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LECTURE

A1
ATHEROSCLEROSIS: FROM CAVEMAN TO COMPUTER ERA

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Atherosclerosis is the leading cause of death in the Western World. Since the Framingham Heart Study, hypertension, smoking and hypercholesterolemia are identified as major risk factors. Years later, diabetes was added to the pathogenesis of atherosclerosis. In addition, a widespread progressive weight gain in recent decades has led to the development of the so-called metabolic syndrome. This disease combines hypertension, dysglycemia and dyslipidemia in a process in which insulin resistance is the common pathophysiological mechanism. Homo sapiens is believed to have developed the bases of such process 50,000 years ago by generating a central fat reserve which would enable the species to survive times of food scarcity. Human fossils show that obesity was not prevalent in our ancestors due to the high energy expenditure they were subjected to. However, Western World humans, while endowed with Paleolithic genes, are required low energy expenditures and the available food is constantly abundant, and is frequently of inadequate quality. This has triggered the current obesity epidemics and the resulting atherosclerotic complications. The thrifty gene hypothesis attempted but failed to explain the syndrome. The thrifty phenotype hypothesis, the inflammatory hypothesis mediated by adipocytokines and other hypotheses have been proposed to account for the origin of the epidemics. However, it must be resolved if insulin resistance has cheated the evolutionary mechanisms, or if scientists have not selected the causal hypotheses properly.

SYMPOSIUM

A2
SEARCHING FOR THE NEXUS BETWEEN TRANSMISSION CYCLES OF *Trypanosoma cruzi* IN ARID ECOSYSTEMS

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Trypanosoma cruzi is the etiologic agent of Chagas disease, a vector-borne zoonosis endemic of the Americas that affects 10 million people and kills more than 10000 every year. The pichi (*Zaedyus pichiy*, Mammalia: Xenarthra), reservoir host for *T.* cruzi, is a small armadillo endemic of arid and semi-arid lands of Argentina and Chile. Pichis have semi-fossorial habits, being omnivores that feed primarily on insects. Pichis are one of the most-hunted species by poachers in Mendoza province, Argentina, which has led to their listing as Near Threatened in the IUCN Red List of Threatened Species. Rural people and their domestic animals constantly interact with them. Pichis are intensely used as a protein source by rural people, sheepdogs hunt them, and goats graze in the habitat of pichis and wild vectors (triatomine bugs) of Chagas disease. Due to interaction between different domestic and wild reservoir hosts and vectors, domestic and sylvatic transmission cycles can interrelate and overlap, providing the pathogens the possibility to migrate from one cycle to the other. We are currently carrying out an eco-epidemiological study in Mendoza. Pichis, goats, and sheepdogs are being screened for *T. cruzi* in three different landscape units: north (Chacoan *monte*), south (Patagonian steppe) and center of the province (ecotone between both ecosystems), whilst laboratory analyses are being carried out at IMBECU (Institute of Experimental Medicine and Biology-CONICET). Our main objective is to study possible mechanisms that could cause the overlapping of both cycles. We believe that a real understanding of the problematic by the resource-poor local communities will contribute to better understand complex public health issues that affect them, and it will also decrease pichi hunting increasing its chances of long-term survival. From the point of view of Conservation Medicine, we seek to understand interplays between the health of rural people, and wild and domestic animals living in arid ecosystems.

A3

TRANSMISSION CYCLES OF *Trypanosoma cruzi* IN THE ARGENTINE CHACO

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Understanding the complex structure and spatiotemporal dynamics of sylvatic transmission cycles of *Trypanosoma cruzi* in heterogeneous environments is a big challenge. The transmission of *T. cruzi*occurs in a great variety of domestic and sylvatic habitats, including a wide diversity of mammal hosts and triatomines. The domestic cycle involves humans, domiciliated triatomines, and domestic dogs and cats, which are the major domestic reservoir hosts in the Americas. The sylvatic cycle includes triatomine bugs and up to 180 species of wild mammals, some of which can act as reservoir hosts in different ecological regions. Our research team has been studying the transmission of *T. cruzi* for 20 years in the Argentine Chaco. During 2002- 2004, in Santiago del Estero we studied the variations in the dynamics and the intensity of sylvatic transmission of *T. cruz*i and examined domestic dogs and cats serologically or by xenodiagnosis, in rural villages under surveillance between 1992 and 2002. Between 2008 and 2011, in Pampa del Indio, Chaco, a longitudinal study was conducted to investigate fluctuations in the occurrence of *T. cruzi* infections, the reservoir host competence and parasite discrete typing units (DTUs) in wild and domestic mammals of an endemic rural area. Through kDNA-PCR and xenodiagnosis for parasite detection, our studies documents that *Dasypus novemcinctus* armadillos in Chaco, *Conepatus chinga* skunks in Santiago del Estero and *Didelphis albiventris* white-eared opossums in both areas, were the main sylvatic hosts of *T. cruzi* and had large reservoir competence. The species were infected by different DTUs. TcIII infections were found in skunks and armadillo species, while TcI were found in opossums, suggesting independent parasite transmission cycles. In Santiago del Estero and Chaco, domiciliated triatomines, dogs and cats from a given house compound shared the same DTUs in most cases, supporting the importance of dogs and cats as domestic reservoir hosts of *T. cruzi*. Supported by UBA, ANPCyT, CONICET.

A4

STRESS HORMONES ASSOCIATED WITH DROUGHT TOLERANCE IN SUNFLOWER

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Drought is an abiotic stress with negative impacts on agricultural production both globally and regionally, but there are crops such as sunflower that have a better performance for this stressful situation. The shift toward more extreme agroecological farming areas in Argentina led to the need to elucidate how the mechanisms activate the drought tolerance as well as provide the tools that contribute to the crop genetic improvement. In this approach were characterized inbred sunflower lines as tolerant and sensitive to water stress in both field, during the growing season, and in laboratory at germination and seedlings early growth, which are critical stages for drought tolerance. I am going to expose about the evaluation patterns morphological and physiological of responses to water stress in tolerant (B71) and sensitive (B59) inbred lines on seedling stage. The B71 line showed better "physiological performance". At hormonal level, changes in the endogenous jasmonates and abscisic acid and its catabolites were observed in both lines. These lines also showed polymorphisms in microsatellites evaluation. The gene expression analyses carried out on DNA chip showed 4186 differentially expressed genes in B59 line and 3631 in line B71, some of which corresponded to the biosynthetic pathways of abscisic and jasmonic acids. Finally, growth and physiological variables were evaluated in seedlings from families F2: 3 (B59 x B71) and (B59 x R432) grown under water stress generated by 400 mM mannitol. Also, different endogenous levels of jasmonic, abscisic and salicylic acids were detected by LC-MS/MS.

A5

GENES FROM WILD TOMATOES TO IMPROVE COLOR, FLAVOR AND FRUIT SHELF LIFE

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The genetic variability in the cultivated tomato for fruit quality traits has been reduced due to intense selection, genetic erosion and changes in production and marketing. In contrast, the large diversity in wild tomatoes is an underexploited resource that can extend the genetic basis to improve the productivity, quality and/or adaptation of crop. Wild species exhibit variability in fruit quality traits as flavor, aroma, color and texture. Interspecific tomato crosses (cultivated x wild tomato) have biological and technological advantages. The large molecular polymorphism (differences at level of DNA) between the parental genotypes can locate many molecular markers in genome regions that control morphological, biochemical or productive differences. In the tomato

breeding program carried out by the Department of Genetics at the FCA-UNR various genetic materials derived from crossing between the Argentine Caimanta cultivar of *S. lycopersicum* and the wild genotype LA722 of *S. pimpinellifolium* (P) have been developed. The generated populations (F_2 generations, Recombinant Inbred Lines (RILs), Second Cycle Hybrids (SCH) and Near-Isogenic Lines (NILs)) are an invaluable resource to study the genetic basis involved in fruit quality traits. DNA molecular markers allowed the characterization of these genotypes and detection of QTLs associated with fruit traits. Some genomic regions detected in P were present in the HSC (obtained among the RILs) and maintained in their segregating F_2 generations. On the set of NILs, unique genomic regions have been introgressed from wild genotype (P) using molecular markers. These studies provide basic knowledge about changes in genomic structure of the cultivated tomato caused by the incorporation of wild germplasm.

A6
THE SUSTAINABILITY EVALUATION OF WILD AND CULTIVATED AROMATIC, MEDICINAL, SEASONING AND DYEING PLANTS (AMSDP)

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Nowadays the concept of sustainability is present in almost every ambit where human activities are developed, with an almost obligatory character in developing proposals and productive activities; taking special interest in the approach of high value vegetable species. In the farming management environmentally committed, regulations have been established and carried out research work in order to highlight the pollution responsibility, the minimization of impacts, the reuse of resources and shared social responsibility. Agroecosystem sustainability of AMSDP plants and the harvest of wild species must be evaluated to give an answer to the aims of each singular case. The Ecology department, of Agronomics Faculty in the National University of Tucumán, developed and is using agroecosystems sustainability valuation models based on 4 pillars: environmental, social, economical and political-institutional. In relation to harvest, management, commerce and manufacturing of wild-harvesting AMSDP plants derivatives, an approximation of international standards implementation such as International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants are pursued. In this way the evaluation is a working tool that leads these productive activities to sustainability.

SHORT COMMUNICATIONS

A7
PROBIOTIC LACTIC ACID BACTERIA AND PREVENTION OF HEMOLYTIC UREMIC SYNDROME BY INHIBITION OF *Escherichia coli* O157:H7

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Hemolytic uremic syndrome (HUS) is a clinical syndrome caused by ingestion of low infective doses (100 bacteria/g of food) of enterohemorrhagic *E. coli* (EHEC) present in contaminated food (meat). Argentina is the country with the highest incidence of HUS in children worldwide. Probiotic lactic acid bacteria are used to prevent contamination of food and stimulate the human immune system. In vitro study demonstrates inhibition of *E. coli* colonies by growth of probiotic lactic prior strains through the method of Reid and an *in vivo* study in mice tries to show that colonization by lactic strains through a diet supplemented avoids the development of enterohaemorrhagic *E.coli* and prevents HUS. The aims are: 1- To know if cultures of *E. coli* (method of Reid or crop with lyophilized) suffers a demonstrable inhibition by halos, compared to a control culture. 2- To demonstrate the usefulness of probiotics as inhibitors of the colonization of EHEC. 3- To reproduce HUS in mice and show some kind of advantage over diet supplemented with fermented milk. So far we have studied 6 *E. coli* O157: H7 (EHEC) producing strain of Shiga toxin and we have noted that inhibition halos are larger when the lactic bacteria are coming from infant stools than strains of yoghurt.

A8
EFFECT OF AGING ON THE CIRCADIAN PATTERNS OF ANTIOXIDANT ENZYMES IN HEART

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Age is a critical component of the cardiovascular disease etiology, and oxidative stress is a key element responsible for the development of age-related pathologies. This study aimed to find out whether endogenous rhythms of catalase (CAT) and glutathione peroxidase (GPx) expression and activity, as well asNrf2 expression and GSH levels are modified in the heart of aged rat. Holtzman rats from young (3-months old) and aged (22-months old) groups were maintained under 12h-dark:12h-dark (constant darkness) conditions, during 15 days before the experiment. Nrf2, CAT and GPx mRNA expression and enzymatic activity were determined by RT-PCR and kinetic assays, respectively, in heart isolated every 4 h during a 24h period. GSH levels were measured by colorimetric assay. Temporal patterns were analyzed by the Chronos-fit software. We observed Nrf2,CAT and GPx expression vary significantly in a 24h periodunder constant darkness conditions (from Chronos-fit: p=0,044, p=0.013, p=0.0047, respectively), with maximal mRNA levels occurring at circadian time (CT) 13:55±00:60, 12:07±00:24, and 12:40±00:00, respectively in the heart of young rats. As expected, circadian rhythms of CAT and GPx enzymatic activity were also observed in this tissue (from Chronos-fit: p=0.0012 and p=0.036, respectively) with their rhythm's acrophases at CT 16:21±00:50, and 21:26±00:36, respectively. We also observed temporal variation of GSH levels (p=0.0042) being maximal at CT 12:19±00:20. Interestingly, aging abolishes the oscillation of endogenous circadian patterns of Nrf2, CAT and GPx mRNA levels, antioxidant enzymes activity, and GSH levels. Understanding the age-related loss of circadian rhythmicity of antioxidant defensesin heart, could lead to advancements into preventive and chronotherapeutic treatment of cardiovascular diseases.

A9
EXPRESSION OF NEI, TH AND TRH-R1 IN HYPOTHALAMIC AREAS OF ADULT MALE RATS: REGULATION BY THYROID HORMONES

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Neuropeptide glutamic acid-isoleucine-amide (NEI) is a 13-amino acid peptide. Both low and high levels of thyroid hormones (THs) modify NEI concentration in hypothalamic areas involved in the regulation of reproduction and certain behaviors in adult male and female rats. NEI activates the dopaminergic system inducing locomotor activity and excessive grooming behavior in the rat. In the present study we explored the effects of THs levels on NEI, Tyrosine Hydroxilase (TH, limiting enzyme of dopamine biosynthesis) and TRH Receptor 1 (TRH-R1) mRNA expression in the peduncular part of the lateral hypothalamus (PLH), preoptic area (POA) and arcuate nucleus (ARC) of male Wistar control (Co), hypothyroid (HypoT: 0.1 g/L PTU in drinking water) and hyperthyroid (HyperT: T_4, 250 µg/kg/day, sc) rats, sacrificed after 21 days of treatment or vehicle administration. Hypothalamic areas were dissected and gene expression was measured by real time RT-PCR. One way ANOVA analysis was performed. PLH NEI expression increased in treated groups ($p<0.05$ vs Co) and remained constant in POA and ARC. HypoT-POA TH mRNA was increased compared to HyperT ($p<0.05$). HyperT-ARC TH expression increased compared to Co ($p<0.05$). No changes in TH levels were observed in PLH. HypoT-POA TRH-R1 levels increased compared to Co and HyperT ($p<0.05$). No changes were observed in the other areas. These results show that NEI and TH mRNAs are located in regions with neuronal terminals but their expression is regulated by THs at the soma level (PLH and ARC respectively). Major changes on TRH-R1 were found in POA. This evidences the complex regulation exerted by THs on different hypothalamic areas and support other results obtained by IF showing the complex interaction of different components involved in the regulation of reproduction and certain behaviors.

A10
VARIATIONS OF PRO-INFLAMMATORY MARKERS EXPRESSION IN ALVEOLAR MACROPHAGES AFTER CASTRATION

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Previously, we demonstrated an increased expression of several pro-inflammatory parameters in lung parenchyma after castration. Alveolar macrophages (AM) infiltrate the site of infection or injury to produce inflammatory mediators (chemokines, cytokines, among others). The antioxidant response element (ARE) is involved in the activation of genes codingfor a number of antioxidant proteins, so Nrf2/ARE pathway is critical

for the regulation of intracellular redox status.Alveolar macrophages produceTNF-α, that promotes endothelial dysfunction and after a blunt chest trauma they have a clear proinflamatory role. We studied if the time of castration (30 and 60 days) affectedinflammation and oxidative stress markers. Male Wistar rats (200 ± 20 g) were separated in 3 lots: controls (Co), castrated (Ca), and castratedsupplemented with testosterone(Ca+T) for five days. After 30 and 60 days rats were killed and lungs were obtained. Previously, bronchoalveolar lavage (BAL) was collected and cell count was performed.RNA was extracted by the method of TRIzol. mRNA levels were quantified using RT-PCR and oxidative stress biomarkers were measured.ANOVA was used for statistical analysis. Cells percentage in BAL at 30 and 60 days, showed an increase of neutrophils (23%) and lymphocytes (25%) in Ca group compared to Co. At 30 days the expression of Nrf-2 and Nox-2 decreased in Ca compared to Co group ($p<0.01$, $p<0.001$), while TNFα did not change. However at 60 days after castration, Nrf-2 and TNFα decreased their expression in Ca group ($p<0.01$; $p<0.001$),butNrf-2 increased inCa+T ($p<0.05$) while Nox-2 did not change. We conclude that castration significantly affected the antioxidant status, especially at 30 days post castration and apparently time modifies the inflammatory balance, at cellular level and pulmonary parenchyma.

A11
EVALUATION OF THE PROTEOLYTIC ACTIVITY OF DIFFERENT TYPE A BOTULINUM NEUROTOXINS OBTAINED FROM A MENDOZA (ARGENTINA) SOIL STRAIN AGAINST NEURAL TARGET PROTEINS

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Botulinum neurotoxin (BNTx) type A, produced by *Clostridium botulinum (Cb)*, is a prevalent serotype in strains from Mendoza soils. Soil (Su) is the main reservoir for *Cb*, and is possibly one of the infectious sources for infant botulism (IB). The high incidence of IB in breast-fed infants with no history of complementary feeding, raises the possibility that soil is an important source of infection. In previous studies we found that cultured strains obtained from Su and from IB cases differ in morphology and also in some BNTx biological properties. In this study we evaluated the proteolytic activity of the different BNTx against target proteins. Rat brain homogenates were incubated with BNTx from either Su or IB and compared with that of the A-Hall strain, under controlled conditions. Proteolysis of SNAP-25 and synaptobrevin was analyzed by Western blot with specific monoclonal antibodies, detected by chemiluminescence. The quantitation of each protein was normalized to actin levels. We observed that both proteins are cleaved into smaller fragments by the Su toxin, and to a lesser extent than with the A-Hall. However, the IB toxin showed low activity against these synaptic proteins. The differential effect of the toxins is consistent with the higher toxicity of Su compared to other strains. The differences between Su and IB toxins imply a more powerful proteolysis-based toxic activity in the former. Interestingly, they could also indicate that the bacterium is highly modified during transit in the infant digestive tract.

A12
OXIDATIVE STRESS AND ANTIOXIDANT DEFENSES IN THE APPLE SNAIL *P. canaliculata* EXPOSED TO HEAVY METALS AND URANIUM

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Biomonitoring 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 volumes, for instance in lakes and streams. The apple snail *Pomacea canaliculata* (Architaenioglossa, Ampullariidae) is a freshwater species original from the Plata basin, which has spread to many places around the world, is highly adaptable and resistant to diverse environmental conditions, shows low individual mobility and has a life-cycle of 1–3 years depending on climate, which make them as a sentinel species. We have shown that the digestive gland (and the symbionts contained therein), the kidney, and the foot of this snail may be useful bioindicators of mercury, arsenic and uranium pollution in freshwater bodies. Here, we have studied lipid peroxidation (TBARs), total antioxidant potential (ABTS), and enzymatic (SOD) and non-enzymatic (GSH and uric acid/allantoin) antioxidants in control snails (no exposed) and snails exposed to mercury (2µg/L), or arsenic (10 µg/L) or uranium (30 µg/L) for 60 days. Interestingly, both mercury and arsenic produced a TBARs decrease in digestive gland and foot, respectively. Uranium produced GSH increase in the kidney. The remaining studied variables did not show statistically significant changes in the studied tissues. These results indicate that this snail is able to reach a physiological steady state between oxidative stress and antioxidant defenses after metallic injury.

A13
TEMPORAL PATTERNS OF ANTIOXIDANT DEFENSES ARE MODIFIED IN THE CEREBELLUM OF AGED RATS

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Motor and cognitive performance declines in older adults have been related to the cerebellum and the biological clock alterations. The presence of circadian clock components in the rat neocortex and cerebellum has been shown recently. The molecular clock activity depends on an optimal cellular redox state. The objectives of this study were to investigate whether the catalase (CAT) and glutathione peroxidase (GPx) enzymatic activity as well as reduced glutathione (GSH) levels display a circadian variation in the cerebellum, and to evaluate whether this circadian rhythmicity is altered in aged rat. Holtzman rats from young (3-month old) and aged (22-month old) groups were maintained under 12h-dark:12h-dark (constant darkness) conditions, during 10 days before the experiment. CAT and GPx enzymatic activity were determined by kinetic assays, GSH levels were assessed espectrophotometrically, in cerebellum isolated every 4 h during a 24h period. CAT activity varied significantly in a 24h period in young and aged rats. However, we observed a phase delay as well as higher amplitude in the circadian rhythm of aged rats. We also observed GSH levels oscillate on a circadian basis in the cerebellum of young animals; such rhythm was lost in aged rats. On the other hand, we didn`t observe a 24h period variation in GPx activity, neither in young or aged rats, although we observed an increase in GPx activity in the cerebellum of 22-month old rats. Modifications in 24h-patterns of antioxidant defenses could lead to oxidative stress and alteration of the circadian clock in the cerebellum of aged individuals.

A14
LOSARTAN INDUCES Hsp70/CHIP INTERACTION AND Nox4 UBIQUITINATION IN PROXIMAL TUBULE CELLS (PTCs) FROM SPONTANEOUSLY HYPERTENSIVE RATS

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The chaperone Hsp70 regulates a diverse set of signaling pathways through its interaction with proteins. CHIP (carboxy terminus of Hsp70 interacting protein), is a cytoplasmic protein that U-box domain contains its E3 ubiquitin ligase activity. CHIP regulates the chaperone function in part by regulating the molecular triage decision and determining whether proteins enter the productive folding pathway or results in client substrate ubiquitination and proteosomal degradation. We investigated Hsp70/CHIP contribution to Nox4 regulation after AT_1R receptor blockade with Losartan, in primary culture of PTCs.PTCs from8-week SHR and WKY rats were stimulated with Angiotensin II (100 nmol/L, 15min)(AII), pretreated with Losartan (100µmol/L, 90min) (L) and with Losartan 75 min plus Angiotensin II15min(L+AII).Losartan, increased Hsp70 and decreased Nox4 protein levels in SHR(L) membranes fraction. Decreased Hsp70 inSHR(L) vsSHR(AII)in cytosolic fraction confirm Hsp70 translocation to membranes. Immunoprecipitation and immunofluorescence confocal microscopy proved interaction and colocalization of increased Hsp70/CHIP that contrasts with decreased Nox4 in membranes from SHR PTCs (L) vs PTCs (AII).PTCs (L) exposed to MG132 blocked the degradation of ubiquitinated Nox4. Immunofluorescence analysis demonstrated Nox4 ubiquitination by increased colocalization of Nox4/ Ubiquitin in PTCs (L) exposed to MG132. Conversely, Hsp72 knockdown PTCs (L) reduced Nox4/Ubiquitin colocalization, resulting in Nox4 up regulation due to the proteosomal degradation inhibition, even Losartan treatment. In conclusion, our data suggest that Hsp70 and CHIP interaction mediates the ubiquitination and proteasomal degradation of Nox4 as part of the antioxidative effect of Losartan in PTCs from SHR.

A15
EXPLORING THE POSSIBLE PHYLOGENETIC RELATIONSHIP BETWEEN PLANT CHLOROPLASTS AND THE C-ENDOSYMBIONT OF AN APPLE SNAIL

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The C morph of the intracellular symbiont of the midgut gland of *Pomacea canaliculata* (Caenogastropoda, Ampullariidae) has some morphological similarities to Cyanobacteria, but contains modified chlorophylls *a* and *b* (pheophorbides) and its genome contains a 16S rRNA gene akin to that of some dicots. This is striking since the endosymbiont has a thick electron-dense wall (suggestive of bacterial nature) while plastids have no wall (with the only exception of the "cyanelles" of Glaucophyta). Here we explored the presence in the C-endosymbiont of: (1) starch, by Lugol staining; (2) glycosaminoglycans, by Alcian Blue staining; and (3) chloroplast genes as *trnK/matK* and *rbcL*, the latter encoding the large subunit of Type I RuBisCO. Also the enzyme sensitivity of the

cell wall was explored on C-endosymbionts isolated by flow-cytometry, and exposing them to lysozyme, cellulose and a mix (Macerozyme®) composed of pectinase, hemicellulase, and cellulase. The endosymbionts did not show accumulated starch, which Cyanobacteria accumulate, but they showed Alcian Blue positive glycosaminoglycans, a character which is shared with Cyanobacteria. Enzyme studies indicated the cell wall was sensitive to lysozyme (indicative of a peptidoglycan-containing bacterial wall), but not to cellulase of Macerozyme®. However, we were unable to amplify the 'green' genes *trnK/matK* and *rbcL*. The current data indicate the C-endosymbiont shares characters with both Cyanobacteria and plastids, and reject the possibility of 'kleptoplasty', i.e., the ingestion of living chloroplasts and their internalization into midgut gland cells. They also emphasize the need of finding additional genetic markers that would clarify the phylogenetic position of this unique endosymbiont.

A16
16S rRNA PROBES HYBRIDATE ON INTRACELLULAR PIGMENTED CORPUSCLES IN THE MIDGUT GLAND OF THE QUEEN CONCH, *Lobatus gigas* (LINNAEUS, 1758)

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The queen conch (Littorinimorpha, Strombidae) bears pigmented corpuscles in the midgut gland, which are morphologically similar to those borne by some Struthiolariidae (Littorinimorpha) and Ampullaridae (Architaenioglossa). We have revisited those previous morphological descriptions in which the attention was centered in some large oval bodies (~27 µm long) which are dark brown and have been considered morphs of an apicomplexan-like parasite. Also, in one of those descriptions, some round, Alcian Blue positive bodies have been mentioned as "granules" of unknown function. In addition, this inclusions showed no *in situ* hybridization when exposed to a universal probe for eukaryotic 18S rRNA. We have described, in histological sections (stain with Nuclear Fast Red, Alcian Blue 8GX, eosin trichrome, from individuals collected in the Caribbean Sea, México) three types of corpuscles: (1) large, dark brown corpuscles, which are oval in shape; (2) smaller, round, light brown corpuscles; and (3) round, non-pigmented corpuscles of similar size, which show an Alcian Blue positive outer layer. Also, we exposed tissue sections for fluorescence *in situ* hybridization (FISH) probes that recognize generalized sequences of the 16S rRNA gene from Bacteria (EUB 338) and Cyanobacteria (CYA 361). Digestive gland sections exposed to either probes showed fluorescence on the large, oval pigmented corpuscles, but the label was much less intense on the small round ones when exposed to EUB338 or CYA361. A possible dietary origin of these corpuscles should be considered, because they may be digested remnants of cyanobacteria or chloroplast from the diet. However, an alternative hypothesis, similar to that proposed for species of *Ampullaridae*, is that they may be morphs of a prokaryotic endosymbiont.

A17
WESTERN DIET-INDUCED OBESITY AND NEUTROPHIL-DRIVEN LUNG INFLAMMATION

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Adipose tissue inflammation is the source of a number of proinflammatory mediators that affect insulin sensitivity and may also affect neutrophil traffic in the lung microvasculature. Myeloperoxidase (MPO) is the main protein of neutrophils and the only one that uses H_2O_2 to oxidize Cl^- to HOCl—a powerful oxidant that causes protein chlorotyrosine (Cl-Tyr). Herein we aimed at determining whether the redox/inflammatory profile of the lung is a target of Western diet-induced obesity (WDIO) in a mouse model. To test our hypothesis we compared the redox/inflammatory profile in the lung of mice fed for 16 weeks either a low-fat diet and tap water (LFD) or a high-fat diet (HFD) and 10% fructose in tap water (HFD+F). In relation to LFD mice, the lung of HFD+F MICE showed: reduced antioxidant antioxidant capacity—assessed by bleaching of the $ABTS^{\bullet+}$ ($P<0.0001$) and reduced GSH/GSSG molar ratio ($P<0.0001$); but increased protein carbonyls—a marker of protein oxidation ($p<0.001$), TBARS—a marker of lipidperoxidation ($p<0.0001$), TNF-α ($p<0.001$), Cl-Tyr ($P<0.001$) and MPO specific activity ($p<0.05$).All together our data indicate that WDIO causes MPO-dependent oxidative stress and inflammation in the lung. Neutrophil retention/activation in the lung microvasculature may be involved in increasing MPO content/activity in the lung of obese mice. Supported by PROICO-2-3214(FQBYF)/PROICO10-0414(FCS)-UNSL

A18
AGING MODIFIES CIRCADIAN RHYTHMS OF ANTIOXIDANT ENZYMES AND BDNF IN THE TEMPORAL CORTEX

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The brain-derived neurotrophic factor (BDNF) is a protein with a function in survival, proliferation and synaptic plasticity. It has been shown, that oxidative stress reduces Bdnf mRNA levels leading to cognitive impairments. Previously, we have shown BDNF transcript displays a circadian rhythm in phase with the oscillation of catalase (CAT) and glutathione peroxidase (GPx) enzymes activity, in the rat hippocampus. Our objectives were: to analyze the circadian variation of those antioxidant enzymes activities as well as the Bdnf mRNA levels in the rat temporal cortex and to assess the consequences of aging on those temporal patterns. Three-month-old (young) and 22-month-old (aged) Holtzman rats were maintained under 12h dark:12h dark (constant darkness) conditions during 10 days before the experiment. Temporal cortex samples were isolated every 4 h during a 24h period and immediately frozen in liquid nitrogen. CAT and GPx enzymatic activities were determined by kinetic assays. Protein levels were measure by the Lowry method. Bdnf mRNA levels were determined by RT-PCR. For circadian analysis GraphPad Prism 5.0 and Cronos-Fit software were used. We found CAT and GPx activity fluctuates throughout a 24h period in the rat temporal cortex. CAT activity peaks at the middle of the subjective day while GPx activity is maximal around the middle of the night. Interestingly, Bdnf expression also oscillates in that brain area with its highest level occurring during the subjective day, in phase with CAT activity. Thus, we predict CAT would prevent, among others, Bdnf mRNA from oxidation, favoring the following translation. Noteworthy, aging abolished circadian rhythms of antioxidant enzymes and phase shifted circadian variation of Bdnf mRNA levels. This could be a molecular basis of altered temporal patterns of cognitive functions in aged individuals.

A19
ELECTROPHYSIOLOGIC EFFECTS OF MELATONIN DURING REPERFUSION IN ISOLATED RAT HEARTS

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Melatonin protection against ventricular arrhythmias is attributed to its antioxidant effects, but when administered during reperfusion, it also induces a transient shortening of action potential duration (APD). The mechanisms implicated in the latter remains elusive. This work assessed whether these effects involve melatonin receptors and KATP channels activation. In Langendorff perfused rat heart, the treatment with 50 µM melatonin initiated after 10 minutes of regional ischemia reduced the incidence of ventricular fibrillation (VF) from 83.3 % in the control to 16.6 % (P=0.0033 vs control by Fisher exact test, n=12 each) and the incidence of ventricular tachycardia from 100% in the control to 41.7% (P=0.0046 vs control). The incidence of VF rose to 80 and 50% with the addition of luzindole (melatonin receptor antagonist, 5 µM) and glibenclamide (KATP channels inhibitor, 10 µM), respectively (both P=n.s. vs control, n=10 each). Ventricular tachycardia protection by melatonin was also lost in the presence of the inhibitors (luzindole 100% and glibenclamida 90%; all P=n.s. vs control). Melatonin shortened APD to 31.4 ± 3.5 ms and this effect was inhibited by luzindole (51.7 ± 4.1 ms, P<0.01 vs melatonin by ANOVA). Glibenclamide did not interfere with the APD shortening induced by melatonin (32.3 ± 2.9, P=n.s. vs melatonin) but when perfused alone lengthened the action potential (47.9 ± 3.6 ms, P=<0.01 vs melatonin+glibenclamide). We conclude that the APD shortening induced by melatonin is associated with receptor activation but this is not by itself the antiarrhythmic mechanism, because glibenclamide interfered with the protective effect but had a minimum effect on this shortening.

A20
IDENTIFICATION OF SOLUBLE COMPONENTS IN HUMAN ADIPOSE TISSUE EXPLANTS FROM NORMAL AND TUMORAL BREASTS

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Among other factors, tumor progression depends on the tumoral epithelial-stromal cells interaction. In the breast, adipose tissue is the predominant stromal type. We have recently demonstrated that conditioned media (CMs) from human adipose tissue explants of tumoral breasts (hATT) regulate proliferation, adhesion and migration of breast cancer epithelial cells. In the present work, we have begun to identify soluble components present in CMs by proteomics. We separated in polyacrylamide gels proteins that were present in both CMs.We lyophilized

aliquots from CMs and, by means of two-dimensional nano-liquid chromatography (2D-nanoLC)-mass spectrometry (MS/MS), we began to identify proteins from complex samples. Finally, we analyzed the obtained data with ProteoIQ (Premier Biosoft) software. Compared to CMs-hATN, CMs-hATT had a greater diversity of proteins (at equal total protein) and a higher protein quantity (at equal final volume) ($p<0.05$). In addition, we found that apolipoprotein A-1 (involved in lipidic metabolic processes), C3 complement factor (involved in signal transduction) and vimentin (a glycoprotein frommesenchimal cells recently seen to be related to an invasive breast cancer phenotype), were expressed significantly more in CMs-hATT*vs*. CMs-hATN($p<0.05$).These factors could be involved in the biological effects previously observed.

A21
DIET-INDUCED OBESITY IN MALE MICE IS ASSOCIATED WITH METABOLIC AND HISTOLOGICAL DISORDERS IN TESTIS

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Overweigh and obesity are known causes of infertility in men, but the mechanism involved remains unknown. The aim of the present study was to investigate both reproductive and metabolic features of C57BL/6 mice fed a normal chow (LFD, low fat diet) or a 60% HFD (high-fat diet). At the end of the experiment (18 weeks), both reproductive and metabolic characteristics were assessed. Our data show that HFD caused significant metabolic alterations in mice, including obesity, glucose intolerance, dyslipidemia, and subtle liver steatosis. These findings suggest that HFD induces metabolic features in mice. Moreover, HFD showed differences in the expression of RNA messenger encoding proteins related to the synthesis and metabolism of cholesterol (SREBP-1, SREBP-2 and HMGCoAreductase). Few changes were observed histologically, but marked variations at the molecular level by comparing mice with control diet and fat. These data suggest that the abnormalities foundin spermatogenesis may be caused by changes in metabolic status and the molecular machinery of testis.

A22
CARDIOVASCULAR RISK FACTORS IN PHYSICAL EDUCATION STUDENTS

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This study aims to determine and correlate cardiovascular risk factors in university freshmen physical education students of UNRC. In all cases the subject was identified with a key to maintain the confidentiality of the data and experimental proposal was approved by the Ethics Committee of the UNRC. Anthropometric, biochemical, medical and psychological factors were evaluated. A standardized survey of stress and determination of salivary cortisol in three daily samples, including at awakening, noon and before nightly rest(most significant) were assessed. Our results show an association between increased of salivary cortisollevels (3.29 vs. 2.12 ng / ml - $P <0.05$) and index mass body (BMI) (30.8 vs. 22.5 - $P <0.05$), which shows the possible interaction between the two and the expectation of increased risk of disease in these cases when the student engaging in demanding muscular and cardiovascular efforts. It will serve as an element of judgment in their career progression. We conclude that these risk factors are largely linked to the causes of stress and should be periodically monitored patients to prevent diseases related to stress.

A23
CIRCADIAN PATTERNS OF RORa AND REV-ERB EXPRESSION ARE MODIFIED IN THE HIPPOCAMPUS OF VITAMIN A-DEFICIENT RATS

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In mammals, the circadian clock mechanism involves two interlocking transcriptional-translational feedback loops. BMAL1/CLOCK heterodimer activates the expression of clock and clock-controlled genes while the PER-CRY complex represses it.Some evidence points out retinoids as regulators of clock genes activity through retinoid receptors (RARs and RXRs). Previously, we have demonstrated vitamin A deficiency modifies daily rhythms of clock genes expression in the hippocampus,our objectives were: first, to investigate whether RORa and REV-ERB also displays a circadian expression pattern in the rat hippocampus and second, to evaluate the effect of a vitamin A-depleted diet on those temporal patterns. Twenty one-day old Holtzman rats received a diet containing 4000 IU of vitamin A/Kg diet (Control group), or the same diet devoided of vitamin A (Vitamin A-deficient, VAD, group), during 3 months. In order to study endogenous circadian rhythms, rats were maintained under constant darkness conditions along 10 days before the experiment. RORa and REV-ERB mRNA levels were determined by RT-PCR and RARα, RXRß and BMAL1 proteins by immunoblotting, in hippocampus samples isolated every 4 h during a

24h period. Regulatory regions of RORa and REV-ERB genes were scanned for clock- and retinoic acid-responsive sites. E-box, RXRE, RARE and RORE sites were found. RORa and REV-ERB expression displays an endogenously-generated circadian rhythm in the rat hippocampus, which was modified in the VAD group; probably, as a consequence of alterations in the circadian patterns of BMAL1 and/or retinoic acid receptors proteins.

A24
ANALYSIS OF THE RUMEN BACTERIAL DIVERSITY OF CREOLE GOATS UNDER TWO DIFFERENT DIET CONDITIONS USINGq-PCR.

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Quantitative real time (qPCR) assays was used to determine both the abundance and diversity of bacterial community characterizing rumen of Creole goats for which the diet was switched from alfalfa hay/corn (AH/C) to native forages (NF). Two fistulated goats were routinely fed with AH/Cdiet for a period of 21 days. Then, goats were switched to the NF diet for others 21 days. The ruminal contents were sampled at 2 (Day 0), 10 (Day 10) and 21 (Day 21) days after the beginning of each diet. One sample per animal was collected after feeding and was stored for later DNA extraction. Total bacteria, Firmicutes, Actinobacteria, Bacteroidetes and γ-Proteobacteria were quantified by qPCR using primers targeting 16S rRNA gene specific. In relation to diet, total ruminal bacteria were significantly higher in goats fed NF diet ($11.75 \pm 1.46 \times 10^{10}$ bacteria g^{-1}) than in those fed AH/C diet ($6.39 \pm 2.67 \times 10^{10}$ bacteria g^{-1}). Not significantly differences in concentrations of Firmicutes, Bacteroidetes or γ-Proteobacteria concentrations were observed during feeding experiment. However, Actinobacteria concentration was significantly higher in goats fed FN diet ($24.65 \pm 5.95 \times 10^{5}$ bacteria g^{-1}) than in those fed AH/C diet ($1.73 \pm 0.28 \times 10^{5}$ bacteria g^{-1}). Within this bacterial phylum, *Actinomyces sp, Flavobacterium sp* and *Bifidobacterium* sp have been described in the ruminal ecosystem, which could be selected at a high number in goats fed NF diet. Findings from q-PCR analysis suggest that rumen bacterial community of Creole goats was influenced by diet. New bacterial species have been associated with the degradation of the nutritional components in diets in the rumen of these animals.

A25
RECOVERY OF KERATINOPHILIC FUNGI FROM SOIL OF MENDOZA (PRELIMINARY RESULTS)

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Keratinophilic fungi of dermatophytes group are agents of superficial mycoses in humans as well as in animals. According to the reservoir, they qualify as anthropophilic, zoophilic and geophilic and there is cross transmission between them. The purpose of this study was to determine the isolation frequency of these fungi from the soil of Mendoza's urban area. Samples of ground (n = 33) were collected from different public places. Vanbreuseghem or hair-baiting technique was used, employing as baits both infant hair and kitten hair of less than 6 months of age. Suspicious colonies were studied through direct microscopic examination with potassium hydroxide and heat and were reseeded in Sabouraud honey and then in lactrimel, both with cycloheximide and chloramphenicol, incubating at 28°C for 30 days in a sequential way. The identification was performed by macroscopic study of the giant colony and microscopy of the microculture. The frequency of isolation of keratinophilic fungi was 24/33 (73%). Higher harvest was observed when cat hair (18/33 = 55%) than infant hair (12/33 = 36%) was used. Isolated species were: *Trichophyton* sp 11/24, *Fusarium* sp 3/24, *Acremonium* sp 2/24, *Streptomyces* sp 2/24, *Penicillium* sp 1/24, *Scopulariopsis* sp 1/24, *Trichophyton terrestre* 4/24 and *Microsporum persicolor* 1/24. Studies will continue to extend the number of samples and sampling areas as well as to deepen the search of dermatophytes.

A26
PREVALENCE OF ANAEMIA AMONG PREGNANT WOMEN AT ATILIO LUCHINI HOSPITAL, SAN LUIS

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Anaemia with an estimated prevalence of 30% among pregnant women is a major cause of maternal deaths in Argentina. Anaemia is defined as a decrease in the ability of blood to carry oxygen due to a decrease in the total number of erythrocytes, a diminished concentration of haemoglobin (Hb) per erythrocyte, or a combination of both. A haemoglobin concentration below 11.0 g/dl or mean corpuscular volume (MCV) of less than 33.0% is regarded as anaemia during pregnancy by the World Health Organization (WHO). The aim of this study was to

determine the prevalence of anaemia, associated red cell morphological pattern among pregnant women at Atilio Luchini Hospital, San Luis. A cross-sectional study of 153 women between 14 and 47 years old during period March-August (2014) were studied. The samples of each pregnant woman. MCV, Mean Corpuscular Hemoglobin (MCH) in hypochromic microcytic (HM) and normochromic normocytic (NN), and Erythrocyte Distribution Amplitude (RDW) were determined by Hematology Autoanalyzer ADVIA 60. According to the weeks of gestation (s) the patients were divided in three groups: 1)1- 12 s, 2) 13-28 s and 3) 29-40 s. Hb < 11 g/dl in group 1) and 3); Hb <10.5 g/dl in group 2); MCV <80 fL microcytic (M) and ≥80 and ≤99 fL normocytic (N); MCH <26 pg hypochromic (H) and ≥ 26 pg Normochromic and RDW ≥ 14.5% anisocytosis. We found a 32% of the pregnant women were anaemic, 26.2 % was NN and 5.8% HM. Regarding RDW, no significant differences were observed. In conclusion, every pregnant woman should be encouraged to obtain antenatal care, and could be given haematinic supplementation.

A27
CONTRACTILE FUNCTION ONSET BY ENDOTHELIN-1 IN SEMINIFEROUS CORDS DURING POSTNATAL DEVELOPMENT IN RAT TESTIS

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Endothelin-1 (ET-1) produces seminiferous tubules contraction acting on peritubular myoid cells (MC) in adult rats. MC has a cytoskeleton of α-actin filaments (AF) disposed in two independent layers inside the cell, which are maintained during contraction when MC change their shapes. In neonatal rats the solid seminiferous cords are surrounded by MC, where AF are already present, but in a disorganized way. The two AF layers do not appear simultaneously during the development: first the inner circular layer appears and then the outer longitudinal layer does. The aim of this work is to establish when the ET-1 response begins, which is the grade of advance of the spermatogenesis, and which is the MC cytoskeleton development at this time. To do this, testes from birth to 30 postnatal days (pnd) were removed, histological sections were observed, AF were studied by confocal microscopy, and contraction was registered by video microscopy, measuring the cords or tubular diameters after ET-1 treatment. The first contraction was detected at 15 pnd, when the first spermatogenic wave had already begun and cords had reached the paquitene step. The cords had not lumen yet, and AF were present only in the circular inner layer inside MC. In conclusion, with only one of the AF layers, the MC are able to contract seminiferous cords in response to ET-1 from 15 dpn onward.

A28
METABOLIC EFFECTS OF DIETARY OLIVE AND PISTACHIO OILS SUPPLEMENTATION IN THE OFFSPRING OF DIABETIC RATS

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Changes in the intrauterine environment during pregnancy affect fetal development. Uncontrolled diabetes during pregnancy is highly correlated with metabolic abnormalities in the adult offspring. Inclusion of monounsaturated fatty acids in the diet has shown beneficial effects on certain metabolic parameters. The offspring of an experimental model of streptozotocin-induced gestational diabetics (30 mg/Kgiv) (DO) and controls mothers(CO) were used to assess the effect of the intake of corn oil (MZ), extra virgin olive oil (OL) and pistachio oil (PS). Cholesterol levels (Chol), triglyceride (TG), fasting plasma glucose (Glu), and fructosamine (Fru) were evaluated. Supplementation was performed during 2-62 days of age (dose: 8µl/15g weight). The samples were collected during fast from10 month animals. Results: Significant increase on Chol levels were observed in DO vs CO (1.24 times; $p < 0.01$) as well as 1.4 times increase in females vs males ($p < 0.01$). PS supplement produced a 61% reduction in females ($p < 0.01$). TG levels did not show significant changes due the condition CO-DO, but they were affected by OL or PS supplement in both sexes ($p < 0.01$). Glu levels were partially affected by oils supplementation ($p < 0.01$).Fru levels increased in DO and up regulated by OL in males ($p < 0.01$). Thus, prenatal diabetes causes changes that are partly reversed by the inclusion of OL or PZ in the diet (CONICET, PICTO/UCCuyo2009-0158- CICITCA UNSJ).

A29
THE EFFECT OF NITRIC OXIDE ON TUBULAR EPITHELIAL CELL APOPTOSIS IN RESPONSE TO MECHANICAL STRAIN

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Obstruction to the outflow of urine in the renal pelvis or ureter, leads to retrograde mechanical pressure transfer to the renal tubular compartment. This is the principal injury stimulus that sets in chain the inflammatory and fibrotic processes that characterize obstructive nephropathy. A primary event in this sequence is tubular cell stress and the induction of apoptosis. Wilms tumor 1 (WT-1), a key regulator of mesenchymal-epithelial transformation, is downregulated during congenital obstructive nephropathy. Of great interest, nitric oxide (NO) bioavailability associated with heat shock protein 70 (Hsp70) interaction might modulate WT-1 expression, preventing obstruction-induced cell death during neonatal unilateral ureteral obstruction (UUO).Hence, the goal of this study was to examine the effect of NOon apoptosis in response to mechanical stretch in derived tubular epithelial rat cells (NRK52E). NRK52E (N=3) were exposed to 48 hours of graded mechanical strain using the Flexcell system in the presence or absence of NG-nitro-L-arginine methyl ester (L-NAME,1mmol), L-arginine (1mmol) and sodium nitroprusside (NPS, 2mmol)or combinations and assess thereafter apoptosis by Annexin V/Propidium Iodide based flow cytometric. NRK52E submitted to mechanical stretchshowed anapoptosis induction in relation to control cells.The cells with L-arginine or NPS treatments and the combination of both showed a reduction in apoptosis even in the control cells with no stress. In addition, L-NAME as well as L-NAME/L-arginine treatment, increased the apoptosis in the groups exposed to mechanical strain but not in the control group.We concluded that NO availability protects against mechanical stretch- induced apoptosis.

A30
FLUTAMIDE AFECTS SERPINE 1F EXPRESION IN RAT TISSUES

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Epididymal sperm association is observed in many species of mammals. In rat, these structures are called rosettes and requires a complete maturation of the sperm.. In previous work our group isolated and identified Serpine 1F, a protein involved in this associations: in the epididymal fluid. To identify whether the expression of this protein depend on androgen, Flutamide, a known antiandrogenic agent, was previously administered to animals for 15 days according to previously established protocols in the literature. Using RT-PCR and Inmunohistochemistry, different tissues were analyzed for mRNA and Serpin 1F expression. In analyzed tissues from animals treated with flutamide, Serpine 1F expression was not detected while the expression was observed in control animals. These results suggest that Serpin 1F secretion and the formation of rosettes in the epididymal tail is an androgen dependent phenomenon.

A31
CROSSTALK BETWEEN PROLACTIN AND THYROID HORMONES SIGNALING IN CORPUS LUTEUM AT THE END OF PREGNANCY IN THE RAT.

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The corpus luteum (CL) is an ephemeral endocrine gland that secretes progesterone (P4) throughout the entire length of gestation in the rat and plays a central role in the maintenance of pregnancy. P4 secretion is supported by prolactin (PRL) and placental lactogens (PL) via PRL receptor (PRLR) and JAK2/STAT pathway. On the other hand, PRL signaling suppresses 20 α-hydroxysteroid dehydrogenase (20αHSD) which metabolizes P4 to inactive 20 α-hydroxyprogesterone. At the end of pregnancy, the surge of uterine prostaglandin F2α (PGF2α) induces expression of members of the suppressors of cytokine signaling (SOCS), that in turn inhibit PRLR signal transduction in the CL triggering luteolysis and the inactivation of P4. Our previous results demonstrated that hypothyroidism (hypoT) impairs CL function at the end of gestation delaying the surge of PGF2α and 20 α HSD expression, thus postponing the onset of parturition in the rat. The aim of this study was to evaluate the effect of hypoT on the expression of the components of PRLR pathway in the CL at the end of pregnancy in the rat. Experimental hypoT was induced in Wistar rats by the chronic administration of the antithyroid drug propylthiouracil. The RNAm expression of SOCS1 and 3, CIS, PRLR, STAT5B and 20 α HSD was determined by RTqPCR and SOCS3, STAT5B, pSTAT5B and CIS protein expression by Western blot in the CL at the end of pregnancy. Our results demonstrated that hypoT diminished protein expression of SOCS3 and increased the ratio of pSTAT5B/STAT5B level at $p<0.05$ without changes at transcriptional level. Overall our results demonstrated

that hypoT interferes with the normal process of luteolysis altering SOCS3-PRLR signaling and the metabolization of P4 that in consequence delay the onset of parturition in the rat.

A32
EFFECTS OF PIOGLITAZONE-RETINOIC ACID ON DAILY RHYTHMS IN AN EXPERIMENTAL MODEL OF ALZHEIMER DISEASE

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder associated with impaired clearance of β-amyloid peptide (Aβ), a process normally facilitated by the apolipoprotein E (ApoE). ApoE expression is transcriptionally induced by the peroxisome proliferator–activated receptor gamma (PPARγ) in coordination with retinoid X receptors (RXRs). On the other hand, besides the cognitive deficit, AD shows alteration in the circadian rhythms. The objectives of this study were: first, to analyze the effect of an i.c.v. injection of Aβ(40-42) peptide on the 24h rhythms of Aβ and ApoE protein levels as well as on catalase (CAT) and glutathione peroxidase (GPx) activities in the rat hippocampus; second, to evaluate the effect of the PPARγ agonist, pioglitazone, along to the RXR ligand, retinoic acid, on those temporal patterns. 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 Pio-RA. Rats were maintained under 12h-Light:12h-Dark conditions. Aβ and ApoE proteins levels were analyzed by immunoblotting; CAT and GPx enzymatic activities were determined by kinetic assays; in hippocampus samples isolated every 6 h during a 24h period. We found that injection of Aβ(40-42) phase shifted Aβ, ApoE, CAT and GPx daily rhythms. Noteworthy, Pio-RA reestablished rhythmicity of those temporal patterns indicating PPARg-RXR heterodimer might be a transcription factor involved in circadian regulation and a potential target for restoration of circadian rhythmicity in neurodegenerative disorders.

A33
CHRONIC HYPOTHYROIDISM ALTERS ENERGY BALANCE AND ADIPOCYTE SECRETION ACTIVITY.

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We investigated the effect of chronic thyroid deficiency on food intake, body weight, body fat mass and serum leptin levels in rats. Female Sprague-Dawley rats were divided into two groups at 55 days of age (Day 1): euthyroid (EUT, n=10) and hypothyroid rats (HYPO, 0.01% PTU in drinking water, n=10). All the animals were weighted weekly and food intake was assessed every other week. Blood was collected on days 1, 35 and 70 for hormone determinations. The rats were sacrificed on day 70 by decapitation.Hypothalamus, a piece of intra-abdominal and mammary fat were taken for histological and molecular analysis (ObR, Adipo R1, mTOR, POMC, NPY). Statistical analysis was performed by Student T test and Chi square (IC>95%). Food intake was lower in HYPO rats compared to EUT during the whole study ($P<0.001$). HYPO showed significantly retarded growth reflected in a decreased weight and reduced levels of circulating GH. No differences in serum prolactin and progesterone were observed. However, estradiol was significantly lower in HYPO rats. Serum leptin levels were reduced in HYPO rats even though the body fat mass and the mammary fat pad were similar in both groups. In conclusion, chronic hypothyroidism induced by PTU treatment had significant effects on food intake, body weight and adipocytes secretion on rats, as evidenced by alterations in serum leptin concentrations. Hyphotalamic food intake regulation is currentlyunder study.

A34
FREQUENCY OF ISOLATION OF TOXIN-PRODUCING CLOSTRIDIA FROM ARGENTINE SOIL IN CHAQUEÑO DOMAIN AND RELATION WITH CASES OF INFANT BOTULISM

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Infant botulism (IB) is caused by the ingestion of botulinal toxin producing clostridia (BTPC) spores, which colonize large intestine of children between age 2 and 32 weeks, and later production of toxin in situ, producing hypotonia and a descending, symmetric flaccid paralysis. IB transmission is under investigation. BTPC spores are distributed to a large extent in nature. At present, IB is the most frequent clinical form of the human botulism in the world, even Argentina. The high incidence of IB in breast milk fed infants with history of no any other complementary feeding suggest that soil is an important source of infection. Swallowing BTPC spores from the airborne, which exist worldwide in soil and dust, has been propose as the principal route of exposure. The aims are: 1- to know the presence of BTPC spores in soils from chaqueño domain. 2- to relate the presence of spores with the IB cases in the geographic area in studied. At present we have studied 92 samples of soil. This were

processed by diluting 25 g of soil in 50 ml of a saline solution, inoculated into chopped-meat medium After incubation for 5 days at 31°C broth media were centrifuged at 12,000 _ *g* for 10 min at 4°C, and 0.5 ml was inoculated in duplicate intraperitoneally into mice. Mice were observed for 96 h for characteristic botulinal signs and death. Toxic broth media were cultivated in solid media to ensure that the cultures were pure. The typing were performed using polyvalent sera (ABFG) y specific type (A, B, F y G) by quantitative neutralization in mice at level 1000 DL_{50}/mice. Of 92 studied samples four were positive; 1: 32 of San Juan, 3:46 of La Rioja and 0: 14 of Catamarca. All positive samples were Type A.

A35
SUCKLING MODULATES PITUITARY RECEPTORS EXPRESSION IN RATS WITH DEFICIENT LACTATION (OFA *hr/hr*)

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During normal lactation, sustained hyperprolactinemia is due to suckling stimulus and decreased sensitivity to prolactin (PRL) negative feedback. OFA (*hr/hr*) rats, derived from the Sprague Dawley (SD) strain, have deficient lactation, a high hypothalamic dopaminergic tone and a blockade of milk ejection. In previous studies, we have demonstrated that OFA rats have an increased sensitivity to PRL negative feedback acting at hypothalamic level, also responsible to the decreased PRL release of these rats. However, this evidence does not rule out the hypothesis that pituitary mechanisms may be also involved. Our aim was to investigate the changes in pituitary receptors expression involved in the synthesis and secretion of PRL in response to suckling stimulus in OFA rats compared to SD rats (normal lactation). Using real time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), we measured the changes in mRNA expression of dopamine receptor (D_2 R), estrogen receptor (ERα) and long PRL receptor (PRLR) in anterior pituitary of mid-lactating OFA and SD rats separated from their pups for 12 h and subsequently subject to suckling during 4 h. Serum PRL and estradiol levels were measured by RIA and weight gain of the litters were determined. After suckling, PRL release was higher in SD than OFA rats in correlation with a higher weight gain obtained. Removing the litters induced an increase in ERα expression only in OFA rats. After suckling, a decreased of D_2R was detected in OFA rats compared with SD rats. No change in PRLR was observed in any strain of rats in response to 4 h of suckling. The results show that suckling stimulus modifies differentially D_2R and ERα expression in each strain. Since no changes on PRLR expression were observed, the decrease of D_2R in OFA rats could reflect a compensatory effect rather a primary change of pituitary receptors, thus supporting the hypothesis that PRL negative feedback acting at hypothalamic level is activated in these rats during lactation.

A36
DAILY OSCILLATION OF BDNF AND ITS RECEPTOR (TrkB) EXPRESSION IS MODIFIED IN THE HIPPOCAMPUS BY AN I.C.V. INJECTION OF AMYLOID BETA PEPTIDE

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Alzheimer's disease (AD) is a neurodegenerative disorder. Elevated levels of β-amyloid peptide (Aβ) in the brain and progressive cognitive impairment are characteristics of AD. Besides the cognitive deficit, AD patients also show alterations in their circadian rhythms. Brain-derived neurotrophic factor (BDNF) is involved in learning and memory process by binding to its main functional receptor (TrkB). Previously, we showed circadian rhythmicity of BDNF and TrkB expression is abolished in the hippocampus of aged rats. The objective of this study was to investigate the effects of an i.c.v. injection of Aβ(40-42) peptide on the Aβ protein levels itself as well as on the 24h rhythms of BDNF and TrkB expression, in the rat hippocampus. Four-month-old males Holtzman rats were used in this study. Groups were defined as: control (CO) and Aβ-injected (Aβ). Rats were maintained under 12h-light:12h-dark conditions before the sacrifice. BDNF and Trkb mRNA levels were determined by RT-PCR and Aβ protein was analyzed by immunoblotting, in hippocampus samples isolated every 6 h throughout a 24h period. We found i.c.v. injection of Aβ (40-42) increased Aβ peptide content and attenuated daily variation of BDNF and TrkB expression in the rat hippocampus. Thus, Aβ peptide accumulation might disrupt temporal expression patterns in a brain area related to memory and learning, such as the hippocampus.

A37
A COMPENSATORY ANTIOXIDANT-MECHANISM TRIGGERED BY DIETARY-SOYBEAN PROTEIN AGAINST CADMIUM-INDUCED NEUROTOXICITY: FOCUS IN THE HIPPOCAMPUS

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Cadmium (Cd) is an important environmental contaminant and causes neurotoxicity. Soybean proteins in the diet have health-beneficial effects due to its antioxidant properties.This study was aimed to testing whether Cd in the drinking water causes oxidative stress in the hippocampus (Hp) and that this process can be modulated by a soybean-based diet. For that, we fed 4 groups of female Wistar-rats for 60 days as follows: 1:casein-based diet (CBD) + tap water; 2: CBD + tap water with 15 ppm Cd (as Cl_2Cd); 3: soybean-based diet (SBD) + tap water; and 4: SBD + tap water with 15 ppm Cd. Water and food consumption, and body-weight-gain curves were not different between the four experimental groups. At the end of the diet period we measured parameters of oxidative stress in serum and Hp. In either group Cd exposure did not change the serum concentration of TBARS (thiobarbituric acid reactive substances), a marker of lipid-peroxidation (LPO). In rats fed a CBD, Cd reduced serum total antioxidant capacity—bleaching of $ABTS^{\bullet+}$ ($p<0.05$), whereas a SBD prevented this effect. Compared to casein, SBD enhanced Cd-induced serum protein carbonyls, a marker of protein oxidation. On the other hand, compared to casein, a SBD increased TBARS in Hp and Cd exposure enhanced glutathione peroxidise (GPX) activity ($p<0.01$). We did not observe any change in catalase activity in the Hp between the 4 groups. Taking together, our data show that dietary soybean protein may trigger a compensatory antioxidant-mechanism mediated by GPX against LPO in the Hp of rats chronically exposed to Cd. A soybean-based diet may provide protection against Cd-induced oxidative stress in the Hp.

A38
ENDOGENOUS 24H-VARIATION OF ANTIOXIDANT ENZYMES IN THE PREFRONTAL CORTEX OF YOUNG AND AGED RATS

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Prefrontal cortex plays a key role in memory and learning and is especially susceptible to oxidative stress. Particularly, aging is associated to increased oxidative stress and altered circadian rhythms. It is known, the molecular clock activity is closely dependent on the cellular redox state. The objectives of this study were to investigate whether catalase (CAT) and glutathione peroxidase (GPx) expression and activity display endogenous rhythms in the rat prefrontal cortex (PFC) and to assess the consequences of aging on those temporal patterns. Holtzman rats from young (3-month-old) and aged (22-month-old) groups were maintained under constant darkness conditions, during 10 days before the experiment. Serum TBAR's levels were measured by a colorimetric assay. CAT and GPx mRNA and enzymatic activity were determined by RT-PCR and kinetic assays, respectively, in PFC samples isolated every 4 h during a 24h period. As expected, we observed an increase in the 24h TBAR's levels in the serum of aged rats. Antioxidant enzymes expression and activity vary significantly throughout a day under constant darkness conditions in the rat PFC. Aging abolished CAT and GPx 24h-variation. Loss of the 24h-variation of antioxidant enzymes in the PFC would modify the circadian oscillation of the cellular redox state and, consequently, the endogenous clock activity, in the PFC.

A39
CHARACTERIZATION OF CAVEOLIN 1 AND RAS/MAPK PATHWAY IN SPERMATOGENESIS

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The caveolins are a family of proteins found in many cell types. These proteins are associated with signaling pathways, transport and the control of cell growth and tumor suppressor. These functions involve its association with membrane microdomains which provide a link between cell membrane and the cytosol. Moreover, Ras/MAPK pathway is associated to cell proliferation and differentiation during the spermatogenesis. Caveolin 1 could be associated with meiosis progression by its regulatory effect on the function of Ras / MAPK. We postulate that caveolin 1 acts as an indirect modulator during spermatogenesis. In the present investigation, the objective was to study the presence of caveolin 1 and ERK 1, 2 in spermatogenesis in adult New Zealand rabbits by indirect immunofluorescence (IFI) and Western blotting (WB). Preliminarily, we found caveolin1in the cytoplasm of spermatocytes, while Erk1,2 is present in spermatocytes and round spermatidsby IFI. Erk1,2 was observed principally in the cytoplasm of spermatocytes and in the development of the acrosomal vesicle in round spermatid. We also observed the expression of caveolin 1 and Erk1,2 in testicular protein extracts by WB. These

results could be indicating that both components are defined from early spermatogenesis and they are relevant during the cell proliferation and differentiation.

A40
PAMPAS AND ANDEAN CATS AS BIO-INDICATORS OF WILD ENVIRONMENTS BY MEANS OF BIOMARKERS OF GENETIC DAMAGE

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Mammals are considered to be one of the groups more suitable as biomonitors, faithfully reflecting the contamination present in their habitats. In particular, felines are potentially useful for the study of genotoxicity, with spontaneous micronucleated erythrocytes because of their inefficient immune system in the removal from circulation of genetically damaged cells. Blood smears from Andean cat (*Leopardus jacobita*) (n = 3) and Pampas cat (*Leopardus pampas*) (n = 3) specimens of high Andean region were analyzed. The aim of this study was to determine the level of spontaneous micronuclei in peripheral blood of these 2 species of wildlife habitat in the possibility that they could be used as bioindicators of environmental quality. The smears were fixed with methanol, stained with 0.003% acridine orange and 20.000 cells/ animal were analyzed at 100x fluorescence microscope. *L. jacobita* and *L. colocolo* showed 1.06 ± 0.24 and 0.86 ± 0.36 micronucleated erythrocytes/ 1000 cells (MNE), respectively. No statistically significant differences between both species ($p > 0.05$) were observed. An organism, to be considered as an indicator of micronuclei inducing agent, it must submit a spontaneous MNE frequency greater than or equal to 0.35 per 1.000 cells. *L. jacobita* and *L. pampas* can be potentially used as biomarkers of environmental quality; however *L. pampas* has more advantages because it has a wider distribution, making it easy to obtain samples. Knowing the baseline level of MN in standard or common situations is essential as a tool to assess the future impact of anthropogenic activity or unforeseen environmental disasters with the release of pollutants into the environment.

A41
DIFFERENTIAL PHAGOCYTIC ABILITY OF CIRCULATING HEMOCYTE SUBPOPULATIONS IN THE APPLE SNAIL *Pomacea canaliculata*.

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Invertebrate hemocytes are concerned with innate defense mechanisms such as phagocytosis, pathogen encapsulation and lysis of foreign cells. Three hemocyte morphological 'types' are found in the circulation of this snail, which have been identified as hyalinocytes, agranulocytes and granulocytes, on the basis of their light and electron microscopy characteristics. Nevertheless, the functional roles of each of these cells remain unexplored in *P. canaliculata*. We have developed a flow-cytometric method to sort three hemocyte subpopulations from freshly withdrawn hemolymph, which correspond predominantly to the three hemocyte morphological 'types', and which will allow us to study their functional properties. Here we report an assessment of the phagocytic ability of the sorted hemocyte subpopulations, as compared between them and with the whole population of circulating hemocytes. Both unsorted and sorted hemocytes were exposed to fluorescent latex beads (10:1 beads/hemocyte) for one hour. Cell associated fluorescence was used to determine the percent of phagocytizing hemocytes (phagocytic index) in each sample. Hyalinocytes showed a phagocytic index statistically higher than that of agranulocytes and granulocytes (ANOVA, Bonferroni's multiple comparison test; $p < 0.05$, $n = 6$), and which was not statistically different from that of the unsorted circulating hemocytes. Interestingly, bead exposure resulted in generalized granulocyte degranulation, even though only some of these cells showed phagocytic activity. Also, degranulated cells showed nuclear condensation, which is suggestive of induction of cell death. It is concluded that hyalinocytes are the main phagocytes in the circulation and that granulocytes release their granules in response to foreign particles. Degranulation might be related to the release of antimicrobial substances.

A42
TWO THERAPEUTIC APPROACHES TO TREAT MODERATELY ASTHMATIC PATIENTS WITHOUT BACTERIAL EXACERBATION

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The use of monotherapy with an inhaled corticosteroid (ICS), ciclesonide, and salbutamol (a short-acting beta 2 agonist- SABA) as needed, was compared with a standard treatment of asthmatic patients consisting of salmeterol, a long-acting beta 2 agonist (LABA) plus fluticasone propionate (ICS). Aims: To evaluate the inflammatory process by sputum cellularity, spirometry to measure Forced Expiratory Volume at 1st second

(FEV1) and Forced Vital Capacity (FVC), impulse oscillometry spirometry (IOS) and to rule out microbial exacerbation and oropharyngeal colonization with fungi. In addition, quality of life was assessed using a standard questionnaire (ACQ).We selected 84 subjects with moderate asthma without bacterial infection and assigned: Group I (40 subjects) to salmeterol-fluticasone and Group II (44 subjects) to ciclesonide. FEV1 showed significant improvement during one year, with decrease of R25 (Total airway resistance by IOS) for both groups. The bacterial isolates were not associated with increased asthma exacerbation processes .*Candida* was present in 45.2% and 57.8% of patients, respectively. Eosinophils in sputum decreased significantly with either medications. ACQ is usefuln to show the control of the asthmatic state in addition to the other methods of indirect evaluation of the inflammatory process of the airways to select a pharmacological treatment for asthmatics patients.

A43
PROPER BREAST FEEDING PROTECTS AGAINST MAMMARY CARCINOGENESIS IN ADULT LIFE

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Cancer is one of the most important causes of morbidity and mortality worldwide, and in particular, breast cancer is the most common in women in developed countries. Each year, new evidence contributes to our knowledge on the role of breastfeeding on survival, growth and child development, as well as its benefits reaching adulthood. Our interest was to study if an appropriate breastfeeding protects against mammary carcinogenesis in adult life, for this we used Sprague Dawley female rats that at 24 hours of age were divided into two groups: one holding eight pups per mother and the other holding three, in order to generate greater exposure to breast milk. After weaning, half of the offspring from each group were fed with standard pet food (12.6% fat) and half with a high fat diet (40% fat). The latter was prepared by adding 12.1 g. of fat removed from the first bovine juice per 100g of food standard. At 55 days of age all rats were treated with an oral dose of DMBA (po, 15 mg / rat). The animals were weighed weekly to develop the respective growth curves and observed until the appearance of the first palpable tumor. Latency, incidence, growth rate and multiplicity were recorded. Furthermore intraabdominal fat content was analized and histological and immunohistochemical studies of the tumors were performed. Our results showed that tumoral incidence was lower ($p < 0.005$) in the group that held a proper breastfeeding. A full breastfeeding would protect against the breast cancer development later in life, even when a high intake of saturated fat is maintained.

A44
EFFECTS OF MELATONIN ON MAST CELL ACTIVATION

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Melatonin is a chronobiotic hormone widely distributed in the body. It has a variety of extrapineal non chronobiotic functions such as neuromodulator action, antiproliferative, antioxidant, immunomodulatory and oncostatic. Immunomodulatory effects are exerted on different immune cells. However, the effect of melatonin on mast cell activation is unknown. The aim of this study was to determine whether melatonin inhibits mast cell activation induced by mast cell secretagogues that act by different molecular mechanisms of action.. Peritoneal mast cells from adult male rats were removed and then activated with compound 48/80, calcium ionophore A23187 and neurotensin. Morphological studies by light microscopy and confocal fluorescence microscopy were performed. The percentage of serotonin release was also determined by HPLC as a marker of degranulation. Melatonin inhibited mast cell activation induced by the calcium ionophore A23187 (10 µg/ml A23187: 16.35±1.6% versus 20 µg/ml melatonin: 2.8±0.21%, $P<0.001$) and did not alter mast cell activation induced by compound 48/80 and neurotensin. Granule morphological changes induced by the calcium ionophore A23187 were also inhibited by melatonin at non-cytotoxic doses, suggesting an interaction of the hormone with calcium-binding proteins, among other mechanisms.

A45
ENRICHED DIET WITH OLIVE OIL IMPROVES THE TESTICULAR INEFFICIENCY INDUCED BY FAT DIETS IN HYPERCHOLESTEROLEMIC RABBITS

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It had been observed in hypercholesterolemic rabbits, sperm abnormalities such as decrease in the number of ejaculated sperm and increase in abnormal forms. These disorders are reversed with diets supplemented with olive

oil. A defective spermiogenesis could be the cause of the abnormal shapes observable by transmission electron microscopy and immunofluorescence; and reduced efficiency in spermatogenesis is responsible for the low number, demonstrable by epithelium seminiferous´ differential cell counts. In order to make this work, 3 groups of adult male New Zealand rabbits were generated: control (C), fat (F) by adding 14% animal fat (w / w), and protected (P) supplemented half of the fat of F (7%) with olive oil (OO 7% v / w) after 6 months of being fed with fat (14%) to achieve the hypercholesterolemia´s reversion. Animals were sacrificed and studied its testicular tissue. Ultrastructure showed changes on head formation and sperm nucleus, these results were confirmed with immunofluorescence. Dividing the seminiferous tubule into a proliferative and a differentiation compartment, all cell types were counted. It was observed that groups F and P have a depression of the first compartment. Efficiency in group F is decreased, with less number of spermatids. Olive oil induces a recovery, showing a significant increase in the elongated spermatids´ number at expenses of spermatocytes and round spermatids. These values are consistent with those observed in seminal sperm counts. Results suggest that OO reverses a negative effect of high fat diet on testis.

A46
HYPERTHYROIDISM DECREASES MAMMARY CARCINOGENESIS IN RATS: ROLE OF THE OVARIAN STEROIDS

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We aim to assess whether changes in ovarian hormonal status affect participation of thyroid hormones on the development of mammary cancer (CaM). Female Sprague-Dawley rats were treated *per os* with a single dose of DMBA (15mg/rat) at 55 days of age and divided into euthyroid (EUT, n=28) and hyperthyroid rats (HYPER, 0.25mg/kg/day T4 s.c , n=32). On day 30, rats of both groups were ovariectomized (OVX) or sham operated (SHAM). All the animals were weighted weekly and observed until the appearance of the first palpable tumor. The latency, incidence and progression of tumors were determined. At sacrifice, whole blood samples and a piece of normal mammary gland and tumor were taken for hormone determinations and histological analyses. Statistical analysis was performed by ANOVA I and Chi square (IC>95%). OVX decreased the incidence and increased survival regardless the thyroid status. The estrogen absence in HYPER completely abolished CaM incidence. The latency and the tumor growth rate were similar in HYPER SHAM, EUT SHAM and EUT OVX. However, tumors of SHAM rats were of a more aggressive histological type than OVX. No statistical differences were observed in GH and prolactin levels. In conclusion, hyperthyroidism decreased mammary carcinogenesis and enhanced the protective effect of OVX. The presence of estrogen, regardless of thyroid status, induced tumors with more aggressive behavior.

A47
INFECTION BY *Demodex* SPP IN PATIENTS WITH CHRONIC SKIN DISEASES IN SAN LUIS

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The *Demodex* spp. is a microscopic mite considered to be the most common permanent ectoparasites in humans. *Demodex* feeds on sebum and inhabits skin areas with active sebaceous excretion such as cheeks, forehead and nose. It has been implicated in several skin diseases, for instance, acne vulgaris and rosacea. The aim of the present study was to measure the prevalence of *Demodex spp* in patients of San Luis and its association with skin diseases. The disease was recurrent or chronic with a duration ranging from 8 months to 5 years.

There were 8 male and 23 female patients (age 29-67 years). The skin lesions were acne rosacea-like (n = 24), papulopustular, (n = 3), perioral dermatitis-like (n = 3), and pityriasis folliculorum (n = 1). Direct examination of the skin scrapings with potassium hydroxide and gummed tape with methylene blue, or a combination of these, are used to establish the diagnosis. We conducted the study in order to review clinic-pathologic findings of 31 cases of diagnosed demodicosis. Nineteen cases were positive with potassium hydroxide examination. Twelve cases were excluded, because of insufficient data for a definite diagnosis. Our patients often had a long history of papulopustular or acne rosacea-like eruptions on the face with poor response to nonanti acarid treatments before a correct diagnosis. In patients with facial rosacea, the pathogenesis of skin lesions has been speculated to be caused by an increased density of mites, which triggers inflammatory or specific immune response, mechanically blocking the hair follicle, or acting as a vector to bring in bacteria. *Demodex* is commonly found in skin samples, its number increase when it is complemented with inflammatory reaction in patients with nonhealing skin lesions.

A48
SEROLOGY IN CELIAC DISEASE

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Celiac disease (CD) is an autoimmune, gluten-sensitive enteropathy where intake of foods containing gluten, a protein found in grain species such as wheat, barley and rye, to cause mucosal damage in the small intestine, leading to malabsorption. Selective immunoglobulin A (IgA) deficiency is the most common primary immunodeficiency disorder that is strongly overrepresented among patients (P) with CD. The area of serological testing for CD has developed greatly in recent years. The most commonly used antibodies are anti-tissue transglutaminase, anti-endomysial and anti-gliadin antibodies of both IgA and immunoglobulin G (IgG) class. The aim of this study was to evaluate, in P with clinical suspicion, total serum IgA levels and the presence of some antibody. A retrospective study was performed on 124 P from a private laboratory in San Luis and it was conducted from January 2013 to May 2014. Sera were used for the determinations.Total IgA was determined by radial immunodiffusion. Anti-tissue transglutaminase and anti-gliadin antibodies IgA and IgG were determined by enzyme-linked immunosorbent assay; and anti-endomysial antibodies IgA and IgG by indirect immunofluorescence.In 124P, 61P (49.2%) were adults. Of these, 4P children (6.3%) and 5P adults (8.2%) had low levels of IgA. Among them, 3 P children (4.8%) and 3 P adults (4.9%) had positive IgA anti-tissue transglutaminase and anti-endomysial antibodies. The other antibodies were negative. In conclusion, more than 90% of the P in this study had negative serology, in coincidence with published data. While the gold standard for the diagnosis of CD is intestinal biopsy, the usefulness of serology in screening, follow up and epidemiological data is highlighted in this disease.

A49
TWO DIAGNOSTIC CRITERIA FOR GESTATIONAL DIABETES IN PREGNANT WOMEN OF THE MATERNITY "DRA TERESITA BAIGORRIA"

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Gestational diabetes (GD) is defined as the impaired metabolism of carbohydrates which is firstly detected or starts during pregnancy period. In Argentina, the prevalence of GD is 5%. The aim of this study was to evaluate the prevalence of GD according to Latin American Diabetes Association (A.LA.D.) in the Maternity Dra. Teresita Baigorria, determine the number of patients who had been diagnosed with GD using the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study, and identify the postpartum complications in the mother and children's patients with basal glycemia higher or equal to 92 mg / dl levels. A retrospective study of the database was performed in 443 pregnant women. Plasma was used for glucose determination (enzymatic method). All the patients were screened with 75 grams oral glucose tolerance test. The samples were analyzed on a 250 Metrolab. The percentage of patients diagnosed with GD by ALAD was of a 9.25% (41) vs 12.19% compared to HAPO criteria. The weight of the newborns which gestational age is older or equal to 38 weeks (31), was 3418 g. Additionally, glucose and calcium values, in this group of patients was 51 mg/dl and 9.8 mg/dl, respectively, but the number of samples (6) was not significant. Currently, the HAPO criteria in diagnosis of GD is not used as there is no evidence to show an improvement in the diagnosis of GD causing higher costs to the health system.

A50
LIPID RATIOS AND ATHEROGENIC INDEX: CLINICAL USE AS PREDICTORS OF CARDIOVASCULAR RISK IN PATIENTS WITH TYPE 2 DIABETES.

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Diabetes mellitus (DM) is a metabolic disorder considered as a chronic non-communicable disease that affects millions of people worldwide.DM is responsible for premature mortality and occurs primarily due to renal failure and cardiovascular disease. Abnormal lipid profile as total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-c) and low density lipoprotein (LDL-c) contribute to coronary risk in diabetics. Here, different lipid ratios were analyzed to establish their potential use as indicators of cardiovascular risk (CVR). The lipid profile, non-HDL cholesterol (non-HDL-c); TC/HDL, TG/HDL-c ratios and atherogenic index (AI) log TG/HDL-c in type 2 diabetic patients and control group, were determined. A total of 368 subjects: 208 diabetic patients (107 males and 101 females, 25-75 yearsold) and 160 controls (43 males and 117 females, 25-60 years old) with a similar body mass index: 25.5 ±3.5 kg/m^2, which attended the Juan G. Vivas Hospital(Juana Koslay city, San Luis), were studied. Serum lipids were measured by enzymatic colorimetric method (chemistry analyzer Metrolab 1600 DR). Data were expressed as a mean ± standard desviation. Results from diabetics *vs* controls were: TC:

210±42 *vs* 177±26 mg/dl (p<0.01); TG: 181±95 *vs* 97±27 mg/dl (p<0.01); HDL-c: 49±10 *vs* 53±10 mg/dl; LDL-c: 118±41 *vs* 108±26 mg/dl; no HDL-c: 144±41 *vs* 122±27 mg/dl (p<0.05); TC/HDL-c: 4.2±1.3 *vs* 3.3±0.7 (p<0.05); TG/HDL-c: 4.1±2.5 *vs* 1,9±0.7 (p<0.001); log TG/HDL-c: 0.5±0.2 *vs* 0.24±0.1 (p<0.001). The significant elevation of the lipid ratios, and AI and non HDL-c, without increase of LDL-c, in diabetic patients suggests that these ratios can be considered in the clinical practice as predictors of CVR

A51
SEROLOGICAL MARKERS IN PREGNANT WOMEN OF THE MATERNITY "DRA TERESITA BAIGORRIA"

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The vertically transmitted infections (VTI) are a major problem in public health, with great impact on maternal and infant morbidity/mortality. The prevalence of antibodies against *Treponema pallidum* (Tp), *Trypanosoma cruzi* (Tc), *Toxoplasma gondii* (Tg), Human Immunodeficiency Virus (HIV) and Hepatitis B surface Antigen (HBsAg) was investigated in pregnant women. A retrospective study of prevalence of these infections was performed in hospital from April 2013 to August 2014 on 3.076 pregnant women (average: 26 years old). Patients were divided into four age groups (G) as follows: G1: 11-20, G2: 21-30, G3: 31-40 and G4: 41-50 years old. Serum was used for the determinatios: HBsAg andHIV were determined by enzyme linked immunosorbent assays, Tc by indirect hemagglutination assay. Tp by direct agglutination and flocculation test. The presence of HBsAg was tested in 90.5% of pregnant women, it was only positive in one patient. Prevalence of Chagas disease and Toxoplasmosis was higher in older age groups. Anti-HIV antibodies were determined in 97.4% patients, prevalence was higher in G4 (2,27%). The prevalence of anti-Tp antibodies was in G2 (1.5%). Different information exists about prevalence of the VTI in Argentina, but data from this cohort are lightly lower than those documented. Serological screening of the mother, promoting good personal hygiene and universal immunization are measures that can contribute towards decreasing the incidence and morbidity of congenital and perinatal infections.

A52
METABOLIC RISK FACTORS IN RENAL STONE FORMATION IN WOMEN AND MEN

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A number of different metabolic conditions can increase the risk of developing lead to kidney stones. The aim was to examine the metabolic differences as risk factors in renal stone formation in women and men. 453 patients were studied, 268 women (W) and 183 men (M). The diet hypocalcic-hyposodic/7 days with calcium 1000mg/day was administered. Urine (24h, 2h) and blood were collected. Calcium, phosphorus, creatinine, magnesium, sodium, uric acid, cystine, citrate, oxalic acid and parathormone were determined. Statistical analysis T-Test and Chi- square were used. Body mass index ≥25 (OMS) 37.68% W vs 60.40% M (p<0.0001). Hypercalciuria (>250 mg/24h) 29.85% W vs 32.24% M (p <0.0002). Marginal, fasting and renal hypercalciuria increased significantly in W and absorptive hypercalciuria in M(p<0.001). Hyperuricemia 25.37% W vs 70.49%M (p<0.0001). Hyperuricosuria 42.91% W vs 33.33% M (p<0.05). Hypernatriuria (>150 mmol/24h) 9.70% W vs 16.93% M (p<0.03). Hyperphosphaturia (>1100 mg/24h) 2.23% W vs 7.65%M (p<0.01).Hyperoxaluria (>45 mg/24h) 5.22%W vs 4.37% (ns). PTH (>55 pg/ml) 16.41% W vs 9.83% M (p<0.05).Hypomagnesiuria (<70 mg/24h) was significantly increased in women than men (42.53% W vs 29.50% M (p<0.006). Hypocitraturia (<350 mg/24h) 33.58% W vs 40.98% M (ns). Cystinuria (>100 mg/24h) 0.37% W vs 0.54% M (ns). Low urine volume (<2000 ml/24h) was found in women and men (>65%).Hypercalciuria, hyperuricemia, hypernatruria, hyperphophaturia and hypocitraturia were most common risk factors for stone formation in men, hypomagnesiuria, hyperuricosuria and hyperoxaluriain women.These metabolic parameters were significantly different between women and men. The results show the importance of studying risk factors kidney stone formation by biochemical parameters to achieve a diagnosis of the metabolic abnormality and introduce a specific diet and therapy to prevent recurrence.

A53
PREVALENCE, AWARENESS, TREATMENT AND CONTROL OF HYPERTENSION IN STUDENTS OF SAN LUIS UNIVERSITY

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Hypertension (HTA) is one of the main risk factors for cardiovascular disease. The prevalence of HTA has increased due to greater life expectancy and prevalence of obesity in the population. The purpose of this study

was to estimate the prevalence and distribution, awareness, treatment, and control of HTA in students of San Luis University. The survey was approved by Ethics Committee and the data were statistically analyzed. A cross-sectional study based on surveys performed on subjects randomly selected from biochemistry and nursing carrier of our university. A total of 264 subjects (53.0% nursing, 47.0% biochemistry) were tested. Measuring rods scales and equipment aneroid blood pressure were used. Mean age: 24.54±5.44 years (18-49 years), weight: 70.1±13.0 kg (42.5-130), height: 1.64± 0.08 m (1.45-1.87), BMI: 23.2±4.2 (16.3-44.9), waist circumference: 80.6±15.0 (53-130), mean systolic and diastolic blood pressure: 108.1±11.3 and 69.8±11.0.The prevalence of HTA was 16% (12% women, 4% men, 2.2% treatment), without HTA 73.4 %, unknown 10.6%. DBT: 16.6%, DLP: 4.5%, hyperuricemia: 5.3%, internment for breast pain: 6.4%, IAM: 4.5%, IC: 3.7%, ACV: 3.0%, coronary disease: 2.6%. Nicotinism: 33.3%; alcoholic drinks frequently 3.0%, occasionally 82.1%, never 14.9%; physical activity frequently 33.7%, occasionally 50.7%, never 15.6%, diet fatty acid frequently 18.1%, occasionally 80.3%, never 1.6%, family history cardiovascular disease: 54.1% and 97,7% know about risk factors.Sixteen percent of the population evaluated has HTA, more common in women. It is advisable to identify risk factors early in life, in order to prevent the subsequent development of HTA in adulthood and its future complications. This study suggests the broadening of bases for bio-clinical examination to other sectors of our community in order to more effectively motivate students, parents, and teachers in healthy habits for cardiovascular disease prevention.

A54
MONITORING METHODS FOR COMPLIANCE WITH TOXIC PROLAMIN FREE DIET IN CELIAC CHILDREN IN SAN LUIS.

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Celiac disease (CD) is one of the most frequent chronic enteropathy all the world. It is an autoimmune systemic disease causedby gluten and prolamins intake, and it appears in individuals geneticallypredisposed to it. The treatment for CD is a gluten-free diet (GFD). Thelack of knowledge and family support gives rise to voluntary andinvoluntary transgressions which relate to foodhandling, crosscontamination and thus to the consumption of products that may have gluten. The purpose of this study is to compare antibody (Ab) predictors for CD with GFD compliance from surveys conducted to celiac children and their parents. 50 celiac children among 6 to 10-years old of San Luis city (2013) with diagnosis CD, in maintenance stage, under at least a 6-month treatmentwere studied. Anti-transglutaminase (ATG), Anti-endomysium (AAE) antibodies and IgA were measured by immunofluorescence, a semi-structure interview with open and closed questions with parents and their childrenwere conducted. The differences in relation to compliance according to interviews to parents and children were significant, correlation coefficient ($p<0.05$). We observed a high adherence (61.4 %) of parents reported treatment compliance in relation to 16 % of the children´s answers. In contrast, the 26 % of the children reported no gluten-free diet compliance, while 10 % of them reported partial compliance to their parents' answers. Most of the children (71.8 %) reported full compliance to GFD, and this relate with negative reaction of ATG and AAE. Finally,positive correlation ($p =0.001$) betweenantibodyresponseandparents regardingadherence to treatmentof his childrenwas found. In contrast, there was no correlation between the response of the children from the Ac.

A55
HISTOCHEMISTRY DETECTION OF FETAL HEMOGLOBIN THROUGH KLEIHAUER-BETKE REACTION IN PREGNANT WOMEN OF THE MATERNITY "DRA.TERESITA BAIGORRIA"

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The presence of erythrocytes containing fetal hemoglobin (HbF) in maternal blood of pregnant women (PW) can assume a relevant pathology such as the haemorrhage feto-materna (HFM). The percentage of erythrocytes with HbF allows generating a diagnosis about the degree of fetal hemorrhage; apart from showing the cause in case of fetal blood incompatibility. The aim of our study was to determine the presence of fetal erythrocytes in blood circulation of PW with more than 5 months pregnant and relate it with some pathologies during pregnancy period. Blood was extracted with EDTA to 108 PW. Hematological assessment and a very thin smear were made in order to apply acid elution technique. Giemsa was used as anti-colorant. 2500 cells are counted under the microscope and a percentage of fetal to maternal cells is calculated. The percentage of positivity obtained was 21.3% (23). In PW with previous pathologies (diabetes (9), hypertension (9), premature rupture of membrane (6)) was observed an increase in the positivity, 44.4%, 44.4% and 50% respectively but the number of observed samples in these categories was small for statistic analysis. The percentage of erythrocytes with HbF found in positive samples

(1.8%) was low compared to that reported in literature for documented cases of HFM (>5%). Published studies report that 50% of PW there is passage of fetal erythrocytes into the maternal circulation, without presupposing a pathology. The HFM is a rare but serious event, Kleihauer reaction is a useful tool in order to provide evidence to clinical diagnosis.

A56
EVALUATION OF VITAMIN D STATUS AND BIOCHEMICAL PARAMETERS ASSOCIATED IN AN ADULT WOMEN POPULATION OF SAN LUIS

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Vitamin D (Vit D) is a fat soluble steroid that regulates the calcium homeostasis and bone health, and has important non-calcemic effects in cancer, autoimmune and cardiovascular diseases. Nutritional status of Vit D has gained importance because its deficiency is highly prevalent worldwide.The aim of this study was to determine if Vit D status is associated with its seasonal dynamics and serum levels of calcium (Ca), intact parathyroid hormone (iPTH) and urinary Calcium (Ca u), in women who attended to a private clinical laboratory of San Luis city, during January-December, 2013. Those women who received calcium and/or vitamin D supplement were not included. In 198 women, between 25 and 70 years of age (53.8 ± 10.22), the stratification of Vit D level with age was analyzed (range: 30-49; 50-59 and > 60 years). Serum total 25-hydroxyvitamin D (25-OHD) and iPTH by electrochemiluminescence -Cobas e 411, and Ca and Ca uconcentrations by spectrophotometry, were measured.The average of 25-OHD was 22.6 ± 1.40 ng/ml. Moderate and severe Vit D deficiencies were defined as 25-OHD values of 10-30 ng/mL and < 10 ng/mL, respectively; and values >30 ng/mL are considered sufficient. The prevalence of moderate and severe Vit D deficiencies in the population was 55.5% and 20%, respectively. In women over 50 years old, the increased 25-OHD deficiency ($p<0.01$) was not associated with secondary hyperparathyroidism. Ca and Ca u levels were within reference values. A higher prevalence of hypovitaminosis was recorded in autumn-winter in comparison to spring-summer ($p <0.02$). The high prevalence of Vit D deficiency in San Luis city emphasizes the need to detect the vitamin status at an early age. This could contribute to reduce consequences of vit D deficiency to reach menopause.

A57
EFFECTS OF *Origanum vulgare* (OREGANO) ON THE RESPONSE OF *Helicobacter pylori* TO OXIDATIVE STRESS

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Helicobacter pylori is a microaerophilic, gram-negative human pathogen that causes several gastric pathologies.Despitethe chronic gastritis that develops, the microorganism is able topersist in the stomach for decades.*H. pylori* produces a strong immune response. The oxidative burst with production of reactive oxygen species (ROS) contributes to the microbicidalactivity of macrophages. *H. pylori*is able to protect itself from ROS through induction of antioxidant enzymes such as superoxide dismutase (SOD). The bacterial attachment to stomach epithelium is the initial step for the pathogenesis and *H. pylori* has the capacity to form biofilm. In this state, *H. pylori*exhibits a remarkable protection against antimicrobial agents.Many therapeutic agents are used for its eradication, however, the increase of resistant strains leads to the search of herbs and spices with antimicrobial potential against *H. pylori*. In this study, the effect of oregano on the expression of *sod* gene in *H. pylori* planktonic cells and biofilm was evaluated. The reference strain NCTC146128 was grown in Petri dish with Mueller-Hinton broth plus 5% fetal calf serum and 0.3% glucose, and a glass surface for adherence and formation of biofilmwas added. The culture was incubated in microaerophilia for 72 h at 37ºC. Then,the biofilm was transferred to a new plate with medium added with 1 mg/ml of oregano extract and was incubated for 26h.For RNA extraction, the biofilm and planktonic cells were treated with Trizol, and cDNA was obtained. Oregano significantly suppressed the expression of *sod*gene in both states.The results suggest that oregano could exert a protective effect inhibiting the *sod*-dependentantioxidant mechanismwhereby*H. pylori*survives in macrophages.These pathways represent potential targets for therapeutic exploitation.

A58
IS THE LUNG A NEW SITE OF *Helicobacter pylori* INFECTION?

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Helicobacter pylori is a gram-negative bacterium that colonizes the gastric mucosaof 50% of the world population causing chronic gastritis andpepticulcer, beingthe principal cause of gastric adenocarcinoma. In recent years, it has shownevidence of theparticipation of *H. pylori* in extradigestive inflammatorydiseases, especially in respiratoryillnesseslikeasthma, bronchiectasis and chronicobstructivepulmonarydisease.Little is known about the

impact of *H. pylori* infection on lung. The objective of this work was to evaluate the effect of *H. pylori* infection on lung. Mice were infected by orotracheal instillation with 20 µl of 1 $\times 10^8$ cfu/ml*H. pylori* reference strain. After 3 days of infection, the mice were sacrificed, bronchoalveolar lavage (BAL) was performed and lungs of each mouse were aseptically removed. Uninfected mice were used as controls.The number of bacteria in lung was determined by homogenization of these organs in saline solution, plating of the homogenates on Mueller-Hinton agar, and counting of colony-forming units (CFU) after incubation at 37 °C for 72 h. The bacterial recovery was 2.3×10^3 cfu/mlin infected mice. From the BAL, a smear was made and cell counts were performed after Giemsa staining. The counts showed 85% macrophages, 10% lymphocytes and 5% neutrophils in the infected mice. The lungs from the uninfected animals did not show histopathological features and the counts showed 95% macrophages and 5% neutrophils. Hematoxylin-eosin-stained lungsof infected mice revealed foci of mild inflammatory infiltration of bronchioles and adjacent alveoli. This is the first study that demonstrates an inflammatory response, morphological changes and bacterial recovery after *H. pylori* infection in mice lungs. This may be possible in patients with duodenogastric reflux, when the spilling or inhalation of *H. pylori* into the respiratory tract could occur.

A59
EFFECTS OF ABIOTIC FACTORS AND INTERACTION WITH *Listeria monocytogenes* ON THE GROWTH PARAMETERS OF *Aspergillus flavus* AF54

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In the storage ecosystem of brewer's grains, microorganisms such as aflatoxigenic*A. flavus* and *L. monocytogenes* were isolated. The interaction between them and with respect to environmental factors is important to the prediction of contamination risk of this food with these undesirable microorganisms. Furthermore, biotic factors such as the presence in the same habitat of different microorganisms can develop ecological interactions between them that are positive, negative or indifferent for fungal growth. The aim of the present work was evaluate the effect of *L. monocytogenes* in interaction with a_W, pH and temperature on the lag phase, and growth rate of *A. flavus.* The strains *A. flavus* AF54, *L. monocytogenes* LM1 and *L. monocytogenes* LM6 were assayed at this study. The basic medium used was brewer's grains meal medium, previously adjusted to different a_W (0.95, 0.98 and 0.995) and pH (5, 7 and 9). Through the pour plate method, 100 uL of *L. monocytogenes* suspension (10^8 c.f.u. mL^{-1}) was added. Then, plates were inoculated in the center with the fungal strain and incubated for 21 days at 25 °C. The diameter of the colony was measured and growth parameters were calculated. In general, the presence of *L. monocytogenes* exhibited increased on lag phase and decreased on growth rate of AF54. The lowest a_W (0.95) assayed showed the largest lag phase and lowest growth rate. The pH was less influential than a_W in fungal growth parameters. The highest time of lag phase was observed at $0.95a_W$, pH 9 and co-inoculated with LM1 (53 h) or with LM6 (52 h) and the lowest growth rate of AF54 was 8.2 mm day^{-1}, at $0.95a_W$, pH 5 and co-inoculated with LM1. In conclusion, the presence of *L. monocytogenes* and osmotic stressgenerally increased fungal lag phase and decreased fungal growth.

A60
ISOLATION OF ENDOPHYTIC MICROORGANISMS FROM VEGETABLES FOR CONTROLLING *Botrytis cinerea* BNM 0527

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Endophytic microorganisms living not aggressively inside plant tissues, give to the host several benefits including resistance to fungal diseases by controlling the development of various pathogens.The aim of this work was to apply endophyticmicroorganisms isolated from vegetables to control *Botrytis cinerea*BNM 0527. Eighteen strains of bacteria and yeasts were isolated from grapevine (*Vitis vinifera*), lemon (*Citrus limonun*), parsley (*Petroselinum crispum*), oregano (*Origanum vulgare*), beans (*Vicia faba*) and alcayota (*Cucurbita ficifolia*) obtained from two organic gardens of the city of San Luis (Argentina).Biocontrol ability of endophytes on *B. cinerea*BNM 0527 was tested in two *in vitro* assays: the first test used plates containing Yeast Glucose Mineral medium(YGM) inoculated with thepathogen and then disks with previously isolated endophytes were placed. After incubation of the plates at 27 ± 1°C for 120 h,growth of endophytes was measured in millimeters.In the second test, plates with YGM were inoculated with disks of *B. cinerea*BNM 0527 and each selected endophyte, and after incubation of plates the antagonistic and/or inhibitory action was macroscopically observed. In the first trial, seven strains which grew between 20-40 mm were considered potential biocontrollers through competition for space and nutrients. In the second study, five of seven strains showed capacity to antagonize efficiently the fungal growth. This work got the isolation of endophytic microorganisms from vegetables in the city of San Luis, and demonstrated a very good biocontrol of *B. cinerea* BNM 0527 by five endophytic bacteria in the preliminary tests.

A61
SIMULTANEOUS SULFATE AND CHROMIUM REMOVAL BY *Streptomyces* sp. MC1

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In previous works, *Streptomyces* sp. MC1 showed ability for reducing Cr(VI); moreover, the presence of 5 mM of sulfate ion mitigated the inhibitory effect of Cr(VI) on the bacterial growth and increased its removal from culture supernatants. The main aim of this study was to investigate the influence of increasing sulfate concentrations on the Cr(VI) reduction by *Streptomyces* sp. MC1 and the sulfate removal in absence and presence of Cr(VI). Cells of *Streptomyces* sp. MC1 were grown in liquid minimal medium supplemented with glucose with or without 20 mg/L of Cr(VI) and Na_2SO_4 (ranging from 0 to 10 mM) for 96 h at 30ºC in an orbital shaker. Determinations of biomass by dry weight, Cr(VI) concentration in the medium by 1,5-diphenylcarbazidecolorimetric method, total chromium by inductively coupled plasma-atomic emission spectrometry (ICP-AES), and residual sulfate by Hach DR2800 were performed. In absence of Cr(VI), the *Streptomyces* sp. MC1 growth was practically the same from 0 to 7.5 mM of sulfate concentration while it was markedlyinhibited at 10 mM. In presence of Cr(VI) but in absence of sulfate ions, bacteria showed no growth. Cr(VI) reduction was highest at 5 and 7.5 mM of sulfate, and the sulfate removal was lightly major in presence of Cr(VI). On the other hand, total chromium concentration in the supernatant decreased 40-50% in all assayed conditions. These results indicate that *Streptomyces* sp. MC1 can utilize chromate as an electron acceptor for sulfate reduction and hence, both Cr(VI) and sulfates get removed from the culturebroth.

A62
GROWTH OF *Listeria monocytogenes* UNDER EXPOSURE TO 2-ISOPROPYL-5-METHYLPHENOL

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The manufacture of minimally processed fresh products presents important challenges for quality and safety, whereby antimicrobial agentsare widely usedfor the preservation ofready-to-eat (RTE) foods. The 2-isopropyl-5-methylphenol is a colorless crystalline substance with a characteristic odor that is present naturally in the essential oils of *Thymus vulgaris* (Thyme) and *Origanum vulgare* (Oregano), both used as condiment infood processing. The aim of this work was to compare the growth kinetics of *L. monocytogenes* in the presence of different concentrations of 2-isopropyl-5-methylphenol. *L. monocytogenes* CLIP 74902 was grown in brain heart infusion under aerobic conditions. Overnight cultures were diluted 1:25 in different Erlenmeyer flasks containing 100 ml of abasal medium (g/l): proteose peptone 30, yeast extract 5, trypticase 5, pH 7.6, supplemented with 2-isopropyl-5-methylphenol (µg/ml): 0 (control), 250, and750. Cultures were incubated for 20 h with agitation (80 rpm). Biomasses were estimated periodically by reading the optical density (OD) at 600 nm. Thespecific growth rate (µ) andlag period (L) werecalculated fromthe growth curves. Under exposure to 0, 250 and 750 µg/ml of 2-isopropyl-5-methylphenol, µ (h^{-1}) values obtained were: 0.54, 0.33, 0.28 and L (h): 0.99, 3.91 and 9.96 respectively. These results demonstrate the important inhibitoryeffect exerted by2-isopropyl-5-methylphenol on growth of *L. monocytogenes*, which would beof interest in the development of edible films and coatings with functional properties for food preservation.

A63
THE AIR THAT WE BREATHE: MORE THAN A GAS MIXTURE

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The biological particles of the air are directly related with air quality and people health. Through respiration process, particles with size less than 20 µm can go across lower respiratory branch and elicit allergy phenomena and bronchial asthma, among other illnesses. In order to characterize the diversity of biological particles circulating in the air of Universidad Nacional of San Luis, it was conducted an aerobiological continuous sampling, in outdoor and indoor environments of Facultybuildings. It was used avolumetric spore trap of Hirst type, Lanzoni trademark, model VPPS 2000, and the samples were analyzed using the methodology proposed by the "Red Española de Aerobiología" (REA). Between the biological particles, we identified spores and other fungi structures from *Cladosporium*, *Alternaria*, *Coprinus*, *Helicomyces*genera, etc. It was also registered pollen from many plants (*Morus* spp., *Eucalyptus* spp., *Pinus* spp., *Cupressus* spp., *Fraxinus* spp., etc.), *Lepidoptera* scales, insect hair, feather barbules, phytoliths, algae, arthropod droppings, between other biological particles. Additionally, non-

biological particles were identified. The type and concentration of the particles varied between indoor and outdoor. The maximal peaks for indoor particles corresponded to fungi spores (3,180.31 spores/m^3 of air/day, in April), and for outdoor particles, to pollen grains (5,774 grains/m^3/day, in September). We conclude that diversity of biological particles in the analyzed air samples is high, and varies between the different environments. The knowledge of the identity, concentration and periodicity of bio-aerosols in different times of the year is important, in order to relate these findings with seasonality of allergy and guide people towards an effective diagnosis and treatment.

A64
ANTIMICROBIAL ACTIVITY OF *Curcuma longa* (CURCUMA) AND *Origanum vulgare* (OREGANO) EXTRACTS ON *Clostridium perfringens* STRAINS

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Clostridium perfringens is an anaerobic gram-positive pathogen that causes severe disease in humans and animals. Curcuma and oregano have been consumed with dietary and medicinal purposes for thousands of years and the two species have demonstrated antibacterial, anti-inflammatory and antioxidant activities. The aim was to determine the antimicrobial activity of curcuma and oregano extracts on *C. perfringens* strains. Two enterotoxigenic (E+) and two non-enterotoxigenic (E-) strains were used. The antimicrobial activity was determinedby the liquid-medium method using thioglycolate broth in anaerobic conditions. All strains assayed (inoculum 1 x 10^8 CFU/ml) were treated with the extracts at 2.5 and 5 mg/ml. The effect of extracts was determined by measuring OD at 580 nm after 6 h of incubation. Simultaneously, the antimicrobial activity by the agar diffusion method on Iron Sulphite agar with the extracts at same concentrations was determined. The antimicrobial activity of curcuma and oregano by liquid-medium method showed 90% and 99% growth inhibition in E+ and E- strains, respectively. By the agar diffusion method,curcuma showed antimicrobial activity significantly smaller in the E+ (7 x 10^2 - 1 x 10^3 CFU/ml) strains than E- (7 x 10^3 and 9 x 10^3 CFU/ml) strains; while oregano extracts showed total inhibition. The results presented indicate that curcuma and oregano extracts have significant antimicrobial properties against *C. perfringens* and could represent useful additives for preservation of foods. The liquid-medium method was more sensitive than the agar diffusion methodto determine the antimicrobial activity.

A65
DETERMINATION OF PARASITIC FORMS IN CANINE FECES AND SOIL SAMPLES COLLECTED IN PUBLIC ROADS

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The presence of domestic animals in public roads is a relevant issue nowadays. Defecation and its contribution to environmental contamination is the main cause of parasite infections in our society. The aim of this study was to determine the presence of protozoa and helminths in canine feces and soil samples collected in public roads from different sectors of San Luis city. Telemann centrifugation method and Willis flotation method were applied to 79 canine feces samples, meanwhile 63 soil samples were centrifuged after homogenizing with water and filtering with gauze. The rate of parasite-positive feces was about 21.5% (17/79) from which 58.8% correspond to protozoa and 41.2% to helminths. *Trichuris spp.* eggs 5.1% (4), *Blastocystis spp.* cysts5.1% (4), *Giardia spp.* cysts 3.8% (3), *Endolimax spp.* cysts 3.8% (3), *Toxocara spp.* eggs 2.5% (2), *Uncinaria spp.* eggs 1.3% (1) and *Ascaris spp.* eggs1.3% (1) were found. Larvae were also observed at 1.3%(1). Soil samples were contaminated with parasitic forms in 14.3%(9/63): larvae 11.1% (7), *Toxocara spp.* eggs 7.9% (5), *Uncinaria spp.* eggs 4.8% (3) and *Ascaris spp.* eggs1.6% (1). Results show that the risk of acquiring a parasitic disease in San Luis city compared to other Argentina areas is significantly lower. Nevertheless, preventive measures should be continuously applied in order to a lesser contamination by parasites and to reduce infections with zoonotic and humanimpact.

A66
SYNERGISTIC EFFECT OF TWO BACTERIOCINS PRODUCED BY LACTOBACILLI STRAINS ON *Listeria* spp. GROWTH

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In recent years there is a growing interest in the combination of different antimicrobial substances to enhance the inhibitory effect on pathogen microorganisms.Bacteriocins L23 and L60 produced by *Lactobacillus fermentum* L23 and *Lactobacillus rhamnosus* L60, respectively, have previously demonstrated the ability to inhibit the growth of several pathogen microorganisms including *Listeria* spp. isolated from food samples. The aim of this study was to

evaluate the interactions between bacteriocins L23 and L60 to inhibit *Listeria*spp growth. Minimum inhibitory concentration (MIC) of each bacteriocin alone and combined were evaluated by the broth microdilution method and the checkerboard assay, respectively, on *L. monocytogenes*, *L. innocua* and *L. welshimeri*growth. MIC values were used to calculate the fractional inhibitory concentration (FIC) and results were interpreted as follows: synergy, FIC≤1; indifference, 1<FIC<2; antagonism, FIC≥2. The results of the present study showed that combining both bacteriocins, MIC values were lower than those observed when they were tested independently on each strain of *Listeria* spp. assayed. Combination of L23 and L60 showed a synergistic interaction on all *Listeria* strains tested with FIC values ranged from 0.50 to 0.75. These findings demonstrated that when the bacteriocins L23 and L60 are used together produced an enhanced antibacterial effect on *Listeria* spp. in comparison to each bacteriocin used alone.

A67
ANTIMICROBIAL SUSCEPTIBILITY OF *Yersinia enterocolitica* STRAINS ISOLATED FROM DIFFERENT SOURCES

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The knowledge of the susceptibility of a microorganism to different antibiotics is very important to choose the appropriate therapeutic treatment. The major challenge generated by the use of antibiotics is the development of antimicrobial resistance mechanisms by the involved organisms. The Kirby Bauer agar diffusion method is a technique commonly used in clinical laboratories to establish the susceptibility of bacterial strains to different antibiotics. Drug selection should be based on clinical response patterns and bacterial susceptibility, as well as on newer standards interpreting results. In this work we determined the susceptibility to antibiotics of 34 *Y. enterocolitica* strains isolated from different sources using the agar diffusion method according to Kirby-Bauer. All *Y. enterocolitica* strains showed susceptibility to the following antibiotics: amikacin, cefotaxime, colistin, fosfomycin, furazolidone, gentamicin, and trimethoprim-sulfamethoxazole (TMS). In addition, these strains were resistant to ampicillin, rifampicin, erythromycin and cephalothin. *E. coli* strain ATCC 25299, used as a control of growth medium and antimicrobial discs revealed consistent results with antimicrobial standards. The use of antibiotics as treatment against enterocolitis caused by *Y. enterocolitica* is only recommended in immunocompromised patients, with septicemia or invasive infection, in which mortality can be very high (50% of cases). The antimicrobial susceptibility patterns obtained in this study are consistent with the *in vitro* findings mentioned by other authors.

A68
EVALUATION OF PATHOGENICITY OF *Yersinia enterocolitica* STRAINS BY IN VITRO VIRULENCE TESTS

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Yersinia enterocolitica is an enteropathogen that includes 6 biotypes (B) and more than 60 serotypes (O) being the biotypes 1B, 2, 3 and 4 and serotypes O:3, O:9, and O:8, the most frequently associated with human infection. Its pathogenicity is attributed to virulence factors encoded by the pYV plasmid and the chromosome. The aim of this study was to evaluate virulence markers encoded at plasmidial and chromosomal levels in *Y. enterocolitica* strains of different bioserotypes, from our collection and from reference laboratories, using *in vitro* virulence tests. The following assays were performed: autoagglutination at 37° C in Clark and Lubs broth (CLB); calcium dependence in magnesium oxalate agar (MOX) and trypticase soy agar (TSA); pyrazinamidase activity on pyrazinamide agar (P) and hydrolysis of esculin on bile esculin agar (E). Strains from pathogenic bioserotypes showed presence of turbidity at 22°C and autoaglutination at 37°C in CLB; predominance of tiny colonies on MOX, large colonies in TSA and negative reaction in the P and E media. Our results agree with previously published data, which show a close correlation between *Y. enterocolitica* pathogenic bioserotypes and virulence markers, which become evident by these phenotypic assays. *In vitro* virulence tests can be used in the screening of the pathogenic potential of *Y. enterocolitica* isolates, which subsequently can be confirmed by the use of more expensive molecular techniques as PCR.

A69
ISOLATION OF *Listeria* spp IN SEAFOOD SAMPLES

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Listeria monocytogenes is an emerging pathogen of interest in the food industry. It has shown a high prevalence in environmental media, vegetables andseafood as it has capacity to grow in a wide temperature range, tolerate salt, acidity, low oxygen tension and produce biofilms. Listeriosis mainly affects pregnant women, fetus, newborns and immunocompromised individuals, causing meningitis and septicemia. The objective was to isolate *Listeria* spp. from seafood and characterize biochemically the isolates. Sixty six samples of mussels from different shops in San Luis city were analyzed. Ten grams of each sample were weighed and incubated in Palcam broth (PB)for 24 h at 37ºC. Then,they were subcultured on Palcam agar (PA) for 48 h at 37ºC. When growth was not detected on PA, isolating was retried from cold selective enrichment in PB for 7 days at 4ºC. Suspicious *Listeria* spp. colonies were recognized for being small, black, greenish, with depressed center and blackening of the medium. These colonies were inoculated on trypticase soy agar and incubated 24 h at 37ºC. From these cultures the following tests were performed: Gram staining, motility test, catalase, hemolysis on horse blood agar and sugar fermentation. From the analyzed samples, three*Listeria*species were identified: 10 isolates corresponded to *L. innocua* (15%), one corresponded to *L. grayi* (1.5%) and other to *L. seeligeri* (1.5%). Although *L. monocytogenes* was not detected in these samples, the presence of other *Listeria* species in raw frozen shellfish warns about the importance of proper cooking of these foods before ingestion and the feasibility of these organisms to generate cross-contamination.Hence the importance of standardized sanitation procedures to reduce risks regarding to this emerging pathogen of significant impact on public health.

A70
ASPARAGUS FROM SAN JUAN: A POTENTIAL FUNCTIONAL FOOD

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The global food trends in recent years have shown highlight interest of certain foods, which besides their nutritional value, provide benefits to the physiological functions of the human body. These variations in eating patterns have created a new area of development in food science and nutrition, which is called "functional food". The aim was to evaluate the total phenolics and flavonoids contents, as well as antioxidant and antibacterial activities of methanolic extracts from culture of *Asparagus officinalis* L. The total phenolics content (according the Folin–Ciocalteau method) was ranging from 67 to 70 mg of gallic acid equivalents per 100 g of fresh weight, whereas flavonoids (by Aluminium trichloride method) ranged from 19.3 to 22 mg of quercetin equivalents per mg/100 g fresh weight.In general, the asparagus samples presented high macronutrients contents of K, P, Ca, and Mg, and lowamounts of Na, Fe, Al, Zn, B, Mn, and Cu (FAAS-FAES e ICP-MS). On the other hand, the methanolic extracts showed a moderate free radical scavenging activity with IC_{50} value of 2mg/ml (DPPH method). Regarding to the antibacterial effect, the asparagus from San Juan, presented a moderate activity against *Salmonella enteritidis* and *Escherichia coli* ATCC (MIC = 500 µg/ml);while *E. coli*clinical isolated and *Yersinia enterocolítica*low activity (MIC value between 1000 and 1500 µg/ml). This is the first report of *Asparagus officinalis* L cultivars from San Juan Argentine, enhancement its popular reputation as anutritionally healthy food. (CICICA –UNSJ-CONICET)

A71
IN VITRO EFFECT OF N-(PHOSPHONOMETHYL) GLYCINE AGROCHEMICALS ON TOTAL HETEROTROPHIC BACTERIA AND *Azotobacter chroococcum*

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Excessive use of agrochemicals can produce a disturbance in the natural balance of the soil. Microorganisms can undergo biochemical alterations that affect their activity in the organic matter decomposition, nitrogen fixation and biofertilizers, threatening biodiversity and the soil productive future. It has been found that soils treated with N-(phosphonomethyl) glycine (glyphosate) reduce the number of nitrogen-fixing bacteria, and doses upper to 4 kg ha^{-1} of this product, inhibit nitrogen fixation in free-living nitrogen-fixing bacteria. The aim of this study was to evaluate the action of glyphosate on the feasibility of total heterotrophic bacteria and *Azotobacter chroococcum* in laboratory conditions. Soil samples were collected in Villa Mercedes (San Luis). They were sifted in 5-2 mm meshes. Dilutions- suspensions of soil were performed. Volumes of 0.1 ml of each dilution were plated on culture medium (soil extract, K_2HPO_4 and 1.5% agar) added with 0.1 ml of glyphosate (based on the proportion of the commercially recommended field concentration). The same procedure was performed with *A. chroococcum* on Ashby medium.

All of them were incubated at 28°C for 72 h. Results showed inhibition of up to 50% of total heterotrophic bacteria and fungi presence in most of the plates with glyphosate. There was no significant effect on *A. chroococcum* and the control group. This study allowed visualize the total heterotrophic bacteria and *A. chroococcum in vitro* behavior in presence of N-(phosphonomethyl) glycine agrochemicals. It would be important to increase research on the effects of glyphosate on soil biodiversity.

A72
BACTERIOLOGICAL ANALYSIS OF GROUNDWATER ARROYO LAS LAJAS WATERSHED, CÓRDOBA

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Groundwater in the Arroyo Las Lajas watershed is used for all activities: agriculture (corn-soybean-wheat), in a subordinate develops livestock (mainly cattle and pigs) and human consumption. Hydrogeological and bacteriological studies of total viable mesophilic aerobic bacteria (RT), total and fecal coliform (CT and CF), *E. coli* and *P. aeruginosa* were determined by traditional methodology (Standard Methods, 1998), to define the fitness for human consumption. We analyzed groundwater samples (n=28) from the first 10 m of the unconfined aquifer. CE (electrical conductivity), pH, T °(temperature), OD (dissolved oxygen) were measured in the fieldand in the laboratory were identified majority, minority and some trace elements. The CE waters samples varies from 266-1916 µs/cm and dissolved oxygen concentrations between 2.24 and 8.26 mg/L. Sixteen samples (57%) were not suitable for human consumption since one o more the parameters exceed normed established by the Argentine Food Code. Were observed RT ranged 30 to 6.4 $x10^3$ cfu/mL; in 87% was CT and 56% of CF between 4 and 4.8 x 10^3 NMP/100 mL and the presence of *E. coli*. We observed the presence of *Ps. aeruginosa* in 37% the samples. These data show that human activities in the region affect the bacteriological quality of groundwater in many cases becoming a potential danger to the health of people who consume it, because of the number of detected markers including indicators of pollution fecal origin.

A73
FREEZE-DRYING FOR PRESERVATION OF *Botrytis cinerea* IN STRAIN COLLECTIONS

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The filamentous fungus *Botrytis cinerea* is responsible for gray mold in stored plant products. The study of the control of this important pathogen assumes the availability of conidia (spores). The ability to form or not form conidia/microconidia during propagation by successive cultures supports the availability of spores. This arises the question why is important their preservation in culture collections. The aim of this study was to document the survival, viability and preservation characteristics of three lyophilized strains of *B. cinerea* (B4, B5 and B10). Conidia (spores) of *B. cinerea* were harvested from cultures grown on potato dextrose agar. The spores were suspended in skim milk, frozen at -70°C overnight and then, lyophilized. The lyophilized spores were stored at room temperature. After 10 days of storage, they were re-hydrated and their viability was checked on potato dextrose agar plates. The growth diameter of the colonies was measured. All cultures were done in triplicate. The viability of spores was also tested by infecting wounds on apples. The 100% of the lyophilized products showed viability after freeze-dryingtreatment. All strains recovered retained their colonization potential, generating 100% rot (as Disease Index % DI) in wounds of infected apples.

A74
ANTIBACTERIAL ACTIVITY OF CRANBERRIES CONSUMED IN THE PROVINCE OF SAN JUAN, ARGENTINA

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Urinary Tract Infections (UTIs) affect men and women of all ages, usually resolve without any complications but in somecases persist. The recurrent UTIs are treated in the majority of caseswith antimicrobial profilaxis, increasing the problem ofantibiotic resistance. Generally, it is considered that *Escherichia coli* is the essential causative agent of 80% of the UTIs. In our country, UTIs in women are a frequent reason for medical consultation. Approximately 25 to 35% of women between 20 and 40 years have had an episode of UTIs during her life. The cranberry has been proposed as an effective agent in the prevention and treatment of UTIs. *In vitro*antibacterial activity of acidified methanolic extracts (EMeOH) from Mendoza, Tucuman and Entre Rios cranberry cultivars was determined using the microbroth dilution method according to the protocols of the Clinical and Laboratory Standards Institute CLSI protocols. The EMeOH showed antibacterial activity against *Escherichia coli* ATCC 25922 and *Escherichia coli* clinical isolates from Laboratory de Microbiology, Hospital Marcial Quiroga(San Juan, Argentina) with MICs

values of 2000 µg/ml and 3000 µg/ml respectively.Chemical studies of characterization and quantification of phenolic compounds, flavonoids and anthocyanins are in progress.

A75
BIOACTIVE METABOLITES OF *Lactobacillus fermentum* L23 TO CONTROL THE *Streptococcus agalactiae* BIOFILM

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The aims of this study were i) to study the *S. agalactiae* (GBS) clinical isolates' ability to form biofilms and, ii) to evaluate their inhibitions by secondary metabolites of the probiotic *Lactobacillus fermentum* L23 (GenBankGQ no.455406). This probiotic synthesizes organic acids (OA) and bacteriocin L23, which are released into cell-free supernatants (CFSs).A total of 30 GBS strains were isolated and identified.GBS cultures adjusted to 0.5 and 1 McFarland scale were incubated at 37 °Cfor 6 h and 24 h. The GBS biofilms dyedwith 1% violet crystal were measured by UV-spectrophotometry. The biofilm-producing GBS strains were classified as high, middle and low producers. Although all the clinical isolated GBS were biofilm-producing strains, 93% of them were strong and middle producers.The different concentrations and incubation times tested did not show statistical differences in the levels of biofilm production ($p< 0.05$). With the pure CFS of L23, all strains had an average meaningful decrease in biofilm production (91.6%) in comparison with the untreatedGBS culture. In 20%of the GBS strains,the production of biofilm was totally inhibited. These effects on the GBS biofilm were caused by the joint action of OA and bacteriocin L23.The neutralized CFS of L23 strain produced a larger meanpercentage of GBS biofilm inhibition (97.9%). In 53% of the GBS strains, the biofilmformationwas entirely inhibited, due only to the bacteriocin L23. In conclusion, the anti-biofilm power of these secondary metabolites of *L. fermentum* L23 could constitute a biological alternative.

A76
ANTIMICROBIAL ACTION OF 2',4-DI(TRIMETHYLSILYL)-CHALCONE IN BINARY AND TERNARY COMBINATIONS AGAINST *Staphylococcus aureus* ATCC 43 300 MRSA

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2', 4-di(trimethylsilyl)-chalcone presents an interesting antimicrobial activity against *S. aureus* ATCC 43300. Due to the intrinsic resistance of this Gram positive coccus and in order to enhance the chalcone action, the bacteriostatic effects of binary combinations (chalcone in varying concentration and oxacillin or rutin in constant concentrations) and ternary combinations (chalcone in varying concentration and oxacillin-rutin in constant concentration) against this microorganism, were investigated. A kinetic-turbidimetric method that allowed to obtain microbial growth curves, was employed. One ternary combination assayed was more effective than two binary combinations tested as antimicrobial agents against *S. aureus* ATCC 43 300. Thus, oxacillin did not synergize the inhibitory action of chalcone on *S. aureus* in the binary combination. This system did not meet the mechanism proposed to evaluate the minimal inhibitory concentrations. In the combination 2', 4-di(trimethylsilyl)-chalcone - rutin, the synergizing agent acted indifferently (MIC = 38.8 $\mu g.mL^{-1}$). Experiences with the ternary combination 2', 4-di(trimethylsilyl)-chalcone - oxacillin - rutin, showed synergistic effect as compared to the action evidenced by the chalcone individually. Its minimum inhibitory concentration (MIC = 32.7 $\mu g.mL^{-1}$) shows that the ternary combination would be well suited to treat infections caused by methicillin resistant *S. aureus* ATCC 43300.

A77
SYNTHESIS AND APPLICATION OF A NEW ANTIMICROBIAL AGENT AGAINST *Staphylococcus aureus* ATCC 43 300 MRSA

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Since compounds belonging to the flavonoid family have known biological activities, it is prominent among them the antimicrobial action. In this study the derivatization of 2', 4-dihydroxychalcone was performed by modifying its hydrophobicity from the incorporation of trimethylsilane on the hydroxyl groups. Its antibacterial activity against *S. aureus* ATCC 43300 MRSAwas determined by using a kinetic-turbidimetric method previously developed. The synthesis of the new compound, 2', 4-di(trimethylsilyl)-chalcone was performed in an inert atmosphere (argon). The reaction (2',4-dihydroxychalcone, imidazole, dichloromethane and trimethylchlorosilane in suitable proportions) was continuously stirred during 24 h. The presence of the interest product was detected by thin layer chromatography (hexane: ethyl acetate, 5:5 as solvent) by observing under ultraviolet light. The reaction was quenched with ammonium chloride solution 20%. The product obtained was extracted and repeatedly washed (ethyl acetate, water) and recrystallized. It was identified by chromatographic and spectroscopic techniques (IR, UV-vis, NMR, MS) as 2',4-di(trimethylsilyl)-chalcone. We proceeded to determine the antibacterial activity against *S.*

aureus ATCC 43 300 in culture media added with increasing concentrations of the derivatized compound. Through the relationship ln N = 27.4 – 10.3 x T, ln N values were calculated to plot the corresponding growth curves. From the slopes of the linear region, specific growth rates (μ) were calculated. The graph of μ vs. 2',4-di(trimethylsilyl)-chalcone concentration fitted a straight, complying with a previously proposed actionmechanism.By extrapolating the abscissa (μ = 0), the value of the minimal inhibitory concentration for this compound (MIC = 40.8 $\mu g.mL^{-1}$) was calculated. This result indicates that the silylation potentiates the antibacterial action of 2', 4-dihydroxychalcone (MIC = 59.1 $\mu g.mL^{-1}$).

A78
INFLUENCE OF PHYSICOCHEMICAL FACTORS ON GROWTH AND EXOPOLYSACCHARIDE PRODUCTION BY THE CYANOBACTERIUM *Nostoc minutum*

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The increased demand of natural polymers for various industrial applications in recent years has led to a renewed interest in the exopolysaccharide (EPS) production by microorganisms. *Nostoc minutum* isa diazotrophic filamentous cyanobacterium isolated locally that has the ability to produce EPS. The aim of this work was to study the influence of different physicochemical factors in stirred and still cultures, to improve EPS production at laboratory conditions. *N. minutum* was grown in two culture media: BW_3 and BG11 with (+) or without (-) nitrogen source added as $NaNO_3$. Cultures were incubated at 30°C in a temperature-controlled room for 14 days with continuous illumination of 4.53 Klux (low irradiation) or 7.30 Klux (high irradiation), pH of 7±0.2 and stirred by air bubbled through a diffuser. Growth was determined by optical density (OD) at 580 nm every 48 h and EPS was estimated by dry weight determinations. It was observed that biomass production at the end of the culture (14 days) was higher in stirred conditions with 3.729 OD for BW_3 medium with nitrogen source added and high irradiation.However, the increased production of EPS (2.485 g/L) was obtained with BG11 medium under the same conditions of stirring and high irradiance. The observations of both Alcian blue and India ink staining preparations with the light microscope showed an EPS production according to that determined by dry weight technique. We concluded that stirring and high irradiance were the best conditionsfor both growth and EPS production in the two culture media.

A79
MODULATION BY TUMOR NECROSIS FACTOR OF INFLAMMATORY MOLECULES IN LPS-STIMULATED MURINE MACROPHAGES

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Tumor necrosis factor (TNF) is a pleiotropic primary cytokine synthesized by macrophages. TNF acts through two transmembrane receptors: TNFRp55 and TNFRp75. We previously demonstrated increased interleukin (IL)-6 and nitric oxide (NO) production by TNFRp55 deficient macrophages after stimulation with LPS, compared with wild-type C57BL/6 macrophages. The purpose was to analyze the molecular mechanisms by which TNF might regulate these inflammatory mediators using murine macrophage cell line RAW 264.7. Since this cell line was established in BALB/c mice, we added IFN-γ in the cellular culture and analyzed the effect of TNF on the production of IL-6 and NO by stimulation with LPS. We found that IFN-γ or LPS stimulation triggers TNF production, and that simultaneous IFN-γ and LPS stimulation did not change this effect. Moreover, we observed that IL-6 and NO was induced by IFN-γ (10 ng/ml) plus LPS (100 ng/ml). To analyze the effect of TNF, the cells were pre-incubated at different concentrations and incubation times with human TNFwhich has demonstrated to be specific for murine TNFRp55. We found that in contrast with NO, 30 and 90 ng of TNF reduced the IL-6 production induced by stimulation with LPS/IFN-γ. We conclude that the cell line RAW 264.7 may be used to analyze the molecular mechanisms involved in a possible regulatory role of TNF on other inflammatory molecules produced by murine macrophages.

A80
EXPRESSION OF ANDROGEN RECEPTORS IN PINEAL GLAND OF ADULT MALE VISCACHA DURING SHORT PHOTOPERIOD

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The participation of melatonin in the regulation of reproduction in photoperiod dependent mammals is well established. The aim of this study was to demonstrate the presence of androgens receptors (AR) in the pineal gland parenchyma of adult male viscachas during short photoperiod (winter). Four pineal glands obtained from June to August were used. The glands were processed for light microscopy and the AR was immunohistochemically identified using the antibody AR (N-20) sc:816. Subsequently sections were counterstained with Harris's hematoxylin. Positive staining was evident by a brown deposit in the site of antigen localization. Immunohistochemical results revealed that AR-immunoreactivity(ir) was detected mainly in the nuclei (ARn-ir), but cytoplasmatic immunolabeling (Arc-ir) was also observed in some pineal cells. The positive AR nuclei were oval or spherical in shape, according to pinealocytes. This work demonstrated the presence of AR in pineal gland of adult male viscacha during short photoperiod.In this seasonal breeding rodent, it is probably that activation of these receptors may modulate the melatonin secretion either directly or via the synthesis of other pineal indoles or peptides.

A81
VARIATIONS IN ANDROGEN RECEPTOR (AR) EXPRESSION IN EPIDIDYMAL CAUDA OF VISCACHA ACCORDING TO SEXUAL MATURITY

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Androgens are steroid hormones that play key roles in the development and maintenance of male phenotype and reproductive function. Our experimental model; the viscacha is a South American nocturnal rodent who develop a seasonal reproduction. The adult male exhibit an annual reproductive cycle mainly characterized by a higher gonadal activity period in summer and lower gonadal activity during the winter. The goal of this work was to study AR-immunohistochemical expression in epididymal cauda, relating this to the animal sexual maturity. Males viscacha were captured in their habitat and were classified as immature (n=4), prepubescent (n=4) and adult (n=4) according to their body weight and to light microscopy observations of testis. Tissue samples of epididymis were collected and processed for Optical Microscopy. Serum testosterone levels were determined by chemiluminescent enzyme immunoassay and AR localization was determined using AR (N-20): sc816 antibody. The serum testosterone levels (ng/dl) in immature animals were lowers than 20, in prepubescent animals the mean was 139.74±30.77 and in adults the mean was 490±40.76. The percentage of immunoreactive-epithelial cells in immature animals (79.18±0.48) was significantly higher than that in prepubescent (34.65±6.01) animals and adults (29.85±5.26). Epithelial and stromal cells of immature and prepubescent animals expressed a moderate immunostaining (++) while those cells in adults expressed a weak immunostaining (+). In all cases the AR-immunostaining was located in cell nuclei. Our results suggest that the epididymal cauda have a high testosterone demand during the first development stages for achieve sexual maturity. However, testosterone requirement is reduced in adult animals, this might be due to that epididymal cauda principal function is sperm storage.

A82
EFFECT OF CASTRATION ON THE ADRENAL CORTEX OF THE VISCACHA (*Lagostomus maximus maximus*). HISTOLOGICAL AND MORPHOMETRIC STUDY

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The viscacha is a photoperiod-dependent seasonal reproduction rodent. Its behavior and physiology varies with environmental signals such as light periods, food availability, temperature, rains and social interactions. The adrenal gland is a key element in the synchronization and adaptation of this rodent to its environment. The objective of this study was to investigate the effects of castration on the adrenal cortex of the viscacha. The animalswere divided into two groups: a group control of intact viscachas (n=4); and a group of surgically castrated viscachas (n=4). They were maintained in captivity during six weeks under 14:10 light-dark cycle with food and water *ad libitum*. The adrenal glands were processed for light microscopy and sectioned at 4-5µm of think. The slides were stained with hematoxilyn-eosin and examined under an Olympus BX40 light microscopy. The images were captured by a Sony SSC-DC5OA camera and processed with Image Pro Plus 5.0 software. The *zona fasciculata* and *zona reticularis* of the castrated group showed cellular hypertrophy, abundant cytoplasmic vacuolization, nuclear pleomorphism and

dilation of blood vessels. Besides, both the outermost and innermost *zona reticularis* showed a significant increase ($p< 0.05$) of the nuclear diameter in the castrated group (6.33 µm ± 0.10 and 5.92± 0.10, respectively) in relation to the control group (5.72 µm ± 0.18 and 5.48± 0.13, respectively). Ourresultsdemonstrated that histological changes on the adrenal cortex are produced by castration, suggesting an increased lipid accumulation. These findings might indicate a stimulated metabolism in the adrenal cortex for increased adrenosteroid biosynthesis.

A83
SEASONAL VARIATIONS OF PCNA EXPRESSION IN PITUITARY OF ADULT MALE VISCACHA

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Some researchers have demonstrated that the cell population of anterior pituitary is not static but rather dynamic. The proliferating cell nuclear antigen (PCNA) is an auxiliary protein of DNA polymerase-ä and it has been used for studying cell proliferation. PCNA is expressed mainly in the G1/S phase of cell cycle, decrease in G2 and is undetectable in metaphase. The aim of the present work was to localize and quantify the cells that express PCNA in pituitary pars distalis (PD) of adult male viscacha during summer and winter(long and short photoperiods, respectively). PCNA were detected by immunocytochemistry and morphometrically quantified by image analysis in pituitary PD. Labeled cells were counted and expressed as percentage of the total number of cells per microscopic field. The values were expressed as mean ± SEM and analysed by Wilcoxon test. PCNA-immunopositive cells were found isolated throughout the PD during both photoperiods. The immunostained nuclei were spherical though some nuclei were spindle-shaped or elongated. Some positive nuclei near follicular structures were irregular in shape. Mitotic figures in PD were infrequent. The percentage of PCNA cells during summer was significantly higher ($p<0.05$) than in winter (1.35 ± 0.24 and 0.48 ± 0.05; respectively). These results demonstrated an increase of cell proliferation during summer (long photoperiod). This finding might be due to normal processes of cell renewal or the need to increase the cell number in response to hormonal demand during the long photoperiod, which corresponds to the reproductive period and major activity of pituitary gland of this seasonal rodent.

A84
ESTRADIOL ON COELIAC GANGLION AND OVARIAN INCUBATION MODIFIES IN A DIFFERENTIAL MANNER THE RELATION BETWEEN THE CELL-EXTRACELLULAR MATRIX AND α, β-ACTIN IN OVARY ON DIOESTRUS DAY II

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Cell-extracellular matrix (ECM) interactions are important in the ovaric apoptotic mechanisms. These interactions are mediated by integrins, which participates in the junction betweenECM to cytoskeletonin the cell. The aimswere to evaluate whether estradiol (E_2) on coeliac ganglion (CG) of the *ex vivo* coeliac ganglion- superior ovarian nerve-ovary system and on ovary alone (OV) is capable of modify in ovaryon DII: 1- GnRH and E_2 release; 2) the interaction ofcytoskeletal proteins (α, β-actin) with the extracellular matrix. The *ex vivo*CG-SON-O system and OV were removed and placed in one cuvette containing Krebs Ringer solution at pH 7.4 E_2 added to ganglion in the system or the OV (experimental groups)without (control groups). The ovarian liquid was extracted at 120 min and GnRH and E_2 were determinated by RIA. The presence of α and β-actin in ovary were determinated by immunofluorescence. ANOVA 1 followed by Bonferroni test with a statistical significance of $p<0.05$was used.E_2 in CG caused an increase in the ovarian GnRH and E_2 release respect to the control. Besides,it did not causechanges inα, β-actinimmunoreactivityinthe differentovarian structure,maintaining the structural integrityof the ovary.However, E_2 in the OV caused an increase of GnRH and a decrease of E_2 liberation. Also, it caused aloss ofα-actinimmunoreactivityincorpus luteum and a breakdown of the cell-cell junction in ovary. For the first time, we demonstrate that estradiol is capable of modify the relationship between theintegrin-ECM junction and α, β-actin by modulating the ovarian GnRH and E_2 release. We demonstrated that E_2, through superior ovarian nerve protects the ovary from apoptotic mechanism. However, E_2in ovary alone induces the ovarian apoptosis, favoring the continuity of estrous cycle.

A85
EFFECT OF TNFRP55 DEFICIENCY ON THE PG SYNTHESIS AT THE END OF PREGNANCY

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An adequate rhythm of progesterone (P_4) is crucial for determining the physiological duration of the estrous cycle and for achieving a successful pregnancy. Tumor necrosis factor is a pleiotropic cytokine and TNF receptor p55 (TNFRp55) mediates most of the TNF effects. We previously showed that P_4 levels and its synthetic enzyme (3β-HSD) exhibit a circadian rhythm in the ovary in diestrus. In this work we investigate the consequences of the lack of TNFRp55 in the P_4 metabolism at the end of pregnancy, in C57BL/6 wild type (WT) and TNFRp55-/- (KO) mice. Animals were maintained on a 12-h light: 12-h dark cycle, at 24±2°C, with irradiated food and water available *ad-libitum*. Five days before the experiment mice were kept under constant darkness conditions. Ovaries were isolated every 6 h (24-48 h before delivery) during a 24h period. P_4 and 3β-HSD protein levels were determined by RIA and Western blot, respectively. Similar to that we previously observed in diestrus, P_4 levels are circadian in the ovary of WT mice ($p<0,01$) at late pregnancy; TNFRp55 deficiency abolished that circadian pattern. On the other hand, the expression of 3β-HSD protein is arrhythmic in the ovary of WT and TNFRp55-/- mice at the end of pregnancy. These results suggest that the absence of the signaling system TNFRp55 affects rhythmicity of P_4 levels at the end of pregnancy.

A86
NITRIC OXIDE IN COELIAC GANGLION AND ITS EFFECT ON OVARIAN PROGESTERONE SECRETION IN THE FIRST RAT PROESTRUS

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Using the coeliac ganglion-superior ovarian nerve-ovary system (CG-SON-O), we showed that stimulating the CG with acetylcholine (Ach) decreases ovarian progesterone (P_4) release. Several studies suggest that nitric oxide (NO) modulates cholinergic neurotransmission. The aim of this work was to study if inhibitors of nitric oxide synthase (NOS) and a NO donor in CG, with and without addition of Ach 10^{-6} M, modifiesP_4 release and gene expression of 3β-HSD and 20α-HSD enzymes (P_4 synthesis and degradation enzymes, respectively), in ovary. The system was incubated in Krebs-Ringer buffer at 37°C.The addition of selective and non-selective inhibitors of inducible NOS, aminoguanidine 400 µM (AG) and L-nitroarginine methyl ester 100 µM (L-NAME) respectively, and NO donor, sodium nitroprusside 100 µM (SNP), were studied separately. P_4 was determined by RIA in ovarian compartment at 30', 120' and 180' incubation times. Ovarian gene expressions of 3β-HSD and 20α-HSD were assessed by RT-PCR at 180'. One-way ANOVA and Tukey test were used ($p<0.05$). AG decreased ovarian P_4 release at 30', 120' ($p<0.001$) and 180' ($p<0.05$) and gene expression of 3β-HSD ($p<0.05$) besides increased gene expression of 20α-HSD ($p<0.01$). AG+Ach increased ovarian P_4 release at 180' ($p<0.01$). L-NAME decreased ovarian P_4 release at 30' ($p<0.001$) and 120' ($p<0.05$) and decreased gene expression of 3β-HSD ($p<0.05$). L-NAME+Ach decreased ovarian P_4 release at 30' ($p<0.05$) and decreased gene expression of 3β-HSD ($p<0.05$). SNP decreased ovarian P_4 release at 180' ($p<0.01$) and decreased gene expression of 3β-HSD ($p<0.05$). NPS+Ach decreased gene expression of 3β-HSD ($p<0.05$). These results suggest that inhibitors of NOS and a NO donor in CG may mediate changes in ovarian P_4 release and gene expression of 3β-HSD and 20α-HSD enzymes, this effect is slightly different with addition of Ach.

A87
STEROIDOGENIC ABILITY OF SPLENOCYTE SECRETIONS FROM RATS WITH POLYCYSTIC OVARY IS MODIFIED THROUGH A NEURAL PATHWAY

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Polycystic ovary (PCO) is a complex endocrine disorder associated with hyperandrogenism and ovarian hyperinnervation. We have shown that the ovarian steroidogenic response is differentially regulated by the splenocyte secretions (Ss) through the neural connection involving ovary-superior ovarian nerve (SON)-coeliac ganglion-spleen in rat. Here, we study the effect of SON transection on the steroidogenic ability of Ss in PCO granulose (GC) and interstitial cells (IC). Two groups of 60 days old rats were used: a PCO group, where PCO was induced by estradiol valerate (2mg/rat) and rats killed 2 months later, and, a PCO+SONt group, where PCO rats were subjected to SON transection 7 days prior to sacrifice. $1x10^6$ S from both groups were cultured for 24 h in RPMI medium plus 10% of FBS. The Ss were used to incubate GC and IC from PCO and PCO+SONt rats for 3 h in a metabolic bath. The androstenedione (A2) and progesterone (P4) release were determined by RIA and nitrites (NO) by Griess reaction. In S, the mRNA expression of nerve growth factor (NGF), trkA receptor and interleukin (IL)-12 were assessed by RT-PCR. Ss from PCO+SONt rats decreased P4 ($p<0.01$) and A2 ($p<0.001$) release from PCO+SONt CG and IC cells, and NO release from PCO+SONt GC ($p<0.01$), in relation to PCO Ss ($p<0.01$).

PCO+SONt-S showed lower NGF mRNA and higher IL-12 mRNA levels, without change in trkA, compared to PCO S. SON section modifies the steroidogenic ability of Ss on ovarian cells associated with changes in neural and immune markers of S, decreasing the high characteristic androgen levels of the PCO.

A88
CELIAC GANGLION PRESENTS PROGESTERONE RECEPTORS AT LATE PREGNANCY

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The addition of progesterone (P_4), a luteotrophic hormone, to celiac ganglion (CG) stimulates ovarian P_4 release through superior ovarian nerve (SON) at the end of pregnancy in the rat. The object of this study is to investigate if such effect may be associated to the presence of P_4 receptor (PR) in CG. Female Holtzman rats at 21 days of pregnancy were used. The CG was removed and fixed in Bouin's fluid. The tissue was dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin. Serial sections were cut at 5µm and mounted. Tissue sections were deparaffinized with xylene and hydrated through decreasing concentrations of ethanol. Tissue was rinsed with PBS and then incubated in Na-citrate buffer at 97°C for 40min. Nonspecific binding sites were blocked by incubation for 1 hour with 5% bovine serum albumin. Sections were then incubated overnight in a humidified chamber at 4 °C with antibodies against PR (H-190: sc-7208, Santa Cruz Biotechnology, Inc) and β III-tubulin (Covance). After three washes in PBS, the samples were incubated with the secondary antibodies (Alexa 488-conjugated IgG and Alexa 555-conjugated IgG; Invitrogen) for 1 h at room temperature. After washing, the samples were mounted with FluorSave (Calbiochem). Images were taken with an Olympus FV-1000 confocal microscope. The immunohistochemical analysis showed positive immunoreactivity for the PR in neuronal somas and axons of the CG. In conclusion, P_4 would promote the corpus luteum protection from regression through the peripheral neural pathway by impacting on PR in CG.

A89
ANDROSTENEDIONE IN SUPERIOR MESENTERIC GANGLION STIMULATES THE RELEASE AND METABOLISM OF PROGESTERONE THROUGH NITRIC OXIDE IN OVARY ON ESTRUS

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The superior mesenteric ganglion (SMG) is formed by principal neurons which have androstenedione (A_2) receptors. Furthermore, it has been demonstrated that A_2 has luteotrofic effect in corpus luteum of rat. The objectives were to demonstrate if A_2 in SMG modifies: 1) ovarian progesterone (P_4)release; 2) activity and gene expression of 3 β-HSD and 20α-HSD (P_4synthesis and degradation enzymes respectively); 3) ovarian nitric oxide (NO) release on estrus. The*ex vivo* SMG-Ovarian Nervous Plexus-Ovary system was incubated withflutamide (Flu) (androgenic receptor antagonist) or Flu plus A_2 added in ganglion. For this, we used a cuvette with two compartments with Krebs Ringer solution, pH 7.4, in a metabolic bath at 37 °C. P_4(RIA) and nitrite (Griess method) were determined at 15, 30, 60 and 120 min. The ovarian enzymatic activities were performed by spectrophotometric method, and gene expression by RT-PCR at 120 min. ANOVA-1 followed Tukey test ($p < 0.05$) was used. A_2 in ganglion increased the ovarian P_4 release ($p<0.001$) in concordance with an increase in the activity ($p<0.001$) and expression of 3β-HSD ($p<0.05$) and a decrease of 20 α-HSD activity ($p<0.01$). Besides, A_2 increased the ovarian NO release ($p<0.001$) in all times analyzed. The effects observed on ovarian P_4 release were reversed by the addition of Flu plus A_2 while Flu did not showed changes respect to the control.In this work we demonstrated the importance of the activation of androgenic receptors in neurons of the SMG on the release and metabolism of P_4andovarian NO in normal condition. These results have possibly relevance in female pathologies as polycystic ovary.

A90
MACROPHAGE SECRETIONS MODULATE THE HORMONE RELEASE FROM ANTERIOR PITUITARY OF RATS WITH POLYCYSTIC OVARY

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Polycystic ovarian syndrome (PCO) is an endocrinopathy with elusive origins (has a heterogeneous presentation) characterized by ovulatory dysfunction, hyperandrogenemia and neuroendocrine abnormalities, and is the most common cause of female infertility. We had provided evidence of a functional relation between the ovary and immune cells in PCO rats, where the macrophages secretions (Mø-S) from rat spleen regulate the ovarian androgen production. Now, we studied whether the Mø-S affect the release of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (PRL) from PCO and Control (C) rat anterior pituitary (AP). PCO condition was

induced in adult virgin rats by 2 mg/rat (single i.m. injection) of estradiol valerateand the animals were sacrificed, on estrus, after two months. Spleen Mø, from C and PCO rats, were cultured ($1x10^6$ cells) for 24 in RPMI medium. Their secretions were used to stimulate C-AP and PCO-AP for 3 h in a metabolic bath (37°C, 95% O2-5% CO2) to measure the LH, FSH and PRL release (by RIA). Serum FSH and PRL levels were higher in PCOthan inC rats ($p<0.01$ and $p<0.05$, respectively), without change in LH concentrations. PCO-AP incubated with RPMI medium alone (basal), released lower LH and PRL than C-AP ($p<0.01$), without change in FSH. The incubation of PCO-AP with PCOMø-S increased the FSH ($p<0.05$) and PRL ($p<0.01$) release, without change in LH, compared to C Mø-S. This evidence suggests that Mø-S modulate the AP hormone release, being the effects of PCO Mø-S different than that of C Mø-S. An interaction between gonadotrophs and lactotrophs with mediators of inflammation cannot be discarded.

A91
EFFECT OF NITRIC OXIDE ON OVARIAN ESTRADIOL RELEASE AND NITRIC OXIDE SYNTHASE EXPRESSION IN THE FIRST RAT PROESTRUS

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Nitric oxide (NO) is a gaseous neurotransmitter involved in steroidogenesis and follicular development. In the first proestrus (PE), the stage where is evident follicular maturation which lead to the first ovulation, the ovarian steroid hormone with major participation is estradiol (E_2). Taking into account this information, the aims of this work, in the first PE, were to study the effects of NO synthase (NOS) inhibitors and a NO donor on: 1) the release of E_2 in ovary without neural influence (Ov), 2) ovarian gene expression of P450 aromatase (P450arom, E_2 synthesis enzyme). The Ov was incubated in Krebs-Ringer buffer at 37°C (control group). Inhibitors of NOS: aminoguanidine 400 µM (AG) and L-nitroarginine methyl ester 100 µM (L-NAME), selective and non-selective of inducible NOS, respectively; and NO donor: sodium nitroprusside 100 µM (SNP), were added in Ov. $E_2$was determined by RIA at 120 min and 180 min. One-way ANOVA and Tukey test were used ($p<0.05$). The gene expression of P450arom was determined by RT-PCR at 180 min. AG *vs* control group showed tendency to increase E_2. L-NAME *vs* control group increased E_2 only at 120 min ($p<0.01$). In contrast, SNP *vs* control group decreased E_2 at 120 min and 180 min ($p<0.05$), with tendency to decrease the gene expression of P450 aromat at 180 min. In the first PE in the rat, NO acts as a negative regulator on E_2 release without significant changes on gene expression of NOS at 180 min.

A92
VARICOCELE INCIDENCE AND SEMEN QUALITY IN PATIENTS ATTENDING A PRIVATE LABORATORY OF SAN LUIS CITY

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The varicocele incidence is about 15% in the male population, while the incidence is about 35-45% in men with fertility problems. The aims of this work were to analyze the seminal quality of patient spermograms who consulted for fertility problems in a private laboratory and report our statistical data about varicocele. 186 samples from patients aged from 20-50 years old were analyzed between the years 2012-2013. Macroscopic and microscopic studies were performed and the main spermatic parameters i.e. volume, concentration, vitality, progressive motility and morphology were evaluated according to Krüger strict criteria and WHO 2010. For the statistical analysis Kruskal-Wallis test was used. A P value < 0.05 was considered statistically significant. The mean age was 32 years old. A 15.5% of the processed samples were from patients treated for varicocele which showed the following median of semen parameters: volume 4.0 ml; sperm concentration 56 sperm/ml, vitality 70%, progressive motility (a+b) 50% and morphology 12%. Our results suggest that the most varicocele patients studied were from the 30-39 years old group and have not performed a preventive treatment, stimulating a further development of the pathology. Therefore, the seminal quality of varicocele patients should be carefully evaluated, in order to develop reproductive health prevention and contribute to the most appropriate treatment election.

A93
ISOLATION OF BRUSH BORDER VESICLES FROM SMALL INTESTINE OF *Columba livia*

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A variety of techniques has been employed forthe isolation of small intestinal brush border membranes (BBMV), e.g. divalent cations as Ca^{++} or Mg^{++}, differential centrifugation using sucrose density gradient and recently using polyethylene glycol (PEG) as precipitant.This study is aimed to improve a method to obtain brush border membrane vesicles (BBMV) from pigeon small intestines using polyethylene glycol (PEG) as precipitant. We tested different PEG concentrations and compared purity and enrichment of the obtained BBMV. The samples were obtained from adult birds. The intestine was cut and divided into two portions (proximal and distal) and the mucosa was scraped off with a glass slide. PEG 3350 solution was added (to give final concentrations of 5, 10, 15, 20 and 25%) and stirred for 15 min at 4°C. After several centrifugation bouts we obtain pellet fractions remained after PEG treatment and BBVM in the supernatant. The supernatant was centrifuged at 40,000 g for 45 min and obtained BBMVs were resuspended in buffer HEPES-mannitol. We measure total protein content, activities of sucrase, maltase and aminopeptidase-N as marker enzymes of the apical cell membrane, Na^+/K^+ pump as basolateral cell membrane marker, and protein profile by SDS-PAGE. The procedures yielded a 20-fold enrichment of apical enzyme activities and remaining activity of Na^+/K^+ pump in the BBMV in all PEG concentrations tested. Enrichment of some characteristics bands was found in BBMV profiles of SDS-PAGE. In conclusion PEG method allows obtaining BBMV with adequate purity.

A94
MULTI ELEMENTAL DETERMINATION IN *Salvia hispanica* L., *Linum usitatissimum, Chenopodium quinoa,* AND *Amaranthus caudatus* L. SEEDS USED FOR HUMAN CONSUMPTION

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Some seeds have relevance in the diet. It is consumed as aggregate in dairy baking products. Commonly used are: chia (Salvia hispanica), Flax (Linum usitatissimum) , Quinoa (Chenopodium quinoa) and Amaranth (Amaranthus caudatus L). If well enough is known about their chemical composition, such as high-protein, energy and omega 3 and 6 fatty acids, little is known about its mineral composition. The aim of this work was to evaluate the mineral composition (Ca, K, Mg, Na, Fe and P) in these seeds. The seeds wereobtained from shops in the surrounding area, for human consumption. The organic matter was removed by a microwave by acid digestion. The elements were subsequently determined by inductively coupled plasma atomic emission spectroscopy (ICP-OES). The mean values (mg/Kg): *Amaranthus caudatus L*: Ca: 1577,97 ; K: 5234,29 ; Mg: 3156,53 ; Na: <LC ; Fe: 105,41 ; P: 5813,21. *Salvia hispánica*: Ca: 6114,9 ; K:6474,5 ; Mg: 3673,4 ; Na: 11,3 ; Fe:239,7 ; P: 7895,4. *Linum usitatissimum*: Ca: 2114,33; K: 8041,62 ; Mg: 3655,06; Na: 233,38 ; Fe: 67,42; P: 6281,97. *Chenopodium quinoa*: Ca: 436,45; K:5976,61; Mg: 1736,74; Na: <LC; Fe: <LC ; P: 4044,16. Our results indicated that chia has the highest concentration of Ca, Mg, Fe and P. These data are relevant to the choice of different kinds of breads in the market. This work is an original contributing to the knowledge of the elemental component the seeds used in the baking industry.

A95
CIRCADIAN PATTERNS OF BDNF AND TrkB EXPRESSION ARE MODIFIED IN THE PREFRONTAL CORTEX OF AGED RATS

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During aging, brain undergoes several changes which begin with alterations in gene expression. The brain-derived neurotrophic factor (BDNF), its receptor TrkB and the cAMP response element binding protein (CREB) regulate a wide range of functions, such as synaptic plasticity and long-term potentiation, and are expressed in different regions, including prefrontal cortex. Previously, we showed circadian rhythmicity of BDNF and TrkB expression is abolished in the hippocampus of aged rats. In this work, our objectives were: to investigate whether BDNF, TrkB and CREB expression also displays an endogenous circadian rhythm in the rat prefrontal cortex, and to evaluate the consequences of aging on those temporal patterns. Young (3-month old) and aged (22-month old) Holtzman rats were maintained under 12hdark:12hdark (constant darkness) conditions, during 10 days before the experiment. Prefrontal cortex samples were isolated every 4 h during a 24h period. Regulatory regions of BDNF and TrkB

genes were scanned for CRE sites, by using a bioinformatic tool. BDNF, TrkB and CREB mRNA levels were determined by RT-PCR. As expected, CRE sites were found in the BDNF and TrkB regulatory regions. BDNF and TrkB expression display an endogenously-controlled temporal variation in the rat prefrontal cortex, which is attenuated in the aged group, probably, as a consequence of alteration in the 24h variation of CREB expression.

A96
PRENATAL BLOCKADE OF AT2 RECEPTOR AND ITS EXPRESSION IN THE CEREBELLUM

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Angiotensin II (Ang II) exerts its physiological effects through binding to two receptor subtypes: AT_1 and AT_2, it is differentially blocked by Losartan or PD123319 (PD), respectively. Ang II receptor expression is highly modulated during development suggesting a role of these receptors in growth and organogenesis. In rodents, cerebellum development takes places during 15-20 days after birth. RNA probes (riboprobes) generated by transcription *in vitro* are more sensitive and stable than DNA probes. The aim was to explore the expression pattern of AT_2 receptor prenatal blockade with PD. Wistar rats during late gestation (G13-G21) were administered subcutaneously with PD and the offspring cerebellum were analyzed at eight postnatal ages. The PCR fragments AT_2 receptors were subcloned in the p-GEM T easy vector. The identity of the subcloned inserts was verified by RFLP. The riboprobes were obtained by *in vitro* transcription using SP6 or T7 RNA polymerases in both sense and antisense to provide non-specific control and specific probes. The riboprobes were labeled with non radioactive digoxigenin. In situ hybridization was performed using the synthetized AT_2 riboprobes on sagittal sections cerebellum PD treated rat and control at eight postnatal days. The labeling was successful for control as well as PD treated tissues. We observed specific signal in cerebellar Purkinje cell layer in control and PD treated animals, in coincidence with previous data in our laboratory by autoradiography and immunohistochemistry. In conclusion, the riboprobes generated here allows sensitive and efficient detection of AT_2 receptor gene expression in prenatal PD treated and control animals at eight postnatal days.

A97
EVALUATION OF THREE METHODS FOR *DNA* EXTRACTION FROM COTYLEDONS AND LEAFLETS OF *Prosopis caldenia* Burk

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The study of genetic resources *Prosopis caldenia* Burk, despite its great economic and ecological importance,is still in an early stage and there is little information concerning their structure and genetic variability.The aim of this work was to obtain genomic DNA from *P. caldenia* with optimal quality, purity and amount for its later analysis by molecular techniques based on PCR amplification.To obtain total genomic DNA from cotyledons and leaflets of *P. caldenia*, three different extraction protocols (modified), developed bySaghai Maroof (SM) et al. (1984), Yu and Pauls (YP) (1994),and commercial kit Wizard® Genomic DNA Purification (Promega) (KW), were evaluated. Modifications to the original protocols were conducted in aspects such as plant material starting sample, chemical solutions used, centrifugation and lenght of some of the isolation steps.The effectiveness of each extraction methodology was evaluated by measuring the yield, purity and integrity of the DNA extracted.According to the results, DNA extraction using KW (modified) protocol was the most suitable; since, it provides a quick method by which genomic DNA with quality, purity and optimal amount can be obtained for further analysis by PCR.The KW extraction protocol optimized in this work represents another antecedent that, together with the commercial kit used by Mottura (2006), form a suitable set ofprotocols for the isolation of DNA from species of the genus *Prosopis*.

A98
LACK OF TNFRp55 MODIFIES TEMPORAL PATTERNS OF CHOLESTEROL REGULATION-RELATED PROTEINS IN THE LIVER

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HDL-associated ApoE is the main cholesterol carrier into mice steroidogenic tissues. On the other hand, RAR/RXR heterodimer induces the expression of the cholesterol catabolizing enzyme in the liver. There is evidence that cholesterol metabolism is regulated by the circadian clock and TNF has been proposed as a modulator of circadian rhythms. In this work, our objective was to evaluate the consequences of TNF receptor p55 deficiency on the 24h-patterns of three cholesterol regulation-related proteins, ApoE, RXRα and RARα. Female C57BL/6 wild type (WT) and TNFRp55-/- (KO) mice were maintained on a 12-h light: 12-h dark cycle, at 24±2°C, with irradiated food and water available *ad-libitum*. Five days before the experiment mice were kept under constant darkness conditions. Liver samples were obtained every 6 h during a 24h period. Specific protein levelswere analyzed by Western-

blot.TNFRp55-deficient mice did not show differences in the ApoE temporal expression in liver, in comparison to the WT group. However, 24h-rhythms of RXR and RAR protein levels were phase shifted in the liver of KO mice. Thus, we suggest TNF, through its p55 receptor signaling pathway, could play a role in the modulation of temporal profiles of proteins involved in cholesterol homeostasis in the liver, a peripheral clock with relevant function in metabolism.

A99
AT2 RECEPTOR ANTAGONIST MODIFIES THE CHARACTERISTIC PURKINJE CELLS MONOLAYERING IN DEVELOPING CEREBELLUM

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Angiotensin II (Ang II) is the main effector peptide of the renin–angiotensin system (RAS). Recently, Ang II AT_2receptor has been related to a potential role in neuronal differentiationduring fetal and postnatal development. In cerebellum, AT_2receptors are located only in the Purkinje cells, which orchestrate the process of postnatal cerebellar corticogenesis. The aim of the present study was to analyze the modifications produced indeveloping cerebellar cortex after blockage with antagonist of Ang II type 2 receptor.Treatment was administered subcutaneously during late pregnancy (G13-G21) with PD 123319 (1.0 mg/kg/day) and vehicle (control). The offspring were analyzed at PN3, PN5 and PN8 (n:12). Morphological studies by indirect immunofluorescence usinganti-calbindinantibody and H&E analysis were performed**.**The detailed analysis revealed alterations in cerebellar layering: significative increased thickness of the EGL in the base and medial part of the fissure on treated vs control animals in different stages studied ($p< 0.01$),significant increased thickness of the PCL in the base and medial part of the fissure at PN8 treated animal vs control ($p<0.001$), impaired formation of the characteristic Purkinje cell monolayer and delayed dendritic arborization. Therefore, the present study demonstrates alterations in cerebellar layering and Purkinje cell development on pups born from treated mothers. In addition, we observed important changes on cerebellum development in animals at eight postnatal dayeven if there was no longer exposure to the drug. These observations confirmthe participation of AT_2 receptors on cerebellar cortex organization.

A100
RECEPTORS EXPRESSION IN VITAMIN A DEFICIENT RATS

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Vitamin A is an essential nutrient involved in numerous processes. The pleiotropic effects of retinoic acid are mediated by its receptors (RARs). Each family consists of three receptors (α,β,γ). We observed a relationship between retinoic acid and estrogen hormone receptors (ER) and progesterone (RPr). The aim is to determine whether vitamin A deficiency alters the expression of receptors RAR alpha, RE alfa and RPrB in mammary gland. It works with female Wistar rats at weaning in three separate groups of 90 days. One diet sufficient in vitamin A (8 mg retinol as retinyl palmitate/kg diet), one with deficient Vitamin A diet and the third group will receive diet deficient in Vitamin A for seventy- five days to the end of which will be given adequate Vitamin A diet for 15 days. Other 3 experimental groups of 180 days, with diet sufficient in Vitamin A, another diet deficient in Vitamin A and the third group will receive diet deficient in Vitamin A for 150 days after which you will be given diet sufficient in Vitamin A for 30 days. Expression of RAR alpha receptors by RT –PCR was determined. Expression of RPrB and ER alpha RT-qPCR was determined. Western ER alpha was conducted. It was observed in 6 month deficient rats decreased expression RPrB about 6 months controls ($p< 0.01$) , and this reduction in the group refed 6 months ($p<0.05$). Deficient in 6 months an increase in the expression of ER-alpha ($p<0.01$) was observed, as was seen in the amount of RE Alfa by Western ($p<0.05$). RAR alpha expression shows a decrease in the deficient group 3 and 6 months but not significant difference. We conclude that vitamin A would produce a difference in the expression of ER alpha and RPrB that is accentuated with time of exposure to vitamin A deficiency.

A101
IN VITRO ANTIOXIDANT ACTIVITIES OF AMARANTH SEEDS

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The consumption of grains has been associated with preventing of diseases that depends on oxidative stress (atherosclerosis, cancer diabetes, Alzheimer´s, etc). The natural antioxidants play important role in the inhibiting of free radicals. Amaranths are pseudocereals, which have gained an increasing interest in recent years on the basis of its nutritional values. Considering also, that antioxidant bioactive compounds were detected, the aim of this work was to assess the antioxidant properties the seeds of four varieties of amaranths: *Amaranthus cruentus* G6/17a

(AcG6/17a), *Amaranthus hypochondriacus* x *Amaranthus cruentus* H17a (AH17a), *Amaranthus cruentus* var. *Candil* (Acc), *Amaranthushypochondriacus* var. *Dorado* (Ahd). The seeds were extracted with methanol and their antioxidant activity were evaluated using following methods: (i)DPPH assay, to determine the free radicals scavenging activity (ii) NO test, to determine scavenging activity against nitric oxide. The results of methanolic extracts (2.5□g/□L) were: Inhibition DPPH%: 88.49 (AcG6/17a), 87.61(AH17a), 82.30 (Acc), 80.53(Ahd). Inhibition NO%: 59.70 (AcG6/17a), 73.89 (AH17a), 61.19 (Acc), 47.76 (Ahd). The four varieties of Amaranthus seeds act as important dietary antioxidants, might be an alternative to replace synthetic antioxidants as additives in food, pharmaceutical and cosmetic preparations.

A102
VARIABILITY CHARACTERIZATION OF SOYBEAN GENOTYPES SELECTED BY NUTRITIONAL QUALITY IN THE PROVINCE OF SAN LUIS

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In the multi-environments trials the environment effect is the main source of variation, thus evaluating the same genotypes at different locations is possible to identify differential behaviors associated whit specific environmental characteristics.The aim of the study was to characterize the variability of soybean advanced lines with quality features in different environments in the province of San Luis. In the 2013/14 crop year, genotypes selected for their nutritional quality (triple null for lipoxygenases and anti-nutritional Kunitz factor) were evaluated in two environments of the San Luis province: Villa Mercedes (VM) and Antiguas Estancias Don Roberto (DR). Yield (Y), weight of 1000 seeds (WS), number of days to maturity (NDM) and plant height (PH) were determined and Principal Component Analysis (PCA) was performed.The first two PC account for 98% of the total variability. The CP1 shows that the genotypes evaluated in VM are grouped to the right of factorial plane, while DR genotypes are located on the left. This separation indicates that the variability expression is influenced by the environment effect.Y, WS and NDM were positive and highly correlated between them and associated with the genotypes evaluated in VM, while DR genotypes were associated with PH variable.The variability characterization of the genotypes allowed detection differential behaviors through the test environments, being Villa Mercedes the most favorablefor the expression of WS and Y for evaluated genotypes.

A103
EFFECT OF FERTILIZATION AND AUXIN ON THE PRODUCTION OF *Digitaria eriantha* CV "SINTÉTICO MEJORADA INTA"

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Mega thermal species play an important role in the extensive farming practices in the province of San Luis. The synthetic cultivar *mejorada INTA* (INTA improved), soon to be registered at INASE, was improved from Irene cv. plants having survived September-October frosts and were selected due to its phenological features regarding tolerance to cold weather. The aim of this study was to assess the production of dry matter under different levels of fertilizers and synthetic auxins (2,4- D). The test was performed with a completely random experimental design with three repetitions in 24 lots of 6 square meters each. The treatments were: T 0 (control); T1 (50 kg Nitrogen-N); T2 (50 kg N + 20 kg phosphorus-P); T3 (50 kg N + 20 kg P + 10 cc synthetic auxin-SA); T4 (20 kg P); T5 (20 kg P + 10 cc SA); T 6 (10 cc SA); T 7 (50 Kg N+10 cc SA). Mean contrast was carried out through Variance analysis and Fisher's exact test (p: 0,05). Two cut (january and april) were done; and after the first one, the treatments were repeated. Dry matter was determined in drying oven at 65°C until reaching constant weight. (No relevant differences with auxin applications were detected.The treatments with nitrogen doubled dry matter production in contrast to the control treatment. Mejorada INTA responds to fertilization exceeding 8000 kg/ha production with 100 N kg/ha.

A104
COMPARATIVE MICROGRAPHIC ANALYSIS ON *Larrea cuneifolia, L. divaricata* AND *L. nitida* FROM WEST-CENTRAL ARGENTINA

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The micrographic parameters of three species of the genus *Larrea* are studied:*L. cuneifolia* Cav., *L. divaricata* Cav., and *L. nitida* Cav. commonly known as "jarillas", their leaves and young stems are used in popular medicine as antirrheumatic, anti-inflammatory, diaphoretic, and as emmenagogue. They inhabit in dry, sandy or stony soils.The aim of this work is to differentiate by their morphological and micrographic characters. The materials were collected in the west-central Argentinean region, fixed and preserved in formaldehyde: acetic acid: alcohol (1:1:1). Micrographic parameters were performed on diaphanized leaves and stained with 10%safranin. The three species

differ mainly in the shape of its leaves: *L. cuneifolia* ("jarilla macho") has bifoliolate leaves with leaflets welded in most of its length; *L. divaricata* ("jarilla hembra") presents divergent leaflets, connated only at base; while *L. nitida*("jarilla crespa") has multifoliolate and odd-pinnate leaves with 5-8 pairs of leaflets. Micrographic parameters were decisive for identify these species: 1) *L. cuneifolia***:** stomata number on upper epidermis (SN_{ue})=7.25±1.22; stomata index on upper epidermis (SI_u)=11.22-14.34; stomata number on lower epidermis (SN_{le})=10.41±1.53; stomata index on lower epidermis (SI_{le})=17.27-20.35; Palisade ratio (PR)=4.92-6.12; Vein islets number.mm^{-2} (VIN)=6.33±2.46; Veinlet termination number.mm^{-2} (VTN)=5.66±1.23.- 2) *L. divaricata*: SN_{ue}= 20.02±1.2; SI_{ue}= 22.7-24.28; SN_{le}= 23.03±1.21; SI_{le}= 25.7-28.1; PR= 4.18-5.22; VIN= 1.58±0.66, and VTN= 3.33±0.98.- 3) *L. nitida*: SN_{ue}=14.25±2.33; SI_{ue}=24.35–30.93; SN_{le}= 21.5±0.76; SI_{le}= 28.65–33.79; PR=4.63–5.61; VIN=21.33±1.77 and VTN= 18.66±2.7.
This study contributes to carry out an effective pharmacobotanical quality control, especially on pulverized drugs.

A105

MICROGRAPHIC PARAMETERS ON *Rollinia emarginata* (ANNONACEAE), "ARACHICHÚ"

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Rollinia emarginata Schltdl. is a native of the South of Peru and Bolivia, Southern and Central Brazil, Southern and Easter Paraguay, and North-eastern Argentina. The leaves decoction is used for gargle in the treatment of sore throat (tonsillitis) and swish to relieve toothache. The fruit is edible and is used as antiscorbutic, too. Other important species of the family are *Annona cherimolia* (custard apple), with edible fruits, and *Annona muricata* (soursop) used in anticancer therapy. This work was carried out in order to find the micrographic parameters allowing an indubitable species identification. It worked with samples collected in north-western Argentina, fixed and preserved in formaldehyde: acetic acid: alcohol (1:1:1). Micrographic parameters were performed on diaphanized leaves and stained with safranin 10%. The leaves show a heterogeneous mesophyll. The epidermal characters are essential to distinguish this species. Paracytic stomata occur only on the abaxial surface.Hairiness of the leaves is sparse with simple, eglandular trichomes. The micrographic parameters are as follows: stomata number (lower epidermis) = 30.58±1.83; stomata number (upper epidermis)= 0; stomata index (le)= 17.92–19.96; stomata index (ue)= 0; palisade ratio= 4.38–5.06; vein-islets.mm^2= 30.08±1.8 and vein termination number.mm^{-2}= 10.66±1.3. Both epidermal characters and micrographic parameters are essential for distinguishing fragmentary material in herbalist mixtures and other drug sources.

A106

MONITORING OXIDATIVE STRESS PARAMETERS AS CHLOROPHYLL LEVEL IN CADMIUN TREATED *Glycinemax* L. PLANTS BY MOLECULAR FLUORESCENCE

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Heavy metals induce different types of stress in plants. The monitoring of chlorophyll *a* (*chla*) level is a good indicator of an imbalance in the redox homeostasis and therefore of oxidative stress. The aim of this work was to determine the decrease of chlorophyll level that reveal the oxidative stress caused for Cd exposure. Soybean seeds were germinated and developed under controlled conditions until were used. On the 4th day of germination were placed in hydroponic conditions with Hoagland nutrient solution. On the 10th day, were exposed to Cd (40 µM) during 4, 6, 24, 72 and 144 h. To obtain extracts, 500 mg of leaves were homogenized under ice-cold conditions in ethanol absolute. Homogenates were centrifuged for 10 min and the supernatants were analyzed. To carry out a methodological comparison, the *chla* determination was measured by a-spectrophotometry and b-fluorescence. The reduction in *chla*content measured by spectrophotometry was significantly higher at 24, 72 and 144 h in Cd-treated plant (*p<0.05). Chlorophyll *a* level determined by fluorescence showed a reduction from 24 h to 144 h (*p<0.05), demonstrating oxidative damage in the plant. This parameter is related with a significant decrease in Mg uptake at leaf level indicating chloroplast damage. In this model, Cd exposure alters the normal physiological status of soybean plants, indicating chloroplast damage and it is related to a significant decreases in both methodologies.

A107

CLASSIC CYTOGENETICS IN TRICEPIRO AND TRITICALE ADVANCED STRAINS

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The interspecific hybridization in Triticeae has resulted in new crops. At the UN Río Cuarto, a breeding program develops in order to obtain new germplasm of triticale (wheat x rye) and tricepiro (triticale x trigopiro); moreover, triticale germplasm from CIMMYT is introduced annually. Ploidy level and the meiotic behavior of two tricepiros obtained in the UN Rio Cuarto (53H6 and 65H4) and two promising triticale strains, the introduced (C94/528) and the re-selection HA of the cultivar Genú-UNRC, were analyzed. Bivalent number in pollen mother cells (II/ PMC)

was determined. The strains were compared using t-tests. The ploidy level was 6x. The 53H6 had 20.49 ± 1.74 II/ PMC (RV = 15-24), whereas 65H4 had19.93 ± 1.08 (RV = 18-21); C94/528 and Genú HA had 20.90 ± 0.55 (RV = 19-22) and 20.52 ± 0.55 (RV = 19-21) respectively. In tricepiros greater range of variation of the II/ PMC was observed, with significant difference with triticale (t = -2.93 **). Both tricepiros showed laggard chromosomes, most often in 65H4, probably due to the different genomes originally involved (A, B, D, R and J). Triticale strains showed more cytological stability because no abnormalities were observed in meiosis. In productive evaluation test, these materials proved superior agronomic characteristics and will be described according to the INASE standards.

A108
DIFFERENTIATION OF MEDICINAL SAMPLES OF *Fumaria* BY FTIR and HPLC METHODS

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In order to expand the identification chances of some medicinal plant species by simple, rapid and inexpensive methods, aqueous extract of dried and milled plant samples of *Fumaria officinalis* L. and *F. capreolata* L. (Fumariaceae) underwent to FTIR and HPLC analysis. The first species is known as "fumaria" or "palomita" and is used as anticholinergic, antispasmodic, antiarrhythmic and bile flow regulator; the second is called "flor de la palomita" or "palomita", being used as a tonic, depurative, choleretic and skin diseases. IR spectra were obtained with a FTIR Protégé 460 spectrophotometer with CsI beamsplitter, acting upon pelletized drug (KBr 3% w/w), at a spectral resolution of 4 cm^{-1} in the range 4,000 and 460 cm^{-1}. HPLC profiles were obtained with a Waters chromatograph provided with a diode array detector (DAD) and a C18 column, using the mobile phase acetonitrile:water (70:30). Obtained data were subsequently subjected to chemometrics methods, mainly the principal component analysis (PCA). FTIR spectra and HPLC chromatograms showed significant differences among species, such as the areas under the most representative absorption bands. Therefore, the profiles can be useful as "fingerprints" for the differentiation of these species, in order to contribute to an effective quality control of raw drugs as well as herbal medicines involved.

A109
RESPONSE OF TRANSGENIC AND NON-TRANSGENIC SOYBEAN PLANTS (*Glyicine max.* L) IN FRONT OF OXIDATIVE STRESS PRODUCED BY CADMIUM

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In Argentina, 90% of the soybean plants are resistant to glyphosate, varieties RR (Roundup Ready) (Transgenic soybeans). Finding advantageous properties of non-transgenic (NT) over transgenic (T) plants, against different stressors is an interesting challenge in the way of attempting replacement genetically transformed plants with native ones. Cadmium (Cd) is a contaminant that interferes with the entry, transport and use of essential elements (Ca, Mg, P and K) and water, causing nutritional imbalances and dehydration, which causes changes in oxidative stress parameters. The aim of this study is to determine the difference in the biochemical mechanisms against oxidative stress by Cd. Leaves of transgenic and non-transgenic soybean with Cd contaminationfor 24 hwere used. MDA, H_2O_2 and catalase were determined. The results for NT (Cd) vs T (Cd) were: H_2O_2 showed a significant increase ($p < 0.05$), catalase showed no significant differences and a very significant increase in MDA levels was observed($p < 0.001$), for the T samples in all the cases. With these preliminary results, we could conclude that the NT variety presented an antioxidant defenseadvantage over the oxidative stress caused by Cd.

A110
MORPHOGENESIS AT TILLER LEVEL OF *Panicum coloratum*

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Panicum coloratum (Pc), perennial megathermic Poaceae, is an important feed resource for cattle breeding systems in the province of San Luis (Argentina). In a plot located in the experimental field of Agronomy in UNSL, after a clean cut, 20 tillers were identified in different plants with the objective of evaluate the morphogenetic behaviour of PC. Every seven days, appearance and senescence of leaves and leaf length was recorded. The maximum number of accumulated leaves / tiller, mean leaf lifespan (VMF) and leaf appearance rate (TAH) was determined. VMF is considered as the days elapsed between the appearance of a leaf and the beginning of senescence and TAH to the time interval (days) between the appearance of two successive leaves. Analyzed tillers were those that had the greatest number of fully expanded leaves (10). During the growth cycle, Pc accumulated 11 leaves distributed

between the initial spring regrowth and two leaf parts in summer-autumn. On average, each leaves generation presented 4 ± 0.83 leaves. The fourth leaf (initial regrowth) reached an average 308 ± 73 mm maximum leaf length, the octave (first replacement) 458 ± 185 mm and the eleventh leaf (second replacement) 617 ± 108 mm. VMF was 73 ± 14 days and TAH 19 ± 4 days, calculated between the fifth and eleventh leaf. 40% of tillers remained vegetative. For the study environmental conditions, Pc provides, every 73 days, three leaves generations suitable for grazing.

A111
COMPARATIVE MORPHOLOGICAL STUDY OF THE GERMINATION PROCESS IN TWO CACTI SPECIES OF SAN LUIS, ARGENTINA

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Cereus aethiops and *Cereus forbessi* are two closely related species of native cacti from San Luis, showing different geographical distribution and morphology. *C. aethiops* adopts shrub forms and is widely distributed across the province, whereas *C. forbessi* adopts tree forms and its location is restricted to the northwest highland area. The aim of this study was to provide information on the comparative morpho-anatomy and seed germination process. In order to infer on the establishment and survival of seedlings of both species, seeds were collected in their natural habitat further analyzed under laboratory conditions. In both species, the fruit is a berry elliptical and dehiscente containing numerous seeds with mucilage, transparent in *C. aethiops* and purple color in *C . forbessi*. The seeds are small, about 1 to 2.5 mm, dark brown testa with presence of tannins and crispness. Germination percentages were variable. The start of the germination process requires seed scarification and presence of light, showing epigeous germination. In both species typical of the genus germination observed (testa rupture in a zone of near the junction funiculus placental recessh). Both species are similar in morpho-anatomy and the seed germination process; showing differences, such as greater amount of tannins in the testa of seeds of *C. forbessi* and cotyledons arranged in different angles to the other specie. It was observed that *C. aethiops*, in low nutrient can stay at seedling stage for a long time, taking the color of the substrate, suggesting a survival strategy. The data obtained are of relevance to the understanding for conservation of these native species.

A112
SEED PROPAGATION CONDITIONS OF *Habranthus cardenasianus, Hieronymiella clidanthoides* AND *Rodophiala mendocina* (AMARYLLIDACEAE)

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The Amaryllidaceae include attractive plants with potential use in commercial floriculture mainly as a landscape plant, as well as known for their pharmacologically active alkaloids. An important approach to treat Alzheimer's disease is directed to the inhibition of Acetylcholinesterase enzyme (AChE). Owing to these, seeds of *Habranthus cardenasianus, Hieronymiella clidanthoides* and *Rodophiala mendocina* (Amaryllidaceae) were collected. Seed propagations were investigated in order to determinate the most effective ways to spread these species. Temperature variations were programmed with alternating 20/10 °C, with light-dark cycles of 16-8 hours respectively. The replicates were placed in Petri dishes on germination paper, moistened with sterile distilled water. The plates were kept for 30 days in the germination chamber, with the same heat-light treatment. The percentage of germination (G) was calculated. These seedlings obtained were transplanted in a plastic tray using substrate: 2 parts of fertile soil and 1 part of fine sand and were maintained for 45 days in germination chamber under the same lighting conditions. Later, they were re-transplanted individually in conical plastic pots, and length was measured from the first sheet and radicle. The analysis of the results obtained so far, corroborate that seeds of Amaryllidaceae species selected can be germinated in growth chambers with similar conditions of the natural environment, however they must be recent collection, since over time have a significant loss of G capacity. In relation to the seedlings developed in the second transplant, it is expected to be used on the evaluation of bulbs growth; their ability to flowering development, which is an important ornamental aspect, as well as chemical composition that could inhibit AChE. (CIN-CICITCA-UNSJ- CONICET-)

A113

Digitaria eriantha CV. SUDAFRICANA-*Azospirilumn brasilense:* RESPONSE TO ABIOTIC STRESS

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Digitaria eriantha is an excellent forage resource in arid areas. The aim of this study was to evaluate the *Digitaria*symbiosis with two strains of bacteria *Azozpirillum brasilense*, a commercial one (Az39) and AIA hypoproductor (ipdC-), in control and abiotic stress condition.*Digitaria eriantha* seeds were inoculated and planted in soil: perlite (2:1, v / v) at 23:21 ° C with 16:8h photoperiod and were separate into four groups: 1) 23 °C and field capacity (control), 2) drought 100 ml PEG 6000 1.5 Mpa 3) cold (4 ° C for 72 hours) and 4) salinity (200 mM NaCl). The effects were evaluated in different parameters: foliage length (FL), root length (RL), fresh and dry weight in foliageand root (FWf, FWr, DWf, DWr).In the control treatment were found increases in FWf, FWr, DWf and DWrfor *ipdC*-. Mitigation of both bacteria was positive against drought, being always more effective*ipdC*-than *AZ-39*. Under cold stress, cv. Sudafricana was unchanged. None bacteria caused mitigation on salt stresswhich indicates that they are not very effective in mitigating saline soils.

A114

WATER AND NITROGEN CONTENTS ASOCIATED TO PGPR AND ABIOTIC STRESS IN *Digitaria eriantha*

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Digitaria eriantha has the ability to play different roles in the farming systems of the semiarid region in San Luis province-. The aim of this study was to determine the content of nitrogen and water in plants associated with two strains of *Azospirillum brasilense* (*AZ-39* and *ipdC*-) subjected to abiotic stress. Nitrogen was determined with 8 to 12 mg of plant tissue, placed in a tube with Folin mineral (potassium sulfate, potassium perchlorate, sulfuric acid and water). Dried on a sand bath at 100-200 ° C and 400 ° C was mineralized. The residue was neutralized with sodium hydroxide and Berthelot colorimetric reaction measured the absorbance at 620 nm of stress treatments versus control with ammonium sulfate. The nitrogen content was calculated. The rate of water was determined from 10 to 12 mg of tissue processing in a mortar and the water content was determined by the Karl – Fisher method (transformation of sulfur dioxide by iodine in the presence of water and an organic base). The higher water content in inoculated plants with the strain AZ-39 and ipdC- was recorded under cold stress and the higher nitrogen content was recorded under drought stress with both bacteria.

A115

INFLUENCE OF SOYBEAN FLOUR ON FATTY ACIDS PROFILE IN THE LIVER OF RATS FED WITH NORMOCALORIC AND HYPERCALORIC DIETS

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The diet fatty acids composition is known to influence the fatty acid composition of stored and structural lipids in the body .This study investigates the effects of replacing casein with soybean flour on the fatty acids profile in the liver of *Wistar* male rats that were previously fed with normocaloric and hypercaloric diets based on casein. One group was fed with control diet and another with hypercaloric diet (with 34.15% sucrose, 42% fat calories) for 9 weeks. Each group was then divided into two subgroups and casein was replaced with soybean in one of them, obtaining: CC (control casein), CS (control soy), HC (hypercaloric casein) and HS (hypercaloric soy), which were fed for 6 weeks. Capillary gas-liquid chromatography (c-GLC) of the fatty acid methyl esters (FAME) was performed.The analysis of the fatty acid profiles in rat liver, showed that replacing the casein diets by a vegetable protein, such as soybean flour, induces an increase of unsaturated fatty acids and a decrease of saturated ones ($P < 0.01$), even with the hypercaloric diets. There was a clear decrease in lauric, myristic and palmitic acids in liver of rats fed with soy. The unsaturated : saturated fatty acids ratio (unsat : sat), increased in both soy diets compared with casein. This allows us to assume that soybean flour, even in hypercaloric diets, would have a positive effect on the prevention of simple steatosis. The total omega-6 polyunsaturated fatty acids (PUFA n-6) was significantly higher in the CS and HS groups compared to the casein groups ($P < 0.01$). Similar behavior was observed in total omega-3 polyunsaturated fatty acids (PUFA n-3), and a lower n-6 : n-3 ratio was observed in soy groups compared to casein groups, exerting a positive effect in preventing many diseases, including: cardiovascular disease, inflammatory and autoimmune diseases.We believe that consumption of soybean flour (whole grain) is a very good dietary resource for improving hepatic fatty acid profile, and therefore, have a beneficial health effect.

A116
MORFOLOGICAL AND PHYSIOLOGICAL PARAMETERS IN TWO CULTIVARS OF *Digitaria eriantha*

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Plant growth of *Digitaria eriantha* Steudel cv. Sudafricana and cv. Mejorada INTA was studied for 90 days under controlled greenhouse conditions. Fresh and dry weight (FW and DW) from shoot and root, leaf area, chlorophyll and carotenoids content was recorded. The average values of length of shoot and root showed no significant differences between cultivars. The dynamic growth of both cultivars was similar: the length of roots increased exponentially until day 31 which reached average values of 22 cm; the length of the aerial part grew exponentially until day 53 where it reached average values of 41 cm. The average aerial foliar and average FW and DW values obtained for roots and shoots at 90 days showed similar values with no significant differences between cultivars. The average values of chlorophyll *a* (22 mg g^{-1} FW), chlorophyll *b* (18 mg g^{-1} FW) and carotenoides (40 mg g^{-1} FW) showed no significant differences between cultivars.

A117
AGRONOMIC EVALUATION OF SOYBEAN LINES WITH ABSENCE OF LIPOXYGENASE AND ANTINUTRITIONAL FACTORS

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Soybean has excellent nutritional quality however the raw grain can not be used as feed for monogastric animals because it possesses anti-nutritional factors as the Kunitz (SKTI) which strongly inhibits trypsin leading to lower digestion and food absorption. However a mutation has been discovered that prevents their accumulation in seeds. Furthermore, acceptance and palatability are conditioned by the bitter and astringent taste elicited by compounds that are the result of enzymatic action of lipoxygenases. The absence of these enzymes is determined by the lx1, lx2 and lx3 null alleles. In order to evaluate agronomically soybean advanced lines selected for their nutritional quality. Twelve soybean advanced lines (triple null for lipoxygenases and anti-nutritional Kunitz factor) and a witness were sown in the province of San Luis in Villa Mercedes (VM) and in Antiguas Estancias Don Roberto (DR) in a completely randomized design with 2 replications. Yield (Y) in kg/ha was determined. Combined Analysis of Variance to detect interaction and later partitioned by location ANOVA with Fisher test 0.05 to determine type of interaction and ranking of genotypes were performed. ANOVA detected significant differences in DR ($p < 0.05$) and highly significant in VM ($p < 0.01$) for the variable evaluated. Also genotype by location interaction was highly significant ($p < 0.01$). In DR, 1513.1 line overcame all other lines including the witness with an average yield of 3018 kg/ha. While in FICA 9 lines overcame the witness with means between 3114 and 3584 kg/ha. In both locations the agronomic potential lines was manifested which allowed selecting genotypes with high nutritional quality and superior agronomic traits.

A118
ACCELERATED AGING TO EVALUATE VIABILITY OF *Jatropha macrocarpa* Griseb. SEEDS

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Jatropha macrocarpa Griseb. grows in arid and semiarid areas; it is tolerant to adverse environments and it is an important resource for the production of biodiesel.The aim of this work was to adapt the methodology of the accelerated ageing test in order to evaluate the seed viability (V) and germination percentage (G) of *J. macrocarpa* seeds. In bottles with distilled water, seeds were placed in mesh without touching the water, in an oven at 40-45 °C for 0: control, 24, 48, 72, 96 and 120 h. Seedswerestaining withtetrazoliumsaltsand were evaluated V and PGin eachtime.Seed lots were treated using temperature regime of 40- 45 ºCelsius during each of six periods of time: 0, 24, 48, 72, 96, 120 h as accelerated aging times and a humidity of 100 %. Data was analyzed through a Complete Random Design under a factorial arrangement and three repetitions. Seed V levels showed 76.6% at 24h; 63,3% at 48h; 56,6% at 72h; 36,6 at 96h and 33,3 at 120 h, with significantdifferences between control all time except 24 h. Seed Gwere 10% at 24 h; 3,33 % at 48 h; 0% at 72, 96 and 120 h with differences in all treatment respect the control.*Jatropha macrocarpa* seeds were sensitive to accelerated aging; they preserve viability over time but lost the ability to germinate indicating presence of dormancy.

A119

GERMINATION RESPONSES AND BIOMASS PRODUCTION OF *Jatropha curcas* L

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*Jatropha curcas*L.is useful for restoring degraded areas and theirseeds containoils for biodiesel production. The aim of the work was to determine the morphometric parameters and photosynthetic activity during early and late growth of *J. curcas.*Seeds were placed in land-perlite and were kept in growth chamber at 30°C and photoperiod of 16: 8 light: dark and 60% humidity. During eight months were evaluated the followed parameters by triplicate: length of roots (LR), hypocotyl (LH) and epicotyl/stem (LE/LS), number of true leaf (TL) and cotyledon leaf (CL), fresh weight (FW) and dry weight (DW) of root, hypocotyl, epicotyl/stem, (TL) and (CL). There were not significant differences in LR.LH increased significantly from day 14 when CL disappeared. LE/LS increased significantly from day 220. FW and DW ofR, H and E/S was gradual until day 220, after that there was a marked growth pulse during later stages. The level of chlorophyll "a" was superior to chlorophyll "b" and carotenoids were detected.The study of early growth is important because it determines the successful establishment of the seedling. The knowledge of *Jatropha curcas* growth, as well biomass production, determines the behavior against environmental stress.

A120

ASSESSING THE VIABILITY OF SEED *Achyrocline satureioides* (Lam) DC “MARCELA” IN THE PROVINCE OF SAN LUIS

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Little information is available about the propagation and conservation of *Achyrocline satureioides,* "marcela”, medicinal specie. *A. satureioides* is a very important folk medicine plant from San Luis Province their populations are exploited as extractive and non-rational culture initiatives known. Because there is a lack of information about sexual propagation of this species, the aim was to evaluate rate and percentage of germination from seeds of "marcela". Seeds collected in April 2013 (old seeds=OS) and 2014 (new seeds=NS), were made according to standard method to germinate in Petri dishes. The dishes are placed in an oven at 23 ± 2 ° C for 21 days.Four replicates of 30 seeds were used for each treatment. The number of seeds with radicle emergence was recorded as a percentage of the number of seeds in the experimental unit. The number of germinated seed in each dish was recorder at 4, 7, 14 and 21 days. New seeds (NS) and old seeds (OS) over 12 months of storage were used. A decrease in the rate of germination of 40% at 4 day with OS and the percentage of germination of 14 % new seeds was observed.

A121

DETERMINATION OF ANTITRYPTIC FACTORS, LECTINS AND SAPONINS IN NEW REGIONAL VARIETIES OF AMARANTH

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Amaranth remains an active area of scientific research for both human nutritional needs and foraging applications. Several scientific studies suggest a somewhat conflicting picture on possible antinutritional and toxic factors in amaranth. Based on the abovementioned,the purpose of this work was to study the some antinutrients content of the seeds flour of *Amaranthus cruentus* var. *candil* (Acc), *Amaranthus hypochondriacus* var. *dorado* (Ahd), advanced lines of *Amaranthus hypochondriacus* x *Amaranthus cruentus* H17a (AH17a) and *Amaranthus cruentus* G6/17a (AcG6/17a). The following antinuntrients were determinated: i) saponins (foam index (WHO/PHARM/92559)); ii) hemolytic activity assay (Duarte Correa, A et al. 1986); iii) hemagglutinin or lectins assay (Do Prado, VC et al. 1980; Das Gupta, VR and Boroff, DA. 1968); and iv) antitryptic factors (Kakade, ML et al. 1974).The obtaining results were: foam index < 100; hemolytic activity = 0 during 12 h; hemagglutinin assay (minimum positive concentration 1/32); antitryptic factors values ranged between 5.72 and 2.00. Such results are within the allowed values without risk to human health. According to this study, these new amaranth varieties and lines could be considered an interesting contribution to the human diet with high nutritional quality.

A122
SURVIVAL TRANSPLANTION OF *Cercidium praecox*, "BREA", SEEDLINGS OF DIFFERENTS AGES

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The aim of this study was to evaluate the survival of *Cercidium praecox* seedlings transplantation of different ages. "Brea" seeds, previously scarified with sandpaper and disinfected, were put to germinate in plastic boxes with vermiculite watered with distilled water, 26±2ºC temperature and 16 hlight/8 h dark photoperiod conditions. Two and seven days old seedlings were transplanted into individual pots of 250 ml capacity provided with a 50:50 mixture of garden soil: vermiculite. The survival rate of transplanted seedlings at 7, 14 and 21 days was measured. The 2 days old seedlings had a response to transplantation of 60% at seven days; 35% at fourteen days and remained until 21 days. The 7 days old seedlings reported 25% survival, with similar value at 14 and 21 day. This study show that younger seedlings have greater tolerance to transplantation and it was shown that the decisive factor seedling death was damping-off.

A123
POLYPHENOLS PROFILE AND ANTIBACTERIAL ACTIVITY OF THE *Pistacia vera* cv Kerman FROM SAN JUAN, IN THEIR COMMERCIAL PRESENTATIONS

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Nowadays, consumers' tendency on choosing food is associated to health and wellness.The pistachio (*Pistacia vera* L.) is a member of the *Anacardiaceae* family, habitually are used as a snack food or as ingredient in the food industry. Actually, pistachio nuts are known for their nutritional quality, antimicrobial, antioxidant capacity and antiradical activities. The aim of this study was to evaluate antibacterial activity and polyphenolic profile of *Pistacia vera* cv Kerman from San Juan (Argentina) in their commercial presentations (natural, roasted and salted-roasted).Pistachios (kernels with their skin) were grounded, defatted and extracted with MeOH-H^+, and assayed as an antibacterial. On the other hand, methanol acidic extracts were obtained from skin of natural, roasted and salted-roasted pistachios, and their phenolic compounds were identified and quantified by HPLC-ESI-QTOF. Antibacterial assay was evaluated by microdilution method according to CLSI, against strains ATCC.The major flavonoids identified were(+)-catechin, procyanidin dimer, isoquercitrin, luteolin and (-)-epicatechin. The heat treatment (roasting) affects the composition of the polyphenols profile; watching an increase of 31 % inthe total compounds, while salted-roasted pistachiosshown a decrease of 42 %. In addition, a moderate antibacterial activity of natural pistachio was found against *Staphylococcus aureus*methicillin-sensitive (ATCC 29213) and *E. coli* (ATCC 25922) with MICs values between 1.5 and 3 mg/ml, respectively.Roasted and salted-roasted pistachios were not active (>3 mg/mL).Natural pistachios are a good source of polyphenolic compounds; and contribute more than others commercial presentations to improve wellnessand health.(SECITI, CICITCA-UNSJ, CONICET)

A124
PHENOLIC CONTENT AND ANTIRADICAL ACTIVITY OF *Pistacia vera* cv Kerman, IN DIFFERENT CROP CONDITIONS AND INDUSTRIAL PROCESSES

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In the last years, pistachio crops have been increased in Argentina. In the present, 787 ha are cultivated, of which 485 ha are in San Juan province, becoming the main production center of pistachio. Environmental factors such as temperature, soil properties,and climate are essential for the development and growth of*Pistacia vera* L. cvKerman. The content of secondary metabolites, such as phenolics is influenced by *terroir*.The goal was to analyze and compare the content of total phenolics compounds, flavonoids and antiradical activity between three pistachios producers (A: Piste-Pistachos Argentines, B: Pistachos de los Andes and C: Fideicomiso Polo Pistachero) located in different regions of San Juan. Methanol acidic extracts were obtained from skin of natural and salted-roasted pistachios.The total phenolic (TP) content and flavonoids (FT) were measured using Folin–Ciocalteu and $AlCl_3$ methods respectively. In addition, theextracts were assessed through the scavenging effects on radical DPPH. Also, soil's physicochemical properties were measured (pH and conductivity). The results indicated that, in natural pistachios ano significant difference was observed in TP content between pistachios samples of different procedure (1.4 ± 2 g GA/100 g skin). Furthermore, a slight but also not significant increment was observed in FT content betweencultivars of different procedure or treatment (30 ± 4 mg Q/100 g skin). Related to DPPH radical scavenging, to 0.5 ppm all samples presented values major than 50% of capture capacity. Moreover the best activity was presented by pistachios roasted-salted from A samples 60.21% to 0.125 ppm. The pH average was 8 in all soils,

but there was a significant difference in conductivity values in soil samples A: 159, B: 2500 and C: 3700 µS. This is a preliminary study, other parameters are necessary to make a complete comparative analysis of different pistachios in diverse crops conditions and industrial processes (SECITI-CICITCA-UNSJ. CONICET)

A125
PHYSIOLOGICAL QUALITY OF PEANUT SEED AND ENVIRONMENTAL STRESS OF MOTHER PLANT

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Stressful environmental conditions during seed formation period of peanut crop can negatively affect their growth and development, and to harm the seed behavior during storage and sowing. Physiological seed quality of two cultivars (Utre UNRC, short cycle, T_b: 9.87 °C, and Granoleico, long cycle, T_b: 11.63 °C), developed under different environmental conditions (locations: Río Cuarto, RC and Del Campillo, DC), and sowing date (RC 08/10/10 and 12/12/10, and DC 24/10/07) were evaluated. Climatic data during crop cycle in both experimental locations were available from meteorological station (FAV-UNRC). When seeds reached equilibrium moisture were classified by size (sieves of 8, 7.5, 7, 6.5 mm in diameter). The seed vigor was evaluated for each category of seed size through different test (cold test, accelerated aging, germination energy, electrical conductivity (EC), and field emergence. Besides the physiological seed quality was measured by the pattern germination test. EC and field emergence data were analyzed for each location by ANOVA, and correlations between variables were estimated. Heat and water stress, to which the seed were exposed during their development, reduced physiological quality with differences between cultivars. Smaller seeds were less vigor. CE test is a good indicator of peanut seed vigor and reliable estimator of field emergence.

A126
GERMINATION OF SUNFLOWER GENOTYPES AT LOW TEMPERATURE AND DIFFERENT WATER AVAILABILITY

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The sunflower crop has been moved to less productive environments and generally sowing is performed under suboptimal temperature and water availability. The aim of this study was to evaluate the physiological quality of sunflower seeds of high (HO) and low oleic (LO) in different thermal and water regimes. Seven hybrids, 3 HO (AO MG 100, T 600 and T 700) and 4 LO (DKS 3845, MG 2, MG 50 and MG 60) were evaluated at temperatures of 8, 10, 12, 14 and 16 °C and 4 levels of available water: -0.7, -1.0 and -1.2 MPa (obtained with PEG 6000) plus a control without water limitation. Germination at 5 and 10 days; abnormal seedlings; no-germinated and dead seeds; and the length of the radicle classified into five categories: 1: 0-3 cm, 2: 3-6 cm, 3: 6-9 cm, 4: 9-12, and 5: >12 cm were quantified. Data were subjected to ANOVA for moisture levels at each temperature. In general, all genotypes germinated reaching values >80% in the different water conditions evaluated; but the germination rate was higher at temperatures ≥12 °C (5 days). The differences between the percentage of germination obtained at each temperature and the maximum expected (100%) was due to different causes: abnormal seedlings with 8 °C; non-germinated seeds (dormant) between 10 and 12 °C and dead seeds between 14 and 16 °C. The radicle length and its variability (percentage of different categories) increased with temperature due to the water condition and genotype (regardless of fatty acid composition).

A127
SOURCE-SINK RATIO IN RUNNER TYPE PEANUT (*Arachis hypogaea* L.) CULTIVARS

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The crop yield depends on the ability to accumulate biomass in harvestable organs. Peanuts have a pronounced indeterminate growth habit, with a short vegetative phase. When new fruits appear begins competition for available assimilates between these and vegetative organs which still growing. Therefore, the crop yield is highly dependent on the source-sink (S/S) ratio. The aim of this study was to analyze the variability of the S/S ratio in different runner type cultivars of peanut. To do this, six runner type cultivars (botanical type most widespread cultivated in Argentina) were sown in the 2011-12 growing season. During crop harvest biomass samplings were carried out. The analysis of the S/S ratio was performed by analyzing the relationship between the fruit number and weight at harvest. Peanut crop yield was, in all cases, limited by reproductive sinks under the prevailing conditions in this work. No was trade-off effect among the number and weight of fruits which indicating that was source in excess during the critical period of fruit set. This response was observed in all cultivars evaluated without differences between them. The results for a wide range of genotypes indicate, in accordance with the literature, the possibility

to achieve yield gains by improving the sink size, i.e. fixation of harvestable structures and reproductive efficiency, even at the expense a decreased ability of the photoassimilates source.

A128
ENDOESTEROLYTIC ACTIVITY IN EXTRACT PROTEOLYTIC OF NEP (*Natrialba magadii*) EXTRACELLULAR PROTEASE

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Natrialba magadii extracellular (formerly *Natronobacterium*) belongs to the haloalkaliphilic group, and dominate in hypersaline environments (low water activity).The esterolytic activity of *Natrialba magadii* was determined by the Silverstein´s method (1974), modified according to optimal conditions of the enzyme. The activity was studied using N-α-carbobenzoxy-p-nitrophenyl esters of some amino acids as substrate. Assays were made at 40 ºC in 0.1 M Tris-HCl buffer (pH 8) containing 20 mM cys, 1.5 M NaCl and 0.15, 0.3, and 6.25 mM of each substrate in the reaction mixture. P-nitrophenol was followed at 405 nm in a Cintra 6 UV-Visible spectrophotometer. An arbitrary enzyme activity unit (Ucbz) was defined as the amount of protease that released one μmol of p-nitrophenolate per min in the assay conditions. For determinate the μmol of p-nitrophenolate produced during the reaction, was carried out a standard curve with p-nitrophenol (5-50 μmol) in 0.1 M tris-HCl buffer (pH 8) containing 5% acetonitrile.*Natrialba magadii* was tested against several N-α-carbobenzoxy-p-nitrophenyl esters of different L-amino acids. The preferred substrate of *Natrialba* was the Gln derivative and order decreasing those of Asn, Gly, Lys, Tpr. These results showed the high selectivity of *Natrialba* by polar amino acids.

A129
ENDOESTEROLYTIC ACTIVITY IN PROTEOLYTIC EXTRACT OF *Acacia caven* (Mol) AND *Solanum granuloso-leprosum*

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Proteases are the group of hydrolytic enzymes most used on an industrial scale. The esterolytic activity of *acaciain* and *granulosain* was determined by the Silverstein´s method (1974), modified according to optimal conditions of the enzyme. The activity was studied using N-α-carbobenzoxy-p-nitrophenyl esters of some amino acids as substrate. Assays were made at 40 ºC in 0.1 M Tris-HCl buffer (pH 8) containing 20 mM Cys and 0.15, 0.3, and 6.25 mM of each substrate in the reaction mixture. P-nitrophenol was followed at 405 nm in a Cintra 6 UV-Visible spectrophotometer. To determinate the μmol of p-nitrophenolate produced during the reaction, a standard curve with p-nitrophenol (5-50 μmol) in 0.1 M tris-HCl buffer (pH 8) containing 5% acetonitrile, was carried out. *Acaciain* and *granulosain* were tested against several N-α-carbobenzoxy-p-nitrophenyl esters of different L-amino acids. The preferred substrate of *acaciain* was the Lys derivate and decreasing orders those of Arg, Gly, Asn. These results showed the high selectivity of *acaciain* by basic and polar amino acids. The hydrophobic character of Phe, Trp, Ile and Val avoid the activity expression of acaciain. The preference of *granulosain* was Asn derivative. *Granulosain* showed high activity and low selectivity by basic (Arg, Lys) and polar (Asn, Gly,Gln) amino acids but it showed low preference by nonpolar amino acids (Phe, Ile,Trp, Val).

A130
STABILITY OF ANTIACANTHAIN IN AQUEOUS- ORGANIC BIPHASIC SYSTEMS

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The use of organic solvents as reaction media for enzymatic reactions provides numerous industrially attractive advantages compared to traditional aqueous reaction systems. In organic media, the proteases synthesize peptide bonds due to the shift of the thermodynamic equilibria in favor of synthesis. The aim of this work was to study the stability of proteolytic enzyme of fruit of *Bromelia antiacantha* Bertol, (a plant that growns in Argentina) in biphasic organic media, for its application to the bioactive peptide synthesis. An experimental statistical design allowed to cluster different organic solvents, by its physical chemistry characteristics and to select one of each group. Antiacanthain stability in biphasic system (Tris-HCl (0.1M) pH 8- organic solvent) was assayed by means of incubation of pre-purified crude extract in the reaction mixture (30:70; 50:50 and 70:30) during 24h. Enzyme specific activity was determined using N-α-benzoyl-DL-arginine-ρ-nitroanilide (BApNA) as substrate.

Antiacanthain in biphasic systems (aqueous-organic) at 50% showed increased stability that in buffer Tris-HCl (pH 8) ($t_{1/2}$= 8h). In addition, initial activity and the profile stabilities of antiacanthain were activated for (50%) ethyl acetate and hexane; maintained for benzene, ethyl ether and dichloromethane (50%) and diminished for octane and chlorobenzene (50%), when they was compared whit buffer. Profiles stabilities of antiacanthain in biphasic aqueous-organic systems (30 and 70%) were maintained, except for chlorobenzene (70%).

A131
STABILITY OF ANTIACANTHAIN IN AQUEOUS- ORGANIC MONOPHASIC SYSTEMS

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From a biotechnological perspective, there are many advantajes of employing enzymes in organic media, such as high regioselectivity and stereoselectivity, minimal side- chain protection requeriments. However, the major drawback of using enzymes in organic solvents is their significantly reduced activity compared to that in buffer media. The aim of this work was to study the stability of proteolytic enzyme of fruit of *Bromelia antiacantha Bertol*, a plant that growns in Argentina, in monophasic organic media, for its application to the bioactive peptide synthesis. An experimental statistical design allowed to cluster different organic solvents, by its physical chemistry characteristics and to select one of each group. *Antiacanthain* stability in biphasic system (Tris-HCl (0.1M) pH 8- organic solvent) was assayed by means of incubation of pre-purified crude extract in the reaction mixture (30:70; 50:50 and 70:30) during 24h. Enzyme specific activity was determined using N-α-benzoyl-DL-arginine-ρ-nitroanilide (BApNA) as substrate. *Antiacanthain* in monophasic systems, which were formed by buffer Tris-HCl (pH 8) and a miscible organic solvent, showed low activities ranging between 0.1 and 8 UI/mg de protein. These values were lower than 32% of the residual proteolytic activity in buffer. These results showed that these media will not allow the bioactive peptide synthesis because of them cause inactivation of the antiacanthain.

A132
ESTABLISHMENT *IN VITRO* OF *Aloysia citriodora* PALAU AND *Cymbopogon citratus* (DC.) STAPF

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Whether for medicinal or cosmetic use, approximately 70,000 aromatic species are used worldwide. They also are the economic livelihood of many rural populations. Despite this, the aromatic species are in danger of extinction due to over explotation, deforestation, introduction of exotic species and climate change, among others. This is what inspires us to work on *in vitro* techniques in order to maintain these species' existence. A technique for *in vitro* culture of plant tissue allows the propagation and preservation these species. This study works with two aromatic species: *Aloysiacitriodora*, and *Cymbopogoncitratus*. These species are characterized by their essential oils and by their medical use (digestive, antispasmodic use, expectorant). The objective of this work is to find the most appropriate treatment for explants' disinfection, and then achieve efficient growth *in vitro* culture. The leaves were previously disinfected with alcohol; sodium hypochlorite and fungicide in different concentrations and exposure time. The culture medium used was Murashige and Skoog. Three treatments were performed for each species. The most efficient outcome for *A. citriodora* in 70% alcohol (5 minutes), 20% sodium hypochlorite (8 minutes) and fungicidal 8% (30 minutes). For the species *C. citratus* the most efficient treatments were: alcohol 70% (10 minutes), 30% sodium hypochlorite (5 minutes) and fungicide 12% (20 minutes). Stages of micropropagation continue to achieve conservation and cloning of selected chemotypes.

A133
FUNCTIONAL CHARACTERIZATION OF AFFINITY MACROLIGAND AND CELL-CIBACRON BLUE

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Adsorption is usually described by an isotherm. Adsorption Isotherm represents an experimental methodology that describes the distribution of the adsorbed molecules between the solid phase and liquid phase system in equilibrium at constant temperature. The objective of this work is to cuantitative study and characterization of the affinity adsorption of BSA and Lysozyme (Lys) to macroligand Cell-Cibacron and determination of the efficiency of adsorbent by kinetic and thermodynamic parameters. Affinity adsorbent microparticles for protein separation were prepared. Yeast cells were modified by chemicals and the Cibacron Blue ligand molecule was immobilized to the wall cell by covalent bond. The adsorbent was characterized by determination of the affinity equilibrium constant (Ka) by means of adsorption isotherms (Langmuir and Freundlich) using BSA and Lys as target protein.

The adsorption behavior of Lys was described by the Langmuir model, BSA adsorption was described by the Freundlich model. The influence of pH and temperature was studied. Maximum adsorption was observed at pH 8 (Lys) and pH 5 (BSA). At values lower and higher than pH 8 and 7 the adsorbed amount of BSA and Lys decreased. In the temperature range studied (7ºC, 15ºC, 22ºCand 37ºC). Adsorption of Lys and BSA on the macroligand was significantly increased with increasing temperature indicating that hydrophobic interactions much more significant in the adsorption. Experimental results analyzed by external diffusion kinetics models conclude that the adsorption rate limiting step in Cell-Cib-BSA system is liquid layer surrounding the adsorbent particle. The adsorption kinetics of Lys to macroligand is described by a model pseudo-second-order.

A134
HSA AND LYSOZYME SEPARATION BY AFFINITY CHROMATOGRAPHY

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The most effective affinity purification technique has been affinity chromatography, which combines conventional column chromatography with affinity interactions. The objective of this work is to study the adsorption and selectivity of macroligand Cell-Cibacron immobilized in solid support using the affinity chromatography system. Affinity macroligands were prepared from yeast cells modified by chemicals and with the Cibacron Blue F3GA ligand molecule immobilized to the wall cell by covalent bond. The amount of ligand immobilized on the wall cell was determined by spectrophotometric method. An fixed-bed column system with immobilized agarose-macroligand cubes was prepared. HSA and Lysozyme (Lys) adsorption from Human Serum and hen egg white was studied. The purity of HSA and Lysozyme was assayed by gel electrophoresis (SDS-PAGE). The maximum attachment of ligand on the wall cell was 212 µmol of Cibacron dry/g of dry cell. HSA and Lysozyme were purified with high purity (more than 80%) with the affinity chromatography column system using agarose-macroligand cubes. It is an easily reproducible system, which also demonstrated an adequate sample processing speed. There was no occlusion or blockage of the affinity column during the separation process.

A135
ESSENTIAL OIL OF *Schinus polygama* AS ATTRACTANT OF WILD MALES OF *Ceratitis capitata*

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It is known that essential oils have different effects on behaviours of insects. Previous experiments with the essential oil of *Schinus polygama* showed that this oil might be an attractant of sterile males of *Ceratitiscapitata* (tsl). In this work, our objective was to evaluate whether the same behaviour is showed by wild males of *C. capitata* whichmay indicate that this oil has the potential for future technological developments such as lure and kill.Attractant activity of the essential oil was evaluated at laboratory conditions(24 ± 2◦C, 50 ± 5% RH) in a Y-tube olfactometer. The time (s) flies spent in each arm of thedevice was recorded. Flies' final choice was the arm they werein at the end of a 2 min experimental period. On the other hand, attractancy was also evaluated in outdoor conditions by dual choice test in field cages. Data analyses were preformed with the Wilcoxon signed-rank test and choices were analyzed by the Chi-square test. Both assays showed that wild males of *C. capitata* were significantly attracted by the essential oil ($p<0.05$). Thus, the components in the essential oils of *S. polygama* could be candidate substances for the control of *C. capitata*. Acknowledgements: To FONCYT (PICT2013-2076).LML, DS are research scientists of CONICET. FJB is a fellow of CONICET.

A136
EFFECT OF DAMAGE BY CODLING MOTH (*Cydia pomonella*) ON THE VOLATILE PROFILE OF FOUR VARIETIES OF QUINCE (*Cydonia oblonga*)

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Volatile compounds released by plants are airborne cues used by pest species to recognize their hosts. Codling moth (*Cydiapomonella*: Tortricide)is an oligophagous pest of pome fruits such as apple, pear and quince (*Cydonia oblonga*). Pest attack causes chemical changes in the volatile profile of fruits with a concomitant decline of the fruit quality. In turn, these chemical changes may carry messages to pests indicating the nutritional status of the host. The aim of this work was to evaluate the effect of damage by codling moth on the volatile profile of different varieties of Quince (*Cydonia oblonga*). We performed an experimental design on four varieties of Quince from a collection of the INTA-Pocito (San Juan, Argentina) at spring 2013. At the beginning of the fruit ripening we covered one branch with fabric bags to prevent the pest infestation. At the end of fruit ripening, protected fruits as well as fruits exposed to wild population of codling moth were sampled. Volatiles were extracted by SPME and

analyzed GC-MS. Up to 13 compounds were identified in the volatile profiles amounting from 90.9 to 98.4%. The different varieties of Quince presented similar chemical profiles. Farnesene and Octanoic acid, ethyl ester, were the main volatile components of the four varieties studied. Damaged fruits presented the same components than healthy fruits and slight quantitative differences were observed. Pear Ester (Ethyl (2E,4Z)-2,4-decadienoate), the volatile of apple and pears recognized as the attractant of codling moth was detected in the volatiles profiles of the four varieties of Quince. Acknowledgments. To FONCyT (PICT 2013-2076), To INTA-Pocito-San Juan. LML, DS are research scientists of CONICET. FJB is a fellow of CONICET.

A137
TEST INDUSTRIAL WASTE USED AS SUPPORT *in vitro* CULTURE TECHNIQUES

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The successful development of *in vitro* plant tissue cultures have been linked to the use of agar media, the support most widely used. However, agar has disadvantages, a major cost and it can inhibit the growth of certain plant tissues. In propagating a commercial scale, one of the biggest challenges is finding alternatives to the various media types that make up the nutrient medium, reagents and supplies used to increase the cost of final production plant. That is why was conducted to determine the feasibility of using sodium polyacrylate (non-degradable synthetic polymer used in diapers) as support for the formulation of nutrient media used in micropropagation of species of agronomic interest. The assay was performed with *Medicago sativa* L. in Murashige and Skoog (MS) solutions. The combined treatment evaluated various concentrations of basal MS media (1.5X, 3X and 4X) with various ratios (P / V) of sodium polyacrylate (1/50, 1/75, 1/100). The combination of MS medium with 4 g of agar and 1/100 sodium polyacrylate, is the one with higher germination and seedling growth. It is important consider further evaluation of these treatments to reach the stage of root development and acclimatization, to complete the cycle of micropropagation.

A138
STATISTICAL OPTIMIZATION OF HYDROLYTIC ENZYMES PRODUCTION BY *Aspergillus kawachii* ON RED GRAPE POMACE IN SOLID STATE FERMENTATION

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The grape pomace is the main by-product of winemaking and the most abundant agro-industrial waste in San Juan, Argentine. Red grape pomace (RGP), in particular, has some undesirable features like a low pH, and antibacterial and phytotoxic phenolic substances. Production of an enzymatic complex appeared as an interesting choice for RGP valorisation.The aim of this work was to found the optimal medium and culture conditions for obtaining an enzymatic complex having cellulase (Cel), endo-polygalacturonase (EPG) and xylanase (Xyl) by *A. kawachii*, in solid-state fermentation (SSF) on RGP. In order to find the relevant variables for the production of Cel, EPG and Xyl, a Plackett-Burman design (PBD) was employed for ten variables (inoculum size, culture time (CT), initial moisture content (IMC), initial pH, agitation, temperature and additions of: glucose, urea (U) and tomato peel).Statistical optimization,for the relevant variables found with PBD(IMC, U and CT), was performed using a Box-Benhken design (BBD)at three levels.Fifteen SSF experiences were done, in Petri dishes, fixing the culture conditions according to the BBD and inoculated with 10^8 spores.g^{-1}. Enzymatic activities were determined by spectrophotometric methods. The optimal conditions for maximum enzyme activities were: 0.61g.$g^{-1}$to IMC, 0.0196g.$g^{-1}$to U, and 30.67hto CT.The responses predicted were: 191.14 U/g for Cel, 174.36U/g for EPG and 349.88 for Xil.These enzyme predictions were experimentally confirmed.

A139
ANTIMICROBIAL ACTIVITY OF *Lactobacillus delbrueckii* ssp.*delbrueckii* AGAINST *Listeria monocytogenes*

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Lactic acid bacteria (LAB) produce antimicrobial substances named bacteriocins. These proteins might be used in food preservation. *Listeria monocytogenes* and *Staphylococcus aureus* are important food borne pathogens and *Enterococcus faecalis* is an indicator for faecal contamination. The aim of this study was to determine the antimicrobial activity against these microorganisms of bacteriocins produced by LAB strains isolated from raw goat milk. The strains were isolated in MRS medium (Man, Rogosa and Sharpe), were biochemically characterized by Gram stain, catalase and oxidase tests, and were typified by API 50 CH test. The antimicrobial activity was determined by the agar diffusion bioassay. LAB strains that showed an inhibitory effect were subjected to liquid-medium method (Cabo et al). Two volumes of cell free culture supernatants (CFCS) were added to one volume of

the indicator suspension and one volume of TSB (Tryptone Soya Broth) and incubated for 6 h. In the blank, CFCS was replaced by the same volume of MRS medium. Twelve LAB strains were isolated and characterized. Only two of them (106 and 110) typified as *Lactobacillus delbrueckii* ssp *delbrueckii*, had antimicrobial activity against *Listeria monocytogenes* with inhibition zone of 25 and 14 mm respectively. The liquid-medium method showed that these strains inhibited 15 and 18% the indicator growth. In conclusion, the growth of *L.monocytogenes* was inhibited by two strains isolated and identified as *Lactobacillus delbrueckii* ssp *delbrueckii* by production of antimicrobial substances which might be used in food biopreservation.

A140
STIMULATION OF THE ANTIMICROBIAL ACTIVITY OF *Lactobacillus delbrueckii* ssp. *lactis1* BY MODIFIED CULTURE MEDIUM

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Lactic acid bacteria (LAB) are part of the large group of saprophytic bacteria which are found in products such as fermented milk, yogurt, aged cheeses, meat products and vegetables. LAB produce bacteriocins which are substances with antimicrobial activity against food borne pathogens. The purpose of this study was to identify a LAB strain isolated from raw goat's milk, and to determine the production of bacteriocins and to stimulate its production by making changes in the composition of the culture medium. The isolate was typified by API 50 CH test. Antimicrobial activity was performed by agar diffusion bioassay by using cell freeculture supernatants (CFCS) of LAB and *Enterococcus faecalis* as indicator microorganism. The proteinaceous nature of the antimicrobial substance was determined by treating the CFCS with pepsin and trypsin and then antimicrobial activity was determined. Stimulation assay was performed by using MRS (Man, Rogosa and Sharpe) medium and MRS modified by adding the following compounds separately: 0.01%tryptophan, 0.01 %lysine, 0.01% vitamin C, 0.03% proline and 1% potassium phosphate. The size of the inhibition zone of CFCS in MRS without modification was 8.5 mm. Antimicrobial activity of CFCS was lost by treating with trypsin that suggests the bacteriocin has a cleavage site for this enzyme. The antimicrobial activity was slightly stimulated by growing LAB in MRS modified with tryptophan, proline and lysine (9.5 mm) while there was no stimulation in MRS media modified with phosphate and vitamin C (8 mm). In conclusion, the LAB strain isolated and characterized as *Lactobacillus delbrueckii* ssp. *lactis1* produces a bacteriocin able to inhibit the growth of *Enterococcus faecalis*. Bacteriocin production was increased by the addition of tryptophan, proline and lysine in culture medium. This increase in bacteriocin production might promote the use of this strain in food preservation.

A141
ENDOESTEROLYTIC ACTIVITY IN PROTEOLYTIC EXTRACT OF *Asclepias curassavica*

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Asclepias curassavica L belongs to a milked plant family called *Asclepiadaceae* whose members produce latex usually containing proteases with pharmaceutical, medical and biotechnological applications. In our study we determinated the esterolytic activity of asclepain by the Silvertein´s method (1974), modified according to optimal conditions of the enzyme in order to select the most appropriated substrate for the synthesis of bioactive peptide in non aqueous media. Asclepain was tested against several N -α – carbobenzoxy –p- nitrophenyl esters of some L-amino acids used as substrates. Assays were made at 40ºC in 0.1 M Tris- HCl buffer (pH=8) containing 20 mM Cys and 0.15, 0.3, 6.25 mM of each substrate in the reaction mixture. Liberation of p-nitrophenol was followed spectrometrically at 405 nm using a Cintra 6 UV-Visible spectrophotometer. To determinate the μmol of p-nitrophenolate produced during the reaction, a standard curve with p- nitrophenol (5-50mM) in 0.1M Tris- HCl buffer (pH8) containing 5% acetonitrile was carried out. This enzyme expressed high activities with all amino acid derivates tested, showing low specificity by basic, polar and not polar amino acids. According to this, asclepain offer a lot of possibilities for the substrate selection in order to carry out the desirable peptide synthesis.

A142
BIOACTIVE PEPTIDES SYNTHESIS FOR THE FOOD INDUSTRY, USING NEW PHYTOPROTEASES

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The synthesis of bioactive peptides using proteases as biocatalysts can be performed with high specificity and reactivity, under mild operating conditions, which is characteristic of enzymatic processes. This produces a strong impact on the process economy and minor consequences for the environment. In our study we applied new proteases isolated from *Asclepias* L. (*Asclepiadaceae*), as a catalyst for the partial or total synthesis of bioactive peptides that could apply as nutraceuticals and preservatives food. The synthesis of an antithrombotic tripeptide was conducted in the medium consisting of ethyl acetate (50%, v/v) - buffer Tris - HCl (pH 8) using crude extract of *asclepain* as biocatalyst and samples were taken for 24 h. After that, the product of the reaction synthesis was separated by RP-HPLC using a Gilson UV-Visible equipment (λ=254) as detector.The product of synthesis was obtained at t_R: 9.4 min, after 5 min of reaction. It was extracted from the organic phase and it´s structure was elucidated by mass spectrometry.

A143
BIOMASS GROWTH DURING SOLID-STATE FERMENTATION OF "ALPERUJO" BY *Aspergillus niger*

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Alperujo (AL) – semisolid agroindustrial waste composed by olive pomace, olive husk and water– contains organic matter including highly contaminant polyphenolic substances (PP), sometimes reported as responsible for the toxicity attributed to the AL. Biological treatments have been proposed for the detoxification of AL. In previous works, the PP depletion in "alperujo" by *Aspergillus niger* (strain M9 belonging to IBT-UNSJ) in solid-state fermentation (SSF) was reported. The aim of this work was to know the relation between *A. niger*growth and the phenolic content depletion. SSF was carried out in a bench-scale drum bioreactor (DBR). The DBR was filled with 10 Kg medium composed by AL 67% + olive husk 33%, humidified to 54.5 % (wet base) and initial pH= 4,5, inoculated at 10^7 spore/g. SSF was carried out at 28 ºC, during 60 days, aerated at 2 l air/min and periodically mixed during 3 minutes at 10 rpm, every 90 min. Samples were taken daily, and PP, water content and glucosamine content (Gluc) were determined. Non-linear regression was used to model the Gluc and PP content. From models obtained, it appears that, at least, PP acts as limiting substrate for fungal growth and product formation (enzyme, etc.). Results are encouraging and the kinetics of ligninolytic enzymes present in this SSF are being studied.

A144
ALTERATIONS CAUSED BY HEAVY METALS IN SOYBEAN PLANTS - COMPLEMENTARY STUDIES OF CULTURED AND FERTILIZED-SOILS

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Soybean (*Glycine max* (L.) Merr.) is an important crop that contains high levels of proteins and oil and plays a key role in contributing to soil fertility through N_2 fixation, which has relevant economic implications. The agricultural activities are partly responsible for the pollution of water and soil caused by cadmium (Cd) and other heavy metals. The use of phosphate fertilizers or pesticides containing traces of these compounds over a long period of time increases its concentration in the environment, and hence its ecotoxicological risk. As part of a study of heavy metal stress on soybean, previous *in-vitro* assays which demonstrated morphological and metabolic disorders in Cd or As treated plants (data not shown), are correlated with complementary field studies.Land with shifting and fertilized cultivation was analyzed at "La Petra" San Luis. Thirteen samples of four pits, each 1 m depth were obtained. Soil physico-chemical properties, essential elements, cations and anions were determined. The results obtained showed that soil owns as average values: hygroscopic moisture 1.44%, pH 7.74, Carbonates (CaCO3) 2.38%, organic matter (OM) 1.032%. Mean values of major anions and cations in g / kg: Sulfates 0.24, Chlorides 3.59, P 1.27, total N 0.048 and trace elements in mg / kg: Fe 0.130 Mn 4.412 and not being able to detect Copper, Zinc, Cadmium and Arsenic with this methodology. We conclude that the analyzed soil is classified as moderately alkaline, mineralized, high in P, with high total N, Fe and Mn with variable values , very low sulfates, variable chlorides, undetected Cu, Zn, Cd and As. These preliminary soil analysis allow an advance in the context of study of heavy metal stress on soybean, since soils do not show detectable levels of heavy metals although they have been subjected more than ten

years after fertilizers and herbicides application. More sensitive studies to corroborate these affirmations are anticipated.

A145
SYNOPSIS OF THE SPECIES OF THE ORCHIDACEAE FAMILY PRESENT IN THE PROVINCE OF SAN LUIS (ARGENTINA)

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Seven terrestrial species of orchids are cited for the province of San Luis: *Sacoila lanceolata* (Aubl.) Garay, *Habenaria gourleiana* Gillies ex Lindl., *Habenaria hexaptera* Lindl., *Pteroglossaspis argentina* Rolfe, *Pachygenium bonariensis* (Lindl.) Szlach, R. Gonzalez & Rutk, *Aa achalensis* Schltr. y *Aa hieronymi* (Cogn.) Schltr. The aim of this work was to study the habitat and to estimate the geographic distribution of species of the *Orchidaceae* family present in San Luis province. The region is characterized by a cold humid climate and rainfalls are concentrated in spring and summer. The species were collected in areas of the mountains of San Luis, the Comechingones and El Morro. These saws are erosional mountainous which are composed of phyllites, granites, metamorphic rocks and andesites. Valleys and plains are also present. We conclude that this family has a distribution characterized by: a) having an optimum development generally over 800 m with overlapping habitats, b) develop on litosolic soils or/and soils with high rock contents, loamy or sandy loamy soil textures, c) found in pastures growing on hillsides, valleys and plains composed of different types of rocksthat are highly vulnerable to trampling and overgrazing by domestic animals.Therefore the obtained potential distribution maps are a valuable tool for further studies.

A146
FORAGING LIVESTOCK MOVEMENT ON A PADDOCK DURING FIVE MONTHS GRAZING PERIOD

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Animals in extensive grazing systems manifest a complex behavior, resulting from the incidence of different biotic and abiotic factors. The distance from water that animals will travel to feed, determines the distribution of cattle in the pasture and consequently the effects produced on vegetation. With the aim to describe the movement of breeding cows on sandy grassland paddock, georeferenced locations of 4 randomly selected adults cows were recorded every 15 minutes, between September 2012 and January 2013. The animals, with stocking rate adjusted to forage production, grazed extensively 150 days on a rectangular paddock of 750 ha, with watering point located in the middle of one of its smaller sides. Animal behavior was described for two half periods, by the distance from the watering point that livestock will travel away (linearly). The 4 cows that carried GPS data logger showed a similar pattern, remaining on average 46% of the days of the first half at distances less than 2000 m. By contrast, during the second half (from December, with higher temperatures) they were concentrated near the water only few days, and most days (59%), they travel to distant areas (> 2500 m), without returning to drink, approximately one third of the days in question.Foraging behavior, in addition to help from paddockssizing, can be helpful to define different strategies for livestock management (e.g. animals rotation).

A147
ECOLOGICAL BIODIVERSITY OF SANDY GRASSLAND PADDOCK, WITH BOVINE GRAZING HISTORY IN CENTRAL REGION OF SAN LUIS

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The central-south region of the province of San Luis is into the sandy grasslands area with “chañar” grove and itis characterized by predominance of sandy soil and perennial summer grasses. In a 730 has paddock at 35 km from SW of Villa Mercedes (San Luis) with single watering point and extensive livestock management history, identification and estimation of aerial coverage of native herbaceous species was performed, previous to livestock grazing. The structure of a plant community was quantified by estimating of the species richness, species dominance (Simpson index: SI) and the degree of evenness among the whole set of species (Equitativity Index: EI). One hundred thirsty sampling quadrants of 1/10 m2 were used on transects located in near (C), medium (M) and away (L) areas from watering point. The vegetative aerial coverage was classified according to species longevity. The three sectors showed similar values of SI, indicators of low diversity (0.20 to 0.27) and average values of EI (0.37 to 0.40). Sector C showed, quantitatively, higher SI (0.27) and therefore less diversity or species dominance. The richness, dominance and equitativity of vegetation in different sectors did not allow a clear differentiation of

these, according to the degree of animal use, while the highest annual coverage in C (25%) compared to L (5%) denotes higher important species richness and significant changes in the composition, associated with degradation of the nearest watering point site.

A148
RELATIONSHIP BETWEEN THE MICRORELIEF AND THE SOILS IN SALINE WETLANDS

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Wetlands are flooded environments periodically assessing the development of hidromorphic soils and of hydrophytic vegetation.The objective of this work was to relate the microrelief with the soil profiles of the different physiognomic vegetation types though studies of a saline wetland. The study area is located to the north of Villa Mercedes (33° 36' S and 65° 26' W, with a height of 515 meters above sea level). The soils were characterized by means of observations "in situ" with the morphological characteristics of the profile, jointly with the depth to the phreatic level and the redox potential of the physiognomic types previously established.The morphology of the profiles is sorted according to the physiognomic vegetation types, from the top of the landscape to the bottom, decreasing the depth of the phreatic level and increasing salinity in surface. In the halophyte scrub soils exhibit the greatest depth to the phreatic level.The prairies halophytes were subdivided into dense and open, with the phreatic level to an intermediate depth, presenting dispersion symptoms. The dense prairies halophytes is located in microrelief more concaves flat where the water lasts longer, causing the presence of algae and presenting dispersion and redoximorphic features.In the scrubland patches of halophyte crawling the water table has a least depth and the profiles are flooded by more time appearing to deeper A horizons and with discoloration. In the saline beach soil remains discovered in the presence of salts in surface in the form of scabs and redoximorphic features. These results confirm that the soils genesis is complex and depends on the microtopography location submit profiles

A149
MORPHOLOGY THE SOILS OF THE SALINE WETLANDS CENTER OF SAN LUIS

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The soils that remain flooded seasonally originate from complex environmental gradients that determine the distribution of the vegetation. Due to the scarce local antecedents on these ecosystems, our objective was to characterize the morphopedologic properties of saline wetlands. The study area is located to the north of Villa Mercedes (33° 37' S and 65° 25' W, with a height of 505 meters above sea level) and comprises approximately 33,5 ha. The soils were characterized jointly by means of observations "in situ" with the morphological characteristics of the profile, jointly with the depth to the phreatic level and the redox potential of the physiognomic vegetation types previously established. Each physiognomic type presented different morphologies as it progresses from the halophytic forest (sector higher) toward the saline beach (area lowest), increasing the surface salinity and decreasing the depth of the phreatic level. This last and redox potential indicate significant differences between the physiognomic types for all months. In the halophyte scrub the redoximorphic features are presented in the form of iron pores coated in the depth where it begins to have influence the phreatic level. In the scrubland patches of halophyte crawling occurs but these same traits in the surface horizon due to the high phreatic level. In the prairies halophytes zones are endowed with discoloration of iron in the horizon A. The saline beaches presented an A horizon discolored of iron. It is concluded that the redoximorphic features,morphopedologic characteristics and studied parameters studied show differences for each physiognomic type change and explain the little genesis of soils in this sector that belongs together with the distribution of the vegetation.

A150
COMBINING WATER QUALITY VARIABLES WITH LANDSAT ETM+ DATA FOR REMOTE SENSING OF CHLOROPHYLL-A CONCENTRATION

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Río Tercero reservoir is considered the largest artificial water reservoir in Córdoba province (Argentina). This multipurpose reservoir has experienced toxin-producing blooms of the cyanobacterium *Microcystis* on a number of occasions over the past decades. Conventional monitoring of lakes and reservoirs is time consuming and expensive. Satellite imagery is another source of information with great potential to be used for the study of water quality variables including chlorophyll-a concentration (Chl-a), among others. Different studies propose reliable

relationships between remote sensing data and Chl-a, however, their procedures rely solely on spectral data and do not consider additional factors that potentially affect Chl-a. In this study, we developed a model to estimate Chl-a of Río Tercero reservoir (Córdoba, Argentina) from Landsat ETM+ data, and we improved model performance by including different water quality parameters to explain variability in Chl-a. Water quality samples were taken coinciding with the passage of ETM+ by the study area. Pearson correlation and backward multiple regression analyses were used to investigate the relationship between Chl-a, ETM+ bands and different water quality parameters. Results suggested that visible ETM+ bands (band 1-3), water surface temperature and water clarity were reliable predictors of Chl-a (R^2=0.84). The procedure presented here could become a low cost measurement tool for water management authorities and decision makers, obtaining simpler and practical results for estimation of Chl-a variable, which is considered a reliable indicator of lake productivity and an ideal metric of water quality.

A151
AN APPROACH TO THE STUDY OF RICHNESS AND ABUNDANCEOF SMALL MAMMALSIN LA FLORIDA, SANLUIS, ARGENTINA

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La Florida is located 50 km to the northeast of San Luis city (33°07′00″S; 66°10′00″O). It corresponds to the Chaco Seco eco-region, with a pronounced seasonal climate, broad range of temperature and rains occurrence during the summer. The small mammals fauna of this region is very diverse, although it has been poorly studied at the local level. The small mammals of the Campsite of the Universidad Nacional de San Luis (UNSL), were studied during 2011, 2013 and 2014. Activities took place as part of a practical experience developed in the subject Population Ecology, for students of the UNSL. Sherman traps were distributed in transects and grids. The capture effort was 300 traps-night (2011 and 2014) and 160 traps-night (in 2013). Individuals were identified, classified by sex and released. Richness, diversity (at the genus level), relative abundance and sex proportions were estimated. The data collected in different environments of the camping were compared, relating the mammal communities to microhabitat descriptions (vegetation cover and soil type). Seven species of small mammals were determined, almost 30% of the total cited for San Luis province. We registered the presence of *Calomyslaucha, C. musculinus,Calomys sp, Graomys griseoflavus, Phyllotis sp*and *Thylamys sp.*, that hadnot been citedfor this locality before. *C. musculinus* was the species found in the greatest proportion (0.58 relative abundance). Possible differences in microhabitat characteristics of sites with and without capture were assessed. However, Student test showed no significant difference in between them. We also designed maps of spacial distribution of species found in the different environments studied. This study is an approach to assessing the state of small mammal communities in San Luis and demonstrates the importance of the study area in terms of local and regional biodiversity.

A152
SURVEY OF CYANOBACTERIA IN AGRICULTURAL-LIVESTOCK FIELD IN THE PROVINCE OF SAN LUIS

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The soils of the province of San Luis are susceptible to degradation due to their low content of organic matter, total nitrogen, structural stability and biological activity. The presence ofedaphic Cyanobacteriais essential to improvethe quality and structureof soils. One groupof them hasthe ability to fixatmospheric nitrogen in aerobic conditionsandtoimprove structural stabilityof soil. The aim of this study was to identify fixing and non-fixing Cyanobacteria in three batches of soybean, corn and natural pasture by means of direct plating in an establishment called Virgen de Luján located 25kmnorth ofthe city ofVillaMercedes(Pedernera Department), along 55 Provincial Route. Each batchwas divided into threeblockswith threesampling stations, and from each one of them compositesoil samples was extracted.Planting was performedin the middle of Watanabeunder controlled conditionsto achieve the growthofCyanobacteria. The results obtained for the2013campaignwere78%, 76% and 60% ofnitrogen fixing Cyanobacteriain the batches sowed with soybean, cornand naturalpasturerespectively. The remaining percentages belong to non fixing Cyanobacteria. These resultsrelate with each other becausenatural pasturehas a higher contentof organic matter andnitrogen, which would explain a decreaseoffixing Cyanobacteria.

A153
USE AND NESTING SITE SELECTION BY *Columba maculosa* AND *Columba livia*, TOWN PIGEONS OF THE SAN JUAN CITY

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We identified the abiotic and biotic features of the environment that explain the use and selection of nesting areas at the mesoscale and landscape levels of two large-sized species of urban pigeons that occur in the desert city of San Juan: the feral *Columba livia*and the native *Patagioenasmaculosa.* To address this question, we collected data along line transects. The main featuresincluded the levels of urbanization, type of buildings and the availability of food and vegetation. The best models that explained the probability of nesting site selection for *C. livia*included: height of buildings, number of strata, type of construction, and type of zone, whereasfor *P. maculosa* the best models included tree height, tree diameter, tree species, distance to the Park and nearest food source. Each pigeon species used different places for nesting. *C. livia*selected mostly urbanized areas and buildings with ledges that pigeons use to build their nests in colonies, whereas the solitary species *P. maculosa*selected areas near large green spaces,using mainly *Morusalba*and*Platanusoccidentalis* trees to build their isolated nests. Control of *C. livia* nests should involve modification of the building structures that allow pigeons to nest. On the other hand, green spaces that contain large trees increase the nesting possibilities for *P. maculosa*.

A154
HYGIENIC STANDARDS OF *Apis mellífera* COLONIES IN APIARIES OF SAN LUIS WITH AND WITHOUT EXCHANGE QUEENS

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The genetic management of a colony of *Apis mellifera* influences its social, hygienic and defensive behavior. This behavior determines the productive profitability of colony. No antecedents for genetic management in relation to defensive-hygienic behavior of the hives in the province ofSan Luis. To achieve the stated objective were selected and evaluated different producers with different management (considering transhumance, multiplication and genetics) at random. Monitoring cleaning performance using skin prick test was performed. The presence of visible symptoms of disease incidence breeding were also analyzed defensive behavior found in the colonies surveyed and producer has been conducted a survey on the genetic management of hives and other information relevant to the study described. Total hives surveyed was 93, only 20% showed high levels of defensive behavior, a means 50% value and 30% had low hygienic behavior. The defense behavior of hives showed a frequency of 17% for class III, more defensive category, and a frequency of 58.5% for class II hives with intermediate aggressiveness. The 55% ofproducers surveyed-respondents did not have to spare queens as systematized management practice their hives. The management of brood diseases did not include the use of antibiotics in 100% of the producers, which is very positive from the point of view of the waste products of the hive and also does not mask symptoms, allowing the elimination of susceptible hives or replacement queens skills without disease resistance breeding. A contribution to knowledge about the handling characteristics, genetics and hygienic behavior of apiaries in the province of San Luis was performed.

A155
WEED SPECIES OF *Malvaceae* FAMILY PRESENT AT THE AGRICULTURAL AND HORTICULTURAL AREA OF VILLA MERCEDES (SAN LUIS, ARGENTINA)

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The *Malvaceae* are distributed from the tropics to temperate zones. Recently, Bayer *et al.* extended the family, and included *Bombacaceae, Sterculiaceae and Tiliaceae*. To date the family under study comprises 35 genera and 198 species, 48 species and one variety are considered endemic to Argentina. In order to respond to inquiries from professionals and producers, the species of the Malvaceae family, that it behave as weeds in agricultural and horticultural area of Villa Mercedes, were studied. Considering the importance of proper identification, plant collections were made in the states of seed, seedling and adult, both in rainfedand irrigated crops in different seasons. The material was identified by traditional botanical methods, then deposited in the herbaria of FICA (VMA) and the EEA INTA San Luis (VMSL). As a result, to date 11 species were determined: *Anoda cristata, Gaya parviflora, Lecanophora heterophylla, Malvastrum coromandelianumMalva parviflora, Malva sylvestris, Pseudoabutilon virgatum,Sphaeralcea bonariensis, Sphaeralcea crispa, Sphaeralcea miniata* and *Sphaeralcea laciniata.* An illustrated key, based on systematic characters, to facilitate easy use both field and laboratory, was also developed.

A156
UNSUPERVISED PATTERN-RECOGNITION TECHNIQUES TO INVESTIGATE MICROBIOLOGICAL AND PHYSICOCHEMICAL VARIATIONS IN CORRIENTE RIVER

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The aim of this study was to obtain a deeper understanding of temporal trends and sources of water pollution in different functional zones by using unsupervised pattern recognition techniques with a case study of the Corriente River, Argentina. Microbiological and physicochemical measurement data were obtained from 5 monitoring sites in three categories of functional zones during the period 2012–2014. Microbiological analysis was performed for faecal indicator bacteria (FIB) including *Escherichia coli* (*E. coli*) and *Enterococcus* (ENT). The higher concentrations of FIB determined in the present study were 1.6×10^3 and 2.7×10^3 CFU 100 mL^{-1} for *E. coli* and ENT, respectively. In addition, unsupervised chemometric techniques were applied to the result dataset, namely, Principal component (PCA) and Cluster analyses (CA). The PCA demonstrated that the samples collected in summer shown higher concentrations of FIB strongly correlated with high levels of temperature, ammonium-N, chemical oxygen demand, and electrical conductivity. Finally, some results achieved by the CA were also found by the PCA technique, demonstrating the complementariness of the methods.

A157
RELATIONSHIP BETWEEN ANTHROPIZATION AND DIVERSITY OF PLANTS AND BIRDS IN THE CHORRILLOS RIVER (SAN LUIS, ARGENTINA)

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In recent decades, there has been a rapid expansion of the urban structure of the city of San Luis, which has influenced the decline of the natural areas that protect native biodiversity. The Chorrillos River area has been one of the areas affected by this change. However, there are still well preserved sectors in the riverbank areas that need protection. The aim of this study was to analyse plant communities (PC) and bird diversity (BD) in this area, in relation to anthropization. Phytosociological inventories were conducted in eight sections of the river, according to the Zurich-Montpellier school. With regard to birds, a survey consisting of account per points of a15 to20 m radius was performed. On the other hand, an anthropization index (AI) adapted to the characteristics of the study area was calculated. The relationship between AI and BD, and AI and PC were negative. The lower value of AI was recorded near the Dique Chico, where even a relict forest of tala trees (*Celtis ehrembergiana*) is preserved. This community was structurally more complex, with more BD (86%) and less invaded by exotic plants (10%). In contrast, the hygrophilous community of *Xanthium cavanilliesii* and *Polygonum* spp., only with herbaceous layer, showed lower BD (60%) and high abundanceof exotic plants (70%). The recommendation derived from this analysis is to manage the study area through the creation of an urban protected area to preserve plant and bird diversity and the environmental services they provide to this urban wetland.

A158
NEST BOX USE BY *Troglodytes aedon* IN THE SEMIARID REGION OF SAN LUIS PROVINCE, ARGENTINA

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The pititorra (*Troglodytes aedon*) is the native passerine with greater latitudinal distribution in America. It is a small insectivorous bird (adult body mass: 9-12 grams) that nests in various types of cavities. In Argentina, there are few data about the natural history of this bird species. In the present work we present the preliminary results of a long-term study ofthe breeding biology of *T. aedon*in the semiarid Midwest of Argentina, with emphasis on behavior during nest building, incubation period, parental care, and chicksdevelopment. The study was carried out at 30 km westof San Luis city. This area is located in the ChacoPhytogeographicalProvince. To achieve our goal,twenty nest boxes were located in the study area, with a random arrangement, the distance between boxes varied from 10 to 15 m.The overall nest-box occupancy rate was 55%, and only in 5 cases (25%) we observed nest, eggs and nestlings. The mean clutch sizewas 4 eggs per nest box (ranging from 2 to 5 eggs) and the mean hatching success was 90%. The nest-building and incubation periods averaged 7and 6 days respectively.These results are similar to those found in other nest-box studies of *T. aedon*.

A159
PLANT INVASION AND DESERTIFICATION IN THE SOUTH OF THE MID-WEST WETLANDS OF SAN LUIS (ARGENTINA)

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Degraded areas are most likely to be invaded by exotic plants. Consequently, areas affected by land degradation in arid, semi-arid and sub-wet dried environments, that are desertified, would be susceptible to invasion by plants. In order to assess invasion by plants and the relationship among invader plants and desertification, 25 (10X30 m) phytosociological Inventories were conducted according to the methodology Zurich-Montpelier School and desertification signs were registred, in the south of the Ramsar Site Guanacache, Desaguadero and Bebedero wetlands (Beazley, Desaguadero and Salina del Bebedero). The richness of invasive plants was low (12 taxa). Among invasive plants, Poaceae and Chenopodicaeae were the most represented taxa (42% of all invasive plants); the taxon with the highest coverage was russian thistle (*Salsola kali*), the predominant habit was annual herbs and the principal geographical origin was Eurasian. Sites that showed signs of desertification, such as soil removal, deforestation and overgrazing, were the most invaded. We conclude that desertified areas were more susceptible to invasion by plants than those not desertified and we infer that the low number of invasive plants is a consequence of the strict climatic and soil conditions. However, the effects of this change could be devastating due to the low natural vegetation cover and the characteristics of local invasive plants.

A160
PHYTOREMEDIATION OF ARSENIC WATERS USING *Salvinia minima* AS BIOACCUMULATOR

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Phytoremediation is based on the use of plants to clean polluted environments and although it is in development, it constitutes an interesting strategy, due to the ability of some plant species to absorb, accumulate and tolerate high concentrations of contaminants. The problem of high concentrations of arsenic in freshwater is an issue of worldwide distribution. San Luis, Argentina is not exempt since the superficial and underground waters in the south of the province have high concentrations of this metal. There have been studies that show evidence of arsenic bioaccumulation using an aquatic plant species. The species used is *Salvinia minima* which is an aquatic fern that stays floating over the water and grows at high speed. Experiences were performed in hydroponic systems, using Hoagland hydroponic solution, exposing plants to different concentrations of As. Samples of leaves and roots were extracted at different periods of timeand the total As concentration was measured in different parts of the fern. The bioaccumulation factor was calculated. After 28 days of exposure to concentrations of 5, 10 and 15 ppm of arsenic, found that leaves accumulated higher concentrations ofarsenic. The average absorbed by the roots does not depend on the dose, and they are always lower than averages absorbed and accumulated in leaves, where there is correlation between contact time and absorbed concentration. The calculated bioaccumulation factors show that *Salvinia minima* is a concentrator of arsenic and the results are promising as to achieve an effective treatment of arsenical waters.

A161
IDENTIFICATION AND FREQUENCY OF NATIVE SPECIES WITH APICULTURAL VALUE IN THE ESTABLISHMENT "LA JUANITA" (SAN LUIS)

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The aim of this study was to identify and describe the frequency and periods of the flowering of native species with apicultural value present in the establishment "La Juanita". This establishment is located at 4 km from the capital city of San Luis, on provincial route N° 3, in the department General Pueyrredón. The establishment "La Juanita" has an area of 1083 ha of native forest, where no agricultural activities are performed, except beekeeping. To set the frequency of trees and shrubs species with apicultural value a survey was based on transects, randomly chosen in a lot of native forest. The results showed that tree species most frequently found were Prosopis flexuosa DC. f. flexuosa "algarrobo dulce" and Aspidosperma quebracho-blanco Schltdl. "quebracho blanco", with 25 and 22% respectively and shrub species most frequently were Condallia microphylla Cav. "piquillín", Larrea divaricata Cav. "jarilla" and "Aloysia gratíssima (Gillies & Hook. ex Hook.) Tronc. var. gratissima "usillo", with 20, 15 and 12% respectively. In a smaller percentage Geoffroea decorticans (Gillies ex Hook. & Arn.) Burkart "chañar", Schinus fasciculatus (Griseb.) I.M. Johnst. var. fasciculatus "moradillo" and Ximenia americana L. var. americana "albaricoque" among others. The existing forest has not been intervened, hence the importance of studying the

existing vegetation, which will allow to determine the productive potential of the area and for the beekeeper, predict the behavior and evolution of the hive.

A162
EFFECT OF ACCESSIBILITY TO DRINKING WATER ON THE BEHAVIOR OF GRAZING BREEDING COWS

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The variation solid excrement deposition of breeding cows was determined according to the distance from the watering points. The work was conducted in four productive livestock establishment in San Luis, Argentina. *Digitaria eriantha* deferred pastures in a sandy area (400 ha), sandy grassland seeded with *Eragrostis curvula* and *D. eriantha* (700 ha), only sandy grassland (700 ha) and natural grassland of "bosque bajo de algarrobo" region in the southwest of de province (700 ha) paddock were used. Stratified sampling was performed considering the distance of watering points. The nearest sector was located at about 300-400 m, the medium sector at 1000-1700 m and the furthest at 2200-3000 m from water point, according size of the pasture land. In each sector, three areas of 625 m2 were randomized demarcated. The variation in the number of dungs indicated that in the system with sandy grassland and cultivated species, in which the watering point was bigger and more accessible (lagoon), fewer dungs were found in the nearest and furthest areas (both: 4.10 m2), and more dungs (6.10 m2) in the middle sector indicating a larger animal presence. In the rest of the pastures (with man-made concrete watering point), a gradient of grass utilization was determined according to the distance to the watering point, denoting larger animal presence in the near (variation according to system 0.11 to 1.85 / 10 m2) and less animal presence in the furthest area (range: 0.03-0.43 / 10 m2). Differences in the amount of dungs between systems are due to the intrinsic management characteristics of each one of them.

A163
URBAN TREES INFLUENCE ON SUMMER TEMPERATURE IN VILLA MERCEDES SIDEWALK FLOORING AND STREETS

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Villa Mercedes is in a lowland area which favours extreme temperatures. During the period between 1973 and 2010, 45% of the years the maximum temperature was over 40°C and 59% of the years the annual thermal amplitudes were over 50°C. In summer the flooring of the intersections often explodes. For this reason we intended to assess the influence of five different urban trees species on summer temperature in the sidewalk flooring and streets. The specimen trees are located in the streets running North to South, on the West sidewalk, between Perón and Montevideo streets. The trees were classified into medium and big and their location was at half of the block – to the sun, on the corner – to the sun, at half of the block – in the shade, on the corner – in the shade, on sides of the road and at half of the block – to the sun, on the corner – to the sun, at half of the block – in the shade, on the corner – in the shade on the sidewalk. Also, flooring temperatures at the intersections were taken. The temperature records correspond to January, February and March 2011. Temperature data was collected between two and three in the afternoon. The analysis of the main components was done to interpret the visualization of the relationships between the five-tree species and the thermal variables in an ideal background. Biplot allowed for the variables being used to state that they are interrelated. *Platanus x acerifolia* and *Melia azedarach* proved to have a similar pattern of behaviour that results in the reduction of the flooring temperature.

A164
EFFECTS OF CADMIUM EXPOSURE IN CEREBELLUM – EFFECT OF DIETS AND AGE

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Cadmium (Cd) is a toxic metal and an important environmental contaminant. We studied its effects on the oxidative stress markers and the histoarchitecture of rat cerebelum under different diets. 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water for 60 days. On the other hand, we also measured some oxidative markers in rat fetus´s cerebellum whose mother received Cd during the pregnancy. Cerebellums were isolated and total RNA was obtained with Trizol. Nrf 2, NOX and SOD were determined by RT-PCR. S28 was used as the control. Histological studies were performed in fixed Hematoxilin-Eosin-stained tissues.

In adult rats, nrf2 and SOD decreased in Cd groups (p<0,05) but SOD also showed an increase in Soy-Cd. NOX decreased in Cas-Cd with no differences in Cas groups. The histological studies showed that Cd groups have certain disorganization, especially in the Purkinje cells layer. When we compared the markers with those found in fetuses, we observed that NOX increased significantly in both Cd groups (p>0.05) and that SOD increased only in Soy-Cd (p>0.05). These results suggest that in the fetuses the response to oxidative stress induced by Cd is better than the one found in adult rats, where the antioxidant system is depressed. Further studies are necessary in order to learn the effect of Cd on the fetuses' cerebellum oxidative stress metabolism and architecture.

A165
CYTOTOXIC EFFECT OF A NATURAL SESQUITERPENLACTONE AND DERIVATIVES AGAINST HUMAN TUMOR CELLS LINES

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Cancer is one of the most important causes of death worldwide. Chemotherapy is one of the best options in the treatment of cancer. However, currently used drugs have serious side effects. Sesquiterpenlactones (STLs) have received considerable attention due to their antitumoral activity. In this sense, the aim of the present study was to evaluate the effect of cumanin, a natural STL, and four of its derivatives on three human tumor cell lines. The sylilated derivatives were synthesized using trimethylchlorosilane, dimethylisopropylchlorosilane and terbuthyldiphenylchlorosilane, using imidazol as a catalytic converter. The acetate derivative was synthesized using acetic anhydride and pyridine. The human tumor cell lines were maintained in 25 cm culture flasks in RPMI 1640 medium supplemented with inactivated fetal calf serum and 2mM L-glutamine at 37ºC in a 5% CO_2, 95% humidified air incubator. Chemosensitivity tests were performed using the SRB assay of the NCI. Each agent was tested in triplicates at different dilutions in the range 1-100µm. Cumanin was active with GI50 values between 24 and 32µM, whereas for the most active sylilated and acetate derivative, GI50 values were lower than 5µM. This study demonstrates the importance of natural products in the search of new antitumor agents and suggests that the development of cumanin derivatives could be used for therapeutic application.

A166
BIOCHEMICAL AND MOLECULAR EFFECTS OF DIFFERENT DIETARY PROTEIN ON FETAL BRAIN TO 20 DAYS PREGNANCY

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Cadmium (Cd) is an environmental contaminant known to exert significant neurotoxic effects on both humans and experimental animals. Exposure to Cd during gestation and lactation (the critical periods of neurodevelopment) can induce changes in the offspring central nervous systems antioxidant defense mechanisms. To evaluate the possible protective role of the consumption of soy protein compared to the mechanisms by which Cd exerts its toxicity, 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water during pregnancy period. We determined TBARS, catalase (CAT) and glutation peroxidase (GPx) activity, and nitrite concentration. Fetal tissue total RNA was extracted and RT-PCR was performed using the following primers: MT I; MT II; Nrf-2; NOX-2 and SOD. The concentration of Cd increased in both intoxicated groups ($p < 0.001$). In Soy-Cd group GPx activity; the levels of MDA, nitrite and expression of Nrf-2; SOD and MT I increased ($p < 0.05$, $p < 0.001$, $p < 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.001$). While CAT activity, expression of mRNA NOX-2 and MT II decreased ($p < 0.01$, $p < 0.01$ and $p < 0.001$). It is known, that the presence of cadmium in the tissues induces stress oxidativo. Maternal exposure to Cd in the drinking water during gestation with soy as protein source in the diet, results into significant changes in the activities of antioxidant enzymes in fetal brain.

A167
EFFECTS OF CADMIUM EXPOSURE IN LUNG LIPIDS AND MORPHOLOGY

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Cadmium (Cd) is a toxic metal and an important environmental contaminant. We studied its effects on the lipid profile and the histoarchitecture of rat lung under different diets. 4 lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water for 60 days. Lung homogenates were used for TBA assay, and the levels of lipid peroxidation products - mainly malondialdehyde (MDA) - were determined spectrophotometrically as TBARSs. Lipids were extracted; total cholesterol (TC), triglycerides (TG) and

phospholipids (PL) were determined. Total RNA was isolated with Trizol and cDNA was obtained. Cytidylyltransferase (CT) was determined by PCR. S28 was used as the control.The lungs were fixed, sectioned, stained, and examined for evidence of lung injury. TBARS showed a significant increase (p<0, 01) in Soy-Cd group. PL increased (p<0,002) in Cas-Cd. TG and TC showed no differences. CT expression increased (p<0, 05) in Soy-Cd. Significant morphological changes in lung parenchyma were observed in intoxicated rats when compared to the control group after 2 months of treatment. Morphological changes were less severe in Soy-Cd group. This shows that lung histoarchitecture and PL are altered by Cd, and Soy might confer protection lung against the metal.

A168

GENOTOXIC ACTIVITY OF *Zinnia peruviana* EXTRACT

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Zinnia peruviana (L) (Asteraceae), is used in folk medicine as hepato protective, antiparasitic, antifungal and antibacterial agent. There is limited information about the genotoxicity of the organic extracts obtained from this plant. In an unpublished study of our laboratory, using the Comet assay, *in vitro*, extracts of *Z. peruviana* of 5 and 20 mg/mL produced genotoxic damage at the DNA level. The aim of this work was to determine, *in vivo*, the genotoxicity of *Z. peruviana* organic extract using the cromosome aberrations and micronucleus bioassay in meristems root *Allium cepa*. Ten *A. cepa* seeds were put to germinate in four concentrations of extracts of *Z. peruviana*: 0.1, 1, 5 and 20 mg/mL, for 72 h at 25 °C. Distilled water was used as negative control. The roots obtained were fixed with Carnoy and colored with carmine. Per treatment, about 3000 cells were analyzed. The Mitotic Index (MI) and the percentage of chromosome aberrations (CA) were calculated. The MI negative control was 11.20 % and was decreasing with increasing concentration of the extracts. Significantly, the percentage of CA in treated cells increased in comparing with the negative control. The CA observed were: C-mitosis, multipolar anaphases, lagging chromosomes, fragments, bridges, and micronuclei. At all the concentrations tested, the percentage of occurrence of CA was 12.68 %.MI less than the negative control indicate reduction in cell division of cells exposed. The occurrence of C-mitotic and multipolaranaphaseindicate alterations in the mitotic spindle. The CA as bridges, lagging chromosomes and fragments showsclastogenic effect while the sticky chromosomes, multipolarity and C- mitotic indicate aneugenic effect. At the concentrations studied, these results, in conjunction with the obtained with the Comet assay, confirm the genotoxicity of *Z. peruviana* extract.

A169

ASSESSMENT OF GENOTOXIC EFFECTS PRODUCED BY HYPOXIA AND THERMAL STRESS IN MERISTEMATIC CELLS OF *Allium cepa* GERM ROOTS

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The *Allium cepa* root chromosome aberrations and micronucleus test is a widespread bioassay for genotoxicity assessment. Different protocols of this bioassay employ meristematic tissue obtained from bulbs of *A. cepa*, which are placed in treatment solutions, with continuous aeration. Chromosomal aberrations as C - mitosis, fragments and micronuclei have been observed in meristematic cells obtained from bulbs of *A. cepa* exposed to tap water, under hypoxic conditions. Given the need to assess the genotoxicity of chemicals and plant extracts available in small quantities, a test with germinal roots of *A. cepa* seeds has been developed. The aim of this study was to analyze the genotoxic effect of hypoxia andlow temperature in germ root meristematic cells of *A. cepa*. Seeds of *A. cepa* were placed to germinate in distilled water under three conditions: a) in a BIOESNAKY seed germinator, b) in Petri dish under hypoxia c) in Petri dish with aeration, in all three cases at two temperatures: 19 °C and 25 °C. Roots obtained from each treatment, were fixed, stained, and squashed in order to get mitotic slides. Meristematic cells in all treatments at 19 °C, showed a decrease in the mitotic index (MI) and karyolysis. Cells obtained from roots germinated at 25 °C showed an increase of MI compared with 19 °C and exhibited chromosomal aberrations (AC) as sticky chromosomes, C - mitosis and chromosome loose, among others. From these first experimental resultswe conclude that low temperature stress delays the physiological and biochemical mechanisms involved in the initial stage of germination decreasing the IM and causing karyolysis; also hypoxic conditions cause AC. To perform correctly his genotoxicity bioassay, from seeds, it is necessary to maintaina temperature of 23 °C to 25 °C and optimal oxygen availability. Future studies will assess which is the optimal oxygen availability to avoid false positives.

A170
PROTECTIVE EFFECTS OF POLYPHENOLS AGAINST DNA DAMAGE INDUCED BY OCHRATOXIN A IN MICE

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OchratoxinA (OTA) is one of the most abundant mycotoxins produced by some fungal species of *Aspergillus* and *Penicillium*. This mycotoxin contaminates food and foodstuff and has nephrotoxic, hepatotoxic, immunotoxic, mutagenic and genotoxic properties. Some studies shown that antioxidant compounds extracted from plants were very effective in reducing the toxic effects of mycotoxins. In previous studies, we have demonstrated that the polyphenols luteolin (L), chlorogenic acid (ChlA) and caffeic acid (CafA) counteracted the toxic effects of OTA on Vero cells and rat lymphocytes as well as ChlA decreased the genotoxicity of this mycotoxin on bone marrow cells of mice. The present study aimed to investigate the protective effect of L, ChlA and CafAagainst DNA damage caused by OTA on blood cells of mice. The genotoxic effects of OTA and polyphenols were assayed by the single-cell gel electrophoresis (Comet) assay in Balb/c mice. Animals were inoculated (i.p.) with: 1) PBS as negative control group; 2) methanol or DMSO/PBS as vehicle control group; 3) cyclophosphamide (20 mg/kg bw) as positive control group; 4) OTA (0.85, 1.7 and 3.4 mg/kg bw); 5) polyphenols (2.5, 5 and 10 mg/kg bw). OTA exposure at all doses caused a marked increase in tail moment respect to negative control group ($p<0.0001$ Kruskal-Wallis and Dunns Test). The DNA integrity was not affected by treatment with polyphenols.Tail moment increases observed in OTA-group were significantly reduced (40%) in the animal groups treated with OTA in combination with ChlA and CafA ($p<0.05$). Luteolin did not shown protective effects on OTA-induced DNA damage in blood cells.Given that ROS plays an important role in OTA genotoxicity, we suggest that ChlA and CafA may have acted as effective ROS quenchers.

A171
SPECIES OF THE GENERA *Candida* AND *Cryptococcus* CAN BE INHIBITED WITH 1-TOSYL-1H-BENZO [D] IMIDAZOL-2-AMINE AND METAL COMPLEXES

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Sulfonamide compounds are widely studied for their pharmacological properties. The aim of the present work was the synthesis of new sulfonamides and metal complexes to study their antifungal properties. For this, these synthesized sulfonamides and its complexes were tested for their antifungal activity against eight important pathogenic yeasts strains of clinical relevance of genera *Candida* and *Cryptococcus*. The yeast strains were provided from the Department of Mycology of the National Institute of Infectious Diseases Dr. Carlos G. Malbrán, Argentina. For the assessment of antifungal activity, 200 µL of yeast (2.0×10^6yeast mL^{-1}) of each strain studied were aseptically inoculated into PDA plates. Cavities of 3 mm were made aseptically and were filled with 50 µL of each synthesized sulfonamide. The plates were incubated at 37ºC for 48 h. After incubation, the zones of inhibition were measured.Regarding antifungal activity, synthesized compounds demonstrated effectiveness for inhibiting the assayed yeasts. The maximum zones of inhibition for synthesized sulfonamides (5, 16, 17 and 18) were observed against *Cryptococcus neoformans* ATCC 24067 (18±3, 22±3, 20±3 and 15±1 mm, respectively) compared with the others strains of yeasts and positive controls (voriconazole and fluconazole) assayed in the present study. The current research opens a new way for the synthesis of novel sulfonamides and its metal complexes, the process is easy to scale up for biomedical applications.

A172
PHYTOCHEMICAL COMPOSITION OF *Hydrocotyle bonariensis* "LOCHITA", "REDONDITA DE AGUA", "BERRO MACHO"

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Hydrocotyle (Apiaceae) is a genus consisting annual or perennial herbs, marsh, with creeping underground stems from which the leaves petiolate, peltate or reniform and inflorescences at nodes emerge. Includes several species of medicinal importance being the best known *H. asiatica* L. (= *Centella asiatica* (L.) Urb). In folk medicine, *H. bonariensis* Comm. ex Lam. and *H. ranunculoides* L.f. are used as emetics and combating diseases of the liver, spleen, lung and urinary retention; the leaves are vulnerary and anti-inflammatory. *H. bonariensis*"lochita", "redondita de agua", "berro macho", "paragüitas" is native to warm temperate America and Africa. In the province grows along rivers and ponds and lagoons.This study aimed to analyze the active chemical ingredients in order to elucidate the principles responsible for the bioactivity. The aerial parts (leaves and flowers) were collected in areas surrounding the Paso del Rey, Province of San Luis, dried at 45°C, were crushed and the aqueous extracts

(infusions and decoctions) and ethereal extracts, were made. On the ethereal extract was performed analytical march, and several groups of compounds are detected: antioxidant phenolic compounds like tannins, flavonoids, unsaturated organic acids; basic alkaloids, sterols and circulation enhancing compounds like triterpene saponins, anti-inflammatory fatty acids were detected.It is carrying out the fractionation of the extracts for structural elucidation of majority compoundsby spectroscopic and chromatographic methods.

A173
BIOLOGICAL ACTIVITY OF MONOTERPENES AND SESQUITERPENES PRESENT IN ESSENTIAL OILS AGAINST PHYTOPHAGOUS INSECTS

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Nowadays new restrictions have been imposed by governments all around the world to control and limit the use of synthetic pesticides. Additionally, the extended use of broad-spectrum insecticides has resulted in resistance to a number of common insecticides. As alternatives to conventional pesticides, naturally occurring substances appear to offer several advantages to users. In this context, pesticides developed based on natural products, are advantageous in that they often have novel mechanisms of action and low toxicity to mammals. These include the use of essential oils and/or its major components. In this study we evaluated the toxic and antifeedant activity of α-pinene, α-terpineol, myrcene, limonene, β-caryophyllene and camphene against Spodoptera frugiperda Smith, Rachiplusia nu Guen (Lepidoptera: Noctuidae) and Xanthogaleruca luteola M. (Coleoptera: Chrysomelidae).These experiments were conducted with third-instar S. frugiperda larvae and X. luteola adults. Percent feeding inhibition (%FI) were calculated.For topical application fifth instars of S. frugiperda and R. nu larvae were randomly selected. Acetone solutions of each compound were prepared. Test solutions were topically applied to the ventral surface of the thoracic segments with a Gilson microsyringe (2μl/larvae). Controls were treated with solvent alone. On a contact toxicity bioassay, the compound caused increased mortality was α-pinene in S. frugiperda, and limonene, β-caryophyllene, and α-terpineol in R. nu. Canphene and α-terpineol were the most active as inhibitors of food.

A174
DRUGS PARENTERALLY ADMINISTERED IN A PRIVATE NURSING SERVICE

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Within nursing practices, by which mostly it goes to a Nursing Service are included: to take blood pressure, placement of several types of injections, to do cures and mists, and administer vaccines. The parenteral administration of drugs (PAD), in some cases, is requested because it is really necessary, and in others, only to the quick relief of some types of symptoms or minor health problems. Our objective was to analyze the PAD in a private Nursing Service of the city of San Luis, the route of administration (VA) and prevalent health problems (HP) for which they were requested. An observational, cross-sectional and retrospective study was carried out. The data were collected in an Excel spreadsheet for two months (August and September 2013). Drugs were classified by the ATC system (Anatomical-Therapeutic-Chemical) and health problems by ICD-10 (International Classification of Diseases 10th review). Results (%): Records 779: PAD 76, other practice of nursing 24. Sex F: 51, M: 49 VA: intramuscular 83, intravenous 11, subcutaneous 4, others 2. PAD and HP: J (antimicrobials, 24.5; infections, bronchitis, pharyngitis): ceftriaxone 37; Penicillin 28; Gentamicin 22; other 13; M: (musculoskeletal system, 24) Ibandronate (56.2, osteoporosis); Ketoralac 21.4; Diclofenac 15.3; Glucosamine 5.1; Indomethacin 2 (pain); H (Systemic hormonal18, allergy) Dexamethasone 61.3, Betamethasone 38.7; Fixed Dose Combinations (FDC): Ampicillin+Guaifenesin+Dipyrone 100; Diclofenac+betamethasone+ B12 vitamine 44.2; Piroxicam+pyridoxine+dexamethasone 30.2; Lysine clonixinate+Propinox 25.6. Unwise use of injectables was encountered, considering it involves a greater risk of adverse reactions and a higher cost. On the other hand, has detected an excessive and inappropriate use of antibiotics, corticosteroids, anti-inflammatories, ibandronate, and FDC, that present an unfavorable benefit/risk profile, and that are used in physiological o pathological conditions in which are not justified.

A175
EFFECTS OF *Lithraea molleoides* ON GASTRIC SECRETION IN PYLORUS-LIGATED RATS

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During the past few decades, a widespread search has been launched to identify new anti-ulcer therapies from natural sources. *Lithraea molleoides* (Vell.) Engl. (Anacardiaceae), known popularly as "molle", is used in folk medicine as digestive. Previously, we have demonstrated that *L. molleoides* and metabolites prevent the formation of gastric lesions and have significant antimicrobial properties against *Helicobacter pylori*. In this study, the effects of *L. molleoides* on gastric secretion in acute treatment were investigated. Rats were fasted for 24 hours with access to water ad libitum before pylorus ligation under chloral hydrate anesthesia was carried out. *L. molleoides* (aqueous extract 500 mg/kg, *p.o*.), saline or ranitidine (30 mg/kg) were administered immediately after pylorus ligation. The rats were sacrificed at 4 h after pylorus ligation. The stomachs were removed, the contents were collected and centrifuged. The volume of supernatant was measured and the acid concentration estimated by titration with 0.1N NaOH. When the rats were subjected to pylorus ligation for 4 h, a considerable amount of basal gastric acid secretion was noted (4.30 ± 0.36 ml) in the control group. In the same control group, the titratable acidity was found to be 238.58 ± 20.74 μEq/ml. Ranitidine was used as control and significantly reduced gastric acid secretion and titratable acidity (2.80 ± 0.25 ml and 89.34 ± 25.05 μEq/ml, respectively, $p<0.01$). The values of volume and titratable acid concentration were not modified by treatment with *L. molleoides* (3.21 ± 0.39 ml and 227.75± 15.77 μEq/ml,respectively). In conclusion, the gastroprotective mechanism of *Lithraea molleoides* does not depend on its inhibitory effect of gastric secretion in rats.

A176
INSECTICIDAL EFFECTS OF *Eupatorium buniifolium* HOOK. ET ARN ESSENTIAL OIL AGAINST *Triatoma infestans* KLUG

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The blood-sucking bug *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) is the most important vector of Chagas disease. As part of a program to study the effects of essential oils against insect pests, we have investigated *E. buniifolium* essential oil, against *Triatoma infestans*. The activity of the essential oils has been extensively investigated. These have ovicidal, toxic, repellent and fumigant activities against different insect pests. The essential oil composition was determined by GC-MS. A total of 65 components were identified. The main component was α-pinene (39.13%). Bioassays to determine the insecticidal activity of *E. bunifolium* essential oils consisted of tests for fumigation, and topical application. Test solutions were topically applied to the ventral surface of the thoracic segments with a Gilson microsyringe (2 μl//larvae). For fumigation test, we selected 20 nymphs 4th stage, which placed in 125 ml Erlermeyers whit a plug of cotton and chiffon. A piece of wire in the center by way of hook was placed. A piece of filter paper of 2 cm^2 was taken and a volume of the test solution was applied to obtain a concentration of 30, 50 and 100μl / L of air. Mortality was recorded at 24 and 48 h. In the toxicity test for topical application, the essential oil was more toxic at 50 % of concentration. The fumigant activity, evaluated (percentage of mortality of Abbott) was more effective in all concentrations. These results are good evidence that the toxicity of the evaporated substance is sufficient to knock down and kill all insects, in a period of time as short as 48 h. The fumigant activity *of E. eupatorium* essential oil it could be attributed to main constituent α-pinene, considering that bioactivity of the essential oil depends on the type and nature of the constituents and individual concentration.

A177
EFFECT OF CADMIUM ON SMALL INTESTINE INTEGRITY

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Exposure to cadmium (Cd), toxic metal, has increased today. The use of Cd in industry has resulted in the contamination of water, food and air. The effects of exposure to cadmium in drinking water in subchronic models are not yet studied. Particularly in the intestine that is the first barrier for entry, there is little information about the effect of the metal. Apoptosis is an important defense mechanism of the intestinal mucosa. Previous results from our laboratory showed an increase of Cd, oxidative stress parameters changes and transcription factors related with oxidative stress and apoptotic mechanisms (Nrf-2, TNF-α, PPARα, β and γ) increased in the small intestine of male rats (180g) that received 15 ppm of Cd in the tap water for 2 months. The objective of this study was to determine if Cd administered in the drinking water affects the normal apoptotic mechanism of the intestinal epithelium. For this purpose the degree of intestinal mucosa integrity by measuring the alkaline phosphatase activity, the activation of γ-

glutamyl transferase, as a mechanism of inhibition of premature rupture of membranes in apoptotic cells and the degree of fragmentation of DNA by Diphenylamine method was assessed. Not changes significatives were observed in alkaline phosphatase and γ-glutamyl transferase activity. The degree of fragmentation of DNA showed a significant increased respect to the control ($P<0.05$). These results indicate that the Cd exposure in drinking water would be initiating a process of apoptosis with increase in DNA fragmentation. Enzyme activities would not be affected due to the appropriate functioning of the mechanisms of inhibition of membrane rupture in this model.

A178
MULTIVARIATE ANALYSIS OF THE MINERAL CONTENT OF GLUTEN-FREE SNACKS

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In this work, the mineral concentrations of seventeen elements (Al, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sr and Zn) in commercial gluten-free snacks were determined by using inductively coupled plasma optical emission spectrometry (ICP-OES). Microwave-assisted acid digestion of samples was used to eliminate the organic matrix of samples. The analytical method was validated by linearity, detection limits, precision, and recovery experiments, obtaining satisfactory values in all cases. The multielemental composition results were evaluated using multivariate analysis. Multivariate pattern recognition tools applied to data sets included principal component analysis (PCA) as a visualization method, and hierarchical cluster analysis (HCA) as an unsupervised learning method. In PCA the data matrix is decomposed into scores and loadings matrices. The scores vectors describe the relationship between the samples in the model subspace and the loadings vectors describe the importance of each descriptor within the model. It can represent graphically intersample and intervariable relationships and provides a way to reduce the dimensionality of the data. Similarly to PCA, clustering of samples reveals similarities among the samples while clustering of variables pinpoints intervariable relationships. As a result, PCA demonstrated that the elements that contributed most for the variability inter-samples were: Ca, K, Ni and Na. A trend was observed towards the classification of samples according to the recipe ingredients. In addition, the results obtained by HCA were in a good agreement with PCA results.

A179
PHYTOCHEMICAL, ANTIOXIDANT AND PROTECTIVE EFFECT OF *Aristolochia argentina* (ARISTOLOCHIACEAE) ROOT EXTRACTSAGAINST ETHANOL-INDUCED ULCER IN RATS

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Aristolochia argentina (family Aristolochiaceae) is popular know as "charrúa", "mil hombres". The roots of this plant are used in folk medicine. The *Aristolochia argentina* aqueous (AAAE) and ethanolic extracts (AAEE) were phytochemical studied, *in vitro* tested for their potential antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and *in vivo* evaluated for theirs hability to prevent ethanol-induced gastric ulcer in rats.Plant extract were subjected to phytochemical test using standard methods. Male Wistar rats (200-250g) were employed. We examined the effect of AAAE and AAEE (250 and 500 mg/kg, *p.o.*) on gastric damage induced by oral administration of absolute ethanol (EtOH).A scanner examined the stomachs and the scanned image was analized by using a program developed by National Institute of Health.Phytochemical screening indicated the presence of flavonoids, saponins, tannins, polysaccharides among others compounds in AAAE, while AAEE was rich in flavonoids and alkaloids. Ulcer control group showed extensive lesions of gastric mucosal layer, whereas rats pretreated with carbenoxolone (250 mg/kg, *p.o.*, $p<0.001$vs. EtOH), AAAE and AAEE showed significant reduction in gastric lesions ($p<0.001$ vs. EtOH). The IC_{50} for AAAE and AAEE in the *in vitro*antioxidant studies using DPPH assay were 47,02 and 47,69 µg/ml, respectively. Results of this study showed that pretreatment with AAAE and AAEE provided significant protection against ethanol-induced gastric ulcer. The antiulcerogenic activity of *Aristolochia argentina* might be due, at least in part, to a possible antioxidant effects.

A180
EFFECTS OF CADMIUM EXPOSURE IN SERUM PARAMETERS AND AORTA MORPHOLOGY

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Cadmium (Cd) is a toxic metal and an important environmental contaminant. The goal of this work was to study the effect of chronic exposure to Cadmium in different serum parameters and the histoarchitecture of rat aorta. Male

Wistar rats (21 days of age) were divided into two groups: the exposed group (Cd) which received 15ppm of Cd as $CdCl_2$ in drinking water *ad libitum* for 8 weeks and the control group (Co) which received water without Cd. The rats were killed and aortas and serum were obtained. We measured serum triglycerides, cholesterol and uric acid by spectrophotometric methods.Aortas were fixed, sectioned, stained, and examined for evidence of injury. Serum Triglycerides showed a significant increase in Cd ($p<0.05$) while cholesterol and uric acid did not show differences. Cd serum concentration showed a significant increase ($p<0.05$) while Zn concentration showed a significant decrease in the same group ($p<0.05$). Regarding aortaarchitecture, the aorta intima layer of the control animals was composed of a continuous layer of endothelial cells. In the tunica media, several elastic fibers were seen to be lying parallel to each other, with smooth muscle cells interposed between them. Irregular luminal layers of endothelial cell linings were observed in aortas of Cd-treated animal. In this last group, light microscopy images revealed structural changes in tunica intima cells, exhibiting clearer and bigger cytoplasm than control aortas. Cells of the tunica media in close contact with the intima also showed these morphological alterations.These changes in aorta architecture as well as the increase in serum triglycerides are preliminary data that lead us to further study this model in order to determine if Cd exposition would induce hypertension.

A181
CYTOTOXICITY OF LUTEOLIN, QUERCETIN, CHLOROGENIC AND CAFFEIC ACID PRESENT IN *Achyrocline satureioides* LAM. (DC) ON VERO CELLS

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Achyrocline satureioides is a medicinal plant belonging to the Asteraceae family. Popularly, it is known as "Marcela del campo" and it is widely used as medicinal herb in South America. Several medicinal properties have been attributed to this plant, such as anti-inflammatory, sedative, antioxidant, immunomodulatory and antiviral. Previously, we demonstrated that an aqueous extract of *A. satureioides* showed antiviral activity against an Alphavirus, *Western Equine Encephalitis* virus. Also, we determined the presence of flavonoids as luteolin (L), quercetin (Q) and dicaffeoylquinic acids as chlorogenic (CL) and caffeic (C) acid in the extract of *A. satureioides*. The aim was to determine the cytotoxicity *in vitro* of luteolin, quercetin, chlorogenic and caffeic acid. The cytotoxic concentration 50% (CC_{50}) was determined by Neutral Red Uptake (NRU) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assays. Vero cell monolayers were exposedto increasing concentrations of compounds: Q, CL and C from 5 at 600 µg/mL and L from 5 at 1000µg/mL, and incubated for 48 h at 37ºC. Assays were carriedout in triplicate. Monolayers incubated only with medium were the cellular viabilitycontrols. The CC_{50} values by NRU were >600µg/mL for Q, CL and C, and >1000 µg/mL for L. C was the more toxic, at 600µg/mL it showed 55% of viability in contrast to Q, CL and L which indicated around 85-90% of viability. On the other hand, the mitochondrial test of viability (MTT) showed CC_{50}values of 410µg/mL for C, 690 µg/mL for L and >600µg/mLfor Q and CL. The four compounds showed low toxicity on Vero cells. These results are very relevant to continue in search of antiviral drugs with selectivity of action.

A182
CHEMICALLY MODIFIED EXTRACTS OF *Zinnia peruviana* ANTIOXIDANT AND CYTOTOXIC PROPERTIES

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Plants are potential sources of bioactive molecules. According to the Dictionary of Natural Products, around 84% of the bioactive compounds contain at least a hydroxyl group or an amine group in their structure. Previously, we had investigated the antioxidant properties of *Zinnia peruviana* (L.) L. (Asteraceae), and promising results were obtained. It is well-accepted that the antioxidant activity is markedly influenced by the presence of hydroxyl groups in a chemical structure. In this sense, in the present work we investigate the antioxidant properties and the cytotoxic activity of *Z. peruviana* extracts.*Z. peruviana* "chinita del campo" is a traditional argentinean herb and antimalaric properties have been reported. A series of crude and modified extracts were prepared in order to study the changes in the bioactivities evaluated. Crude extracts were obtained from aerial parts of *Z. peruviana* using different organic solvents at room temperature. Modified extracts were prepared by chemical transformations. Acid hydrolysis was performed with HCl 2 M for 30-40 min. at 80º C and sulfonylation reaction was carried out with *p*-toluene sulfonyl chloride and K_2CO3 in refluxing acetone for 24 hs. The changes produced were monitored by TLC. The radical scavenging activity was evaluated using DPPH assay. Chemosensitivity tests were performed using the SRB assay of the NCI. Significant changes in antioxidant properties and cytotoxic bioactivity were observed. These results show how a particular biological activity can be affected by chemical modification of an organic extract, and represent a possible alternative strategy for producing natural product-like libraries from natural products libraries.

A183

SEXUAL DIMORPHISM IN TORTOISE (*CHELONOIDIS CHILENIS*) IN CAPTIVITY, SAN JUAN, ARGENTINA

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Sexual dimorphism in nature is present in a wide range of organims. In general males and females differs not only on reproducttive organs, also in external estructures not directly linked to reproduction.. The main aim of this work is to adquired different morphological variables of local tortoise *Chelonoidis chilensis*. 10 variables has been measured with a calibrator, considering Medem (1976) and Loaiza (2006) method, plus it has been considered 7 more standard variables for reptiles. Of the total individuals measured, 27 were females and 21 were males, in captivity situation. From this variables measured, 14 showed significant differences, for instance, height, width and long of the carapace and femur large, among others; desmonstrating strong sexual dimorphism, being the females larger than males. This strong differences in morphological characters associated to sex, could be probably related to defensive strategies or to the species survival.

A184

SEASONAL VARIATION OF FIELD BODY TEMPERATURE OF *Liolaemus darwini* AND *Liolaemus acostai*: A BIOLOGICAL PARAMETER WITH ASTRONOMICAL O CLIMATE CRITERIA?

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The weather seasons can be defined according to numerous criteria such as mean daily temperature, mean minimum and maximum temperatures, cloudiness, frost frequency, etc; these variables take into account the criteria of weather seasons. The aim of this study was to perform a comparative analysis of body temperature of *Liolaemus acostai* and *L. darwini* with astronomical and climate seasons criteria to determine whether the change of these points of view generates significant changes in the results. Data from body temperature (Tb) of *L. acostai* and *L. darwini* were used, both populations from the province of San Juan, Argentina. The data were taken over an entire annual cycle and grouped according to the astronomical criteria (winter, spring, summer and fall with three months per season) and climate criteria according Poblete (2006). *L. acostai* and *L. darwini* Tb showed variations in climate criteria only. It is suggested that the climate criteria best represents climate variations that affect certain biological parameters such as body temperature.

A185

EFFECT OF HYPOTHYROIDISM ON THE MOLECULAR MECHANISM OF MILK EJECTION OF MAMMARY GLAND DURING LACTATION IN THE RAT

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During lactation the mammary gland function involves synthesis, storage and ejection of milk to the offspring. Previous results of our group showed that in the rat, hypothyroidism (hypoT) decreases milk ejection and oxytocin secretion in response to suckling, generating milk stasis and stunted offspring growth. It has been shown that oxytocin receptor (OXTR) transcription is activated by estrogen receptors (ER) mediated ER binding on ERE elements on oxtr promoter. To elucidate the mechanism by which hypoT alters mammary function we studied its effect on day 2, day 7 and day 14 of lactation (L2, L7 and L14 respectively)on serum estradiol (E2) levels by radioimmunoassay and the mammary gland ability to respond to E2by estrogen receptors α and β expression (ERα and ERβ) measured by western blot and the nuclear receptor co-regulators: nuclearco-repressor 1,nuclear co-repressor 2 (ncor1 and ncor2), nuclear co-activator 1,nuclear co-activator 2 (ncoa1 and ncoa2) and oxtrby real time PCR. Our results show that hypoT decreased estradiol serum levels throughout lactation. Also hypoT decreased mammary ERβ, ncoa1, ncoa2andoxtr expression and increased mammary ncor1 expression altering mammary gland response to$E2(p< 0.05)$. The decrease in E2 and ERbeta signaling may cause decrease oxtr expression that in turn, may explain the decrease in milk ejection in response to suckling.

A186
PARICALCITOL REVERSED MYOCARDIAL INJURIES INDUCED BY DEFICIENCY OF VITAMIN D RECEPTORS IN HEARTS

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Cardiovascular diseases are often associated with chronic kidney disorders. Myocardial vitamin D receptors (VDRs) could be a link between them. Paricalcitol (vitamin D receptor activator, Pari) protects against some of those complications, but its mechanism has not been study yet. We try to determine if obstructive nephropathy (ON) linked to VDRs deficiency induce changes in structure or electrophysiological properties (EP) and/or in the incidence of ventricular arrhythmias. Rats were underwent to ON or sham operation. Both were treated either Pari or vehicle. In some hearts we evaluated molecular and structural changes. In the other hand, in isolated hearts submitted to regional ischemia and reperfusion, EP were evaluated. ON showed a reduction in VDRs, an increase in angiotensin II type 1 receptor, fibrosis, myofibrils reduction and an increase in mitochondrial size. All these changes were reversed by Pari. Also, Pari reduced the incidence and duration of ventricular fibrillation during reperfusion, meanwhile vehicle-treated hearts maintained high incidence. Pari lengthened the action potential duration in both treated groups. Amplitude and resting potential were very similar in all groups. We concluded that the reduction in VDRs might be associated to myocardial remodeling and increasing arrhythmogenesis. Pari protects against these changes by restoring myocardial VDRs and prolonging action potential duration.

A187
EFFECT OF AGING ON THE CIRCADIAN PATTERNS OF ANTIOXIDANT ENZYMES IN HEART

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Age is a critical component of the cardiovascular disease etiology, and oxidative stress is a key element responsible for the development of age-related pathologies. This study aimed to find out whether endogenous rhythms of catalase (CAT) and glutathione peroxidase (GPx) expression and activity, as well as Nrf2 expression and GSH levels are modified in the heart of aged rat. Holtzman rats from young (3-months old) and aged (22-months old) groups were maintained under 12h-dark:12h-dark (constant darkness) conditions, during 15 days before the experiment. Nrf2, CAT and GPx mRNA expression and enzymatic activity were determined by RT-PCR and kinetic assays, respectively, in heart isolated every 4 h during a 24h period. GSH levels were measured by colorimetric assay. Temporal patterns were analyzed by the Chronos-fit software. We observed Nrf2, CAT and GPx expression vary significantly in a 24h period under constant darkness conditions (from Chronos-fit: p=0,044, p=0.013, p=0.0047, respectively), with maximal mRNA levels occurring at circadian time (CT) 13:55±00:60, 12:07±00:24, and 12:40±00:00, respectively in the heart of young rats. As expected, circadian rhythms of CAT and GPx enzymatic activity were also observed in this tissue (from Chronos-fit: p=0.0012 and p=0.036, respectively) with their rhythm's acrophases at CT 16:21±00:50, and 21:26±00:36, respectively. We also observed temporal variation of GSH levels (p=0.0042) being maximal at CT 12:19±00:20. Interestingly, aging abolishes the oscillation of endogenous circadian patterns of Nrf2, CAT and GPx mRNA levels, antioxidant enzymes activity, and GSH levels. Understanding the age-related loss of circadian rhythmicity of antioxidant defenses in heart, could lead to advancements into preventive and chronotherapeutic treatment of cardiovascular diseases.

A188
INFLUENCE OF DAILY CONSUMPTION OF VEGETABLE OILS ON LIPID PROFILE IN TYPE 2 DIABETIC PATIENTS

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Fats are calorie-laden lipids that provides 9 calories/gram. Cells use fatty acids and glycerol as a source of energy. Excess of fat is stored and deposited under the skin causing weight gain and later obesity. There are two types of fat: saturated and unsaturated. Saturated fats are found in animal products, they cause increase of cholesterol and triglycerides blood levels, increasing the risk of arterial vascular diseases. On the other hand daily consumption of unsaturated fats from vegetable prevent vascular pathologies. The objective was to know the daily consumption of vegetable oils in type 2 diabetes patients and its influence on total cholesterol (TC) and triglycerides (TG) blood levels. 40 adult type 2 Diabetes patients, with a diagnosis of five years or more, were interviewed. The reported data are referred to the last 3 months. 90% of interviewed does not consume at least 3 servings of crude vegetable oils per day (wo), being the minimum recommendation, and the remaining 10% consume (w), choosing firstly sunflower oil, corn and finally olive oil. Plasma levels of TG and CT showed significant differences between both

groups (wo vs w), TG: 254.5 ± 37.5 mg/dl vs 173.1± 26.3 mg/dl ($p < 0.01$) and TC: 222.8± 28.6 mg/dl vs 176.5± 20.3 mg/dl ($p < 0.004$). None of the interviewed referred to consume: olives, alligator pear and nuts. We can conclude that consumption of vegetable oils would improve the lipid parameters studied in diabetes type 2 patients, which might reduce the risk of cardiovascular disease.

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