations reported that the Salí-Dulce river basin is considered one of the most polluted in the country, its main collector is the Salí river, which during its passage through Tucumán receives the effluents from more than 80 industries directly or indirectly. Within this water system, the Colorado river constitutes one of the tributary basins of the Salí river with a smaller surface area, but with a high pollution load. In this research, we studied in the fertilization and different stages of the embryonic development of the *Rhinella arenarum,* the incidence of different samples of water collected in influents and effluents of the sugar-alcohol and citrus industries of Tucumán, which discharge their waters to the Colorado river. During the harvest, as an indispensable measure for the collection of the waters, a study of the area was carried out and the intake points were located in the tributary beds of the Colorado river. Three physical-chemical parameters were analyzed to establish the water quality in a preliminary way: pH, electrical conductivity, and total phosphorus content. Fertilization percentages and analysis of embryos at different stages of embryonic development were determined by "in vitro" fertilization tests. Using the google maps app, the satellite area and location of the industries involved with the effluents to be analyzed, the complete route of the Calimayo stream and the collection points of the different water samples in the Calimayo and San Miguel streams, tributaries that discharge to the Colorado river in the south of the province. The water samples were collected: MA1 (influent that supplies the paper industry from the Lules river), MA2 (industrial effluent that leaves the paper mill), MA3 (effluent that leaves the San Miguel citrus farm through the San Miguel stream), and MA4 (effluent that comes from the paper mill and from Arcor-Misky through the Calimayo stream). The MA2 and MA4 presented turbidity, abundant yellowish / brown foam on the surface, industrial solid waste, and a strong irritating odor in the respiratory mucosa, similar to the hydrogen sulfide chemical. The MA1 and MA3, unlike the previous ones, were clear and odorless. The parameters of pH, conductivity and phosphorus of the MA presented values within the standards. The MA2 reported conductivity and total phosphorus values above the standard. In all MA, the fertilization percentages remained similar with respect to the control with Ringer's 10% solution (R10: 97%; M1: 97%; M2: 97%, M3: 98% and M4: 100%). The embryos developed in R10, M1 and M3 did not show modifications in the stages analyzed: 14 (neural sulcus) and 17–18 (caudal sprout and muscular response). The embryos developed in R10, M1 and M3 did not show modifications in the stages analyzed: 14 (neural sulcus) and 17–18 (caudal bud and muscular response). However, in MA2 and MA4, the embryos exhibited significant changes from stage 14 on, most of them delayed in gastrula and others with signs of degradation. Unlike the control, the jelly that surrounds them does not disappear. These embryos remained arrested and undeveloped at stages 17–18. Later studies continue with the analysis of the waters and the components that affect normal embryonic development.

A222
PRELIMIMARY STUDY OF DIVERSITY OF DYE PLANTS IN SAN PEDRO DEPARTMENT, JUJUY, ARGENTINA

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In San Pedro Department of Jujuy Province, there is a great biological diversity, with a varied flora that constitutes an important source of natural resources. Different types of plant communities stand out, some of them with significant economic potential, due to their coloring or dyeing characteristics. Currently, interest in natural colors has been revalued, especially in food, object designs and clothing. The objective of this work was to carry out a preliminary study of the diversity of dye plants in the aforementioned Department. Field trips were made to collect specimens in the following locations: La Mendieta, Sauzal, El Quemado, San Pedro, and Arroyo Colorado. Species were taxonomically identified, and a list was prepared taking into account their dye characteristics. 141 species were collected between originals and duplicates. So far, 42 taxa have been identified as dyes, distributed in 17 families, 15 of them belonging to the Magniopsids with 39 taxa and 2 families of the Liliopsids with 3 taxa. Among the dye species native plants of the region were observed such as: Guaran-Guaran (*Tecomas stans*), Tusca (*Acacia aroma*), Guayacán (*Caesalpinia paraguariensis*), Seibo (*Erythrina crista-galli*), Chal chal (*Allophylus edulis*), Palán-Palán (*Nicotiana glauca*) and other introduced species were observed. The specimens identified as dye plants have the characteristic colorants in leaves, flowers, barks, fruits, seeds, and/or roots. Taking into account the bibliographic review, it is observed that in the species identified as dyeing, there is a wide variety of colors ranging from brown, gray, yellow, red, green, and intermediate colors. From the results obtained, the importance of the diversity of dye plants in the Department of San Pedro is revealed, as well as the contribution of information to future research on local flora, its application in ethnobotany and other science.

A223
GENETIC DIFFERENTIATION AND AFFINITIES IN THE LOCOTO CHILE (*Capsicum pubescens*): EXPLORING DISPERSAL AND DIVERSITY PATTERNS THROUGH RAD-seq DATA

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The locoto or rocoto chile (*Capsicum pubescens*, Solanaceae) is cultivated mainly in the highlands of Central-South America, from Mexico to northwest Argentina. Despite being a species of economic and cultural importance, the knowledge of its evolutionary history and genetic diversity is incipient. Although different varieties or cultigens defined on the basis of fruit characteristics are informally recognized, the relationships and genetic structure within the species throughout their distribution/cultivation range have yet not been studied. The knowledge in genetic diversity of crops

allows to promote actions for management and conservation of the agrobiodiversity, as well as using this diversity to strengthen food production. Therefore, the objectives of this work were to investigate the intraspecific relationships in *C. pubescens* and to explore the spatial distribution of its genetic variation. Using the RAD-seq methodology (Restriction-site-Associated DNA sequencing), a total of 67 individuals were studied, from cultigens of different Latin American countries, with emphasis on Bolivia, and also material conserved in germplasm banks (*ex situ*). Different alignment hypotheses of the RAD-seq sequences were generated and then phylogenetic and genetic structure analyses were performed. The results obtained using both approaches were congruent. Globally, two large genetic groups were recognized: 1) individuals from Bolivia and, 2) cultigens from Peru, Ecuador, and Central America, up to Mexico. At the same time, the germplasm cultivated in Argentina is mostly represented within Group 1, while the material conserved *ex situ* belongs to Group 2. According to the obtained phylogenetic reconstructions, the area of origin of *C. pubescens* could be located in central-west Bolivia. Given the presence of a greater diversity/genetic groups in the Bolivian territory, according to the wide morphological variation of the crop in that region (i.e., flowers, fruits, pubescence, etc.), it could be inferred that the species would have been primarily domesticated in Bolivia, more precisely, in the highlands around La Paz. In addition, the presence of reticulate relationships and genetic admixture among the individuals from this region was detected, so it could represent an initial area of cultivation and dispersal of the species by humans, which later expanded to the north and south of the continent. Overall, the results allow us to increase our knowledge of the locoto chile affinities and the distribution of its genetic variation, giving new insights about its origin and expansion. It is planned to incorporate other analytic approaches that allow a better understanding of its evolutionary history and diversity, relevant information for the use and conservation of the species.

A224
NEW CONTRIBUTIONS TO THE KNOWLEDGE OF THE MARSUPIALS FROM THE SOUTH OF SANTA FE BASED ON THE ANALYSIS OF THE *Tyto furcata* PELLETS

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The marsupials represent a group of mammals with a long evolutionary history in South America. In Argentina, there are 27 different species, and they are mainly located in the northern ecosystems: the 'Yungas', the 'Paranaense' Forest, and Chaco province. Three species have been historically recorded in Santa Fe province, most of these recordings come from the north and center of the province. In the south of the province, these recordings of the marsupials are almost exclusively restricted to the 'overa' weasel (white-eared opossum) (*Didelphis albiventris* Lund, 1840), which, historically, is one of the most typical mammals of this region. This contribution is aimed to present the first recordings of two new species of the marsupials preyed by the bell-tower owl (*Tyto furcata*) in the south of Santa Fe province. The field work was carried out between January and June 2020 in four different types of environments (urban, peri-urban, anthropicrural, non-anthropized rural, or natural) located in the Caseros department (33º03'00''S 61º10'00''W). The pellets (ball-shaped no-digested substance) were collected monthly in previously established locations, where the *T. furcata* used as a perch. The samples were put in paper bags, identified beforehand, and then put in hermetically sealed polyethylene bags following all the biosecurity measures. The material was processed using tweezers to extract the remains of jaws and skulls from the animals found. The identification of the preys was done comparing the identified samples in osteological collections with specialized literature. 2055 pellets were obtained, and they contained 4808 vertebrate preys. It is widely documented that most of the preys are rodents (main prey items in this bird's diet). However, three records of two species of marsupials were found: *Lutreolina crassicaudata* (Desmarest, 1804) (N = 2) and *Monodelphis dimidiate* (Wagne, 1847) (N = 1). Although the number of specimens is low, the results become relevant since these species are not studied in depth in Argentina and with few or no records in this area. The transformation or degradation of the natural habitat by the expansion of the agricultural frontier is the main threat that these species face. In this work, the records were presented in a non-anthropized rural or natural environment which is consistent with what several authors state: some members of certain communities have fragmented themselves and occupied isolated patches of native vegetation or not intensively used patches. Another important fact to highlight is the importance of the study of communities of micromammals from the analysis of the *T. furcata*'s diet since it could be considered a worthy methodological tool to determine the distribution of micromammals with low density avoiding other procedures (such as intensive traps) potentially risky as regards health and costly as regards time and effort.

A225
SPECIFIC RICHNESS AND RELATIVE ABUNDANCE OF NATIVE RODENTS (RODENTIA: CRICETIDAE) PREYED BY *Tyto furcata* IN THE SOUTH OF SANTA FE PROVINCE, ARGENTINA

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The bell-tower owl *Tyto furcata* (Temminck, 1827) is a predatory night bird which belongs to the Strigiform order, Tytonidae family, and it is widely spread over the American continent. It is able to live in rural areas as well as in urban areas because it can nest in different places even in man-made nests. Its eating habits have been studied in many regions through its wide range of distribution. In South America, this predatory bird was characterized as being an opportunist predator specialized in small mammals (mainly rodents and small marsupials). In addition, the review of the studies done on its diet suggests that its choice is determined by the availability and vulnerability of the preys in the habitat. This lets us know, indirectly, the assemblage of micromammals which make up a community. The aim of this work is to present the specific richness (S) and relative abundance (%) of Cricetidae native rodents preyed on by this bird in the south of Santa Fe province. The field work was done between January and June 2020 in four different kinds of environment which belong to the land ecosystems (urban, peri-urban, anthropic, rural, non-anthropized rural or natural) near Casilda town (33-02-39''S 61-10-05''W). This categorization was established according to the type of soil usage based on the

relationship that is needed to develop the different anthropic activities, since we consider them a representative measure of the degree of disturbance or ecosystem simplification. The samples were collected monthly in previously established locations that *T. furcata* uses as perches. The pellets were put in paper bags identified beforehand and then in hermetically sealed polyethylene bags following all the biosecurity measures. The material was processed using tweezers to extract the remains of jaws and skulls from the animals found. The identification of the preys was done comparing the samples identified with osteological collections and specialized literature. From 2055 pellets, 4808 recovered preys were obtained. Of the total, 98.5% belongs to micromammals, being 92% native rodents of the Cricetidae family. On the basis of the results obtained, it was possible to establish a specific richness of S = 10, represented by three species of the Akodontini Tribe (*Akodon azarae*, *Necromys lasiriurus*, and *Oxymycterus rufus*) three species of Oryzomyini (*Holochilus chacarius*, *Oligoryzomys flavescens*, and *Oligoryzomys nigripes*) and four species of Phyllotini (*Calomys laucha*, *Calomys musculinus*, *Calomys venustus*, and *Graomys cf. Chacoensis*). The *Calomys* genus, with N = 2223 (50%) constituted the most consumed group followed by the *Akodon azarae* species N = 1280 (28%) and the *Oligoryzomys* genus N = 877 (19.8%). On the other hand, *Holochilus chacarius* and *Oxymycterus rufus* contributed the least to the diet with N = 8 (0.18%) and N = 4 (0.09%), respectively. This preliminary analysis makes it possible to establish new locations to record some species and aims to set the basis for future monitoring allowing the implementation of population studies for the involved species.

A226
PHYSICOCHEMICAL AND BIOLOGICAL WATER QUALITY OF LOS ANGELES RIVER

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Los Angeles River runs through a longitudinal valley in the homonymous locality, in the department Capayán province of Catamarca. The water is collected for human consumption, irrigation, and recreation in summer. The objective of this research was to evaluate the quality of the water for different uses, combining physicochemical and biological parameters: bacteriological and biotic indices based on benthic macroinvertebrates. The sampling station was established in "La Toma" (28º26'45,17''S 65º57'01,89''W; 1.779 MASL), where the water is captured and diverted to the water treatment plant for its treatment. On site, with a digital multimeter for water it was determined: temperature (T), electric conductivity (CE) y pH. In the laboratory, it was determined: alkalinity (Al), hardness (D), calcium (Ca) magnesium (Mg), chlorine (Cl), organic material (MO) and sulfates (S). Bacteriological analysis included: Total Aerobic Mesophilic Heterotrophs (TAMH), Total Coliforms (TC), Fecal Coliforms (FC) and presence/absence of *Pseudomonas aeruginosa* and *Escherichia coli*. Physicochemical and bacteriological analyzes of water were carried out following standardized norms. The macroinvertebrates were collected with a Surber type sampler (900 cm^2 of surface; 300 µm-mesh opening); two integrated samples for analysis, in winter 2019. Indices were obtained: IBMWP' (Iberian Biological Monitoring Working Party) adjusted for NOA; ASPT' (Average Score Per Taxon) and FBI (Family Biotic Index). The values of the physicochemical variables of the water were: T = 9ºC; CE = 0.252 µS/cm at 25ºC; pH = 6.87; Al = 119 ppm; D = 86 ppm; Ca = 26.8 ppm; Mg = 3.78 ppm; Cl = 7.9 ppm; MO = 0.8 ppm; S = 0.8 ppm. The results of the bacteriological analyzes were: TAMH = 100 CFU/mL; CT = 23 NMP/100 mL; CF = 9 CFU/100 mL; Presence of *E. coli*; Absence of *P. aeruginosa*. The biotic indices reached the following values: IBMWP' = 175 (very clean waters); ASPT' = 5.83 (no impact water); FBI = 4.19 (very good quality, light organic contamination). The water can be used for irrigation, recreation, and human consumption, after purification. The knowledge generated may be used to enhance and manage the water resource.

A227
CHARACTERIZATION OF THE BENTONIC ENTOMOFAUNA OF LOS ANGELES RIVER, CAPAYÁN, CATAMARCA

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Benthic insects are a vital functional component in lotic ecosystems. Los Angeles River runs through a longitudinal valley in the homonymous locality, in the department Capayán province of Catamarca. Its biota has not yet been studied. The objective of this research was to characterize the benthic entomofauna of the river through simple biological metrics and ecological indices. Sampling station was established at 28º26'45.17"S 65º57'01.89"W; at 1779 MASL. Samples (N = 2) were taken with a "Surber" type sampler (900 cm^2 surface; 300 µm-mesh opening), integrated for analysis, in winter 2019. Taxonomic determinations were made at the lowest level possible to discern. The abundance was 1159 benthic insects (larvae and adults), of nine orders, 30 families and 28 genera. Percentage composition of orders showed that the most abundant were: Diptera (38.31%), Coleoptera (27.27%), Trichoptera (14.06%), Ephemeroptera (10.87%), and Plecoptera (6.56%). The remaining orders were poorly represented (range: 0.43–0.86 %). The most abundant families were: Chironomidae (28.65%) and Elmidae (19.67%), Hydropsychidae (8.37%), Baetidae (7.42%), Psephenidae (7.08%) and Perlidae (6,56%). In terms of wealth, the most diverse order was Trichoptera (6 families and 9 genera), Diptera (7 families and 6 genera), followed by Coleoptera (5 families and 6 genera) and Ephemeroptera (3 families and 6 genera). The most abundant genus was Austrelmis (16.73%), followed by Smicridea (8.37%), Psephenops and Baetodes (7.08% each). The diversity index Shannon-Wiener (H'$\log_2$) was: 2.92. The Simpson dominance was 0.19. The set of simple metrics and H' index was obtained for the first time for the sampled river site; They are compatible with results obtained for rivers and mountain streams in the Chaco Serrano ecoregion of the Capayán department, in the same altitudinal range and contribute to the knowledge of its rich aquatic biota.

A228
CHARACTERIZATION OF PLANTS' COMMUNITIES IN "THE TRAPAL" NATURAL RESERVE FROM GENERAL ALVEAR, MENDOZA

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The inventories and descriptions of plants' communities are considered a basic requirement to orient all the activities and decisions of certain areas to be managed and preserved. The ecological contribution that can be offered from these studies is fundamental, as the different vegetation structures also respond to specific ecosystems as well as to changes in it. In relation to this last aspect this work is done oriented to order the native forests of "The Trapal" reserve located in the south- east of General Alvear city and to describe their current situation. A systematic stratified sampling was carried out to survey the vegetation. The visual analysis allowed to interpret 4 zones (stratification) considering its homogeneity, which constituted 4 inventoriable polygons. The size and shape of the sample units (SU) were suited for dasometric measurements of forest inventory. The SU is integrated by 2 concentric circular subplots designated as A and B. Subplot A has a surface of 500 m^2 (12.62 m radius), while sub-plot B is 12.5 m^2 (2 m radius). The description of the herbaceous layer needed another plot corresponding to one tenth of a square metre (0.1 m2), systematically arranged at two metres from the centre of subplot A in direction to the closest tree. To identify the plants' species, the classical method of Systematics was applied through the use of dichotomous keys and specific bibliography. The nomenclature of the quoted taxa was corroborated with the on-line version from the Darwinion Botanical Institute, as well as the distribution of them in Argentina. The species were grouped by families according to their origin (natives, endemic, introduced, adventitious and cosmopolitan) and habit (trees, bushes, sub-bushes, herbs and climbing plants). The conservation status of the species was corroborated in the web page PlanEar, endemic plants from Argentina. The comparisons among treatments were made by the Kruskal-Wallis test where significant differences were observed among the studied polygons. The amount of the resulting taxa corresponds to one hundred and forty-five (145), belonging to forty-two (42) botanical families. The families with greater representation are Asteraceae (23), Poaceae (17), Chenopodiaceae (13), Fabaceae (12), Solanaceae (11), Verbenaceae (8), Malvaceae (7), and Boraginaceae (5); the rest of the families possess between 1 and 4 species. 44 endemic taxa were identified. The results of the plant cataloguing indicate a 30% of endemism and a 59% of native species in the zone studied. A predominance of herbs (59%), followed by bushes (22%), trees (6%), and in lower percentage, the sub-bushes, climbing trees, aquatic, succulent, parasites, and epiphytes. Plant structure in the four forests is made up of three strata with different wealth and physiognomy. Tree density in polygons I and II is relevant in relation to the other zones with a great predominance of *Prosopis flexuosa*, *Geoffroea decorticans*, and *Larrea divaricata* (tree height) as tree species; regarding shrub and herbaceous species the highest density is located in polygons III and IV with a great predominance of *Atriplex crenatifolia*, *Allenrolfea vaginata*, *Baccharis spartioides*, and *Suaeda divaricata.* Tree and shrub species with the highest height and size are in Polygon II and with less height are in Polygon I. Floristic cataloguing and the wealth represented in the area of study are relevant factors to consider for the development of strategies in the maintenance and management of "The Trapal" reserve. It becomes relevant the preservation of native species and that 30% of endemic species which include 27 endangered ones among them. It also becomes essential to expand the management strategies considering that it is an area recently affected by fire and to a lesser extent by deforestation. The results presented in this work constitute a fundamental basis for future research.

BIOTECHNOLOGY AND GENETICS

A229
DISTRIBUTION OF ANTHOCYANINS PRESENT IN BLACK FIGS AND RED PLUMS BY EXTRACTION WITH AQUEOUS BIFASIC SYSTEMS

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Anthocyanins are a large family of polyphenols in plants and are responsible for the colors for many fruits and flowers observed in nature. Recent research has focused on the health benefits of these pigments, especially their antioxidant activity. The objectives of this study were to quantify the concentration of anthocyanins present in black fig and epidermal tissue of red plums in extracts obtained in traditional form and in aqueous biphasic systems formed by ethanol and ammonium sulfate (SBA). These systems are prepared from the binodial curve obtained in previous work. A spectrophotometer was used to quantify anthocyanins and absorbance was measured at 520 nm, wavelength at which anthocyanins are most absorbed in pH buffer: (1) In fig epithelial tissue extracts, obtained by grinding and extraction with ethanol with a concentration of 0.6 g/mL, the anthocyanin concentration was 16.3 mg/100 mL, similar to values reported by other researchers, for radibas. Similar values were obtained for red plum epithelium. In the case of SBA, the best values were obtained with SBA consisting of 24% ammonium sulfate, 24% ethanol and 5% fruit tissue. With these systems, the amount of ethanol used is reduced, increasing extraction performance. The concentration of anthocyanins in the upper phase was 3.7 more than in the lower phase for black figs and 2.3 times more for red plums. In these systems the volume ratio of the upper and lower phases was 0.5, being able to concentrate most of the anthocyanins in the upper phase facilitating the obtaining of them by evaporation of ethanol, with lower energy expenditure. In turn, epitheliar tissue is used directly, which is retained between the two phases, when the systems are formed and finally discarded, by filtration.

A230
GENETIC ASPECTS ASSOCIATED WITH THE SEED PER POD TRAIT IN SOYBEAN

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Numerical traits related to yield have different sensitivity to the environment, being the seed per pod (SPP) one of the most stable, providing an opportunity to increase it genetically if lines with high SPP were available. The SPP is a weighted mean that depends on the relative quantity of pods with different number of 2-, 3-, and 4-seeds (2SP, 3SP, and 4SP, respectively). In our Laboratory, lines with SPP > 3.7 (i.e., with +70% 4SP) were developed. In the Argentine market there are no soybean commercial varieties with high % 4SP. The aim of this study was to characterize genetic aspects related to SPP and associated traits such as pod number per plant (PNP), seed number per plant (SNP), % 2SP, % 3SP and % 4SP, and their possible association, during the growing seasons (GS): 2015/16 and 2016/17. A set of 131 recombinant inbred lines (RIL) derived from the cross of two experimental lines: one with SPP = 3.53 (54% 4SP) and the other with SPP = 2.25 (0% 4SP), were used. The RIL population with their parents, hybrids and their reciprocals were planted in a single-line plot of 2 m in length and 0.52 m in width, in a randomized complete block design with three replications. Six plants of each inbred line (genotype) were phenotyped at full maturity for SPP, PNP, SNP and the % of 2SP, 3SP and 4SP. All the screened traits showed variability in the RIL. Mean values for each trait in both GS were: SPP = 2.8 (range: 2.0–3.9); PNP = 68 (range: 13–187); SNP = 189 (range: 20–532); % 2SP = 32% (range: 0–100%); % 3SP = 55% (range: 0–91%); % 4SP = 13% (range: 0–78%). For variance components, genotype explained >85% of the total variation for SPP, as well as % of 2SP, 3SP, and 4SP. Conversely, genotype only explain <5% of the total variation for PNP and SNP. These results show high narrow sense heritability values (h^2) for SPP, % 2SP, % 3SP and % 4SP ($h^2 > 0.95$); and low heritability for PNP and SNP ($h^2 < 0.23$). Correlation coefficients among the different traits were: SPP and % 4SP (r = 0.9, $P < 0.01$); PNP and SNP (r = 0.9, $P < 0.01$); SPP and PNP (r = −0.1, $P < 0.01$). Results proved the strong genetic regulation and limited environmental influence that presents the SPP trait, providing the possibility to improve it through the increase in the % 4SP. Additionally, even though SNP is highly associated with PNP, the low correlation between PNP and SPP proved the lack of tradeoff between these two components. Thus, the positive effect of incorporating the high % 4SP trait on SPP will be maintained regardless of variations in PNP.

A231
STUDY OF THE PROTEIN PRODUCTION OF *Aspergillus sp.* V1 USING SUGARCANE VINASSE AS SUBSTRATE

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Vinasse is an acidic effluent with a high organic load, which result from ethyl alcohol production. This residue represents a potential hazard for the environment if not responsibly managed. Filamentous fungi can adapt to a wide variety of substrates and grow in large quantities on organic wastes. In turn, bioconversion of residues into protein-rich fungal biomass is of great interest since it can be used as an alternative nutrient source to the expensive aquafeeds such as fishmeal and soybean meal. In a prior study, a filamentous fungus isolated from northwest of the Argentine, *Aspergillus sp.* V1, was able to grow on sugarcane vinasse. The objective of the present work was to evaluate the protein content of *Aspergillus sp*. V1 biomass cultivated on vinasse, with and without supplement of exogenous nutrients. The optimal vinasse concentration for the growth of *Aspergillus sp*. V1 was determined making dilutions of the residue in distilled water (10% to 100%, v/v) at a final volume of 10 mL. Each dilution was inoculated with 1×10^6 spores/mL and incubated at 30ºC (150 rpm) for 96 h under sterile conditions; then dry weight of biomass at 105°C was determined. Biomass production was carried out in 200 mL of sterile vinasse at the selected concentration, with and without supplementation of nitrogen and phosphorous in the following combinations: vinasse without nutrient supplementation (B_1); vinasse supplemented with 2 g/L of $(NH_4)_2SO_4$ (B_2), or 2 g/L of $CO(NH_2)_2$ (B_3); vinasse supplemented with 2 g/L of $(NH_4)_2SO_4$ and 1 g/L of KH_2PO_4 (B_4), or 2 g/L of $CO(NH_2)_2$ and 1 g/L of KH_2PO_4 (B_5). The biomass produced was separated by filtration, lyophilized, and weighed. In each case, percentage of total proteins (Kjeldahl-Arnold-Gunning method using the universal factor of conversion to protein 6.25) and productivity (in terms of milligrams of protein per liter of culture per h) was determined. The highest growth of *Aspergillus* sp. V1 was observed in 100% vinasse, with a biomass production of 41.55 g/L thereby following assays were conducted witn undiluted vinasse. The weight of lyophilized biomasses was 0.89; 0.61; 2.84; 1.00 and 2.99 g/L, with protein percentages of 33%; 49%; 41%; 38% and 36%, and a productivity of 3.0; 3.1; 12.0; 4.0 and 11.1 mg/L h for B_1, B_2, B_3, B_4 and B_5, respectively. According to literature, aquafeeds should contain between 26% to 55% protein. In all cases, protein percentages of *Aspergillus sp*. V1 biomass were within the desirable range. However, B_3 was selected as the most promising biomass for future assays due to its higher productivity (12.0 mg/L h). Our findings demonstrate that the mycelium of *Aspergillus sp*. V1 grown in vinasse could be a promising and inexpensive protein source to be used as aquafeed.

A232
FUNGAL ENDOPHYTES AS BIOCONTROL AGENTS: IDENTIFICATION OF BIOACTIVE SECONDARY METABOLITES

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The use of endophytes as biocontrol agents is a sustainable, affordable, and eco-friendly alternative to deal with plant diseases of high agricultural importance. The potential lies on the pathogen resistance that endophytes confer to the plant and the mutual benefits generated by the symbiotic association such as the increase in nutrients uptake, the growth promotion, and the higher stress tolerance. With the aim of discovering new biological control agents, the inhibitory activity of three endopthytic fungal strains isolated from the endemic plant *Eupatorium buniifolium* was

assessed. The fungi were identified as *Fusarium solani* Eb01, *Alternaria alternata* Eb03, and *Neofusicocum sp.* Eb04. Firstly, we tested the antagonistic effect of the three endophytic strains against each other and later we faced them toward a panel of well-known phytopathogens by confrontation experiments carried out on solid media. *F. solani* Eb01 not only inhibit the growth of the other two endophytic strains but also inhibit the mycelial development of *Aspergillus flavus*, *A. niger*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, and *Penicillium chrysogenum.* Further, we addressed the identification of the *F. solani* metabolites potentially responsible for the antifungal activity. Therefore, the fungus was cultured in PDB liquid media, and the organic extract was obtained. By Gas Chromatography and Mass Spectrometry analysis (GC-MS), we detected two δ-lactones as major components, namely 5,6-dihydro-6-pentyl 2*H*-pyran-2-one or massoia lactone and tetrahydro-4-hydroxy-6-pentyl-2*H*-pyran-2-one. These results suggest that *F. solani* Eb01 and/or its metabolites are good candidates to propose biological control strategies for agriculture.

A233
ADHESION OF STALLION SPERMATOZOA ON POLY(ACRYLAMIDE)-BASED HYDROGELS: EFFECT OF HYDROGEL IONIC CHARGE

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Polymeric hydrogels are soft materials used in several biotechnological applications. We demonstrated that poly(acrylamide) (PAAm) based hydrogels are devices for equine sperm selection; however, the molecular mechanisms responsible for this interaction are largely unknown. We hypothesized that the net ionic charge of the hydrogel surface may be involved in the sperm attachment. Therefore, we copolymerized acrylamide with 10% in moles of (3-acrylamidopropyl) trimethylammonium chloride (10%APTA, cationic monomer), N-[tris(hydroxymethyl)methyl] acrylamide (10% HMA, neutral monomer) and acrylic acid (10% AA, anionic monomer at pH > 4) to achieve different ionic charges on the hydrogel surface. Then, we performed the sperm binding experiments in presence or absence of bovine serum albumin (BSA), a protein that is negatively charged at pH 7.4. For this purpose, each hydrogel was incubated in a culture dish containing sperm-TALP medium (pH 7.4) with and without BSA (6 mg/mL) at 37°C in 5% CO_2 for 30 min. Finally, an aliquot of raw stallion sperm suspension was added (1×10^6 sperm/dish) and incubated at the same conditions mentioned before. Sperm incubated in culture dishes without hydrogels served as controls. The percentage of sperm attached to the surfaces was determined as the difference between the number of sperm initially added into the culture dish and the recovered non-bound cells after incubation. Data (mean ± SD) were analyzed by two-way ANOVA/Bonferroni post-test; a $P < 0.05$ was considered to be significant. The results evidenced that the percentage of equine spermatozoa attached to the hydrogel surface varied according to hydrogel physicochemical characteristics and BSA supplementation ($P < 0.05$). Thus, for hydrogels cultured in BSA free-medium, the percentage of attached spermatozoa was higher in 10% APTA surface than in 10% AA and 10% HMA surfaces; and also respect to the control (10% APTA: 74.5 ± 8.7% > 10% AA: 37.2 ± 16.3% > 10% HMA: 22.6 ± 2.3% > control: 7.1 ± 1.1%; $P < 0.05$). Interestingly, for hydrogels cultured with BSA supplementation, the spermatozoa attached mainly to 10% HMA and 10% AA surfaces and only few sperm bonded to 10% APTA surface (10% HMA: 57.1 ± 9.6% ≈ 10% AA: 48.6 ± 3.3% > 10% APTA: 8.1 ± 1.7% and control: 15.0 ± 3.1%; $P < 0.05$). In fact, the percentage of sperm bound to 10% APTA surface was similar to that in the control ($P > 0.05$). Additionally, in all experiments independent of BSA supplementation, spermatozoa attached to 10% AA and 10% HMA surfaces remained motile; whereas spermatozoa bound to 10% APTA surfaces were immotile. In conclusion, the ability of bonding between copolymeric hydrogels based on PAAm and spermatozoa is influenced by the hydrogel net ionic charge. The supplementation with BSA modifies the hydrogel superficial property and the ability of equine spermatozoa to bind on hydrogels without affecting the motility of bounded spermatozoa. This information might contribute to assisted reproduction in stallions with low reproductive efficiency.

A234
BIOTRANSFORMATION OF ESTAFIATIN WITH EXTREMOPHILE ACTINOBACTERIA

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Sesquiterpene lactones (SLs) constitute a large and diverse group of biologically active plant chemicals that have been identified in several plant families. On the other hand, the phylum Actinobacteria composes a diverse group of Gram-positive bacteria, which are abundant in soils and present in various special and extreme habitats. Actinobacteria have made a significant contribution to the health and well-being of people throughout the world and produce structurally diverse bioactive natural products, such as enzymes. Extremophilic actinobacteria selected as biocatalysts were: *Rhodococcus pyridinivorans* PDB9, *Micrococcus yunnanensis* YIM 65004, *Streptomyces pratensis* ch24, *Streptomyces sparsus* YIM 90018, *Streptomyces luridiscabiei* NRRL B-24455, and *Streptomyces anulatus* NRRL B-2000, isolated from Laguna Diamante (Catamarca, Argentina) and Laguna Socompa (Salta, Argentina). Estafiatin (1), a guaiane-type sesquiterpene lactone, was selected in the present work for the biotransformation in order to obtain potentially bioactive derivatives. Biotransformation experiments were carried out under the whole-cell modality, cultivating the microbial species in liquid medium with orbital agitation (25°C; 180 rpm). After ten days of bioconversion, the media were extracted and purified by chromatographic methods affording three majority derivatives. The corresponding compounds were elucidated and characterized by spectroscopic methods, showing modifications related to epoxide hydrolysis and double bond stereoselective reduction at C-11. The high percentage of bioconversion from *Rhodococcus* was highlighted. Finally, these procedures, which used microorganisms isolated from extreme environments, made it possible to establish comparisons with respect to the amounts of product and bioreaction times obtained with previously tested filamentous fungi, which demonstrates the potential of actinobacteria to effectively produce xenobiotic modifications by environmentally friendly methods.

A235

Cordyceps tenuipes, ENTOMOPATHOGENIC FUNGUS WITH ANTIPATHOGENIC ACTIVITY AGAINST *Staphylococcus aureus*

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In recent years, the potential of entomopathogenic fungi (EF) has been explored in human health due to the number of bioactive metabolites that they synthesize and their use in the oriental folk medicine. EF applicability is limited since it is still unknown if the presence of the host insect is necessary to trigger the synthesis of these compounds. In this study, we evaluated the entomopathogenic fungus *Cordyceps tenuipes* capacity to synthesize antipathogenic metabolites against *Staphylococcus aureus* strains, in the presence and absence of insect. *C. tenuipes* (CEP 425) culture were prepared on potato glucose broth medium, under two conditions: without insect and with 2% w/v of *Spodoptera frugiperda* remains. From supernatants (S) and mycelia (M) were obtained 4 extracts with ethyl acetate (MAcOEt, M + I AcOEt, SAcOEt, and S + I AcOEt) and 4 ethanolic extracts (MEtOH, M + I EtOH, SEtOH and S + I EtOH). The effects on bacterial growth, biofilm formation and the production of bacterial coagulase and hemolytic enzymes were evaluated in two concentrations (100 and 500 µg/mL) against three ATCC *S. aureus* strains: 6538, 700698 MRSA and 700699 VISA. Insect-free AcOEt extracts reduced bacterial growth (65–90%) and biofilm production (42–100%), as well as inhibited hemolytic activity and delayed or inhibited coagulase activity. The ethanolic extracts affected considerably the biofilm formation (42–94%) without altering bacterial growth. Our results revealed that *C. tenuipes* synthesizes antipathogenic metabolites independently of the presence of the host insect and, in general the activity decreases in the insect condition, suggesting that the extracted metabolites could be used by the fungus during the infection process.

A236

ANTIOXIDANT CAPACITY OF COMPOUNDS SYNTHETIZED BY ENTOMOPATHOGENIC FUNGUS *Cordyceps tenuipes*

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Many species of entomopathogenic fungi of the *Cordyceps* genus are used in traditional oriental medicine as food additives or supplements and for the treatment of various ailments. The interest in exploring them as a source of new drugs lies in the great variety of biologically active metabolites they synthesize. Among them, compounds that could act as antioxidants have been reported. These molecules can work as a double ally: protecting normal eukaryotic cells against oxidative stress and intervening in the resolution of infectious and inflammatory processes. Finding compounds capable of achieving the modulation of the redox system of pathogens and hosts as an antimicrobial and antioxidant therapeutic strategy is a major challenge. In this work, it was determined the antioxidant power of ethyl acetate and ethanolic extracts from *Cordyceps tenuipes* cultures (CEP 425) grown in potato-glucose liquid medium under two conditions: without insect and with 2% w/v of *Spodoptera frugiperda* larvae. It was evaluated the capacity of the extracts to purify the radical $ABTS^{\cdot+}$, to purify the nitric oxide and to reduce the Fe^{3+} ion. All the extracts showed purifying activity of $ABTS^{\cdot+}$ with a concentration of 50% of the radicals (CD50) between 72 and 201 µg/mL for the extracts without insect and from 136 to 365 µg/mL in the condition with insect. The highest capacity to purify nitrites was registered in the extracts without insect. The ethanolic extracts showed higher Fe^{3+} reducing power. In general, a loss of antioxidant activity was registered when adding insect remains to the culture medium, suggesting that antioxidant metabolites are synthesized by the fungus *per se* and then could be used during the insect infection. We consider that the chemical nature of the metabolites with antioxidant capacity is diverse since both ethanol and ethyl acetate extracts proved to be active.

A237

CHARACTERIZATION OF ANTIFUNGAL METABOLITES PRODUCED BY *Bacillus atrophaeus*

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Biological control can be defined as the use of living organisms or their metabolites to reduce the population density of other pest organisms, contributing to the decrease in the use of chemical pesticides. The microorganism ceparium of Microbiología ambiental del INQUISAL has a bacterium of the *Bacillus* genus isolated from bat guano, which in previous works showed chitinolytic activity and capacity to inhibit phytopathogenic fungi. The objective of this work was to characterize the nature of the metabolites involved in the antifungal activity present in the cell-free supernatant (CFS) of *Bacillus atrophaeus* A14 with antifungal activity. *B. atrophaeus* strain A 14 was cultured in Standard Nutrient (SN) during 72 h. CFS was obtained by centrifugation at 4°C for 15 min at 10,000×*g*. For the characterization of the metabolites of interest, different tests were carried out: (i) extraction with *n*-butanol saturated in water; obtaining two fractions: a butanolic and an aqueous fraction; (ii) acid precipitation, obtaining two fractions: one soluble in acid and the other insoluble in acid, with subsequent dissolution in methanol; (iii) precipitation with ethanol and subsequent dissolution in water. The antifungal activity of each of the fractions was determined by diffusion in agar on Petri dishes containing the potato-glucose agar, using *Colletotrichum acutatum* (a causal agent of anthracnose), as a reporter fungus of the antifungal activity in several crops. The plates were cultured for 72 h at 30°C. The activity was visualized by the absence of fungi growth around the well with fraction under study. In parallel, the following controls were used: n-butanol; methanol; ethanol and acidic water (pH 1.0). None of the controls presented antifungal activity indicating that the activity obtained by the fractions were due to metabolites present in the CFS of *B. atrophaues* sp. A14. The antifungal

activity was found mainly in the butanolic fraction and in the acid precipitate dissolved in methanol. Subsequently, the fractions that presented antifungal activity were used in the bioautography assay on silica plates. *C. acutatum* spores were used as reporter fungus. After incubating the plate at 72 h and 30°C, the inhibitory effect of the metabolites was observed and the Rf was estimated. Parallel these fractions were analyzed by means of ^{1}H-NMR. From the results of this work, it is concluded that the metabolites with antifungal activity of *B. atrophaeus* A14 CFS belong to the lipopeptide family due to their solubility in butanol and methanol, and insolubility with HCl. The results obtained by ^{1}H-NMR and bioautography agree with those reported in the bibliography for Fengycin. Fengycin is a cyclic lipopeptide that can adopt and mimic secondary structures (loops and even vesicular) with surfactant nature, conferring selective antimicrobial activities. It is planned to deepen these studies in the future and apply them to the control of diseases caused by fungi in the context of an integrated pest management strategy.

A238
BACTERIAL NANOCELLULOSE APPLICATION IN TRANSDERMAL THERAPEUTIC SYSTEMS DESIGN

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Transdermal Therapeutic Systems (STT) are pharmaceutical forms designed to achieve a prolonged therapeutic effect at a single dose, through the continuous release of the drug in a certain period of time. Excipients play a key role as release control is regulated by the materials that make up the system. The pharmaceutical industry evolution demands the exploration of new biomaterials for the production of drugs with efficiency *in vivo* performance. Products of bacterial origin have a plethora of advantages over natural, semi-synthetic and chemical synthetic products. They represent a cleaner and more ecologically friendly production. Among them, bacterial nanocellulose (NCB) stands out, which has improved physicochemical properties to its vegetal origin homonym, in which it has a greater swelling capacity, mechanical resistance and biocompatibility. Within this framework, we develop controlled release carrier matrices to convey Active Pharmaceutical Ingredients (API) from NCB excipients. The matrices will comply with the premises: low cost, stability, security, ease of handling and application, ensuring a successful transfer of the product. *Pseudomonas fluorecens* SBW25 was used as the NCB-producing strain. Growth and production conditions were optimized. In the pre-formulation study, the components of the STT matrices were characterized and those with desirable physicochemical and technological properties were selected. To perform the release studies, a pure drug model was designed. To assess API release, the *in vitro* release assay of Sesto Cabral *et al*. (2015). The versatility of NCB made possible the vectorization of APIs in wide pH ranges. *In vitro* release assays and a theoretical study of the pharmacokinetic behavior of API, the concentration at the site of action was adjusted at the appropriate time for treatment and a sustained release was foreseen. Due to its high chemical stability, biocompatibility, and adaptability to the combination with other NCB polymers, it is an interesting, novel and versatile alternative for the manufacture of pharmaceutical matrices.

A239
EFFECT OF VARIOUS PLASTICIZERS ON BACTERIAL NANOCELLULOSE BASED FILMS

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The use of edible films and coatings based on biopolymers gained popularity in the food industry due to their biodegradability, their potential to avoid food alteration and the generation of products derived from renewable natural sources. Bacterial nanocellulose (BNC) provides mechanical resistance and low oxygen transmission rate in edible coatings. Objective: to elaborate and characterize edible films based on hydroxypropylcellulose (HPMC) and BNC using a variety of plasticizers. BNC was obtained by static culture of *Pseudomonas fluorescens* SBW25 in KB medium, from 72 h at 28°C. BNC was washed with 2% NaOH solution, autoclaved and dried at 105°C. Filmogenic solutions were designed using glucose (GLU), glycerol (GLI) and polyethylene glycol (PEG) 400 as plasticizers in concentrations of 0.2, 0.5, 1, and 1.5% with and without plasticizer. Films were formed by a casting method at 37°C. Solubility, humidity, swelling, optical and organoleptic properties were measured. The addition of the 1% PEG and 1% GLI plasticizers improved the flexibility and integrity of the films, they presented better percentage of Humidity than control, 33% and 20%, respectively. Films with 1% GLI showed 24% swelling over control. Opacity values were below the control and the UV barrier capacity increased 17% compared to controls. All the films presented high percentages of solubility. Edible films and coatings are an innovation within the concept of biodegradable active packaging; they interact with food, extending its shelf life, improving their sensory or functional properties.

A240
MORPHOLOGICAL CHARACTERS SEGREGATION STUDY IN *Cynara cardunculus* L.

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Cynara cardunculus (2N = 34) is an economic important species in both cultivated forms: var *scolymus* L. (Fiori) (globe artichoke) and var *altilis* (DC.) (Cultivated cardoon). Among the distinctive characters we can mention the presence of spines, intensity of leaf lobing and flower color. Even when some research has been conducted for molecular markers segregations and linkage map constructed, no information is available regarding the joint inheritance of these characters. A uniform material for absence of spines in leaves, entire lamina, and blue flowers (Cultivated cardoon Florensa Seeds Co.) was crossed with a uniform stock for spines in leaves, lobulated lamina, and white flowers. The F1 progeny of lobulated lamina, inermis and blue flowers was selfed obtaining 72 F2 plants that were scrutinized for the three traits. The presence of spines and flower color adjusted the

monogenic inheritance segregation previously reported. Lobed / entire lamina segregated according to a digenic inheritance of a double dominance/recessive epistatic (13:3) leaves ($\chi^2 = 1.11$; $P < 0.05$; 1 df). The joint inheritance was only of independence between lobed/entire lamina and presence of spines ($\chi^2 = 3.68$; $P < 0.05$; 3 df). Between presence of spines and flower color, a 35 UM distance was calculated. Among these characters, presence of spines in leaves is the only located in the linkage group (LG) VIII in a consensus map. It is inferred that flower color should also belongs to this LG.

A241
EVALUATION OF ADVANCED PEANUT LINES (*Arachis hypogaea*) FROM THE NATIONAL UNIVERSITY OF RIO CUARTO, ARGENTINE, FOR PEANUT SMUT TOLERANCE (*Thecaphora frezii*)

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Smut is a disease caused by *Thecaphora frezii* with a significant yield impact in the main crop peanut region of central Argentina. Fungicides commonly used in peanut cultivation show little or no effect on disease control, therefore, it is a priority to obtain tolerant genotypes. The objective of this research was to identify smut-tolerant peanut genotypes, within the group of cultivars and higher lines created in the Faculty of Agronomy and Veterinary Medicine, National University of Río Cuarto (FAV-UNRC), by using genotypic values and the GGE biplot. Seventeen peanut genotypes were planted: 12 advanced lines from the FAV-UNRC and 5 commercial varieties (including 2 varieties with high diffusion and proven susceptibility to the disease). Genotypes were evaluated in 5 experimental trials conducted in different environments (combination of locality and crop season: Las Acequias–2016/17, General Deheza–2017/18 and 2018/19, Las Peñas–2017/18 and Reducción–2017/18). A randomized complete block design with three replications was used in each environment. For each genotype, the disease incidence (percentage of affected pods) and severity (estimated as an index on the scale 0–4 scale, which considers the degree of affectation of the pods) were quantified. Linear mixed models were used to estimate genotypic values (BLUP), of genotype (G) and genotype–environment interaction (GE). The first two main components explained 97.5 and 97.4% of the variation in incidence and severity, respectively. The correlation between the BLUPs of the variables was positive and significant. The extreme genotypes were FAVar1, FAVar2, FAVar3, LAx1, LAx7, LAx8, and LAx10. The Reduction and General Deheza environments presented the highest values for the disease variables. The FAVar1, FAVar3, Granoleico, LAx5, LAx2, and Pepe ASEM INTA genotypes showed the highest incidence and severity values, while LAx8, LAx9, FAVar2, LAx10, LAx11 had the lowest values and the greatest stability across the different environments. These materials have the potential to become new varieties or as a source of smut-tolerance in breeding programs.

A242
BIOENGINEERED MATRIX TO REGENERATIVE BONE THERAPIES. BIOLOGICAL EVALUATION

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Bone tissue engineering promotes different materials acting as scaffolds for cell adhesion, facilitating growth and differentiation for the new bone formation. The frames from the third generation have osteoinductive, osteoconductive and/or osteo promoter potential. They are degradable, non-toxic and allow bone defects regeneration. Our work's aim was to evaluate in vivo the regenerative ability from a novel biomaterial created in our lab, to treat critical bone defects (CZD), at calvariae from rabbit. Twenty New Zealand rabbits were used; 6 months old, 3.5 ± 0.500 kg, pre-medicated (acepromazine 1 mg/kg) and anaesthetized (Ketamine 35 mg/kg). A central incision, mucoperiosteal flap and CZD were made by 15 mm trephine, under constant cold sterile solution irrigation. Animals were randomly divided into control group (CG) and experimental group (EG). The EG defects were treated by the novel biomaterial. Euthanasia was performed at 45 and 90 days, respectively. Samples were evaluated by Cone Beam computed tomography (CBCT) XG Sirona, 14" exposure time, 83 kV and 8 Ma, window 12 × 8 cm. They were analyzed at 1mm thick sections and then adapted for multiplanar reconstruction. Histopathological light microscopy studies were carried out on preparation obtained after oriented sections, from tissues decalcified and stained by H&E. Digital images were obtained with Sony SC50 camera adapted to Olympus BX43 microscope, using Soft CellSens 1.16 (Life Science Imaging Software). In addition, a morphometric analysis was performed using Image ProPlus software. The statistical analysis of radiometric and histomorphometric data was made by Kruskal Wallis test (Minitab 17). The CBCT results at CG 45d and 90d were 20% and 32% of bone regeneration, respectively. While at EG were 43% at 45 days and 73% at 90 days, the new bone formation obtained. Histomorphometric bone neoformation results from CG at 45 days and 90 days, were 16% and 22%, respectively. And EG obtained 45% at 45 days and 85% at 90 days, new bone formed. No significant differences were found between both selected groups ($P > 0.05$). The results validate the bone regenerative potential from our bioengineered matrix, supporting its use for bone absence treatment from various origins. *Ethics Committee CICUAL-UNT approval. Res 23/2017. Subs. PDTS N12, PIP 864, PIUNT J615. Key Words: rh-PTH/collagen membrane, bone regeneration, rabbits.*

A243
EFFECT OF SPERM SELECTION BY ATTACHMENT TO HYDROGELS ON BIOLOGICAL PARAMETERS OF PIG SPERMATOZOA

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PNIPAM hydrogels copolymerized with APTA-15% (N-isopropylacrylamide co-3-(acrylamidopropyl) trimethyl ammonium chloride) which gives it positive charges. These biomaterials are gaining great importance in the biomedical field due to their low cellular toxicity and versatility of application. The aim of this work was to evaluate the effect of the sperm selection process using PNIPAM co-APTA-15% surfaces on pig sperm. To accomplish this, the pig sperm were exposed to the surfaces of PNIPAM co-APTA-15% hydrogels, in TALP-Ca^{++}–Albumin medium for 30 min, and subsequently the medium was replaced by TALP-medium without Ca^{++}–Alb. TALP-Ca^{++}–Alb medium was used to stimulate sperm binding to the hydrogel and TALP- without Ca^{++}–Alb, to promote the release of spermatozoa from PNIPAM co-APTA-15% surfaces. The effect of sperm manipulation on motility, morphology, sperm membrane integrity, viability, acrosomal reaction and sperm cell death was evaluated throughout the selection process. The analysis of these parameters was included in the initial sample, postcentrifuge, sperm not adhered to the hydrogel and sperm cells released from the biomaterial. Results were statistically analyzed by one-way ANOVA and Bonferroni as a post-hoc test ($P \leq 0.05$). These results suggested that the sperm quality is maintained with high quality to be used in assisted reproduction techniques. Sperm motility was maintained in an acceptable range from good to very good (70–80%). Furthermore, it was found that the PNIPAM co-APTA 15% hydrogel does not cause any alteration in viability, maintaining 66.67 ± 6.67% of viability in released sperm, a hypoosmotic swelling of 73 ± 3.54% after its release, a low percentage of sperm with an acrosomic reaction and sperm morphology within the desired parameters. In conclusion, these results suggest that 15% APTA hydrogels are a biomaterial with the capacity to be used as a method of swine sperm selection for subsequent use in assisted reproduction techniques

A244
IN SILICO DESIGNING OF gRNAs TO GENERATE KNOCKOUT BETA-LACTOGLOBULIN GENE IN BOVINE FETAL FIBROBLASTS

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Gene knockout mutations can create specific gene silencing at the DNA level and play an important role in biological research. It has been shown that CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISP associated system) technology can be an effective tool to edit livestock's genome. To fulfill CRISPR experiment's goals, two components are important: an endonuclease and a guide RNA (gRNA). Both components form a ribonucleoprotein. The gRNA targets a preselected genome site to be edited, so the success of the experiment depends mainly on a correct gRNA design, which will lead to high mutation efficiency and minimize off-target events. Our hypothesis proposes that it is possible to generate cows with beta-lactoglobulin (BLG) gene inactivated by the CRISPR/Cas9 system. Thus, the removal of the BLG in milk could reduce allergic disorders associated with milk consumption by humans. The aim of the present work was to *in silico* suitable gRNAs design that would match DNA sequences on the bovine BLG gene. Related to this goal, the selected region of the gene, to which the gRNAs will be directed, was exon 2. Therefore, a mutation in this exon would produce a truncated BLG, avoiding in this way the production of the allergenic effects when cow's milk is consumed by humans. Co-expression plasmid pSpCas9(BB)-2A-Puro (PX459) V2.0 was chosen (Addgene #62988) for the CRISPR/Cas9 system. PX459 plasmid allows Cas9 and gRNA sequences to be transfected in one-step. CRISPOR program (http://crispor.org) was selected to design the gRNAs. It finds guide RNAs on an input sequence and ranks them according to different scores that predict potential off-targets in the genome of interest and estimates on-target activity. In addition, it provides primers sequences needed for testing guide activity and potential off-targets. It has been shown that CRISPOR is an effective online tool for genome editing experiments with the CRISPR/Cas9 system. Gene Runner program (www.generunner.net) enables the analysis of the gRNAs sequences previously designed by CRISPOR. The secondary structures of the gRNAs, like hairpin loops, dimers, bulge loops and internal loops were studied. The best four of the list of gRNAs obtained by CRISPOR were selected to continue. No secondary structures were present in the selected gRNAs. These results allowed predicting the best gRNAs options before conducting in vitro experiments. Finally, the gRNAs obtained will be used to test knock out efficiencies of this system in bovine cell cultures. The bovine cells can then be used as donors of nucleus in somatic cell nuclear transfer technique.

A245
PHENOTYPIC CHARACTERIZATION OF THE PEANUT (*Arachis hypogaea* L.) GERMPLASM COLLECTION OF THE NATIONAL UNIVERSITY OF RIO CUARTO

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Argentina is the world's leading exporter of edible peanuts and the second largest exporter of peanut oil, being worldwide recognized for the quality of the grain that produces. Germplasm collections are a reservoir of potentially useful genes for research and genetic improvement. Most peanut breeding programs aim to improve the genetic potential for quantitative traits. The use of a small number of populations, lines and/or obsolete cultivars in breeding programs leads to a varieties pool with narrow genetic base. It is essential to detect and incorporate genes from germplasm with high variability to make more efficient the improvement from agronomic traits of interest. However, the available germplasm in genebanks remains

still largely unexplored, therefore the use in breeding programs is limited. The objectives of this study were to phenotypically characterize the peanut germplasm collection of the UNRC genebank and to determine the relationship between accessions, between traits and between accessions and traits. Seventy-nine accessions were evaluated by 17 descriptors under the field conditions from Río Cuarto (Córdoba, Argentine), during the 2018/19 crop season. For the characterization, the list of peanut descriptors suggested by the IBPGR-ICRISAT was partially used. Coefficient of variation showed variability within the collection, corresponding the highest values to the stem, fruit, and seed traits. Principal component analysis (PCA) showed that the first three components explained 70% of the variation for the quantitative plant attributes. The traits that explain the greater variation in PCA include both leaf and plant morphological descriptors and maturity, fruit, and seed descriptors. Cluster analysis resulted in a dendrogram where the accessions are grouped into two main groups. This research work showed a wide variation for the evaluated traits, at the same time that it allowed to identify potential parental genotypes for the development of new advanced commercial cultivars.

A246
DIFFERENTIAL EFFECT OF LIGHT ON THE SYNTHESIS OF INDOLE ACETIC ACID AND PHENAZINE-1-CARBOXYLIC ACID IN *Pseudomonas aurantiaca* SR1

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The development of biological products containing beneficial microorganisms for agriculture tends to use various strategies to broaden their spectrum of action and improve inoculation under unfavorable environmental conditions. The objective of this work was to evaluate the effect of light, such as different wavelengths on growth and the ability to synthesize indole-3-acetic acid (IAA) and phenazine-1-carboxylic acid (PCA), two metabolites of great importance for *Pseudomonas aurantiaca* SR1, a plant growth promoting bacteria (PGPR) used as an active principle in the formulation of inoculants in Argentina. To do this, *P. aurantiaca* SR1 was incubated in TSB liquid medium (25% v/v) for 24 h at 30°C and 180 rpm and darkness until reaching an initial growth of OD_{595} 0.1. At this time, the culture was fractionated under sterile conditions into 20 mL aliquots in sterile Petri dishes that were incubated at 30°C without shaking, in a culture chamber under exposure to white light (56 $\mu W/mm^2$); PAR38 blue (11 $\mu W/mm^2$) and PAR38 red (13.9 $\mu W/mm^2$). A treatment maintained in dark conditions was used as a control. At exposure intervals of 24, 48, and 72 h, biomass production (OD_{595}) and growth (CFU/mL) were determined. IAA concentration was evaluated by spectrophotometry (µg/mL) and PCA production was evaluated quantitatively. The results indicated that only the presence of blue light caused an increase in the number of cells (CFU/mL) of *P. aurantiaca* SR1 after 72 h of exposure, while at 48 h no significant difference was observed with the other treatments. The biomass production did not undergo modifications in any of the evaluated conditions. At the level of IAA and PCA biosynthesis, a higher production of the hormone and a lower production of the pigment were determined by exposure to white and blue light, compared to the treatment exposed to red light and darkness, where the biosynthesis of the PCA pigment was stimulated. These results suggest that white and blue light act as positive effectors for IAA biosynthesis while red light and dark do for PCA. Understanding the bacterial response to light is a poorly studied model in non-photosynthetic bacteria such as *P. aurantiaca* SR1. The use of this microorganism as an active principle for the formulation of bio-inputs in Argentina requires a greater understanding of the bacterial response to different environmental effectors, including light, as a necessary strategy to improve the formulation and functionality of such products under agronomic conditions. In this work, we were able to establish that different wavelengths determine a differential physiological response on the production of a phytohormone (IAA) and a pigment (PCA), considered key to the functionality of the microorganism and its ability to promote growth in interaction with plants.

A247
PROXIMAL ANALYSIS OF THE FUNGUS BIOMASS CULTIVATED ON SUGARCANE VINASSE AND ITS POTENTIAL USE IN AQUACULTURE

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The need to find a comprehensive solution to the problem of contamination with vinasse in the province of Tucumán is a priority that concerns both public and private organizations. Vinasse is an acidic effluent with high organic load and salinity, and its use for obtaining microbial biomass could be an excellent strategy to improve the sustainability of bioethanol plants in the long-term. Filamentous fungi biomass contains large amounts of crude protein with essential amino acids that could be used in aquaculture for feed formulations. Therefore, the objective of the present work was to conduct the proximal analysis of the mycelium of a fungus cultivated on sugarcane vinasse to estimate its possible use in fish farming. The microorganism was isolated from a soil contaminated with vinasse recollected in the province of Tucumán. The sequence analysis of the 18S rRNA gene showed 100% identity with different species of the genus *Aspergillus* (accession number NCBI MT165899.1) thereby microorganism was named *Aspergillus* sp. V2. The 96-h-biomass produced on 50% vinasse added with 1 g/L of KH_2PO_4 and 2 g/L of $(NH_4)_2SO_4$ was washed with distilled water and was lyophilized to determine total proteins by the Kjeldahl-Arnold-Gunning method using the universal factor of conversion to protein 6.25, total fat (or lipids) by the Soxhlet gravimetric method, crude fiber by the official AOAC method (OMA-Official Methods of Analysis), moisture by heating under reduced pressure, ash by weight difference after calcining the sample, and in carbohydrates indirect form: Total carbohydrates = 100 – (Proteins + Total Fat + Moisture + Ash). Biomass analysis revealed a protein content of 31.7%, 4.72% fat, 15.8% ash, 4.04% crude fiber, 0.1% humidity, and 43.64% carbohydrate. The results obtained demonstrated that the fungus mycelium complies with the basic nutritional properties for aquafeed formulations, with a protein content within the desirable range (from 26% to 55%). The lipid, crude fiber, humidity, ash, and carbohydrates levels were within the standards for aquafeeds reported in the literature. Based on this study, it is concluded that the mycelium of *Aspergillus* sp. V2 produced from vinasse with the addition of nitrogen and phosphorus, constitutes an alternative and low-cost nutrient source giving added value to a local residue and thus helping to care for the environment.

A248

ANTIBIOFILM ACTIVITY OF *Schinus fasciculatus* ANTIBACTERIAL EXTRACT AND ITS COMPONENTS

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Phytopathogenic bacteria have the ability to adhere to and colonize plant tissues using biofilms. The formation of these bacterial films can be affected by the presence of bactericides in sub-lethal concentrations, something that commonly happens when the application of these compounds is not homogeneous on the surface of the plant. In this context, the antibiofilm activity of an antibacterial compound could contribute to the control of plant diseases that produce large losses in crops. We evaluate the biofilm inhibition capacity of an extract of *Schinus fasciculatus* with antimicrobial activity and its components in sub-lethal concentrations. The fAcet foliar extract of *S. fasciculatus* and its components, the flavonoids agatisflavone, quercetin, and kaempferol were tested in previously established sub-lethal concentrations (125–1.9 µg/mL) to determine their ability to inhibit the biofilm formation of 5 strains plant pathogens, *Pseudomonas syringae* pv. *tomato*, *Pseudomonas corrugata*, *Xanthomonas campestres* pv. *vesicatoria*, *Erwinia carotovora* var *carotovora*, and *Agrobacterium tumefaciens*, using the violet crystal microplate assay described by O'Toole. The results were statistically analyzed using the Shapiro-Wilk, ANOVA and Kruskal-Wallis tests using the STATISTICA software, version 7. The inhibition of biofilm was dependent on the bacterial strain and, in a lesser extent, to the compound tested, where *E. carotovora* var. *carotovora* and *A. tumefaciens* were the most susceptible, with inhibitions between 40–80%, while *P. corrugata* and *X. campestres* pv. *vesicatoria* were the least susceptible with a maximum inhibition of 39%. The extract and flavonoids inhibited by 40 to 80% the biofilm formation of the tested bacterial species, so that in sub-lethal concentrations these compounds would be able to attenuate the pathogenicity of the investigated phytopathogenic bacteria.

BIOCHEMISTRY, PHYSIOLOGY AND NEUROCHEMISTRY

A249

BUILDING AN EXPERIMENTAL NUTRITIONAL MODEL OF OBESITY. EVALUATION OF ANTHROPOMETRICAL AND NUTRITIONAL PARAMETERS.

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The etiology of obesity is multifactorial, and includes genetic, environmental, and dietary factors, where hypercaloric diets play a central role in the development of the disease. It is known that obesity in adulthood can increase the risk of suffering neurodegenerative diseases. As a part of an institutional project that studies obesity as a base disease for the development of chronic age-associated diseases and the search for early biomarkers with predictive potential, one of our general objectives is to establish a nutritional model of obesity in rat. Particularly, the objective of this work was to evaluate the effects of a high saturated fat diet on different anthropometric and nutritional parameters. Male Wistar rats weaned at 21 days of age were fed with a normocaloric diet (ND) containing 366 kcal from lipids/kg diet. At 2 months old, they were randomly separated and fed with the NC diet (Control group) and a high saturated fat diet containing 1570.7 kcal from margarine/kg diet (HFD group) for the following 14 weeks. Animals were maintained under 12 h light:12 h dark and 22–24°C conditions, with food and water *ad libitum*. The anthropometric profile included the evaluation of food intake, body weight, body mass index (BMI), weight gain, dietary consumption, and Lee index, throughout the entire treatment period. The following nutritional parameters were also calculated: energy-intake, and feed efficiency. Statistical differences between groups and throughout the treatment period were analyzed by two-way ANOVA, followed by Bonferroni *post-hoc* test, with $P < 0.05$ to confirm significant differences between groups and weeks. Our results show that feeding HFD resulted in significant increases in the following anthropometric parameters: body weight ($P < 0.001$ from the 9th to the 21st week), BMI ($P < 0.05$ from the 14th to the 22nd week), weight gain ($P < 0.05$ from the 15th to the 22nd week) as well as in the nutritional parameter: energy-intake from lipids ($P < 0.001$ from the 9th to the 22nd week). We did not observe significant changes in food intake, Lee index, feed efficiency nor in the total energy intake. Thus, we could conclude that a high saturated fat, from margarine, diet modifies key anthropometrical and nutritional parameters, and it could be used to establish a nutritional model of obesity in rat.

A250

CAN CALORIC RESTRICTION IMPROVE COGNITION IN AGING RATS?

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Caloric restriction (CR) consists of reducing daily calories intake without causing malnutrition. CR is the most effective non-pharmacological intervention in increasing longevity and reducing the effects of normal and pathological aging. Memory loss and cognitive impairment are one of the main features of aging and the effect of CR on these cognitive functions are still under study. Previous behavioral and molecular studies of our group showed that old animals had a low cognitive performance and loss of temporal expression of BDNF and TrkB, two proteins strongly linked to memory and learning processes, in hippocampus. Furthermore, we also demonstrated that CR treatment in old animals restored these temporal patterns of BDNF and TrkB in the hippocampus. Due to these antecedents, in the present work our objective was to evaluate if this restoration we previously observed at a molecular level is related to improvements in the cognitive performance of older animals under CR. Male Holtzman rats

were separated into three experimental groups: young *ad libitum* (3-month-old, Y-AL group, N = 10), older *ad libitum* (22-month-old, O-AL group, N = 10), and older subjected to a 40% CR treatment during the last 3 months prior to the 22 months of age (O-CR group, N = 5). Cognitive performance was assessed using the Barnes Maze (BM) test for spatial learning and memory and the New Object Recognition (NOR) test for contextual learning. In the BM test, we observed that the O-CR rats presented a shorter distance traveled on the platform, similar to Y-AL group. We did not find significant differences between O-CR and O-AL animals in the rest of the parameters analyzed with BM test (exploratory frequency of the target region, total exploratory activity, numbers of errors in reaching around the target hole, escape box latencies, percentage of exploration of the meta holes). In the NOR test, again we did not find significant differences between old animals and those subjected to CR. To date, the studies carried out on the effects of CR on cognitive functions are inconclusive and depend on the used protocol. The effects of CR depend on its intensity, the period of life in which the treatment begins and its duration. Our studies are preliminary, with a first group of animals in CR (N = 5), therefore increasing the number of studied animals could provide more conclusive data. CR could be a non-pharmacological alternative for maintaining mental and cognitive health during aging.

A251
CONTENT OF ZINC, MACRONUTRIENTS AND FIBER IN MENUS OFFERED IN ELDERLY HOMES. "MACA ANDINA" AS A SUPPLEMENTATION PROPOSAL IN THE FACE OF ZINC DEFICIENCY

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The biochemical and physiological changes that accompany the aging process, associated with the components of institutionalized life, have clear implications for the nutritional status of the elderly, making them more susceptible to nutritional deficiencies. The purpose of this research was to know the average nutritional values of zinc, macronutrients and total fiber of lunches and dinners offered to the elderly between 75 and 90 years old, who reside in long-term accommodation centers in the province of San Luis, Argentina, and furthermore, in case of zinc deficiency, suggest supplementation with "maca andina" (*Lepidium meyenii*). The study was carried out in two stages, one of them included an observational design with a cross-sectional correlational descriptive scope, and the other one involved an experimental design, being the sampling of probabilistic and multistage type. The sample was made up of 44 menus, which were classified into menus without meat (N = 22) and menus with meat (N = 22). The nutritional composition of all the menus (with and without meat) was analyzed using the SARA software. In addition, an experimental analysis of the meat-free menus was carried out (for which 3 types of menus were taken) and "maca andina" was also analyzed in triplicate, using the corresponding analytical techniques. The composition per serving of the meat-free menus was: 497.69 kcal, 58.26 g of carbohydrates (CHO), 15.81 g of proteins, 21.59 g of total lipids, 6.31 g of total fiber, and 2.29 mg of zinc. In the menus with meat, the average nutritional composition per serving was: 542.97 kcal, 49 g CHO, 27.76 g of proteins, 22.23 g of total lipids, 5.29 g of total fiber, and 4.31 mg of zinc. In relation to the recommendations established for dining rooms for the elderly, the meat-free menus covered 86.88% of kcal, 70.12% of CHO, 95.91% of proteins, 142.1% of lipids, 77.01% of total fiber, and 80.09% of zinc. Menus with meat contributed 86.17% of kcal, 53.72% of CHO, 160.7% of proteins, 113% of lipids, 63.69% of total fiber, and 151.3% of zinc. It was observed that both the composition and the percentage of protein and zinc adequacy was significantly higher in the meat menus ($P < 0.05$). Zinc deficiency was observed on meat-free menus. The experimental composition of the analyzed meat-free menus was as follows: menu n° 1 showed a deficit of all its components except for lipids, menu n° 2 showed adequate fiber and zinc coverage and menu n° 3 showed adequate caloric and zinc intake. Finally, when analyzing the zinc content in "maca andina", it was found that it provides 18.58 mg zinc/100 g; therefore, zinc deficient menus would cover the recommendations for this trace element with one tablespoon (15 g) of it. Due to its easy access and high nutritional value, "maca andina" could be beneficial to supplement diets deficient in this trace element.

A252
EFFECT OF A PPARγ SYNTHETIC AGONIST ASSOCIATED WITH RETINOIC ACID ON 24-HOUR RHYTHMS IN THE HIPPOCAMPUS OF AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most frequent cause of dementia in the older adults. The main pathogenic mechanism in sporadic AD is the decrease in amyloid beta peptide (Aβ) clearance. It is known that Apolipoprotein E (Apo E) modulates Aβ deposition and clearance. ApoE expression is transcriptionally induced by PPARγ in coordination with RXRs. Previously, we found that an intracerebroventricular injection of Aβ(1-42) modified the daily rhythms of Apo E, Bmal 1, and Aβ in the rat hippocampus. Taking into account those observations, the objective of this work was to investigate the effects of synthetic PPARγ agonist, pioglitazone, and retinoic acid (Pio-RA) on the 24-h rhythms of Apo E, BMAL1 and Aβ protein levels, as well as on the daily rhythms of brain-derived neurotrophic factor (Bdnf) and its receptor (TrkB) expression in the rat hippocampus. In this study, male Holtzman rats from control, Aβ-injected (Aβ) and Aβ-injected treated with Pio-RA groups were euthanized throughout a 24-h period and hippocampus samples were isolated every 6 h. Apo E, BMAL1 and Aβ proteins levels were analyzed by immunoblotting and Bdnf and TrkB mRNA levels were determined by RT-PCR. Regulatory regions of Apo E and clock genes were scanned for E-box, RORE, RXRE and PPRE sites. We observed that the treatment of Pio-RA reestablished the daily rhythms of Apo E, Aβ, BMAL1 protein, and Bdnf mRNA levels. This treatment also increased Bdnf and TrkB levels. We found E-box, RXRE, and PPRE sites on regulatory regions of Apo E and Bmal1 genes. The results of the present study could suggest that the treatment of Pio-RA would not only restore the altered rhythmic patterns of the clock genes and their target genes observed in animals injected with Aβ aggregates, but also, interestingly, would increase the levels of cognition-related genes, which are decreased in Alzheimer's patients.

A253

EFFECTS OF PIOGLITAZONE-RETINOIC ACID ON THE 24H RHYTHMS OF COGNITION-RELATED FACTORS IN AN EXPERIMENTAL MODEL OF ALZHEIMER DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive decline of cognitive function and also disruption of circadian rhythms. Synthetic PPARγ agonists such as pioglitazone have been shown to improve cognitive performance in patients with AD. Previous studies indicate that retinoic acid rescues memory deficits in an Alzheimer's disease model. Previously, we found that an intracerebroventricular injection of Aβ(1-42) modified the daily rhythms of cognition related factors in the rat temporal cortex (TC). We also found E-box sites on regulatory region of Bdnf and TrkB genes. Taking into account those observations, the objectives of this study were: first, to investigate the effects of pioglitazone-retinoic acid (Pio-RA) on the daily rhythms of Bdnf and Trkβ expression, as well as on the 24-h rhythms of clock protein levels; second, to evaluate the effect of Pio-RA on cognitive performance. Four-month-old male Holtzman rats were divided into three groups defined as: (1) control, (2) Aβ-injected, (3) Aβ-injected treated with Pio-RA. Rats were maintained under 12 h light:12 h dark conditions and received water and food *ad libitum.* Bdnf and Trkβ mRNA levels were determined by RT-PCR and clock protein levels were analyzed by immunoblotting in TC samples isolated every 6 h throughout a 24-h period. Regulatory regions of Bdnf and TrkB were scanned for RXRE and PPRE sites. The cognitive function was evaluated by Barnes test. We found that the day-night oscillation of factors related to cognition was maintained in the Aβ-injected animals treated with Pio-RA and that the treatment increased the levels of Bdnf and its receptor mRNA levels. Such an increase could be fundamental for synaptic plasticity. We also observed that treatment of Pio-RA reestablished the daily rhythms of the clock's protein and improved cognitive disorders. These findings suggest that the administration of Pio-RA would be a novel therapeutic strategy for Alzheimer's disease.

A254

GLUCOSE HOMEOSTASIS IS ALTERED IN KISS1-GABAB1RKO ADULT MALE MICE

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Kisspeptin (Kiss1) neurons co-express GABAB receptors (GABABR) and GABA is an important regulator of their physiology. *Kiss1* expression is a key factor in the control of reproduction and is involved in metabolic control. We developed a new strain of mice with specific deletion of GABAB1R in *Kiss1* cells/neurons (KO). KO males have similar body weight compared to WT; however, they have an increase in postnatal ano-genital index (AGI), increase in arcuate and decrease in anteroventral periventricular nucleus *Kiss1* expression. Here, we confirmed the specific deletion of GABAB1R by double immunofluorescence and we determined different parameters in WT and KO adult males: (a) AGI and fertility; (b) non-fasted (NFG) and fasted (FG) glycaemia; (c) glucose (GTT) and insulin (ITT) tolerance tests. AGI and fertility in KO were similar between genotypes. While KO males had normal NFG, they had higher FG, altered response to glucose (GTT) and lower insulin sensitivity (ITT). In conclusion, the lack of GABABR in *Kiss1* cells alters glucose homeostasis in adult male mice. We need to investigate whether these alterations have a central or peripheral origin, or both. *(CONICET/ANPCYT/ISN-CAEN/UBA/ René Barón F./ Williams F.)*

A255

THE PLANT UREASE "JACK BEAN UREASE" ALTERS OOGENESIS IN THE CHAGAS DISEASE VECTOR *Rhodnius prolixus* (HEMIPTERA: REDUVIIDAE)

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Ureases are enzymes that catalyze the hydrolysis of urea in carbon dioxide and ammonia. In the last decades, it was postulated that plant ureases are also defense proteins against phytophagous insect species, thus presenting biotechnological potential. Previous reports of our group and collaborators demonstrated that the injection of "Jack Bean Urease" (JBU), the main urease isoform of the leguminous *Canavalia ensiformis* in the hemocele of triatomine insects elicited different toxic effects, including the activation of the immune response. Even though the insecticidal effect of JBU was described more than fifteen years ago, several aspects of its mechanism of action and target organs are not completely understood. In particular, the effects of this urease on the reproductive system of females and the consequences of sub-lethal doses were not studied yet. In this work, we employed the Chagas disease vector *Rhodnius prolixus* as a model in order to study the effects of JBU on the survival, ovarian development and oviposition of females. Firstly, it was standardized an injection protocol to obtain control insects without affecting their survival and oviposition capacity. For that purpose, control individuals without injection *vs*. control individuals injected with vehicle were compared after receiving a blood meal. Thereafter, the assays were conducted comparing control insects injected with phosphate buffer *vs*. problem insects injected with different doses of JBU in phosphate buffer. The results showed for the first time that a sub-lethal dose of JBU alters different reproductive parameters of *R. prolixus* females. All tested doses (0.01, 0.025, and 0.05 µg of JBU/mg of body weight) significantly diminished the number of eggs and the highest tested dose of 0.05 µg/mg delayed the beginning of oviposition and hatching. Nevertheless, only the dose of 0.01 µg of JBU/mg did not result in insect mortality. In the case of this sub-lethal dose, the diminution of the number of eggs resulted in an extended longevity of the insects. On the morphological level, it was shown that the ovaries of JBU-treated insects were less developed and presented atretic follicles. Future studies will be performed to unravel the cell death mechanism(s) that participate in JBU-induced follicle degradation. Taking into account the relevance of reproduction and oviposition in the population dynamics of pest insects and disease vectors, our findings reveal a new aspect of the entomotoxic effect of JBU that reinforces its importance as a promising tool to control harmful species.

A256
LONG-TERM EFFECTS ON HIPPOCAMPAL PROBDNF FOLLOWING SUCROSE CONSUMPTION IN JUVENILE VERSUS ADULT RATS

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Excessive consumption of sucrose in early stages of development has deleterious neurobiological and behavioral effects in adulthood. We previously reported difficulties in memory retrieval. Here, we examined the proBDNF expression in the ventral hippocampus (vHIP) and media prefrontal cortex (mPFC) of animals exposed to sucrose during youth (SY) or adulthood (SA) by Western blot. Two-way ANOVA showed significant differences among groups in the vHIP ($Fi(1,16) = 13.456$, $P = 0.003$). Animals SY showed a decrease pBDNF levels (Fisher's LSD post hoc test, $P = 0.035$) while animals SA showed a raise of these values (Fisher's LSD post hoc test, $P = 0.013$).When all animals were considered, the proBDNF levels correlated positively with the exploration ratio in two memory tasks T3 and T4 (one-way ANOVA, $FT3(1,19) = 5.470$, $P = 0.0334$, $r^2 = 0.268$; and $FT4(1,19) = 14.617$, $P = 0.0034$, $r^2 = 0.3076$) indicating that higher levels of proBDNF corresponds to better memory response. No differences in proBDNF levels were found in the mPFC (two-way ANOVA, $Fi(1,16) = 0.539$, $P = 0.4743$). Taken together, these results show that sucrose affects long-term BDNF expression in vHIP and these abnormalities are different depending on the age of exposure. In addition, it also demonstrates that animals exposed to unlimited consumption of sucrose during youth require higher levels of proBDNF to achieve the same memory response as a control animal in adulthood.

A257
CONSEQUENCES OF PIOGLITAZONE-RETINOIC ACID ADMINISTRATION ON DAILY RHYTHMS OF TNFα, IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder. The neuronal dysfunction and cell death mechanisms that are commonly found in this disease are due to the production of high levels of cytokines, TNFα among them, the formation of amyloid plaques and the alteration of the circadian rhythms. Due to the etiology of AD, multi-target therapies could be more effective. Both PPARγ agonist and retinoids are good candidates for this approach, since they regulate a large number of keys genes and proteins in various pathways, including neurotransmission, Aβ, inflammation, neurogenesis and circadian synchronization, among others. Previously, we found that an intracerebroventricular injection of Aβ (1-42) modified the daily rhythms of TNFα and clock proteins in the rat prefrontal cortex. Taking into account those observations, the objective of this study was, to evaluate the effect of the PPARγ agonist, pioglitazone, along to the RXR ligand, retinoic acid, on the 24-h rhythms of Aβ, ApoE, and clock protein. Four-month-old males Holtzman rats were used in this study. Groups were defined as: (1) control, (2) Aβ-injected, (3) Aβ-injected treated with Pioglitazone-Retinoic Acid (Pio-RA). Rats were maintained under 12 h light:12 h dark conditions with food *ad libitum*. Aβ, ApoE, BMAL1, and RORα proteins levels were analyzed by immunoblotting in prefrontal cortex samples isolated every 6 h during a 24-h period. We found that the treatment of Pio-RA reestablished rhythmicity of clock and TNFα proteins and decreased Aβ levels in the rat prefrontal cortex. These findings could indicate that PPARg-RXR heterodimer might be a potential target for restoration of circadian rhythmicity in neurodegenerative disorders.

A258
BUILDING AN EXPERIMENTAL NUTRITIONAL MODEL OF OBESITY. EFFECTS OF HIGH FAT DIETS ON LIPID PROFILE AND SERUM ENZYMES ACTIVITY

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Obesity is the most common nutritional disorder and is associated with a cluster of chronic metabolic disorders such as dyslipidemia, atherosclerosis, and type 2 diabetes. As a part of an institutional project that studies Obesity as a base disease for the development of chronic age-associated diseases and the search for early biomarkers with predictive potential, one of our first main objectives is to establish a nutritional model of obesity in rat. Particularly, the objective of this work was to investigate the effects of high saturated fat diets on anthropometrical parameters, lipid profile, serum enzymatic activity of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and glucose levels, in rats. For that, male Wistar rats weaned at 21 days of age were randomly separated and fed with a normocaloric (NC) diet containing 366 kcal from lipids/kg diet (control group) or one of two high saturated fat diets, one containing 1570 kcal from margarine/kg diet (HFM group) and other with 1698 kcal from pork fat/kg diet (HFP group), for 12 weeks. Rats were maintained under 12 h light:12 h dark and 22–24°C conditions, with food and water *ad libitum*, during the whole treatment period. Food consumption was recorded daily while animals' weight and body mass index (BMI) were registered weekly. After 12 weeks animals were euthanized, and blood samples were collected. Serum ALAT and ASAT enzymatic activity were determined by kinetic assays while glucose (G), triglycerides (TG), total cholesterol (TC), HDLc and [LDLc+VLDLc] were determined by colorimetric assays. Statistical differences between groups and throughout the treatment period were analyzed by two- or one-way ANOVA, depending on data, followed by Bonferroni *post-hoc* test, with $P < 0.05$ to confirm significant differences between groups and weeks. We observed HFM and HFP diets did not modify anthropometrical parameters nor serum glucose levels, during the whole treatment period, in comparison to the control group. However, interestingly, HFM and HFP significantly increased TG ($P < 0.01$ and $P < 0.05$, respectively), TC ($P < 0.001$ in both cases) and [LDLc + VLDLc] levels ($P < 0.001$ and $P < 0.01$, respectively) as well as ASAT activity ($P < 0.05$), in the rat serum. Our results also show decreased circulating HDLc levels in the HFP group in comparison to the NC group ($P < 0.05$). Thus, we can conclude that feeding rats with HF diets (~400–450% higher

fat's kcal in comparison to NC) during 12 weeks from weaning, induces early metabolic alterations; though, the treatment length, or the animals age, was not enough to generate a nutritional model of obesity.

A259
NAV1.8 RELATION WITH CHRONIC INFLAMMATORY PAIN ON AGING

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Treatment of pathological pain (resulting from damage and inflammation of peripheral nerves and tissues innervated by them) is less effective in older adults. There is little research about the pain mechanisms involved, although there is evidence in rodents that nociceptors excitability differs between young and old ones. Nociception is mediated by primary nociceptive dorsal root ganglion (DRG) neurons, and pathological chronic pain is believed to arise from increased excitability in these nociceptors. This hyper excitability may be attributable to changes in the expression and regulation of voltage gated sodium channels (especially Nav1.7, 1.8 and 1.9). The aim of this work was to determine the expression pattern of Nav1.8 in primary sensory neurons of the DRG in young adult rats (aged 3 to 6 months) and compare it with aged rats (12 to 18 months) and correlate the expression pattern of this ion channel with the behavioral changes observed in a model of chronic pathological inflammatory pain. We quantitatively evaluated the expression of Nav1.8 by ABC/DAB immunohistochemistry in 7-µm serial cryostat sections of L4 and L5 DRGs from Wistar rats aged 3, 6, 12, and 18 months. We induced inflammation with a single intradermal injection of Complete Freund's Adjuvant solution (CFA) in 8 3-month-old and 12 14-month-old rats. We evaluated and followed during 120 days after CFA two types of pain: spontaneous pain, using the spontaneous foot lifting test (SFL) and evoked pain, which results in hypersensitivity to mechanical stimuli (mechanical hyperalgesia) using the von Frey test. Nav1.8 staining intensity in small neurons (area <400 µm^2) was lower in 3-month-old rats compared to 6-month-old rats (36.5 ± 0.9% *vs*. 49.9 ± 1.3%, $P <$ 0.0001), and it was similar when comparing 12 against 18 months (55.3 ± 1.4% *vs*. 55.1 ± 1.4%). There were no differences in staining intensity in medium neurons, while in large neurons, it was lower at 12 months compared to 18 months. On the other hand, the proportion of Nav1.8 positive neurons (intensity ≥40%) tended to increase with age, from 35% at 3 months to 69% at 18 months (P = 0.0368). We observed that aged rats showed a faster reversal of the SFL phenotype compared to young ones (21 *vs*. 28 days for young rats), although its intensity was higher to begin with. On the other hand, the reversal of mechanical hyperalgesia was slower in aged rats (49 *vs*. 21 days in young rats). In both groups, hypoesthesia manifested after 77 days. Based on these findings, lower Nav1.8 expression in young rats associates with lower intensity of SFL events along with faster reversal of mechanical hyperalgesia. We propose that a higher expression of Nav1.8 would be related to the persistence and intensity of pain in aged individuals.

A260
IN VITRO EFFECTS OF BPA, BP2 AND BP3 ON CELL PROLIFERATION IN A MATURE GNRH NEURONAL CELL LINE, GT1-7 CELLS

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Previously we showed that the in-vitro exposure to BPA, BP2 y BP3, endocrine disruptors, and E_2, (1×10^{-7} and 1×10^{-9} M, 24 h) increased cell proliferation in an immature GnRH cell line, GN11 cells (Susan Wray, USA). The aim of this study us to evaluate the effects of the in-vitro exposure of the aforementioned compounds on cell proliferation in GT1-7 cells (mature GnRH neurons, Pamela Mellon, UCSD, USA). Cell proliferation was evaluated using a Non-Radioactive Cell Proliferation Assay, MTS (Promega, WI, USA), after BPA, E_2, BP2 and BP3 exposure (1×10^{-7} and 1×10^{-9} M, 24 h). We also evaluated if the estrogen receptor antagonist ICI 182780, (1×10^{-6} M) was able to block the effects. Results were recorded as Abs490/Abs490 (Control), presented as mean ± SE and analyzed by repeated measures ANOVA with a Fisher post test (Statistica, StatSoft, OK, USA). Neither BPA nor E_2 modified cell proliferation (ANOVA ns, N = 5). BP2 did not modify cell proliferation either, but BP3 increased cell proliferation compared to control values [Control = 1 ± 0.03; $BP2^{-7}$= 0.92 ± 0.12; $BP2^{-9}$ = 0.94 ± 0.11; $BP3^{-7}$ = 1.29 ± 0.13; $BP3^{-9}$ = 1.29 ± 0.09; Repeated measures ANOVA $P < 0.05$, $BP3^{-7}$ and $BP3^{-9}$ different from Control $P < 0.05$, N = 5]. The estrogen antagonist ICI 182780 only blocked the effects of $BP3^{-9}$ (Repeated measures ANOVA $P < 0.05$, N = 5). The results obtained show that exposure to ED have different effects on mature and immature GnRH neurons. This reinforces the notion that effects of the exposure to ED depend on the developmental period, among other factors.
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A261
PRENATAL D-AMPHETAMINE EXPOSURE ALTERS THE HYPOTHALAMUS PITUITARY AXIS RESPONSE THAT REGULATES PRL SECRETION IN ADULTHOOD. INVOLVEMENT OF STRESS AND SEXUAL STEROIDS

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Prenatal amphetamine exposure (PEA) induces long-lasting changes that are evident even in adulthood. D-amphetamine is a stimulant of CNS and acts on the dopaminergic and noradrenergic systems. Prolactin (PRL) synthesis and secretion is regulated by an inhibitory hypothalamic tone exerted by tuberoinfundibular dopaminergic neurons (TIDA). Dopamine (DA) is synthesized by tyrosine hydroxylase (TH) and released into portal blood to act on pituitary dopaminergic receptors (D2R) to inhibit PRL. Stress and sex steroids modulate PRL release, and PRL regulates its own secretion

through a negative feedback that acts on hypothalamus and pituitary gland. Our aim was to evaluate the effect of PEA on PRL secretion in adult male and female ovariectomized/ovariectomized plus estradiol (OVX/OVX + E2) Wistar rats under basal and stress conditions and its interaction with the dopaminergic system. Female rats were treated daily with D-amphetamine 2.5 mg/kg i.p./saline (SAL) during days 15 to 21 of pregnancy. Their female offspring were OVX at day 60 under anesthesia (ketamine/xylazine) and treated 15 days later with estrogen/oil (E2; 2 × 5 µg/rat/24 h). Male and (OVX/OVX + E2) female offspring, were exposed to immobilization stress for 30 min. After sacrifice, blood and tissue samples were collected for PRL measurement by RIA, and pituitary PRL content, D2R and prolactin receptor long isoform ($PRLR_L$) by real time PCR and Western blot (WB). Phospho- tyrosine hydroxylase (p-TH-Ser 40) expression was determined by WB in medial basal hypothalamus (MBH) extracts. Comparative CT method was used, and RNA expression was normalized with respect to S16 gen (mean ± SEM; N = 6–8). Data were analyzed using two-way ANOVA and Student's *t*-test. Serum PRL levels increased significantly in response to stress in male and OVX + E2 *vs*. control (SAL) rats, and PEA prevented this rise. E2 increased pituitary PRL expression (PCR) in basal and stressed conditions, and PEA reduced PRL content (WB) in OVX and OVX + E2 rats. Basal male D2R expression was lower than in females, and E2 treatment of OVX rats reduced D2R expression in PEA groups in terms of RNA and protein both in basal conditions and in response to stress. PEA and stress did not modify male $PRLR_L$. However, stress reduced $PRLR_L$ expression in OVX and OVX + E2, and this effect was prevented by PEA. Moreover, stress reduced MBH p-TH in PEA OVX + E2 rats. In conclusion, prenatal amphetamine exposure may deregulate hypothalamus-pituitary axis, affecting PRL synthesis and secretion. E2 treatment may sensitize this effect.

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