



## **CUYO BIOLOGY SOCIETY**

**Abstracts from the XXIII Annual Scientific Meeting**

## **ARGENTINEAN MICROSCOPY SOCIETY (SAMIC)**

**III Annual Meeting**

**December 15 - 17, 2005**

**Gran Hotel Uspallata**

**MENDOZA - ARGENTINA**

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### 1. IMMUNOHISTOCHEMICAL STUDY OF FOLLICULO-STELLATE CELLS IN THE ADENOHYPOPHYSIS OF VISCACHA (*Lagostomus maximus maximus*)

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The folliculo-stellate cells (FS) of the adenohipophysis were initially described as agranular cells with support functions. Recent studies have shown other functions: phagocytic activity, production of colloid, synthesis of peptides, growth factors, cytokines and nitric oxide. These cells express S-100 and glio fibrillary acidic (GFAP) proteins enabling their morphological study. The aims of this work was to study the FS of viscacha using anti-S-100 and anti-GFAP antisera. The FS are distributed in the pars intermedia (PI) and pars distalis (PD). They are elongated or stellated in shape. Their cytoplasmic processes are extended around follicles, neighbouring endocrine cells and close to blood vessels. During the gonadal activity period the FS (ir-GFAP) are localized mainly in the periphery of the PD and limiting of Rathke's pouch. The immunolabeling is lower in the follicles in PI. In this period the immunolabeling with anti-S-100 is observed in PI and PD. During gonadal regression period a decrease in the staining with anti-GFAP is observed in PI. Thus, these cells express differentially both proteins throughout the year. A correlation between the observed changes in the FS and the photoperiod is possible. The regional distribution observed in PD suggests a functional relation between the FS and the endocrine cells.

### 2. *Staphylococcus* SPECIES IDENTIFICATION, SLIME PRODUCTION AND PRESENCE OF *ica* GENES

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*Staphylococcus* has many components accounting for its pathogenicity, such as extracellular polysaccharide (slime), whose production is encoded by *ica* genes. The purpose of this study was to identify staphylococci species, to evaluate the production of slime and the presence of *icaA* and *icaD* genes. Microbial isolates derived from respiratory secretions and wounds. *S. aureus* was identified by conventional tests and *S. epidermidis* by the method of De Paulis et al. Detection of slime production was performed with Congo red agar (CRA). Slime-producing strains appeared as black (B) and Bordeaux almost black (Ba/B) colonies, whereas non-producing strains were red (R). The *ica* genes were determined by PCR. Results are shown in the Table. Twenty-three percent (11/48) *S. aureus* and 27% (12/44) *S. epidermidis* formed slime. There were no significant differences in *ica* genes or CRA positivity among the strains tested. Rapid identification of virulent properties of strains responsible for staphylococcal infections is crucial for therapeutic decision-making.

<i>Staphylococcus</i> strains (n)	<i>S. aureus</i> (48)		<i>S. epidermidis</i> (44)	
	CRA test	<i>ica</i> genes	CRA test	<i>ica</i> genes
Wounds (66)	B = 5 Ba/B = 3 R = 32	5 3 1	B = 3 Ba/B = 4 R = 18	3 4 0
Respiratory secretions (26)	B = 2 Ba/B = 1 R = 0	2 1 0	B = 2 Ba/B = 2 R = 14	2 2 0

### 3. STUDY ON THE BIOTRANSFORMATION OF ISOCHROMAN BY FITOPATHOGENIC FUNGI

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We look for organic compounds with novel structure as well as potential antioxidative activity. This time we have chosen to work with fitopathogenic fungi, because of their capability of transforming complex compounds such as terpenoids found in plants. As substrate isochroman (**1**) was chosen since there are few bibliographical data on its biotransformation, and it has a formal structural relationship to many compounds which shows a wide variety of biological activity. The biotransformation was carried out in a two stage protocol. Biotransformation was achieved after 17 days at 25°C. Only one oxidation compound was obtained. It was isolated, purified and analysed by means of spectroscopic methods. This compound was identified as 3-hydroxyisochroman (**2**). This biotransformation was achieved using a strand of *Aspergillus niger* from the Department of Organic Chemistry of the Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Unfortunately, this oxidation product of compound **1** gave a very low yield. Nevertheless, the method is promising to obtain this kind of compounds. The fungus is able to perform regioselective hydroxylation of substrate **1**. Using fitopathogens proves to be a successful approach for the transformation of complex compounds and might lead to the discovery of novel compounds with potential biological activity.

### 4. PERCENT BACTERIOSTATIC EFFICIENCY (Pbe) OF FLAVONOID ANTIMICROBIAL COMBINATIONS

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Synergistic combinations of flavanones (5,7,4'-trihydroxy-flavanone (I); 5,7,3',4'-tetrahydroxy-6-methoxy-flavanone (II)) with flavones (3,5,7,3',4'-pentahydroxyflavone (III); 5,7,3',4'-tetrahydroxy-3-O-rutinosyl-flavone (IV)) against *Staphylococcus aureus* ATCC 25 923 and *Escherichia coli* ATCC 25 922 were evaluated. Growth kinetic experiments were performed in a Rosi 1000 incubator with constant stirring (35°C, 180 rpm). Aliquots were extracted at 20-min intervals during 5 h, recording transmittance measures (T) at 720 nm. The combinations assayed were: \* I variable - III constant and II variable - IV constant against *E. coli*; \* I variable - III constant and I variable - IV constant against *S. aureus*. Specific growth rate values were obtained for different drug concentrations for each flavonoid combination from the curve of  $\ln N_t$  vs.  $t$  in the exponential growth phase, and the minimal inhibitory concentrations (MIC) were determined. To facilitate the comparison of inhibitory activity of the combinations assayed, it was appropriate to define drug Percent bacteriostatic efficiency as  $Pbe = 100/MIC$ , where 100 is an arbitrary factor. A comparison of combination Pbe values with individual compound Pbe showed that the combination I-III ( $Pbe\ I-III = 3.39 \gg \gg Pbe\ I (1.48) + Pbe\ III (1.29) = 2.77$ ) showed a clear synergism against *E. coli* and an evident antagonism against *S. aureus* ( $Pbe\ I-III = 1.58 \ll \ll Pbe\ I (0.936) + Pbe\ III (2.96) = 3.90$ ), the other combinations assayed being indifferent against both bacteria.

**5. CHRONIC EXPOSURE TO CADMIUM AT 100 ppm INDUCES PROSTATE APOPTOSIS**

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Cadmium (Cd) is a toxic metal, widely distributed in the environment. We have previously shown that, after 3 months of oral exposure, Cd induces oxidative stress and involution in rat prostate. The present aims were to study the expression of apoptosis markers and to look for signs of cell death in the prostate. Male Wistar rats were divided into a Cd group, which received 100 ppm of Cd in drinking water for 12 weeks, and a control group which received Cd-free water. A portion of the prostate was processed by conventional electron microscope (EM) techniques and total RNA was isolated using the Trizol method. RNA was transcribed to cDNA using random hexamers and RT-MMLV (Moloney Murine Leukemia Virus). PCR amplification using specific primers for Bax, SMAD3 and Clusterine was performed. Beta-actin served as an internal control. The EM study showed nuclear changes and abnormal cytoplasm in prostate exposed to Cd, compatible with apoptosis. On the other hand, the expression of the gene that codifies for Bax, a pro-apoptotic protein, increased as well as SMAD3 (a protein that participates actively in the apoptotic process), while the expression of clusterine, an earlier marker of apoptosis, decreased. These results suggest that after a 3-month Cd exposure, the prostate suffers apoptotic events which have been initiated earlier in the treatment.

**6. INTRACELLULAR TRAFFIC OF *Brucella abortus* S19 AND RB51 IN MACROPHAGES**

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Brucellosis is a zoonosis with worldwide distribution. *Brucella* is a facultative intracellular bacterium able to infect its hosts through skin and mucosae. *Brucella* intracellular survival within macrophages is particularly important. The maturation process of phagosomes containing *B. abortus* of diverse virulence was monitored in macrophages belonging to the J774 cell line. The intracellular transport of the virulent strain 2308 and attenuated vaccinal strains S19 (smooth) and RB51 (rough) through the endocytic pathway of macrophages was studied. Macrophage lysosomes were marked with 60-nm colloidal gold particles (CGP) through incubation for 3 h at 37°C. Afterwards the strains were internalized and macrophages were incubated at 37°C for 2 h and 18 h. Early endosomes were marked by exposing them to 20-nm CGP for 15 min before fixation. Transmission electron microscopy allowed monitoring of lysosomal and endosomal markers. Phagosomes containing 60-nm CGP, 20-nm CGP or no CGP were counted. After 2-h incubation, 60 to 70% of bacteria were inside lysosomes. Only 10% of virulent *B. abortus*, but a significantly higher fraction of vaccinal strains (50-60%), were in endosomes. After 18-h incubation, 95% of attenuated strain bacteria were in phagolysosomes. These results clearly indicate that vaccinal strains are located only in phagolysosomes which interact with *de novo* formed endosomes, and are absent from modified phagosomes. The RB51 strain always locates within large phagosomes and shows signs of marked digestion in early periods.

**7. DIFFERENTIAL BIOACCUMULATION OF METALS IN THE AMPULLARIID SNAIL *Pomacea canaliculata* AND ITS MIDGUT GLAND ENDOCYTOBIONT**

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Two morphs of a bacterial endocytobiont (identified as C and K corpuscles) are found within cells of the midgut gland in *P. canaliculata*. Since previous evidence suggested that the K morph was able to accumulate iron, preliminary observations were launched to measure toxic metals concentrations in both isolated morphs, as well as in samples of the midgut gland, the posterior kidney and the foot of cultured snails. Neutron activation technology was used to determine metals. We were surprised by the ability of both the C and the K morphs to accumulate uranium (in concentrations about 300 and 80 times that of aquarium water, respectively). Uranium levels were also found in the midgut gland (which includes the endocytobiont), and were much lower in the posterior kidney and foot of laboratory cultured snails. Mercury accumulation also occurred in the endocytobionts (about 100 and 90 times above aquarium water for C and K morphs, respectively) but it was also significantly accumulated in the midgut gland and the posterior kidney (about 75 and 100 times above aquarium water, respectively). Arsenic was also accumulated, and predominated in both C corpuscles and the midgut gland (nearly 180 times above aquarium water). These preliminary observations indicate that this freshwater snail and its endocytobiont may be used as bioindicators of metal pollution in limnic environments.

**8. LYMNÆID MOLLUSCS BIOTIC AND ABIOTIC DISTRIBUTION IN ENDEMIC ZONES OF FASCIOLOSIS, RENGO AND COIHUECO SECTOR, CHILE**

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The epidemiological interest of fasciolosis in South America has increased in recent years. Highly human endemic areas, that may not be necessarily correlated to areas of animal prevalence, have been reported. The distribution of the disease seems to be more related to the hydro-geographic and climatic conditions as well as to the presence of its intermediate host. In this study, a biotic and abiotic analysis of the distribution zones of lymnaeid molluscs from the collection sectors of Rengo (34.3744S; 70.8577W) 600 m ASL and Coihueco (36.5494S; 71.7672W) 800 m ASL, was performed. Every 21 days, during spring-summer, 4 similar biotopes were sampled for each sector under study, 2 with presence of lymnaeids and two without. The following parameters were evaluated: water (temperature, pH, conductivity, chloride and hardness), soil (pH, organic matter, N, P, K, Na, Ca and Mg concentrations) and botanical (grasses and legumes). There were no significant multiple differences between the two sectors, neither between biotopes nor between biotopes with and without lymnaeids. The importance of complementary ecological and bio-molecular studies for the ecological characterization of the lymnaeids present in Chile is evident, especially for the epidemiological description of a disease incorporating a high number of hosts, currently recognized to be present in a broad variety of climates, locations, conditions, and colonized biotopes.

**9. THE COMMISSION TO WHICH ANATOMY II STUDENTS ARE ASSIGNED DOES NOT AFFECT THEIR FINAL EXAMINATION MARKS**

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First-year students of Veterinarian Sciences of the University of Buenos Aires are assigned to one of six groups or commissions, which work two days per week (Monday and Tuesday or Thursday and Friday), at different hours (morning, afternoon or evening). Each commission has a different professor, who must comply with general guidelines but has freedom regarding teaching style and methods. We assessed whether different commissions show evidence of homogeneous learning proficiency. Our null hypothesis was that the average of correct answers in final examinations are not significantly different among commissions. The marks of tests taken in 2001, 2002, and 2003 were analyzed using an ANOVA test for a fixed factor. The F value obtained was 0.9957 ( $p = 0.4230$ ). Since there was no significant difference, the null hypothesis is not rejected. We conclude that learning objectives are similarly reached despite the differences in teaching style and methods among the professors in charge of each commission.

**10. THE SCAPULAR DORSAL, A FORGOTTEN NERVE?**

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The scapular dorsal nerve is a well-known structure, which formerly was frequently referred to in human medical literature (specially anatomical reports). A specific disorder known as *trapping of scapular dorsal nerve* has been described. In this specimen, it is a part of cervical (brachial) complex (brachial), where it stems from the ventral branches of spinal nerves C3 y C4. It is therefore a wholly motor nerve, which supplies the rhomboid and angular (elevator) muscles of shoulder blades. Our work included different aspects of the scapular dorsal nerve: 1) A comparison with literature from the nineteenth and twentieth centuries describing the nerve itinerary, relations and structure; 2) whether it innervates rhomboid and serrate ventral cervical muscles and we should regard these as hyposomatic, and 3) if these muscles are hyposomatic, the layer they form has laterally the branchiomic musculature—derived from branchial arches and innervated by cranial nerves—while those located medially are episomatic, innervated by spinal nerves. We conclude that this nerve, forgotten in the recent literature, becomes very important because it innervates a transition group of muscles between branchiomic and episomatic muscles.

**11. PHYSIOGNOMIC TYPES OF VEGETATION AND SOILS OF THE “BAJO LAS SALADAS”(SAN LUIS)**

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Combining photointerpretation and field procedures, the physiognomic types of vegetation was determined. Their relationships with the soils are reported here. The “Bajo las saladas” is located to 33° 37' of South latitude and 65° 25' of West longitude, at 505 m ASL; its area is about 200 ha. Physiognomically it includes the “monte halófilo”, “arbustal halófilo alto”, “arbustal halófilo bajo”, “pradera halófila” and “playa salina”. The “monte halófilo” with woody species, mainly “chañar” (*Geoffroea decorticans*). The “arbustal halófilo alto”, is constituted by “zampa” (*Atriplex undulata*), and other woody and herbaceous species. The “arbustal halófilo bajo”, dominated by “jume” (*Sarcocornia perennis*), succulent species that appears quite sparse. The “pradera halófila” integrated for graminaceous by low grass where it dominates the “pasto salado” (*Distichlis spicata*). The “playa salina”, is the lowest area in the concave depression; it has a salty surface when dry and lacks vegetation. The soils of these sectors were characterized and all them classified as saline-sodic. The superficial salinity increases and the depth to the phreatic mantle decreases from the “monte halófilo” (highest sector) toward the “playa salina” (lowest). The physiognomic types appeared in strips, and the zonation the halophilous vegetation is roughly related to salinity and depth of the phreatic mantle.

**12. GROWTH STUDY OF FOUR SPECIES OF *Atriplex* FOR PLANTINGS PRODUCTION**

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The genre *Atriplex*, with 32 species cited, is widely distributed in Argentina. Its species are an important source of fodder, specially in critic periods. They are also used for re-vegetation of abased areas. We studied the growth of four species of the genre: *Atriplex lampa* (Moq.), *A. crenatifolia* (Chodat & Wilczek), *A. argentina* (Sphegazzini) y *A. nummularia* (Lindl), under greenhouse conditions. The mechanically scarified seeds were planted in 700-cm<sup>2</sup> bags using a 1:1 mixture of sand and peat. The planting were watered with complete solution of Hoagland every 7 days. The design was random, with 6 repetitions. Height and number of leaves was measured monthly during 4 months and the last reading was taken after 6 months. Data were analyzed with MANOVA and Hotelling's test. The analysis showed that the species grew in a different way under the same conditions. The highest growth values were seen on *A. crenatifolia* and *A. lampa*. The lowest growth was seen on *A. argentina*, whereas *A. nummularia* had an intermediate response, showing significant differences with the other 3 species. These results show that, for the production of plantings followed by transplantation to soil during re-vegetation tasks, *A. crenatifolia* and *A. lampa* grow faster than the other two species.

**13. AFFINITY ADSORBENTS FROM YEAST CELLS AND THEIR APPLICATION TO LYSOZYME PURIFICATION BY AFFINITY FILTRATION METHOD**

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Affinity adsorbents from yeast cells modified by chemicals and with the Cibacron blue 3GA ligand molecule immobilized to the wall cell by covalent bond were prepared. Nucleotide-dependent enzymes bind most avidly to dye-ligands, as Cibacron blue, due to a superficial resemblance between the dyes and the nucleotides. These particles or macroligands of affinity were characterized by determination of adsorption capacity, binding constant and selectivity using egg white lysozyme as a target protein. Results indicate that the macroligands obtained have selective and reversible adsorption with lysozyme ( $K_d: 10^{-5} - 10^{-6}$  M); high adsorption capacity for lysozyme and low, non-specific interactions with other proteins of the sample. The affinity particles have an appropriate size (mean diameter 5  $\mu$ m), and chemical/mechanical stability. These characteristics of the macroligands are required to be used in affinity filtration. From these results, an application model of the macroligands to egg white lysozyme purification at a larger scale process was designed. The steps, operational variables, membrane and equipment that take part in affinity filtration technology was analyzed, showing that the choice of macroligand is of upmost importance in the purification process. The use of heat-killed yeast cells (without dye) as affinity macroligands to concavalin A purification by affinity ultrafiltration are described in the literature.

**14. ORIENTATION OF THE LEAVES OF *Larrea cuneifolia* UNDER LABORATORY CONDITIONS**

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*Larrea cuneifolia* is an endemic species of the shrub-land of the Argentinean west. The phenolic nordihydroguararetic acid component of *Larrea*, is applied as an antioxidant in foods and pharmaceuticals, lubricant products and rubber. Additionally, it has fungicidal and tumor-inhibiting properties. *L. cuneifolia* leaves are oriented from east to west; this orientation, called paraheliotropic, is associated to the increase in light intensity, high temperatures and drought. The cause of the movement is to avoid the loss of water, for what a stomatic occlusion would be expected to avoid it. The behavior of samples was analyzed *in vitro* and *ex vitro* in environmentally controlled conditions, respecting the photoperiod. The positions of the faces of the leaves were modified, with the objective that they were led from east to west or the whole plant north to south. The measures of the leaves angles and the percentage of the hydric saturation showed that the leaves were not guided by water deficiency as it was expected. The orientation of the leaves was observed during the first days before beginning the water stress.

**15. IDENTIFICATION OF CLAUDIN-2 AND SGLT1 IN *Galea musteloides***

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Several protein complexes govern the absorption of water-soluble solutes from the intestinal lumen into the organism. Claudin-2, a transmembrane protein of the tight junction complex has been reported as a major component involved in the permeability of the paracellular pathway. On the other hand, SGLT1, the Na<sup>+</sup>/Glucose co-transporter located at the brush border membrane of the small intestine, is a relevant component of the glucose uptake through the transcellular pathway. The aim of our study was to identify claudin-2 and SGLT1 in a very precocial rodent, *Galea musteloides*. Animals used in this study were randomly chosen from our own in-captivity colony at UNSL. SDS-PAGE and Western blot analysis were performed in duodenal and jejunal brush border membranes isolated from the small intestine. We also assayed Wistar rat tissues to validate the specificity of the antibodies. Immunoblotting was developed by an ECL chemiluminescence system. Characteristic bands for claudin-2 and SGLT1 were obtained. This technique will provide an useful tool for to study the development of intestinal absorption function in *G. musteloides*.

Supported by CyT-UNSL 22/Q451 grant to ECV.

**16. NORMAL AND PATHOLOGICAL ULTRASTRUCTURE OF DENTAL TISSUES FROM THE COLLECTION OF PAMPA GRANDE (SALTA)**

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Macroscopic and microscopic analyses of dental tissues is important and necessary to identify valuable individual data allowing inferences for reconstruction of a the population life habits. In the present study a register of the disorders found in the collection Pampa Grande (Salta) curated in the Museo de Ciencias Naturales de La Plata was performed by standardized methods. All dental samples were registered by age, sex, teething, maxilar and dental groups, and were observed by stereoscopic microscopy. Some of them were first metallized with gold, then fractured with shear and afterwards observed with scanning electron microscopy (SEM). From 289 teeth studied, the findings were: 45 caries (16%), 6 coronary fractures (2%), 6 hypoplasias (2%) in adults of both sexes. Hard and soft tissues with well-preserved structure were detected by SEM (pulp fossilized sulcated by conspicuous fibrils). The cavities of the caries presented fossilized yeast-shaped microorganisms. No significant morphological differences could be observed in the microscopic structure of the teeth as compared with the fossilized ones. Soft tissue preservation is due to an "instantaneous phosphatization," occurring immediately after death as a result of the presence of calcium phosphate deposits.

**17. CHARACTERIZATION OF POLYPHENOLOXIDASE FROM RED DELICIOUS APPLE**

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Plant polyphenoloxidases (PPO) are responsible for the enzymatic browning reaction occurring during the handling, storage and processing of fruits and vegetables. PPO catalyze the hydroxylation of monophenols which are oxidized to quinines, and subsequently polymerized to brown or black pigments. Polyphenoloxidase was extracted from apple (cv. Red Delicious). Experiments were performed to evaluate kinetics characteristics of polyphenoloxidase. PPO was analysed spectrophotometrically at 25°C and 400 nm using catechol as substrate. One unit of PPO activity was defined as the change in absorbance of 0,001 min<sup>-1</sup>. The optimum pH for enzyme activity was 7 with 50 mmol/L catechol in phosphate buffer. At pH 7 and 25°C, K<sub>M</sub> and V<sub>max</sub> values were 232.8 mM and 5464 U/min.mL E. Optimal temperature was 30°C. Three putative inhibitors were tested. Ascorbic acid was effective at 0.02-0.5 mmol/L. Neither NaCl (5-20 mmol/L) nor benzoic acid (0.1-1mmol/L) showed inhibitory effect. Thermal stability of apple PPO extract was assessed at pH 7, at 6 constant temperatures from 30 to 80°C using various incubation times (5-30 min). At 40°C, 25% of PPO activity was lost after 30 min, whereas at 70°C, more than 90% of the PPO activity was lost after 30 min. After 10 min at 80°C, apple PPO extract was completely inactivated.

**18. PERITUBULAR MYOID CELL MYOSIN FILAMENTS ARE LABILE TO LOW ATP CONCENTRATION**

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Testicular peritubular myoid cells (PMCs), from seminiferous tubules, are contractile cells expressing the cytoskeletal markers of true smooth muscle, such as alpha-isoactin, F-actin, and myosin. We found that a PMCs protein, TMC-myosin, resembling smooth muscle myosin (SMMII), is soluble in the cell cytoplasm. *In vitro*, PMC-myosin assemblies in filaments at 37°C only at low ionic strength. Once a PMC-myosin filament is assembled, the same ionic strength is necessary to disassembly it than for SMMII filaments. This work aimed at analyzing whether PMC-myosin filament can be solubilized by ATP, in the way to infer the behavior of PMC-myosin in PMC cytoplasm. PMC-myosin from rat testis and SMM II from chicken gizzard were purified, assembled in filaments and incubated with different concentrations of ATP (0-32 mmol/L). After incubation, the suspension was centrifuged and the fraction of disassembled myosin was measured. At 0.2 mmol/L ATP, 30 ± 3% of PMC-myosin is released from filaments. At 2 mmol/L ATP, 80 ± 7% of PMC-myosin, but only 0.9 ± 1.0% of SMMII, are released from filaments; SMMII release reached just 20 ± 3% at 32 mmol/L ATP. Thus, PMC-myosin filaments are much more labile to the ATP disassembling effect than SMMII filaments, and this might be the reason why PMC-myosin is soluble in PMC cytoplasm.

**19. SURFACE TENSION AND ELASTICITY IN THE NEONATAL RESPIRATORY DISTRESS SYNDROME**

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The neonatal respiratory distress syndrome (NRDS) is a disorder which ranks first as a cause of neonatal death, and fourth in global mortality rate for any age. NRDS is related to decreased release or deposit of pulmonary surfactant, which is a very complex tensioactive agent lining the alveolar surface of the lungs. Lung surfactant is constituted by 90% of lipids and 10% of proteins. The most abundant lipid is phosphatidylcholine, regarded as the major tensioactive component which promotes alveolar stability by decreasing the surface tension of the alveolar interface. The goal of this work was to simulate the NRDS. A respiratory apparatus simulator was designed and constructed. It allowed to obtain an *in vitro* pressure volume curve for a rat lung. Surface tension was measured by removing the lungs of a rat and filling them alternately with saline solution and air. Different curves were obtained modifying the tensioactive concentration with an organic solvent. From the results obtained it is concluded that saline solution reduces surface tension down to zero, while the curves obtained when surfactant concentration was changed by an organic solvent simulate those expected in NRDS cases.

**20. FATTY ACID COMPOSITION IN MEAT FROM CREOLE AND ANGLONUBIAN KIDS. 1. DESCRIPTIVE RESULTS**

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Meat fatty acid composition from Creole (C) and Anglo Nubian (AN) kids was determined. Twenty kids (BW 10-13 kg) were slaughtered from 4 groups: 5 males AN and 15 C from two different farms and both sexes. Diets: AN: milk and corn grain; 5 male Creole (SIM): mother milk and a calf concentrate; 5 male and 5 female (SIQ) milk and grass. *Longissimus dorsi* (LD) and *semimembranosus* (SM) muscles were dissected from the carcasses and kept at -20°C until the analysis was performed. Intramuscular fat % (IMF), cholesterol content and fatty acid composition were measured. Lipids were extracted using the Folch et al (1957) technique. Fatty acids were determined using capillary gas chromatography with a column coated with CPSil 88 and a temperature program. Saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), conjugated isomers of linoleic acid (CLA), n-3 and n-6 fatty acids were determined. IMF % from both muscles varied between 0.90 and 1.52 %, which are low values compared with other domestic species. Cholesterol content (56-67 mg/100g) was similar to those found in other meats, but it was higher than that found in C kids from other regions (La Rioja: 25-45mg/100g). Analyzed muscles showed the following fatty acid composition: SFA 32-34%; MUFA: 28-35%; PUFA: 15-21%; n-3: 3-3.6%; n-6: 11-15% and CLA: 0.70-1.80%.

**21. FATTY ACID COMPOSITION IN MEAT FROM CREOLE AND ANGLO NUBIAN KIDS. 2. STATISTICAL ANALYSIS**

*Bonvillani A<sup>1</sup>, Freire V<sup>1</sup>, Rossi D<sup>1</sup>, Garcia PT<sup>2</sup>, Casal JJ<sup>3</sup>.*

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Meat fatty acid composition was determined in meat samples from Creole (C) and Anglo Nubian (AN) kids. Twenty kids (BW 10-13 kg) from 4 groups were slaughtered: 5 males AN and 15 C from two different farms and both sexes. Diets: AN: milk and corn grain; 5 male Creole (SIM): mother milk and a calf concentrate; 5 male and 5 female (SIQ) milk and grass. *Longissimus dorsi* (LD) and *semimenbranosus* (SM) muscles were dissected and kept at -20°C until analysis. Intramuscular fat % (IMF), cholesterol content and fatty acid composition was determined. Lipids were extracted using the Folch et al (1957) technique. Fatty acids were determined using capillary gas chromatography. Saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), conjugated isomers of linoleic acid (CLA), n-3 and n-6 fatty acids were determined. PUFA:SFA and n-6:n-3 ratios were in agreement with those required for human consumption. The first ratio should be higher than 0.4, and the second, less than 7. No significant differences (ANOVA) were found for SM muscle in MUFA and n-3 PUFA. Differences in SFA, PUFA n-3 and n-6 and CLA were found in LD muscles. Diet affects significantly n-3 PUFA in both muscles and LD CLA in AN and SIM compared with SIQ. Differences in total PUFA in SM and LD muscles were found between SIM and SIG. AN and SIM present differences in SFA.

**22. MODULATION OF HORMONALLY INDUCED PROLIFERATION BY ARYL HYDROCARBON RECEPTOR LIGANDS IN GRANULOSA CELLS**

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that, besides mediating toxic responses, may have a central role in ovarian physiology. This study aimed at determining the effect of different AHR ligands on granulosa cell (GC) proliferation. Primary cultures of rat GC were treated with increasing doses of the ligands 3-indol-acetic (3IA), indigo, indole-3-carbinol (I3C), tryptophan (TRP), 3-methylcholanthrene (3MC),  $\alpha$ -naphthoflavone ( $\alpha$ NF), or  $\beta$ -naphthoflavone ( $\beta$ NF). Each ligand was added in control conditions, in the presence of FSH, and FSH plus estradiol ( $E_2$ ). Cell proliferation was assessed by [<sup>3</sup>H]-thymidine incorporation and cyclin D2 expression. None of the tested ligands had an effect on proliferation when added alone or in the presence of FSH. However, I3C, TRP and  $\alpha$ -NF dose-dependently inhibited the proliferative response elicited by the combination of FSH and  $E_2$ . However, the AHR agonists 3MC and  $\beta$ NF showed a co-mitogenic effect with FSH and  $E_2$ . The synergism between  $E_2$  and  $\beta$ NF was greater in cells that overexpress the AHR; it was reversed by co-treatment with an AHR antagonist and abolished by a pure antiestrogen. Thus, our results indicate that AHR ligands modulate rat GC proliferation, and show that AHR agonists can amplify the co-mitogenic action of classic hormones through a mechanism that might involve a positive cross-talk between the AHR and the estrogen receptor pathways.

**23. LOCATION, QUANTIFICATION AND ISOLATION OF HIGH THIOL LEVEL SPERM PROTEINS EXPRESSING DURING EPIDIDYMAL TRANSIT**

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During epididymal transit, the sperms undergo several changes, collectively known as sperm maturation. It allows the gamete to achieve capacitation and ability to fertilize the oocyte. One major change affects sperm protein redox status, namely that thiol groups are oxidized to form disulfide bonds. Disulfide bonds are responsible of chromatin condensation and sperm tail structure stabilization. In rats, the epididymal fluid is crucial in this process, due to the redox properties of this milieu. Based in our previous experiences in protein isolation and cellular fractionation, we isolated and localized subcellular structures rich in thiol groups. Sperm cells from epididymal caput and cauda were split in head and tail by 4 bursts of sonication at 30-s intervals. After sonication, no more than 1% of sperms remain intact. Heads and tails were isolated by sucrose gradient (40-80 %). The presence of sperm head in the fraction containing sperm tail was 20%, while the presence of sperm tails in the head fraction was 50%. Both fractions were treated with monobromobimane, a specific fluorescent thiol label. Afterwards, proteins were extracted with sample buffer (Laemmli UK) and analyzed by SDS-PAGE. Some minor differences were observed, mainly in proteins from tail fraction. Further work must be done to improve the isolation method.

**24. ULTRASTRUCTURE OF PITUITARY GLAND OF RATS EXPOSED TO CADMIUM IN THE DRINKING WATER**

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Exposure of adult male rats to 15 ppm of cadmium (Cd) in the drinking water for two weeks, modified the lipids synthesis of pituitary gland. Here, in the same experimental model we assessed whether Cd (II) induces pituitary ultrastructural changes. Anterior pituitary glands were excised and immersed overnight in a fixative solution prepared according to Mollenhauer (1972) and containing 3% glutaraldehyde and 10% v/v saturated picric acid in 0.05 mol/L phosphate buffer (pH 7.2). Then samples were dehydrated through graded ethanol and three changes of absolute acetone. Finally, they were embedded in Epon 812. Thin sections were made with an ultramicrotome and observations were performed in a Zeiss 900 electron microscope using reagents from Pelco (Redding, USA). Thin sections with the classical ultrastructural aspects of anterior pituitary cells were observed. In general, cells were well preserved and their granules and organelles presented the characteristic distribution and electron density of the main types of adenohypophysial cells. The ultrastructure of somatotroph cells from rats exposed to Cd (II) showed partially condensed chromatin and an undulated nuclear membrane. A high number of vacuoles and a confluent membrane system were clearly observed between the characteristic dark granules, compared with the absence of vacuoles in normal cells. Exposure to Cd (II) modifies the ultrastructure of rat anterior pituitary gland.



**25.**  
**HYDROLYTIC CAPACITY OF TREHALASE IN *Bufo arenarum***

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Several studies in endotherms examined how digestive capacity is adapted to different life conditions, such as dietary switches, hibernation or growth. This problem has not received enough attention in ectotherms. Consequently, this work aimed at assessing the enzymatic activity of an intestinal carbohydrase – trehalase - in an insectivore amphibian. This enzyme is important since it breaks down trehalose, a sugar present in insects. In this study, we explored if trehalase activity scales with body size. We captured 30 toads (14 males and 16 females; body weight range: 63.13 to 158.35g) in Río Cuarto (Córdoba) and La Florida (San Luis). We assayed trehalase activity in homogenates of small intestines. Total trehalase activity scaled with body weight ( $p < 0.01$ ). A tissue-specific activity pattern is apparent along the small intestine (proximal > medial > distal,  $p < 0.05$ ); the same profile was also found for other carbohydrases. Thus, during growth the digestive capacity of trehalase increases as a result of the growth of the small intestine, while tissue-specific trehalase activity remains constant. These results suggest that the increment in hydrolytic capacity needed for matching increased energy requirements is provided by enlargement of the intestine.

Supported by a UNSL CyT grant to EC-V.

**26.**  
**FOCAL CHEMICAL HYPOXIA INDUCES NEURONAL DEATH**

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Hypoxia is one of the pathological factors inducing neuronal injury. Cobalt chloride ( $\text{CoCl}_2$ ) is a pharmacological activator of hypoxia-inducible factor-1. Adult male Wistar rats were intracerebrally injected with 2  $\mu\text{L}$  of 50 mmol/L  $\text{CoCl}_2$  in right hemisphere (layer 2-3 of frontoparietal cortex, Bregma  $-1.30\text{mm}$ ) and with 2  $\mu\text{L}$  of saline in the left hemisphere. Animals were killed and their brains fixed at 1 h, 6 h or 5 days after treatment. Both the ipsilateral and contralateral areas were analyzed using haematoxylin-eosin, methylen blue, Hoescht 33342, TUNEL techniques and electron microscopy. Few cells with abnormal morphology were seen 1 h after  $\text{CoCl}_2$ , but a large number were altered after 6 h, and a still larger damage appeared after 5 days. We observed dense neuronal chromatin, nuclear segmentation, disorganized cell distribution and astroglial reaction. These changes were confined to the lesion area, were minimal at the neighbouring area and not apparent in the control side. After 5 days nuclear cleavage, multilamellar vesicles and altered mitochondria were found in the penumbra area. Outside the penumbra limits and in left sites, brain tissue presented normal morphology. The core of the lesion presents a early neuronal death (maximal at 6 h), while the penumbra area shows delayed neuronal death (5 days).

Supported by UBACYT-M072 and PIP 5034 CONICET grants.

**27.**  
**MICROBIOLOGICAL ASSAY OF AZITHROMYCIN**

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Azithromycin is a macrolid antibiotic, which is indicated for patients with infections of the upper and lower respiratory system, skin and soft tissues caused by susceptible microorganisms. A microbiological assessment technique was assessed as an alternative to the official one for quantification of azithromycin (High Performance Liquid Chromatography). The assay used azithromycin (960 mg/g) as a reference, and as a test sample pharmaceutical azithromycin 500 mg pills. The initial solvent was methanol and the final diluent, phosphate buffer 0.1 mmol/L (pH 8). In order to develop the microbiological assessment, the plate diffusion (medium 11 Farmacopea Argentina, 7° Ed) with random block design, and as sensitive test organism *Micrococcus luteus* ATCC 9341, were used. The results from the microbiological assessment technique were statistically validated according to the parallel stretches model. This technique is advantageous not only as a quantification tool for a product or raw material, but also to make evident the actual antimicrobial activity of this macrolid, which depends on its structural integrity.

**28.**  
**EVALUATION OF ANGIOTENSIN CONVERTING ENZYME (ACE) EXPRESSION DURING DEVELOPMENT IN THE RAT HEART**

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Angiotensin-converting enzyme (ACE) is primarily localized in various tissues and organs. Pathologic activation of local ACE can have deleterious effects on the heart, vasculature, and the kidneys. Endothelial dysfunction (hypertension, diabetes mellitus, hypercholesterolemia) disrupts the balance of vasodilatation and vasoconstriction, the inflammatory and oxidative state of the vessel wall, and is associated with activation of somatic ACE (sACE). Thus, variations in the renin-angiotensin system could be crucial in the aetiology of cardiovascular diseases. The aim of the present work was to investigate the heart sACE expression during development in rat. sACE expression in heart from Wistar rats was determined at different postnatal stages: PND1, PND15, PND30, PND60. The sACE expression was semi-quantified by multiplex RT-PCR. mRNA was obtained from heart tissue and we set up a procedure for co-amplification of both ACE and GAPDH sequences, by using two sets of primers during the RT step. Heart sACE expression varied significantly during the experimental period with a maximum at PND15, as compared to GAPDH amplification under the same conditions. Thus, we show that in the heart, sACE expression changes during growth suggesting that integrity of the RAS is required for the normal development of the heart.

**29. TUMOR NECROSIS FACTOR RECEPTOR P55 DEFICIENCY AFFECTS THE SEVERITY OF POST-*Yersinia* REACTIVE ARTHRITIS**

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Alpha- tumor necrosis factor ( $\alpha$ -TNF) is associated with protection against *Yersinia*. TNF receptor (TNFR) p55 is the dominant pathway in the majority of known TNF effects. The objective of the present work was to study the role of tumor necrosis factor receptor (TNFR) p55 in the *Yersinia*-associated arthritis by the use of a murine model of oral *Yersinia* infection. TNFRp55 deficient (TNFRp55<sup>-/-</sup>) and C57BL/6 mice were orally infected with 10<sup>7</sup> colony-forming units (CFU) of *Y. enterocolitica*, serotype O:3. Mice were daily observed and arthritis development was monitored by clinical score, joint assessment, x-rays and examination of histopathology of joints. Higher susceptibility to the infection was observed in TNFRp55<sup>-/-</sup> mice. In addition, TNFRp55<sup>-/-</sup> mice developed arthritis since day 14 postinfection (p.i.) with severe inflammation and tarsus deformation on day 55 p.i. Erosion of cartilage and bone was observed in knockout mice on day 39 p.i. Differences in the local and systemic expression of cytokine mRNA was found in TNFRp55<sup>-/-</sup> mice. The results suggest that TNF signaling through TNFRp55 has a protective role in arthritis induced by oral *Yersinia* infection. A better understanding of the host factors predisposing to the disease will help to the development of specific treatments for reactive arthritis.

**30. CONCENTRATION OF TBA-REACTIVE SUBSTANCE IN LUNG OF CASTRATED MALE RAT**

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Oxidative lung damage may be associated to the destruction of alveolar cells. Thiobarbituric acid reactive substances (TBARS) were measured as marker of lipid peroxidation of alveolar epithelial cells (AECs).

**Objective:** To determine if castration changes the concentration of lung TBARS of castrated male rat.

**Methods:** Adult male rats weighed 200-250 gr were separated in two group: control (G1) and castrated (G2). 21 days after castration the rats were sacrificed under a light anesthesia with ether, the trachea was cannulated and the extracellular surfactant extracted with NaCl 0.5M. After that, the lungs were extracted, washed, and homogenized in PBS solution pH7.4. In one aliquot of lung homogenate the concentration of TBARS, through the synthesis of a red adduct between TBA (Thiobarbituric Acid) and MDA (Malonaldehyde) was measured.

**Results:** The results were expressed as nmol/g de tejido; G1:  $1.62 \times 10^{-4} \pm 0.03$  and G2:  $1.2 \times 10^{-4} \pm 0.01$ ,  $p < 0.01$ ,  $n=20$ .

**Conclusion:** We think that castration increases the production of oxidative stress, what would cause the alterations in lung morphology and lung metabolism previously found (Ojeda MS *et al.*, 1998).

**31. ORNAMENTAL VALUE OF NATIVE CLIMBING PLANTS IN SAN LUIS PROVINCE, ARGENTINA**

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In our country there is a general tendency to undervalue native flora due, in most cases, to lack of knowledge. The use of native plants for ornamental purposes is relatively new, as most parks and gardens are made using exotic species. Textures, colors, forms and fragrances are some of the outstanding qualities native plants offer. The goal of this work is to promote scientifically native climbing plants with ornamental value from Argentina by characterizing and evaluating them. Their use by the community will help promote our patrimony. Different species of native climbing plants from natural areas and cultivated in parks and gardens in San Luis province, were photographed, collected, herborized, and identified. Phenologic registers were studied and the morphologic descriptions were made with fresh and herborized material. The scientific name, common noun, botanical family, synonymous, morphologic description considering the most excellent ornamental characters, geographic distribution, phenology, photographic registry and uses for each species are indicated in this work. 32 species belonging to 17 families are described.

**32. VIABILITY OF HUMAN COLONIC EPITHELIUM IN VITRO**

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Despite its physiological and clinical importance, the energy demands of ion transport by human colonic epithelium have not been adequately characterized *in vitro*. Of course, this kind of study demands, first of all, obtaining viable preparations of human tissues. We therefore secured samples of human colon from surgical procedures, which were transported in a modified Ringer solution (previously gassed with O<sub>2</sub>) at 2-3°C and mounted, within 30 min after excision, in a chamber specially designed for microdissection. Histologically checked mucosal samples were obtained and mounted in a water-jacketed (37°C) Ussing chamber stirred with a gas lift. Out of 12 samples, 8 were successfully mounted and found to remain functionally stable for 5 h. Means  $\pm$  SEM of electrical measurements were: transepithelial resistivity  $111.1 \pm 5.9 \Omega \cdot \text{cm}^2$ ; short-circuit current  $57.1 \pm 5.6 \mu\text{A} \cdot \text{cm}^2$ ; and potential difference  $6.9 \pm 0.9 \text{ mV}$ . Values are within the range reported by other authors. In pilot experiments, the response to acute hypoxia, induced by switching the lifting gas from O<sub>2</sub> to N<sub>2</sub>, was found to be qualitatively similar to that previously reported by us for rat colonic epithelium.

**33. ANTIBACTERIAL EFFECT OF *Baccharis salicifolia* AND *Flourensia oolepis* ESSENTIAL OILS ON *Listeria monocytogenes***

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*Listeria monocytogenes* is an important foodborne pathogen due to its wide environmental distribution. Several kinds of food have been implicated as sources of animal and human listeriosis. The objective of this work was to assay the antimicrobial effect of *Baccharis salicifolia* and *Flourensia oolepis* essential oils against fourteen *L. monocytogenes* strains, and their potential application as antimicrobial in foods. We have used ten regional strains isolated from clinical and food samples and four strains (reference strains) were provided by the Institute Pasteur, Paris, France. Each strain was grown in Tryptic Soy broth supplemented with 0.6% yeast extract for 24 h at 37°C. The cultures were appropriately diluted in PBS to obtain an inoculation level of 10<sup>8</sup> CFU/mL. Assays were performed by a standard disc diffusion technique using gentamicin discs (10 µg) and essential oils discs (10 µL). We observed for gentamicin, zones of inhibition from 33.3 ± 2.4 mm to 25.8 ± 2.2 mm, for *F. oolepis* from 16.0 ± 2.4 mm to 8.0 ± 0.0 mm and for *B. salicifolia* from 17.0 ± 0.7 mm to 8.0 ± 0.0 mm. We conclude that both oils have moderate antimicrobial activity against *L. monocytogenes* strains.

**34. BIOLOGICAL AND ENVIRONMENTAL PARAMETERS IN VITRO CALLUS PRODUCTION OF *Prosopis caldenia* BUKART**

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*Prosopis caldenia* Burk. (caldén) is a key species for the planning of strategies of reforestation to short and long term, being of great importance to achieve massive propagation of plants, germ plasm conservation and improvement genetics. The *in vitro* callus formation, through the somatic embryogenesis, is considered an efficient form of clonal multiplication, of great potential in the forest investigation. The objective of this work was to optimize the means of cultivation and to select the type of appropriate explants for the induction of organogenic or embryogenic callus in caldén. The basic Murashige and Skoog medium was used, with sucrose (30 g/L), agar (6.5 g/L), and 2,4-dichlorofenoxyacetic in different concentrations and combinations. The explants were cotyledons (at 30 days from germination), shoots, leaves and roots. The samples were incubated at 25 ± 2°C, half of them under conditions of darkness and the other half with a photoperiod of 12 hours of light. After seven days of culture, the formation of friable callus was observed in the abaxial of the cotyledons, generally of yellow-greenish color, only when samples were cultivated in darkness and half nutritious with 2,4-dichlorofenoxyacetic (5 mg.L<sup>-1</sup>). Under light conditions, the deformation of the cultivated explants was observed.

**35. PRODUCTION OF BOTULINIC TOXIN BY COCULTURES OF *Clostridium argentinense*- *Pseudomonas mendocina*. EFFECT OF GLUCOSE AND DEXTRIN**

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*C. argentinense* in coculture with *P. mendocina* produces enhanced levels of botulinic toxin, which in turn are influenced by the nature of the carbon source. This work aimed at characterizing the metabolic effect by kinetic analysis of cocultures using carbon substrates of fast and slow assimilation. *C. argentinense* G 89 and *P. mendocina* were cocultured in a 800 mL fermenter containing a basal medium of the following composition (g/L): proteose peptone 30, yeast extract 5, trypticase 5, glucose 11 or dextrine 15, cystein chorhydrate 0.5 (pH 7.6). Analytical techniques included biomass by dry-weight, toxin by intraperitoneal mouse injection and glucose by an enzymatic method. The level of botulinic toxin by cocultures were higher with dextrin. Specific toxigenic activity (DL<sub>50</sub>/g) and toxin productivity (DL<sub>50</sub>/h) were respectively 2.5 and 2.86 times higher than with glucose. The kinetic analysis of growth and toxin production indicated that, with dextrine, cocultures developed at slow specific growth rates (µ=0.03 h<sup>-1</sup>), showing a kinetic production typical of secondary metabolites. This specific growth rate was similar to that displayed by cocultures using glucose when the carbon source was totally consumed. It is concluded that complex compounds produced by *P. mendocina* under anaerobic conditions are responsible for the higher toxin levels produced by the cocultures.

**36. A RAPID METHOD FOR BOVINE LACTOFERRIN QUANTIFICATION**

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Bovine mastitis is the most prevalent and costly disease in dairy farms. Due to its bacteriostatic and bactericidal activities bovine milk lactoferrin (BLF) seems to play an important role in non-specific response against mastitis infections. It has been reported that milk BLF concentration may increase upon mammary gland infection. Also, nutraceutical properties of BLF have been demonstrated. The objective of this work was to settle the basis for the development of a rapid and accurate method for measuring BLF concentration in bovine milk. To achieve this goal, we decided to use a competitive enzyme linked immunosorbent assay (cELISA) approach. We purified BLF from bovine milk by ion exchange chromatography. One fraction of the resulting protein was biotinylated and the other was used to generate specific rabbit antiserum. High affinity antibodies were subsequently isolated from this antiserum by affinity chromatography. Optimal concentrations of antibody and biotinylated BLF were empirically determined and BLF calibration curves were generated. Finally, potential application of this method to quantify BLF concentration in milk was evaluated in samples of milk from mastitic and healthy animals. The method presented in this work will allow rapid and simple milk BLF quantification and has potential application in udder health assessment and evaluation of milk nutraceutical properties.

**37. A METHOD TO INCREASE LIFE SPAN OF BOVINE FIBROBLASTS FOR THEIR UTILIZATION IN TRANSGENIC TECHNOLOGY**

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One challenge of manipulating and introducing genes in primary cells is that they have a finite proliferation capacity, which makes identification and expansion of transgenic clones very difficult. This study aimed at establishing a system that allows bovine fetal fibroblasts (BFF) to reach their maximal proliferation capacity *in vitro*. We explored the utilization of a conditioned medium from the bovine granulosa cell line A4. It was found that A4 conditioned medium is able to dose-dependently increase BFF proliferative life span. Cells could be cultured for over 90 population doublings when conditioned medium was added, compared with 60 cell divisions in DMEM-F12/10% FBS control culture. Conditioned medium also improved BFF clonal capabilities, as observed in single cell adherent cultures and methylcellulose colony forming assays. Finally, we assessed the potential efficacy of this conditioned medium in transgenic generation experiments. When conditioned medium was added to BFF transfected with a neomycin resistance plasmid, fast growing resistant colonies were observed after 7 days of selection. These cell clones reached confluence in 96 wells plates at day 10 and in 35 mm dishes after 21 days. These results show that A4 conditioned medium is effective in prolonging the life span and in enhancing the colony forming capabilities of primary cultured BFF. Our findings highlight the potential utilization of this medium in the generation of transgenic somatic cells.

**38. SEASONAL MORPHOLOGICAL STUDY IN SEMINAL VESICLES OF VISCACHA (*Lagostomus maximus maximus*)**

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The seminal vesicles (SV) are morphologically and functionally dependent on the levels of circulating testosterone. The viscacha is a wild South American rodent exhibiting a seasonal reproductive cycle with active (summer-autumn), and regression (winter) periods. The aim of this work was to perform a light microscope (LM) study of the viscacha SV and to describe its morphological changes during the reproductive cycle. SV samples obtained in both periods were processed by conventional techniques for LM. SV samples corresponding to end of the active period showed three layers of covering: a mucous membrane with scarce folds covered by low cylindrical pseudostratified epithelium; a tight media layer constituted by smooth muscular and collagen fibers; and a narrow external layer with presence of blood and lymphatic vessels. The glandular lumen presented abundant secretory material. SV corresponding to end of the regression period presented a mucous membrane with numerous folds covered by a greater height epithelium, while the media and external layers were more developed with respect to previous period. In the glandular lumen, the secretory material was scarce. These results show the presence of morphological changes in the SV of viscacha, revealing a variation of their functional activity along the seasonal reproductive cycle. This coincides with the fluctuations of circulating testosterone shown in previous work.

**39. MULCH AND POPULATIONAL LEVELS OF WHITE GRUBS (COLEOPTERA: SCARABAEIDAE) IN CROPS UNDER NO-TILLAGE IN SAN LUIS WEST**

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A no-tillage system benefits the development of a diverse population of organisms. Soil remains relatively undisturbed and surface is covered by residues of plants ("mulch"). "White grubs" (Coleoptera: Scarabaeidae) are associated with the no-tillage system. Earlier studies suggest a relationship among the quantity of mulch and the density of white grubs population. This study aimed to contribute to the knowledge of white grubs species in crops under no-tillage system in center-west San Luis and to determine if some relationship exists among the quantity of mulch and the population density of these species. A simple random soil sampling was used in two plots with more than 8 years under no-tillage, each with different levels of mulch (high and low), and in a grassland plot regarding the absence of soil removed through the time. There were four species of white grubs: *Cyclocephala signaticollis*, *C. putrida*, *C. modesta* and *Phylochloenia bonariensis*. When comparing the three plots with different covering conditions (high/low/control), there were no significant differences ( $P > 0.05$ ) in the density of white grubs. Although the studies should be continued to validate the obtained results, the utilized methodology is effective to investigate the specific composition in other farming areas, since the margins of the agricultural activity are enlarging due to the implementation of the no-tillage system.

**40. ANALYTIC INTERFERENCE: EFFECT OF HEMOGLOBIN IN ENZYMATIC ANALYSIS**

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The effect of hemoglobin in defining enzymes of clinical interest was analyzed in serum using an auto-analyzer (CCX ABBOTT), in order to establish correction factors for this analytic interference. The enzymatic activity (Ao) of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK) and  $\gamma$ -glutamyltransferase (GGT) was determined in samples of non-hemolyzed serum. An hemolysate was added in increments to five fractions of these sera to obtain hemoglobin (Hb) concentrations of 1, 2, 5 and 6 g/L. Later, the studied enzymes activity (Ai) was determined. The presence of Hb caused the ALK and GGT Ai values to drop, and the AST and ALT to increase in proportion to serum Hb concentration ( $p \leq 0.00001$ ). The interference was regarded as clinically significant when the difference between Ai and Ao is  $\geq 10\%$ . The observed Hb-dependent variations (Ao-Ai) were analyzed through regression tests to estimate the appropriate correction factor (fc). Linear variations were obtained with good correlation rates (r). The parameters obtained for ALK in  $n=60$  were:  $fc = -22.48$ ,  $S_d = 1.21$ ,  $r = 0.94$ . The fc of GGT, AST and ALT were -4.29, 5.40 and 1.70 respectively. *In-vitro* samples of hemolyzed serum can be analyzed considering the interference caused by [Hb]. Thus the actual value of enzyme activity (Ac) becomes  $Ac = Ai + fc [Hb]$ .

**41. RELATION BETWEEN LEVELS OF CHOLESTEROL AND TBARS IN UNIVERSITY STUDENTS**

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There is an increasing interest in the study of the health effects of cigarette in the mechanism of disease initiation and progression following exposure to smoke. In the present study we compared the occurrence of oxidative stress and the possible correlation with the level of cholesterol and the blood pressure in university smoker (2-20 cigarette per day) students and no-smoker (younger adults). The occurrence of lipid peroxidation was measured as thiobarbituric reactive substances (TBARS) in serum. We also measured serum cholesterol and blood pressure. In the study, 39 students participated: 24 women and 15 men. The levels of TBARS did not change between the groups and they were between normal parameters. Only 2 smoker men had higher pressure values and only one had serum cholesterol over the normal values. Regarding women, 29% were smokers and 71% were no smokers, but 35% were passive smokers. In men group, 40% were smokers and 60% no smokers but 33% were passive smokers. These results suggest that the age and the diet attenuate lipid peroxidation directly or indirectly. On the other hand, the percentage of passive smokers (35% and 33% respectively) is very important, because it would conduce to an equal level of TBARS and does not show differences with the smoker group. *Technical Assisstants: Ferrari C.*

**42. MEASUREMENT OF NICOTINE IN BIRD BLOOD BY REVERSE PHASE HPLC: PRELIMINARY DATA ON NICOTINE ELIMINATION IN PIGEONS**

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Wild animals are exposed to xenobiotics, which are absorbed in the intestine by transcellular and paracellular routes, depending on the size and charge of the molecule, its water solubility and the presence of transporters on the apical membrane of enterocytes. Nicotine is a water-soluble toxin present in several plants consumed by birds. An interesting problem in ecotoxicology is to know how much nicotine is absorbed by the bird intestine. We present here an adapted technique to assess nicotine in blood samples of pigeons (*Columba livia*) using RP-HPLC with electrochemical detection based on the method of Mahoney and Al-Delaimy (2001) designed to determine the toxin in hairs. As an application example, the kinetics of nicotine elimination from blood after intramuscular injection was described. Nicotine was extracted from plasma samples with dichloromethane. The solution was evaporated and the residue, in phosphate buffer, was analyzed by HPLC with a reverse phase C18 column in isocratic condition and a solvent flow of 1 mL/min (50 mmol/L  $\text{KH}_2\text{PO}_4$ : 2.5% MeOH: 4% acetonitrile; pH 4.8). An electrochemical detector was used (oxidation potential: +1.10 V, sensibility: 200 nA.V<sup>-1</sup>). The detection limit of nicotine was 1.2 ng/mL of plasma. Nicotine elimination from blood shows apparent first order kinetics. This method is suitable for assaying low nicotine levels in plasma.

*Supported by CyT-UNSL 22/Q451 to ECV.*

**43. PREVALENCE OF ANEMIA IN CHILDREN UNDER 12 YEARS OLD IN THE URGENCE LABORATORY FROM SAN LUIS HOSPITAL**

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Anemia in children is associated to a retardation in the psychomotor development and behavior alterations when children are younger than 2 years old, alterations that can be irreversible. Hemoglobin (Hb) values of 862 children (from 0 to 12 years old) that concurred to the urgency laboratory of "San Luis Hospital" were evaluated. The patients were divided in four groups: A-Newborn (until three days old), B- children from 4 days old to three months old, C-children from 3 months old to 1 year old, D-children from 1 to 12 years old. Groups B, C and D were also divided in boys and girls. Patients with Hb values lower than 14 g/dl (in group A), 9.5 g/dl (in group B), 11 g/dl (group C), and 12 g/dl (group D) were considered anemic, in accordance to the OMS(1972). A hematological counter Advia 60 (Bayer) was used. From the 862 children, the higher percentage corresponded to children included in group C (48%), while the smaller group was B (8%). In all the groups, the higher percentage corresponded to boys and the most notable was group B (boys: 66%, girls: 34%). 28% of the total number of patients were anemic. A 56% of the anemic patients belonged to group D and 32% to C. These results show that anemia has more prevalence in children from groups C y D, what is consistent with previous results. The prevalence of anemia in newborns and breastfed children was significantly lower, maybe because of their lower possibility of suffering of iron deficiency.

**44. ISOLATION OF *Clostridium perfringens* FROM DEHYDRATED SOUPS IN SAN LUIS**

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*Clostridium perfringens* is an important pathogen agent which causes, among other disorders, enteritis in humans and enterotoxemia in domestic animals. This bacterium can produce more than 13 toxins, one of which is the enterotoxin (CPE) which causes human food poisoning, in which *C. perfringens* is transmitted by polluted food. The disease can be caused by ingestion of food containing  $\geq 10^5$  vegetative cells per g food of an enterotoxigenic strain. The aim of this work was to isolate strains of *C. perfringens* from dehydrated soups sold in retail stores in San Luis city. Thirty samples of dehydrated soups were processed. The following determinations were carried out: count of total aerobes in agar medium count, count of *C. perfringens* in milk iron medium (MPN), determination of *C. perfringens* in agar triptosa sulfite cycloserine (TSCA) and determination of enterotoxigenic strains of *C. perfringens* by RPLA. *C. perfringens* was detected in 15 samples. No enterotoxigenic strain was found. The 15 positive samples gave counts  $<3.0-43 C. perfringens/g$  by the MPN method, 3 samples exceeded the limits suggested by the Alimentary Code (CAA). Total aerobe counts in the 30 samples were  $10^2 - 10^4$  cfu/g. Although the incidence of *C. perfringens* in these samples was low, a potential risk exists in the event of improper handling during food preparation.

**45. ENDOPHYTIC FUNGI FROM SAN JUAN FLORA**

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Endophytic fungi are able to produce bioactive metabolites. Some of these could be synthesized by the symbiont or by development inside the plant. Isolated endophytic fungi might be used as biocontrol agents. Fungi were isolated from wood, flowers and healthful fruit samples. Plants samples were collected on December 2004 in San Juan Province, Argentina. The collected genera were: *Larrea*, *Zuccagnia*, *Senecio*, *Baccharis*, *Adesmia*, *Maytenus*, *Oxalis* and *Leucheria*. The samples were surface-disinfected with 5% sodium hypochlorite and 70% ethanol. After that, they were placed in PDA (potato dextrose agar) and YMA (yeast-malt-agar) media, supplemented with antibiotics (cefotaxime 50 mg/l) and cultured at 25 °C. Identified microorganisms are kept at the microbial strain collection from the Valparaíso University and from Biotechnology Institute (San Juan University). Isolated endophytic fungi were: *Penicillium pupurogenum*, *P. verrucosum* (*Leucheria*); *Aspergillus ochraceus*, *Alternaria alternata*, *A. tenuisima*, *P. purpurogenum* (*Maytenus viscifolia*); *Sporormiella minima*, *P. citrinum*, *P. verrucosum*, *P. glabrum*, *A. alternata* (*Oxalis erythroriza*); *P. purpurogenum* (*Adesmia*). This is the first report about endophytic fungi isolation from plants of San Juan Province, Argentina. Supported by ANPCyT PICT 102/10-1278, grant to MLC and a UNSJ grant to GEF.

**46. THE INTERNAL MILIEU OF *Pomacea canaliculata* (CAENOGASTROPODA, AMPULLARIIDAE)**

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In the course of studies dealing with various aspects of *P. canaliculata*'s biology, we have been many times confronted with lack of data on its internal milieu composition. In the current study we obtained hemolymph from the heart of adult specimens, and we studied some aspects of their cellular and biochemical composition. EDTA-treated hemolymph has a concentration of  $2976 \pm 597$  cells/ $\mu$ L,  $9 \pm 1\%$  of which are eosinophilic granulocytes, while the remaining are hyalinocytes showing different degrees of vacuolization. Without EDTA treatment, hemolymphatic cells aggregate and cannot be counted. Living hemolymphatic cells have been seen emitting numerous long pseudopodia when hemolymph is observed under the polarizing microscope. Hemolymphatic plasma is faintly violet, probably reflecting the occurrence of dissolved hemocyanin. Both protein ( $16.4 \pm 1.5$  mg/L) and glucose ( $28.5 \pm 4.1$  mg/L) concentrations were low. Plasma osmolarity =  $224.2 \pm 2.8$  mOsm/L; plasma pH =  $7.60 \pm 0.02$ . The main circulating cation was sodium ( $59.8 \pm 2.2$  mmol/L), followed by calcium ( $4.25 \pm 0.21$  mmol/L), magnesium ( $3.74 \pm 0.11$  mmol/L), potassium ( $1.74 \pm 0.06$  mmol/L) and iron ( $0.02 \pm 0.01$  mmol/L). Circulating ammonia was  $95.1 \pm 9.3$  mg/L, while urea was undetectable. Uric acid, which is not excreted in this snail, but is deposited in specialized urate cells throughout the body, had a plasma concentration of  $5.1 \pm 1.1$  mg/L.

**47. EPILITHIC ALGAE FROM NOGOLI RIVER (SAN LUIS PROVINCE)**

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The study deals with qualitative results of epilithic diatoms from The Nogolí river basin for complementing the phytoplankton study. The area presents as rocky substratum plutonic-metamorphic rocks, that constitute the igneous-metamorphic basement of the Mountain ranges of San Luis. The samplings had been made in different times during years 2000 to 2004. The sampling was carried out according to the altitudinal and longitudinal gradient, during an annual cycle. The quantity of samples varies between 12 and 22, and spaced among them it was of 500 m, approximately. Samples were collected from the planktonic and epilithic communities, and physicochemical and hydraulic registries were taken. Each epilithic algae sample was obtained by scraping from a known surface of about 100 cm<sup>2</sup>. For this sampling small rocks from the bottom were selected, according to a randomly stratified sampling. The scraped material was transferred to the laboratory in containers with sterile water. In this first stage 10 samples were analyzed and a total of 50 taxa was identified. The genera better represented were *Cymbella* and *Navicula*, with greater number of species. *Cymbella affinis* was the most frequent species in all the samplings. It is emphasized that this species is the predominant one also of the phytoplankton.

**48. NITRIC OXIDE PRODUCTION AND EXPRESSION OF LPS RECEPTORS OF MACROPHAGES TREATED WITH *Larrea divaricata* EXTRACTS**

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*Larrea divaricata* Cav. (jarilla) is a plant commonly used in folk medicine in Argentina. It is important to find out which *in vivo* effects stimulate the immune innate system. This work aimed at evaluating the *in vivo* effects of two aqueous extracts of jarilla on murine peritoneal macrophages (MPM). Mice were sensitized with proteose peptone (10%), and then treated with 0.5, 5 and 50 mg/kg of decoction (D) or infusion (I). MPM were incubated for: a) 48 h, with 100  $\mu$ g/mL of D or I, with or without LPS (10  $\mu$ g/mL); and b) 24 h, with 100  $\mu$ g/mL of D or I. Next, NO production and the expression of LPS receptors were evaluated in MPM. Results showed a significant increase in NO production when MPM were treated with D ( $p \leq 0.05$ ) and with I ( $p \leq 0.03$ ). MPM treated with D + LPS and with I + LPS showed a decrease in NO production ( $p \leq 0.03$  and  $p \leq 0.04$  respectively). MPM treated with D and I bound more LPS than untreated MPM. D and I showed identical effects. However, D produces the same effects at lower concentrations, probably because D contains a higher amount of active compounds than I.

49.

**DIFFERENTIAL RESPONSE OF PREPUBERTAL POLYCYSTIC OVARY TO ADRENERGIC AND CHOLINERGIC INFLUENCES**

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Adrenergic and cholinergic receptors have been found in rat ovary (O). Polycystic ovary (PCO) is a complex endocrine disorder associated with ovarian hyperinnervation. This work assessed the *in vitro* modulation of ovarian androstenedione ( $A_2$ ) and nitric oxide (NO) response in prepubertal rats (32-day old) with polycystic ovary (PCO), induced at 4-day old by a single sc injection of estradiol valerate, 1 mg/rat in 0.1 mL corn oil. Controls were injected with vehicle alone. The left O were isolated and incubated in Krebs-Ringer-glucose buffer in a metabolic bath in the presence of norepinephrine (NE) or acetylcholine (ACh) ( $10^{-6}$  mol/L). Aliquots from incubation medium were taken at 60 and 120 min to measure  $A_2$  by RIA, and NO by Griess reaction. Statistical significance by ANOVA, at  $p < 0.05$ . Control O in basal condition released 29.1 and 28.3 pg/mg ovary of  $A_2$ , and 17.3 and 24.7 nmol/mg ovary of nitrites at 60 and 120 min, respectively. Values were significantly lower in PCO. NE increased  $A_2$  release ( $p < 0.001$ ) from control O, but not in PCO, while ACh increased  $A_2$  release ( $p < 0.005$ ) in controls and decreased it ( $p < 0.001$ ) in PCO O, compared with their respective basal values. In PCO, NE increased NO release. The different steroidogenic response of the PCO to adrenergic and cholinergic agents indicates a potential contribution of the neural system to the PCO etiology since neonatal age.

50.

**ENAMEL TYPES IN TEMPORARY AND PERMANENT DENTITIONS**

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The microstructure of mammalian enamel is complex, showing a variety of patterns among different species. These variations correlate with biomechanical and phylogenetic factors. Koenigswald and Clemens defined five hierarchical levels of complexity in the structure of mammalian enamel: crystallites, prisms, enamel types, schmelzmuster, and dentition. Mammalian variability is evident at the last three levels. This hierarchical system is recognizable from the orientation of crystallites in a limited area of the enamel up to the variation and distribution of patterns of enamel microstructure throughout the entire dentition. The purpose of this study was to describe the types of enamel present in the human dentition -both permanent and deciduous- at different planes, in order to build a three-dimensional model that allows comparisons with other species' patterns. Extracted human healthy pieces were used and imbedded in resine. They were polished with grinding powder and studied with scanning electron microscopy. Radial prismatic enamel was observed, with prisms directed away radially from the EDJ, as well as aprismatic enamel with parallel crystallites. The prismatic enamel shows prisms decussated in layers. Thus, human enamel presents variability at the enamel type level, both in the temporary and permanent dentition, as well as compared to other mammalian species.

51.

**CHEMICAL COMPOSITION AND VIRUCIDAL ACTIVITY OF *Heterothalamus alienus* ESSENTIAL OIL**Duschatzky C<sup>1</sup>, Almeida N<sup>1</sup>, Lampasona M<sup>2</sup>, Schuff C<sup>2</sup>, García C<sup>3</sup>, Talarico L<sup>3</sup>, Damonte E<sup>3</sup>.<sup>1</sup>FICES, Univ Nac San Luis, Villa Mercedes, San Luis; <sup>2</sup>Inst Quím Org, UNT; <sup>3</sup>Lab Virol, FCEN-Univ Buenos Aires. E-mail: cduscha@fices.unsl.edu.ar

Plant products provide an interesting source for the discovery of bioactive compounds. Among the structurally diverse plant components, the essential oil fraction has been recently started to be analyzed for antimicrobial properties. In the present study, we determined the chemical composition of the essential oils isolated from *Heterothalamus alienus* (Asteraceae) at different harvest periods and the relationship between their composition and their virucidal activity against herpes simplex virus type 1 (HSV-1), dengue virus type 2 (DENV-2) and Junin virus (JUNV). The plants were collected in Loma Bola (Córdoba Province) and the oils were isolated from leaves (S1) or leaves and flowers (S2) by steam distillation and analyzed by GC-MS technique. The major components detected were beta-pinene, spathulenol and germacrene-D. The percent of oxygenated sesquiterpenes was found to be higher in S1 than in S2, while the percent of monoterpenes was higher in S2. The oils were screened for cytotoxicity in Vero cells and virucidal activity by incubation of each virus, with different oil concentrations. The cytotoxic concentration 50% values obtained were in the range 96-148 ppm. No virucidal activity was observed against HSV-1 and DENV-2, while S1 was found to inactivate JUNV more efficiently than S2, with a virucidal concentration 50% value of 44 ppm.

52.

**EFFICACY OF WASHING PROCEDURES IN REMOVING *Yersinia enterocolitica* FROM THE SURFACE OF TOMATOES**

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Different washing solutions could reduce partial or totally the presence of pathogenic microorganisms on fresh vegetables. In this study, several chemical agents were used to determine their efficacy in removing *Yersinia enterocolitica* from the surfaces of tomatoes. Produce were inoculated with a cocktail of two *Y. enterocolitica* O:9 strains. After drying overnight at 25°C, tomatoes were washed with: 50 mL sterile distilled water (DW, pH 7.03), 200 ppm chlorine (C, pH 7.87), 0.1 mg/mL benzalkonium chloride (BzC, pH 7.07) or 0.2% lactic acid (LA, pH 2.57). Nontreated samples were used as a control of *Y. enterocolitica* initial population ( $8.28 \pm 0.23$  log cfu/tomato). All tomatoes were rinsed with 100 mL DW for 1 min and dried with sterile paper towels. Counts of the remaining microbial population was assessed by submerging each tomato in 20 mL Dey-Engley neutralizing broth, serially diluting, and spread plating on trypticase soy agar and MacConkey agar. *Y. enterocolitica* reductions of 4.04, 4.21, 4.77 and 5.08 log with DW, BzC, C and LA, respectively, were observed. C and LA produced significantly higher reductions than those observed with DW and BzC ( $p < 0.05$ ). C and LA could be useful for the control of *Y. enterocolitica* on fresh vegetables.

**53. MULTIPLEX PCR FOR DETECTION OF VIRULENCE GENES IN *Yersinia enterocolitica* FROM FOOD AND FAECAL SAMPLES**

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Of the six biotypes (B) of *Y. enterocolitica*, five (1B, 2, 3, 4 and 5) are considered pathogen for humans, and contain markers associated with virulence. These are located in the chromosome (*ail*, *inv*, *yst*, *htrA*) and in the pYV virulence plasmid (*yadA*, *VirF*). Multiplex PCR assays for detection of plasmid- and chromosome- borne virulence genes of *Y. enterocolitica* isolated from a variety of foods and human patients in Argentina and Chile were developed. Specific PCR primers were obtained from sequences available in the GeneBank database (GeneDoc and Primer3 softwares). Each multiplex PCR assay was performed in 50 µL of reaction mixture containing 3 µL of DNA, 1mmol/L dNTP mix, 1.5 mmol/L MgCl<sub>2</sub>, 1 X reaction buffer, 10 pmol of each forward and reverse primer and 0.25 U of Taq DNA polymerase. The samples were amplified for 35 cycles. Each cycle consisted of 1.5 min at 94°C, 1.15 min at 58°C, and 1.5 min at 72°C. *Y. enterocolitica* B2 strains from Argentina and Chile were positive for *yadA*, *VirF*, and *invA* genes, while *Y. enterocolitica* B4 strains from Chile and *Y. enterocolitica* B1A strains from Argentina were positive for *invA* and *yst*, respectively. Multiplex PCR assays showed simultaneous amplification of virulence genes from different biotypes of *Y. enterocolitica*.

**54. EFFECT OF EDAPHIC ALGAE ON THE GERMINATION AND INITIAL GROWTH IN WHEAT**

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*Cyanophyta* (cyanobacteria) increase polysaccharide concentration and secrete enzymes, modifying plant growth. In the semiarid Argentine region, with sandy loam, organic material content is less than 1% and available nitrogen in soil is low. Nitrogen fixation *Cyanophyta* (*Anabaena azollae*) provide nitrogen for rice cultivation. The goal of this work was to select and multiply *Cyanophyta* edaphic algae from perennial cultivation and to inoculate them in wheat seeds. Compound samples were obtained; 0 to 20 cm deep in soil, in *Eragrostis curvula*, *Poa ligularis*, *Digitaria eriantha* and *Sorghastrum pellitum* cultivation. Algal biomass was isolated and purified for 6 months, with liquid cultivation medium for *Cyanophyta* development (Watanabe). Seeds were inoculated in 4 treatments with 800, 400, 200, 100 cells/mL and controls in a completely random design with 4 repetitions: Statistical analysis was performed with SAS software. In the repetition, 50 inoculated seeds which were sown in 10 x 14 cm plastic trays on germination paper, in a cultivation chamber at 30:20 °C and 8:16 h (light-darkness) during 21 days with countings every 7 days. No significant differences was found in the germination percentage above 97%. The leaf length was superior in 800 and 400 cells/mL but no difference in the radicle length was detected. Fresh weight of plantule and dry weight of leaf favoured treatment with 800 cells/mL. Thus, the selected edaphic blue-green algae bring about a differential stimulus in the initial growth of the plantule.

**55. EFFECT OF MICRONUTRIENTS IN THE DIET ON THE AMOUNT OF LIPIDS IN RAT PROSTATE**

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Micronutrients are essential in the diet, in order to maintain cellular integrity. We previously reported that Zn deficiency modifies phospholipids and cholesterol concentration in lung and epididymis. In the same manner, deficiency of vitamin A (vit A) modifies the amount of these lipids in aorta, heart and liver. Here we present the results of phospholipids and cholesterol measurements in rat prostates. Rats were separated in six groups: I- control Zn (+), II- Zn (-), III- Zn (-) refeed with Zn (+), IV- control vit A (+), V- vit A (-), IV- vit A (-) refeed with vit A (+). Groups I, II and III were fed during 2 months; groups IV, V, VI, were fed during 3 months. In the case of groups III and VI, they were refeed during two weeks with the respective control diet. Afterwards rats were killed under light ether anesthesia. Their prostates were removed, weighted and kept at -70Y C until processed. The lipids were separated by Folch method, cholesterol was measured according to Zak et al, and phospholipids as described by Bartlett et al. The results show that these micronutrients did not change phospholipids and cholesterol concentrations in prostate. Thus, Zn and vitamin A deficiencies selectively affect lipid concentration in different rat organs.

**56. SEASONAL STUDY OF THE GROWTH HORMONE CELLS IN THE PITUITARY PARS DISTALIS OF VISCACHA**

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In species that undergo seasonal reproduction a strong correlation between reproductive status and GH secretion was demonstrated, supporting the physiological importance of GH in the reproductive function. Androgens stimulate the secretion of GH. The present aim was to study the GH cells (somatotropes) during the annual reproductive cycle of adult male viscacha by immunohistochemistry and image analysis. The percentage immunopositive area and major cellular and nuclear diameters have been considered as a measure of the cellular activity. The GH cells were densely distributed throughout the pars distalis (PD) parenchyma. These cells were not observed in a sector delimited by a great blood vessel in the caudal region. These cells were not found in contact with the colloidal lumen, whereas they were situated in a basal position. The GH cells were oval, pyramidal or spherical in shape with a large nucleus. Homogeneous immunolabeling was observed in all the cytoplasm. During the gonadal regression period (winter) the percentage immunopositive area and the cellular diameter showed a significant decrease in relation to the values found in the reproductive period (summer-early autumn). These results demonstrate that the cells GH change seasonally, in agreement with changes in the hypophysis-gonadal axis, and suggest that serum androgens may act through a direct or indirect mechanism on the cellular activity.



**57. PARTIAL CHARACTERIZATION OF TEREBINTH PROTEASE (*Schinus areira*)**

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In our country there is an important native flora. However, data are lacking about plant protease isolation. The aim of this work is the characterization of protease of *Schinus areira*. The plant tissue (leaves) are processed at 0–4°C in a homogenizer with a phosphate buffer 0.1 mol/L (pH 7.0), and centrifuged at 10,000 g. The total protein content was determined through the Bradford method. The effect of both pH and temperature on proteolytic activity was determined, using azocasein as a substrate. Likewise, the remaining activity of the preparations that were exposed to different temperatures (20–80°C) and various pH conditions (pH 5 a 9) was verified during various time periods. Highest proteolytic activity was observed at pH 7 and the remaining activity was 51.7% at pH 5 and 56.5% at pH 9. The optimal temperature was 50°C and remaining activity was 69% at 20°C, 91.6% at 40°C, 99% at 60°C and 79% at 80°C. A 50 KDa molecular mass was estimated by SDS-PAGE, using a 7.5% polyacrylamide gel.

**58. REGULATION OF OVARIAN FUNCTION FROM CELIAC GANGLION STIMULATED WITH NEUROPEPTIDES**

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We have previously shown that ovarian function is modified by neural influences from the celiac ganglion (CG) through the superior ovarian nerve (SON). Here we examined the *in vitro* effects of neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), and substance P (SP) acting on the CG on ovarian progesterone (P) release of Holtzman rats on diestrus 2 (D2). The CG of the integrated CG-SON-Ovary system was incubated with 50 ng/mL of each neuropeptide. Samples from the ovarian compartment were collected at different times to measure P release by RIA. Statistical significance was assessed by ANOVA, at  $p < 0.05$ . In basal condition O from the integrated system released 0.015, 0.027, 0.028 and 0.040 ng P/mg ovary, at 30, 60, 120 and 180 min, respectively. These values were lower ( $p < 0.001$ ) than those released by O alone, indicating an inhibitory effect from CG. CG stimulation with NPY, VIP or SP increased ovarian P release at all studied times, compared with the respective basal values. When O was disconnected from the CG by SON section after 60 min of CG stimulation, ovarian P release was equal to that of O in the intact system, indicating that influences from CG reach the ovary in the first 60 min of stimulation. NPY, VIP and SP in CG reverse the inhibitory effect of CG on ovarian P release.

**59. VITAMIN A DEFICIENCY DECREASES SERUM TESTOSTERONE AND PARAOXONASE ACTIVITY AND INCREASES AORTIC ANDROGEN RECEPTOR EXPRESSION**

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Androgen receptor (AR) is expressed in key vascular tissues, including endothelial and vascular smooth muscle cells. In addition, antiatherogenic and vasodilator effects of testosterone have been suggested. Paraoxonase-1 (PON-1) is a HDL-associated enzyme that confers antioxidant activity on HDL. Its activity in serum has been correlated with protection against atherosclerosis. Previously, we showed increased oxidative stress in serum and aorta of vitamin-A deficient rats. Now, we investigate the effect of vitamin A deficiency on PON-1 activity and testosterone concentration in serum, and AR expression in aorta. Wistar male 21-day old rats were fed during 3 months with vitamin A-free diet (-A) and the same diet plus 8 mg of retinol palmitate/kg of diet (+A). A group of -A rats received control diet 15 days before killing (-A refed). PON-1 activity toward phenyl acetate (arylesterase activity, AE) was determined by an enzymatic assay. Circulating testosterone was assessed by RIA. Rat aorta proteins were resolved by SDS-PAGE (8%) and AR by Western blot. The -A group showed decreased AE activity and testosterone levels in serum, and increased AR expression in aorta compared with +A and -A refed. The AR expression and testosterone levels could be associated with oxidative stress and PON-1 activity, since these effects induced by Vit A deficiency are reversed by vitamin A refeeding.

**60. THE “BOTONES DE ORO” (“BUTTERCUPS”) FROM FOLK MEDICINE OF CUYO REGION, ARGENTINA**

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Some species of Asteraceae : Helenieae used in folk medicine in Cuyo region (central-western Argentina) under the vernacular name “botón de oro” (“buttercup”) are studied. The capitula and –exceptionally– leaves of *Gaillardia megapotamica* var. *radiata* (Griseb.) Bak., *G. m.* var. *scabiosoides* (Arn.) Bak. and *G. m.* var. *megapotamica* (Spreng.) Bak., as well as *Helenium donianum* (Hook. & Arn.) Seckt, *Helenium argentinum* Ariza and *Hymenoxys anthemoides* (Juss.) Cass. are used. Only one of them (*G. m.* var. *megapotamica*) integrates herbal remedies that are marketed in local shops for antiallopecic and anticephalalgic treatments. The present study was performed because the areas of all species are approximately coincident, and these plants present a few differential morphological characters among them. With the aim to contribute to the quality control of the regional phytomedicines, samples of these taxa were collected, fixed and conserved in formol:acetic acid:alcohol (1:1:1). Stems and leaves were paraffin-included, cutted and mounted in DPX. The diacritical micrographic parameters were counted in diaphanized samples.

Character	<i>Gaillardia</i>	<i>Helenium</i>	<i>Hymenoxys</i>
Receptacle	paleaceous	without palea	without palea
Vein-termination	2.1 ± 0.4	4.8 ± 0.75	4.33 ± 1.22
Vein-islets	2.4 ± 0.3	13.5 ± 2	18.66 ± 4.12
Indument	pubescent	pubescent	glabrescent

**61. URIC ACID AND STRESS: THE APPLE-SNAIL *Pomacea canaliculata* AS AN EXPERIMENTAL MODEL**

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Animals are able to respond to different stress types, but mechanisms involved may be different. Most defense systems studied involve the expression of enzymes participating as scavengers of free oxygen radicals or the production of molecules that neutralize them. In previous studies we have shown that *P. canaliculata* accumulates uric acid in large and ubiquitous stores throughout its body, and we have hypothesized that *P. canaliculata* could use such molecule as an antioxidant compound. This snail may undergo a period of seasonal quiescence when the limnotopes they inhabit dry up (usually called "estivation"). This is a kind of combined hydric and fasting stress that occurs "physiologically", and is followed by a reperfusion oxidative stress when the snail raises from dormancy. We wanted to know if this condition is associated with changes in uric acid accumulation. Adult *P. canaliculata* individuals were left outside water for 75 days. Then the animals were killed and uric acid concentration was measured in different tissues. Estivating animals lose water as compared with normally active controls (5.3% to 18.1% of their body weight,  $P < 0.05$ , Student's *t* test), but estimated "total" uric acid concentration was not significantly different ( $206.3 \pm 36.4$ ,  $N=10$  vs  $198.0 \pm 30.1$ ,  $N=18$ , respectively;  $P > 0.05$ ). A significant diminution of uric acid concentration was only observed in the lung. Such observations indicate that uric acid stores are probably not consumed during estivation stress.

**62. IS THERE ANY INTESTINAL PROTEASE ACTIVITY IN THE DIGESTIVE SYSTEM OF THE APPLE-SNAIL *Pomacea canaliculata*?**

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The interest in the biology of this invasive snail has increased in the last decades. However, its digestive physiology has remained virtually unexplored. In the 1950's and 60's, both Meenakshi and Andrews were unable to detect any protease activity in the snail's gut and hypothesized that nitrogen requirements were met by the absorption of dietary aminoacids. Andrews also hypothesized that midgut gland (MGG) was secreting enzymes for non-protein digestion that were contained within pigmented corpuscles we now know are endosymbiotic (cyano?)bacterial cells (identified as C and K corpuscles). These pigmented bodies reproduce within the acinar MGG cells and are released in large quantities to the gastric vestibule. Adult female *P. canaliculata* were exposed to a high-protein diet for a 2 days acclimation period and then protease electrophoretic motility, molecular weight and activity were estimated simultaneously by a zymographic technique (PAGE without reductor agents and copolymerized with gelatin at 0.1%). A 130 KDa protease was found in salivary gland (SG) tissue and in the crop sac content, while a 32 KDa protease was found in the stomach content and MGG tissue, as well as in isolated C and K corpuscles. We conclude that the 130 KDa protease originates in the SG, while the 32 KDa protease appears to originate in the MGG's endosymbionts.

**63. PREVALENCE OF INTESTINAL PARASITES AMONG CHILDREN IN PRIMARY SCHOOLS OF SAN LUIS CITY**

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Intestinal parasitoses are common in developing countries and affect mostly children's growth and development. This work aimed at estimating the prevalence of intestinal parasitoses, and the social risk factors associated with them, in primary school children of San Luis. A comparative analysis of the intestinal parasites detected among children aged between 5 and 7 years was carried out in 3 urban schools. The samples were obtained together with a cross-sectional descriptive and analytical study based in personal and social data from questionnaires. Ninety samples were collected from April 2004 to September 2005 in three consecutive days, and were studied by means of coprological analysis using modified Carles-Barthelemy enrichment technique and Graham method. The analysis of the results showed that 52% of tested children were infected with one or more species of enteroparasites. *Enterobius vermicularis* was the most common helminthic parasite detected (34%) followed by *Hymenolepis nana* (2%). *Giardia duodenalis* was the most frequently encountered protozoon (9%), followed by *Entamoeba coli* (7%). Sexual distribution of helminths and protozoan infections showed higher rate in males (32%) than that of females (20%). These results show the role of health education for disease prevention in schools of San Luis city as a natural place to reach the community living next to it.

**64. CORRELATION BETWEEN LDL FRACTIONS OF TYPE 2 DIABETIC PATIENTS AND THEIR FIRST GRADE RELATIVES IN CONCARÁN, SAN LUIS PROVINCE**

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A high level of low-density lipoprotein (LDL) is a major risk factor for atherosclerosis. New studies have demonstrated that LDL is an heterogeneous family of particles that vary in size, density and chemical composition. This work aimed at detecting whether there is a correlation between cholesterol (Chol), triacylglycerides (TG), and phospholipids content (PL) and proteins from LDL fraction of diabetic patients (DP) and their first grade relatives (F1°G). LDL was isolated from fasting serum of DP and its F1°G by sequential ultracentrifugation in a density range of 1.019-1063 g/mL. An LDL aliquot was used to determine Chol, TG, PL and proteins. Lipid determinations were performed with commercial kits, while proteins were determined by the Lowry method. Results are expressed as DP vs F1°G. LDL-Chol.%:  $33.1 \pm 4.3$  vs  $34.3 \pm 4.8$ ; LDL-TG%:  $34.3 \pm 2.3$  vs  $30.8 \pm 1.6$ ; LDL-PL%:  $20.5 \pm 0.8$  vs  $29.1 \pm 1.4$ ; LDL-Proteins%:  $18.26 \pm 1.2$  vs  $18.4 \pm 0.9$ . It is concluded that there are no significant differences between both groups, a finding that suggests a direct correlation of the respective values between the patients and their relatives.

65.

**DETECTION OF APOPTOSIS INDUCED BY *Yersinia enterocolitica* IN IMMUNODEFICIENT MICE**

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*Yersinia enterocolitica* is a Gram negative pathogen that causes enteritis and enterocolitis in humans and rodents. Interleukin 12 (IL-12) mediates protective mechanisms against the *Yersinia* infection by induction of IFN- $\gamma$ . Interleukin 4 (IL-4) antagonizes the protective response against this pathogen. Apoptosis can play a role in infections. The purpose of this work was to study the impact of IL-12 and IL-4 deficiency in apoptosis induced by *Yersinia* infection. IL-12 knockout (IL-12  $-/-$ ), IL-4 knockout (IL-4  $-/-$ ) mice and wild type C57BL/6 mice were infected with  $2 \times 10^8$  colony-forming units (CFU) of *Y. enterocolitica* 0:3 by gavage. Mice were sacrificed at day 3 postinfection. Fluorescent dye staining with ethidium bromide-acridine orange (early and late stages of apoptosis), DNA fragmentation (late stages of apoptosis) and the mRNA expression of pro-apoptotic (Bax) and anti-apoptotic (Bcl2) genes were measured. We observed differences in the induction of apoptosis between the groups of mice and in different organs and methods. Less apoptosis was detected in the spleen of IL-4  $-/-$  mice ( $p < 0,05$ ). Higher DNA fragmentation and Bax expression were observed in Peyer's patches of IL-12  $-/-$  mice. We conclude that IL-12 deficiency increases apoptosis induction in mucosa of mice orally infected with *Yersinia*.

66.

**ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL FROM *Baccharis grisebachii***

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Infusions of aerial parts of *Baccharis grisebachii* Hieron (Asteraceae) are used to treat gastric ulcers, as a digestive, antiseptic and wound-healer in San Juan folk medicine. Chemical analysis and antimicrobial activity of its essential oil constituents are lacking. Thus, the aim of this work is the identification of the volatile components of its aerial parts by gas chromatography – mass spectrometry (GC–MS). In addition, essential oil antimicrobial activity was assessed by the agar dilution method. Fresh aerial parts (300 g) collected in San Juan province, were submitted to hydrodistillation for 4 h in a modified Clevenger apparatus. The oil yield was 0.20% (0.6 mL of oil) and showed a broad spectrum of antimicrobial activity. Methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus* and *Escherichia coli* were strongly inhibited (MIC = 1  $\mu\text{g/mL}$ ). *Candida albicans*, *C. tropicalis*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae*, filamentous fungi *Aspergillus flavus*, *A. fumigatus*, *A. niger*, and dermatophyte *Microsporum gypseum* with MICs ranging from 62.5 to 250  $\mu\text{g/mL}$ . Also, *Trichophyton rubrum*, the most common agent of dermatophytic infection and *T. mentagrophytes* were inhibited (MIC = 62.5  $\mu\text{g/mL}$ ). Our results support the traditional use of infusions of *B. grisebachii* as an antiseptic by the Andean population in western Argentina.

Supported by ANPCyT PICT Redes 260 and UNSJ grants to GEF and BL.

67.

**A BDELLOID ROTIFER ASSOCIATED WITH THE SOUTH AMERICAN APPLE-SNAIL *Pomacea canaliculata***

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*P. canaliculata* hosts a variety of eukaryotes, such as ciliates, arthropods, platyhelminths, nematods and annelids. We describe here a bdelloid rotifer inhabiting the lower gut (contorted gut and rectum) of cultured adult snails. The rotifers are eliminated in the feces and they are also found in the mantle cavity (in much smaller numbers than in the gut). They proliferate in feces from adult *P. canaliculata* individuals, but are not found either in hatchlings or their feces. The rotifer has a corona with two large lateral trochal disks and a cingulum underlying them. A circular sulcus separates corona and trunk. The iloriccate trunk is 2-segmented; the elongated cephalic segment bears a dorsal antenna, two ocelli above the ramate mastax, and a large stomach lumen which separates the bilateral germovitellaria; the caudal segment is much shorter and bears intestine, cloaca and anus. The foot is retractile and is 3-segmented; the longer first foot segment contains the pedal glands, whose ducts terminate at the junction of the second and third foot segment; the latter bears two lateral spurs at its origin and three toes at its end. The stomach and intestinal lumina are filled with a granular, brownish/greenish material, whose color is that of the putative cyanobionts inhabiting the snail's midgut gland and which passes in large quantities into the lower gut. It is hypothesized that the granular material is made of the daughter cells of the putative cyanobiont and that the rotifer predate on them.

68.

**BIOLOGICAL EVALUATION OF THE PHYTOESTROGENIC ACTIVITY OF *Prosopis torquata* PODS IN PREPUBER RATS**

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The phytoestrogenic activity of the powder of *Prosopis torquata* pods (ptp) was evaluated in rats. This work is part of a broader analysis of the role of legume fruits in the reproductive cycle of wild animals, given the concentration of phytoestrogens in legumes and the high presence of these fruits in the diet of wild animals. Twenty five prepuber Wistar rats were randomly distributed in five groups: Control 1 (C1: no ptp powder), Control 2 (C2: 0.05% of 17-beta estradiol), and three treatments [T1, T2 and T3] including 5, 10 and 50% ptp in the rat chow. Rats were treated for 15 days. Time to vaginal aperture (VA), percentage of VA, fresh and dry weight of uterus, oviducts, ovaries, and macroscopical evaluation of organs, were measured as indicators of estrogenic activity. All fresh organs showed edema, except C1. The statistical differences in fresh or dry weight of organs were given by C2. VA aperture was 100% for C2 (days 28 and 29 of age), 40% for C4 (days 38 and 39 of age), 20% for C3 (day 26 of age) and 0% for C1 and C5. These results indicate a functional response of ptp. We are currently evaluating the effect of lower doses of ptp, blood estradiol levels, as well as different ways of ptp administration.

**69. EVALUATION OF HORTICULTURE SEEDS GERMINATION IN TOP SOIL FROM EUCALYPTUS PLANTATION**

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Eucalyptus produces allopathic substances that prevent germination and growth of some species within and near the implantation area. The objective of this work was to use soil from eucalyptus plantation and evaluate the germination percentage of some horticulture species. The chemical parameters of the soil used, from "Complejo Los Robles", were: conductivity 4.520 ds/m, pH 7.8, organic carbon 1.21%, organic matter 2.09%, phosphorus 28 ppm, nitrogen 0.091 g % and humidity 11.4%. Ten seeds were seeded in plastic trays (10 x 15 cm) containing 150 g of dry soil and 30 mL distilled water. The trays were kept at 25°C and exposed during 14 days to 8:16 h of light/darkness. Controls were carried out in fine sand with 30 mL of distilled water. We used a random design with 3 repetitions. Comparison of means was performed by Duncan's multiple range test. The species seeded were: beet Bressane, radish Net Tip Blanca, cabbage Brunk, beet Detroit, beet wide penca, basil wide leaf, eggplant Florida M., pepper Calafyuco, beans yellow chaucha, squash Verones. There were no significant interactions in the germination percent among treatments and controls. Also, there was a low germination percentage in both cases. With these results we cannot talk about the influence of the allopathic substances in the germination process of the analyzed species. Treatments must be continued using high quality seeds and whether they affect the yield should be assessed.

**70. CHRONIC HYPOBARIC HYPOXIA INCREASES SODIUM CURRENT IN RAT HEART CELLS: A WHOLE-CELL PATCH-CLAMP STUDY**

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Chronic hypoxia elicits a host of physiological adaptations, partly mediated by changes in gene transcription caused by hypoxia inducible factors (HIFs). The effect of chronic hypoxia on cardiac cell sodium current was studied by the whole-cell patch-clamp technique. Adult male rats were submitted to hypobaric hypoxia (about 0.52 atm) in a chamber, 8 h per day during 9 days. The control group was exposed to normal ambient pressure. Under ether anesthesia, animals had their hearts removed. Hearts were treated with a mechanical and enzymatic protocol for obtaining isolated cardiomyocytes. Heart cells were harvested and mounted in a set up for electrophysiological measurement. Cells were monitored with an inverted microscope and records were obtained with pClamp6 (Axon Instruments, Inc.). Results were analyzed with a Student *t* test; values are reported as mean  $\pm$  SEM. Compared with control heart cells, cells from hypoxic rats showed higher surface ( $7,725.6 \pm 62.7$  vs  $7,325.6 \pm 12,7 \mu\text{m}^2$ ,  $p < 0.0001$ ), lower membrane potential ( $49.6 \pm 6.2$  vs  $69.6 \pm 1.2$  mV;  $p = 0.0085$ ), and higher sodium current ( $850 \pm 30$  vs  $500 \pm 30$  pA;  $p < 0.0001$ , 95% CI for the difference 278-442 pA). Although the cardiac electrophysiological effects of hypoxia have been previously studied, this is the first report clearly showing an hypoxia-induced increased permeability of voltage dependent heart sodium channels.

**71. LIPID METABOLISM IN LIVER OF RAT EXPOSED TO CADMIUM**

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The present study was undertaken to assess whether chronic exposition to cadmium (Cd, 15 ppm for 8 weeks) in drinking water affects lipid metabolism in the liver of adult male Wistar rats. As compared to rats not exposed to Cd, serum triacylglycerides (TG) and LDL+VLDL cholesterol concentration increased, but total cholesterol and HDL-cholesterol did not. High TG levels were associated with an inhibition of lipoprotein lipase, as was observed after injection of Triton WR1339, a detergent that prevents intravascular triglycerides catabolism by inhibition of LPL. On the other hand, we did not find changes in apoB mRNA. The high content of TG in liver of Cd-treated rats were due to increased synthesis, as indicated by the increment in the amount of hepatic mRNA of mitochondrial glycerol-3-phosphate acyltransferase. In addition, liver fatty acid synthesis was increased, as determined by the increment in the fatty acid synthase activities, and [ $^{14}\text{C}$ ]-acetate incorporation into the liver saponifiable lipid fraction and proportion of palmitic acid (16:0). Cd exposure also resulted in a higher relative percent of saturated fatty acids, without changes in polyunsaturated fatty acids and unsaturation index. Chronic Cd exposure may directly or indirectly alter the serum lipid content and liver lipid metabolism.

**72. FREE RADICAL SCAVENGERS AND MINERAL CONTENT OF PROPOLIS FROM SAN JUAN PROVINCE, ARGENTINA**

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Flavonoids and phenolic compounds derived from plants are potent free radical scavengers. These compounds from medicinal plants, fruits, vegetables, and propolis and included in dietary supplements or medicines aiming to prevent or treat diseases. Also, propolis contains many trace minerals elements essential for health (Zn, Cu, Ca, Fe, K, Mn, Mg). This study evaluated the free radical scavenger as well as mineral, phenolic and flavonoid content of propolis collected from different locations of San Juan province. In addition, representative HPLC DAD chromatograms of propolis were obtained. Total phenolic content of the plant was determined by the Folin-Ciocalteu technique using a calibration curve with tannic acid with percent value ranging from  $17.50 \pm 0.56$  to  $26.62 \pm 0.10$ . Total flavonoid content was estimated by the aluminum chloride method and showed percent values ranging from  $5.70 \pm 0.05$  to  $10.57 \pm 0.80$ . Free radical scavenging activity in propolis methanol extract, measured by decoloration of a methanolic solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). At 50  $\mu\text{g/mL}$  all showed a percent decoloration from  $88.54 \pm 0.06$  to  $94.74 \pm 0.20$ . Mineral content determined by atomic absorption spectrophotometry showed values ranging from 12-35, 2-6 17-19, 1100-2000 and 800-1200 ppm for Mn, Cu, Cr, K and Ca respectively. Supported by ANPCyT PICT Redes 260 and UNSJ grants to GEF and BL.

73.

**ANTIMICROBIAL ACTIVITY OF PROPOLIS FROM SAN JUAN PROVINCE, ARGENTINA**Lima B<sup>1</sup>, Tapia A<sup>1</sup>, Rodriguez J<sup>1</sup>, Juarez S<sup>2</sup>, Wunderlin D<sup>3</sup>, Zacchino S<sup>2</sup>, Feresin GE<sup>1</sup>.<sup>1</sup>Instituto de Biotecnología-ICB, U.N. San Juan. E-mail: gferesin@unsj.edu.ar; <sup>2</sup>Farmacognosia, Fac. Cs. Bioq. y Farmac., U.N. Rosario; <sup>3</sup>CEQUIMAP, U.N. Córdoba, Argentina.

Propolis is a resinous and a balsamic material with a complex composition, which is collected by bees from sprouts, exudates of plants. Chemical and pharmacological information on the reputed medicinal properties of the propolis from San Juan province is limited considering the potential chemical and pharmacological possibilities they offer. Six raw propolis samples purchased from San Juan's apiculture were assessed for antimicrobial activity against bacteria and fungi with the agar dilution method. All methanolic propolis extracts showed the broadest spectrum of action against fungi, inhibiting all of the tested dermatophytes *Microsporium gypseum* *Trichophyton rubrum* *T. mentagrophytes* and yeast *Candida albicans* *C. tropicalis* *Cryptococcus neoformans* and *Saccharomyces cerevisiae* with MICs ranging from 62.5 to 250 µg/ml. Regarding the antibacterial activity, all propolis extracts were active on methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* as well as against *Escherichia coli* with MIC from 125 to 1000 µg/ml. None of the propolis extracts was active towards filamentous fungi *Aspergillus flavus* *A. fumigatus* and *A. niger*. This is the first report about antimicrobial activity on propolis from San Juan province, Argentina.

We are grateful to ANPCyT PICT Redes 260 and CYTED Proy. X.7. We also thank UNSJ for financial support & grant to GE Feresin and B Lima.

74.

**CHANGEABILITY IN FOLIAR MORPHO-ANATOMY OF *Lippia turbinata* GRISEB. (VERBENACEAE)**

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Medicinal plants with mesomorphic and xeromorphic characteristics are a significant component of the indigenous flora of San Luis Province. In popular medicine, *Lippia turbinata* ("poleo") has emmenagogic, diuretic, digestive and sedative properties. The morphological and anatomical aspects of foliar leaves were studied according to their environmental conditions to contribute to the identification of samples of commercial drugs. Young and mature leaves of fresh and fixed material on FAA (formol, alcohol, acetic acid) were analyzed. Cuts from the middle of the leaf were embedded in paraffin and then sectioned with a rotatory microtome. The colorations used were safranin-fast green and safranin aqueous solution. For the securing of the epidermis, typical techniques of microhistology were used. Photomicrographs were taken from the sections, and measures were carried out with a graduated ocular of a light microscope. From the studied sections, differences in the following aspects were detected, namely the form and type of epidermic cells, the presence of bulliform cells, density of stomata, thickness of cuticle, density of nonglandular trichomes, and in the structure of the mesophyll. The study of the foliar morpho-anatomy of *Lippia turbinata* turns out to be an important contribution to a better understanding of its intraspecific changes, and to detect possible adulterants in commercial samples.

75.

**PREVALENCE OF *Yersinia enterocolitica* IN CHICKEN AND PORCINE MEATS**

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*Yersinia enterocolitica* is a food-borne bacterium that can cause gastroenteritis in humans. This work assessed the prevalence of *Y. enterocolitica* in chicken and pork products acquired in San Luis city. Twenty five g of sample were homogenized in 225 mL phosphate buffer saline (PBS) with addition of 1% sorbitol and 0.15% bile salt, and incubated for 21 days at 4°C. Characteristic colonies were analyzed by culture, biochemical and serological tests. The isolated strains were genotyped by random amplification polymorphism – polymerase chain reaction (RAPD-PCR), using the primer A08, and plasmid isolation was also performed. From 117 chicken samples studied, 7 strains (6.9%) of *Y. enterocolitica* and 8 strains (6.8%) of *Yersinia intermedia* were isolated. From 144 pork samples studied 3 strains (2%) of *Y. intermedia* were isolated. The *Y. enterocolitica* strains belonged to biotype 1A, 2 of them showed a plasmid with a molecular size < 72-76 Kb. RAPD-PCR showed heterogeneity among the studied strains, showing to be an acceptable subtyping method. Although the biotype 1A is considered non-pathogenic, epidemiologic and experimental data suggest that *Y. enterocolitica* biotype 1A is able to cause illness by independent pYV mechanisms.

76.

**INVESTIGATION OF *Staphylococcus aureus* IN ARTISAN FRUIT SALAD OBTAINED IN SAN LUIS CITY**

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*Staphylococcus aureus* is a pathogenic bacterium that can cause alimentary intoxication. Fruit juices and their derivatives are vehicles of intestinal diseases. This study aimed at investigating the presence of *S. aureus* in fruit salads prepared and sold in San Luis city. Seventy one samples were studied: 25 g of the sample were homogenized in stomacher during 60 s, 0.1 mL of the homogenate was spread onto Bair-Parker agar (BP) and incubated at 35°C for 48 h. Characteristic colonies of *S. aureus* were confirmed by Gram stain, thermonuclease, catalase and coagulase tests. The agar diffusion method of Kirby-Bauer was used to determine antibiotic susceptibility. The strains were tested against amikacin, ciprofloxacin, fosfomicin, gentamicin, rifampicin, sulfamethoxazole-trimethoprim, tetracycline, and vancomycin. Eleven strains (15.49%) were isolated; the counts varied between 20-300 FCU/g. All strains gave a 4+ coagulase reaction and were positive to thermonuclease and catalase. Also, they were sensitive to every antibiotic tested, showing a similar inhibition halo. It seems that all the strains isolated could come from the same origin. A strict hygienic control during the manufacturing step of this kind of food is suggested.

**77. MITOCHONDRIAL ALTERATIONS AFTER A CHRONIC TREATMENT WITH A CB RECEPTOR AGONIST**

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The duality neuroprotection-neurotoxicity assigned to cannabinoids is today unclear. The goal of the present work is to analyze the mitochondrial alterations after a chronic treatment with a synthetic cannabinoid agonist (WIN 55,212-2) in the cerebellum, where there is a high density of cannabinoid receptors (CBR). Mitochondrial alterations are one of the most striking indicators of neuronal injury. Six Wistar rats were separated in two groups: one received 6 mg/kg/day of WIN dissolved in DMSO subcutaneously during 14 days in two daily doses, and the control group received the same volume of the vehicle. At day 14, rats were fixed by perfusion and brains were processed for electron microscopy study. The results showed that in treated group mitochondria exhibited swelling, loss of limits between the internal and external membranes, disruption of the external membrane, heterogeneity and increase of electron-opacity of the matrix, deposits of electron-dense material in the mitochondrial matrix, and vacuolization. In addition, endoplasmic reticulum appeared expanded with a minor quantity of attached ribosomes and the plasmatic membranes are disrupted. We conclude that chronic treatment with CBR agonist produces neuronal damage.

*Supported by UBACYT-M072 and PIP 5034 CONICET grants.*

**78. GANGLIOSIDE BINDING AND ANTIMICROBIAL SENSIBILITY IN *Clostridium botulinum***

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Infant botulism is a severe neurological disease caused by botulinum neurotoxin (BoNT). Most cases are due to *Clostridium botulinum*, but toxigenic strains of *C. baratti* and *C. butyricum* have also been identified. Specific characteristics of these strains are unknown. Our aim was to determine antimicrobial resistance pattern and ganglioside binding of BoNT in *Clostridium botulinum* isolated from infant botulism cases. We studied 27 serotype A strains: 13 from infant botulism cases and 14 from other origins. Minimal Inhibitory Concentration (MIC) was determined by agar dilution (NCCLS) for ampicillin (AMC), amoxicillin (AMX) and clindamycin (CLI). Ganglioside binding was determined by immunostaining thin chromatography layer (TLC). TLC plates were incubated with BoNT and primary (antitoxin) and secondary antibodies (peroxidase conjugated). AMC and AMX MIC were between 0.06 and 2 µg/mL and CLI MIC were between 16 and 32 µg/mL. All BoNTs bind to GD1a and GM1. Binding to others gangliosides was relatively specific for each BoNT. These results suggest that antimicrobial susceptibility does not present significant differences to characterize these strains. For this purpose ganglioside binding could instead be used.

**79. OCCURRENCE OF INFANT BOTULISM IN ARGENTINA AND ITS RELATION WITH PRESENCE OF *Clostridium botulinum* IN SOILS**

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Infant botulism has presently become the most common form of botulism. However, its transmission has not been completely elucidated. The main aim of this work was to assess the occurrence of infant botulism in Argentina and its relation with the presence of *C. botulinum* in the soil. In Argentina, 336 infant botulism cases were reported between 1982 and 2004. The average annual incidence was 2.1 per 100,000 live births. In our laboratory, 97 cases of infant botulism were confirmed by the bioassay method. Type A was identified in all cases. Cultures were positive in all stool specimens. Botulinum neurotoxin testing was positive for 75.6% (59/78) of sera and 97.8% (90/92) of stool specimens. A correlation between incidence of infant botulism and prevalence of *C. botulinum* in soils was observed in Western (Mendoza, San Juan and San Luis) and Northeastern (Chaco, Corrientes, Formosa and Misiones) regions. Moreover, type A was the most prevalent in soil (60.2%) and it was detected in all infant botulism cases, indicating an association between the distribution of spore type in soils and the causative toxin type of infant botulism. Biochemical tests, antimicrobial susceptibility and haemagglutinin-positive botulinum neurotoxin production showed uniformity among strains isolated from cases and soil samples.

**80. NEONATAL UNILATERAL URETERAL OBSTRUCTION (UO): NITRIC OXIDE IN MITOCHONDRIAL APOPTOSIS PATHWAY**

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Neonatal obstructive nephropathy, a main cause of chronic renal failure in infancy, is characterized by epithelial cell apoptosis leading to tubular atrophy. We have studied nitric oxide (NO) involvement in the mitochondrial apoptosis pathway in unilateral ureteral obstruction (UO). *In vivo* experiments were performed in control and neonatal rats with UO for 14 days. *In vitro* experiments were done in control and obstructed cortex homogenates (CC and OC), in the presence of L-NAME 20 mmol/L or sodium nitroprusside (SNP) 200 µmol/L for 24 h. Apoptosis induction, demonstrated in UO by an increased number of apoptotic cells in cortical collecting ducts, increased expression of Bax/Bcl<sub>2</sub> ratio, and caspase 3 activity, was shown to be associated with decreased mRNA iNOS expression and lower nitrite generation. CC in the presence of L-NAME decreased nitrites release with an increase in caspase 3 activity and proapoptotic Bax/Bcl<sub>2</sub> ratio expression. On the contrary, in the presence of the NO donor, SNP, increased nitrite generation with a simultaneous decrease in the expression of the proapoptotic Bax/Bcl<sub>2</sub> ratio and caspase 3 activity near baseline levels were demonstrated, in control and obstructed cortex homogenates. From our results, a cytoprotective role of nitric oxide for mitochondrial apoptosis induction in neonatal ureteral obstruction may be suggested.

**81.  
URINALYSIS IN CLINICAL PRACTICE: EFFECTS OF DRUGS**

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The establishment of normal values for urine physiological parameters throughout urinalysis is essential in clinical practice. However, a large number of chemicals, such as food and drugs, can produce a physiological variation among normal results. Here we refer to substances which can possibly modify the physiological urinalysis values. Bilirubin: false negative results are obtained if there are large amounts of ascorbic acid and nitrites. Leucocytes: esterase method is used. Presence of glucose, albumin, ascorbic acid, tetracycline, cephalixin, cephalothin or large amounts of oxalic acid can inhibit the reaction. Glucose: Ascorbic acid, ketones and aspirin in urine can cause a false negative reaction. Ketones: False positive results may occur with highly pigmented urine or samples containing large amounts of levodopa metabolites. Ascorbic acid and phenazopyridine can yield false positive results. Compounds containing sulfhydryl groups, such as mesna, may cause atypical colours or false positive reaction. Occult blood: Elevated specific gravity and captopril may reduce the reactivity of the test. Protein: Quaternary ammonium compounds (from antiseptics) or chlorhexidine can also give false positive results. A summary of the substances which can markedly alter the normal clinical chemistry data from urinalysis has been produced. It is very useful to know the list of several substances that modify the values. The positive and negative values given in this section are intended therefore to serve only as a guide.

**82.  
DISTRIBUTION PATTERN OF INTERSTITIAL CELLS OF CAJAL IN THE FORESTOMACH OF NEWBORN CALF**

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C-kit is the tyrosine kinase receptor of stem cell factor expressed in different tissue layers and organs. By light microscopy, the specific marker of interstitial cells of Cajal (ICC) in gastrointestinal tract is c-kit/CD117. These ICC have a functional relation with the neurons of myenteric plexus and smooth muscular cells, playing an important role in the control of gastrointestinal motility. The present study analyzed the distribution of the c-kit positive cells in the normal forestomach of newborn calves. C-kit immunohistochemistry was performed on  $4 \pm 1 \mu\text{m}$  sections paraffin embedded of rumen, reticulum and omasum obtained from six 2-day old calves. The density of c-kit positive cells was significantly greater at the myenteric plexus, circular and longitudinal smooth muscle layers. Around the myenteric plexus they present short processes. In muscular layers, a network organization was found, in which thin processes of positive cells overlapped with processes of other positive cells. Rumen and reticulum showed high density in longitudinal muscle layers while the omasum displayed more positive cells in circular muscle layers. Omasal lamina showed large c-kit positive cells. These morphological data show for the first time that in forestomach of the newborn calf there are cells phenotypically similar to ICC.

**83.  
PEPERINA OF THE HILLS (*Hedeoma multiflorum* Benth.): DOES IT NEED POLLINATION WITH INSECTS TO PRODUCE VIABLE SEEDS?**

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*Hedeoma multiflorum* Benth., an aromatic native plant, has very small seeds (more than 7000 individuals/g), with relatively low germination rate and a state of primary dormancy surmountable six months after the crop. Moreover, the germination *in vitro* with these seeds, with special conditions of cultivation, gave better results. However, we have observed that many of the seeds are empty (vain), from lack of fecundation lack. This could be another cause of the low germination rate. As in the cultivation of this species, during the flowering period, it has been observed visit of insects, specially Dipteral - family Sirfidos, tests were carried out, under experimental cultivation conditions: 1 - with the use of closings; 2 - without the use of closings, to determine if the "peperina" requires pollination with insects to obtain viable seeds. In the seeds gathered in both tests, the determination of the germinative power was performed, achieving very similar values among the seeds coming from plants with closing and of plants without closing. These results suggest that, in this species, the visitor insects observed do not influence the production of viable seeds.

**84.  
FREQUENCY OF *vacA* GENOTYPES AND *cagA* STATUS OF *Helicobacter pylori* CLINICAL ISOLATES FROM SAN LUIS**

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*Helicobacter pylori* is genetically diverse and certain strains are more virulent and cause more severe diseases than others. Such diversity is reflected on the clinical outcome. The genotypes of *H. pylori*, *cagA* and *vacA*, are important virulence factors involving gastric diseases and distribution of the *vacA* allele varies between geographic regions. The aim of this work was to investigate the *vacA* mosaicism and the presence of *cagA* gene in local isolates of *H. pylori*. DNA was isolated from 80 patients with chronic gastritis or peptic ulcer in San Luis and the genotypes *cagA* and *vacA* were determined by PCR with primer pairs from each virulence gene. Fifty seven strains (71.25%) were common *vacA* genotype s1/m1, and only 23 (28.75%), were s2/m2. Presence of *cagA* was detected in 20 strains (25%). We observed high percentage of s1/m1 strains with chronic gastritis and *cagA* gene was not found to be associated with gastritis or ulcer. In conclusion, as in other populations, *cagA* and *vacA* genes not only can be used as predictive markers in *H. pylori* clinical isolates to identify a particular strain as a gastritis or ulcer producer. Perhaps new virulence factors should be described with more power to discriminate among *H. pylori* strains.

**85. CROSS-REACTIONS BETWEEN *Larrea divaricata* CAV. PROTEINS AND BACTERIAL CELLULAR PROTEINS**

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*Larrea divaricata* Cav. ("jarilla") has been used to treat a number of conditions. Nevertheless, so far no information is available regarding its immunological properties. The study aimed at immunologically characterizing proteins from a crude extract of *L. divaricata* Cav and assessing antisera for recognition of bacterial cellular proteins identified by immunoblotting technique. The crude extract was obtained from leaves in PBS (pH 7.4) 24 h at 4°C. *Pseudomonas* sp., *K. pneumoniae*, *P. vulgaris*, *E. coli* O:157 were the tested bacteria. The extract was concentrated by ultrafiltration using Microcon-10 (Amicon) concentrators. The extract proteins were used as immunogens. Extract and bacterial proteins were electrophoresed through a 10% separating polyacrylamide gel and electroblotted onto nitrocellulose. The extract protein patterns and the protein profiles of the microorganisms showed many bands (17-23). Sera from mice immunized with extract proteins showed homologous and heterologous antigen-antibody reactions. The bacterial proteins gave a strong immune reaction to *Pseudomonas* sp: 16(80%); *P. vulgaris*: 14(61%); *K.pneumoniae*: 7(37%); *E. coli* O:157: 7(30%) and extract: 10(59%) of immunoreactive bands. There was an elevated cross reactivity of extract proteins specific antibodies against bacterial proteins, indicating that *L. divaricata* Cav extract proteins are immunogenic candidates for development of a vaccine against bacterial infections in immunodepressed patients.

**86. IMMUNOREACTIVITY OF PROTEINS RELEASED FROM *Clostridium chauvoei***

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*Clostridium chauvoei* causes blackleg, a fatal disease of cattle and sheep. The control of this disease is done by vaccines, generally prepared from formalinized whole cultures. Immunity against *C. chauvoei* is considered principally anticellular, but our results showed that extracellular proteins (EP) elicit a good response of protective antibodies. Here the immunoreactivity of *C. chauvoei* proteins released to culture supernatants was determined. Mice were immunized with EP from *C. chauvoei* ATCC 10092 and two local strains identified as 17 and 8. Reactive bands were obtained by Western blot. To determined cross reactivity, cellular antigens were made to react with EP antisera. Three common bands of relative molecular masses 130, 72 and 34-kDa were recognized by sera raised against EP in all strains, which were also present in SDS-PAGE. Thus, these three proteins seem to be conserved extracellular antigens of *C. chauvoei*. The ATCC 10092 strain showed 8 immunoreactive bands, while local 17 and 8 strains showed a lower number of bands, 5 and 4 respectively. Sera raised against extracellular antigens also recognized cellular antigens with the same molecular masses, except for the 74 kDa band of 17 strain. Similarities between cellular and extracellular immunoreactive patterns ranged from 80% to 30%. Thus, the major soluble antigens are also present in the cells, and are released to the culture medium.

**87. DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR MEASURING THE POTENCY OF *Clostridium chauvoei* VACCINES**

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*Clostridium chauvoei* is the ethiological agent of blackleg, a serious disease that affects sheep and cattle. The disease is controlled by vaccines, generally prepared from formaldehyde-treated whole cultures. The immunity is regarded as antibacterial, but results from our laboratory showed that extracellular proteins elicit a good response of protective antibodies. Potency tests for vaccines containing *C. chauvoei* antigens are based on a challenge assay in guinea-pigs, but animal welfare considerations and the cost of maintaining laboratory species provide incentive to search for alternative methods. Here, an enzyme-linked immunosorbent assay (ELISA) was developed for measuring the mice response to *C. chauvoei* extracellular antigens by IgG titer determination and compare to mice challenge potency test. Extracellular partially purified proteins (EPP) by ultrafiltration of three *C. chauvoei* strains were used as sensitizing antigen in ELISA. Correlation coefficient between IgG titer and % protection (R) was determined by Pearson test. A protective IgG titer for each strain could be determined. The R obtained for the three strains ranged from R=0.71 to R= 0.81 ( $p \leq 0.0005$ ). ELISA can be considered an alternative method to measure the potency of vaccines containing extracellular *C. chauvoei* antigens and has the advantage of allowing a significant reduction in the number of laboratory animals used in the test.

**88. CONSTRUCTION AND CALIBRATION OF A LIOFILIZATOR FOR VEGETABLE TISSUE CONSERVATION**

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In this work a pilot system was designed and constructed for the conservation of food sensitive to temperature by lowering its aqueous activity. The process is developed in two phases, a sublimation phase, called "primary dehydration", in which about 90% of water is eliminated and a desorption phase or "secondary phase" in which the 10% of bound water is eliminated. As the sublimation can only occur at temperatures lower than 0°C and a pressure lower than 4.00 mm Hg, in the first phase frozen food is utilized and vacuum is obtained by a vacuum pump (SIEMENS-SCHUCKERT: 220V, 25 A). In the second phase, evaporation is done in vacuum but at temperatures of 20°C and 60°C. At the pressures applied in lyophilization, the volume of water vapor is very high and as it cannot be absorbed by the pump and it is indispensable to eliminate it, a distillation bottle was placed with a copper serpentine with NaCl solution circulating through it at 0°C. For the calibration of the apparatus kinotos were utilized. This lyophilizator was able to extract more than 51% of water which represent a substantial reduction in aqueous activity and allows food to be conserved for a long time.



**89. PEANUT-RHIZOBIA: CELLULAR COMMUNICATION AND RESPONSE TO ENVIRONMENTAL STRESS**

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Lipids and proteins seem to be implicated in the adaptive response of rhizobia interacting with peanut crops. Here we analyzed LPS and fatty acid (FA) patterns of control and stressed bacteria. We also tested the presence of quorum-sensing signal molecules. The strain used was *Bradyrhizobium* SEMIA 6144 grown in control and stressed conditions. LPS were subjected to SDS-PAGE and silver stain was used for staining procedure. Fatty acids methyl esters were obtained by means of BF<sub>3</sub> in methanol and analyzed in a HP 5890 series II gas chromatograph with Agilent Cerity NDS software. Bacteria were screened for QS inhibitor and AHL production using a bioassay protocol onto Petri plate and indicator bacteria. LPS pattern modifications were not detected under stress conditions. The main FAs detected were 16:0, 16:1, 18:0, 18:1  $\Delta^9$  and 18:1  $\Delta^{11}$ . Temperature stress increased saturated fatty acids (16:0 and 18:0), decreased 18:1  $\Delta^9$  and eliminated 18:1  $\Delta^{11}$ . Thus, the U/S ratio decreased. *Bradyrhizobium* SEMIA 6144 did not present AHL. This absence may indicate that a different quorum sensing system is present in this bacterium. The results suggest that modification of unsaturation degree of FA is an adaptive and compensatory mechanism to environmental stress and implies that its proportion is regulated in response to that challenge and/or cell function. Thus, bacterial cell maintains an optimal membrane physical state for normal functioning.

**90. IDENTIFICATION OF SUPERIOR HONEYBEE (*Apis mellifera*) COLONIES OF THE UNRC APIARY**

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The preliminary results obtained in the 2003-2004 season of evaluation of 50 colonies belonging to the Universidad Nacional de Río Cuarto (UNRC) apiary were previously reported. Characters measured were Population Density, Temperament, and Health Conditions. Honey production estimation for each family was made later to complete the total character series. The evaluation of honey production was made on the field by counting filled combs in each colony during December 2004-February 2005. To obtain the amount of honey produced by each family, the total honey harvest of the apiary was weighed and the mean weight of honey in each comb was obtained by multiplying for the number of combs harvested. With these data, a selection index was made by giving relative index values to all characters, being 0 the lowest and 5 the highest value. Each of these values was multiplied by a factor related to the economic importance assigned to each character. There was a fair number of colonies with similar high values. Nevertheless, the methodology of evaluation applied allowed to discriminate among the best of them and select the five superior families comprising two of Río Cuarto (Córdoba), one of Sampacho (Córdoba), and two of Amenábar (Santa Fe). The selection differential applied was 10% of the base population.

**91. ALTITUDINAL DISTRIBUTION OF LYMNAEID MOLLUSCS IN MENDOZA PROVINCE, ARGENTINA**

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Lymnaeid molluscs are the intermediate hosts of the trematode *Fasciola hepatica* which causes fasciolosis, a parasitic disease that affects livestock and a very important zoonosis. The studies related to the distribution of lymnaeid snails in Mendoza are scarce. Due to the orographic characteristics of Mendoza, our objective was to describe at which altitude the lymnaeid snails are found, since most of the data relates that the animal and human cases originate in the Andean valleys. Samples were collected at the different river basins of Mendoza (Mendoza, Tunuyán, Diamante, Atuel and Llacanelo), both in the lowlands and at higher altitudes in the Andes. The altitude was determined by means of a GPS, lymnaeid snails identified according to Castellanos & Landoni. Sampling was done at altitudes ranging from 645 to 3900 m ASL. Lymnaeid snails were found at 13 sampling points (6 Mendoza river basin, 3 Tunuyan river basin, 2 Diamante river basin, 2 Llacanelo basin) at an altitude range from 1526 m ASL to 2638 m ASL. Even though other molluscs were found at lower altitudes, no lymnaeids were found in the plains region. The absence of lymnaeid molluscs above 2700 m ASL could be due to the extreme climatic conditions. Further studies are necessary to confirm and explain why they are not present at lower altitudes.

**92. ABATTOIR CONDEMNATION OF BOVINE LIVERS DUE TO *Fasciola hepatica* IN TUPUNGATO, MENDOZA**

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Lymnaeid molluscs are the intermediate host of *Fasciola hepatica*, trematode that causes fasciolosis. This disease produces great losses to the cattle industry due to the condemnation of livers and diminished meat and milk production. *F. hepatica* has also shown to be an increasingly important zoonotic disease, especially in the Andean regions. Even though it is endemic in Mendoza province, reports are very scarce. The department of Tupungato has valleys which are suitable for the development of *F. hepatica* cycle. Tupungato abattoir slaughters exclusively animals from its department, enabling thus to trace the origin of the animals. Our goal was to quantify at the slaughterhouse the amount of livers with *F. hepatica* which would be a direct indicator of the importance of the disease in the region. All bovine livers were inspected by the veterinarian, condemning those which presented lesions due to the presence of *F. hepatica* flukes. From June 2002 until August 2005, 653 cattle were slaughtered, of which 441 (67.5%), had hepatic fasciolosis. This prevalence of fasciolosis in slaughtered bovines is amongst the highest in Argentina, which indicates that it is a relevant disease in Tupungato and that cattle are an important reservoir. Control measures and further studies should be implemented.

**93. WATER CONDUCTIVITY, pH, HARDNESS, AND TOTAL IONS IN NATURAL BIOTOPES OF LYMNAEID MOLLUSCS IN MENDOZA, ARGENTINA**

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Lymnaeid molluscs are the intermediate hosts of the trematode *Fasciola hepatica* which causes fasciolosis, a parasitic disease that affects many mammals including humans. Its distribution is determined by biotic and abiotic factors, neither of which has been well described for the province of Mendoza. The objective of this work is to describe the water parameters related to the presence of lymnaeid molluscs in the endemic zones of fasciolosis. The river basins of Mendoza, Tunuyán, Diamante and Llanquanelo were prospected. Water temperature was recorded and the following parameters measured in the laboratory: pH, conductivity, and total hardness. Lymnaeid snails were found in 13 sites of 43 studied. In sites with presence of lymnaeids, the following parameters were found: T° 6-17 °C ( $\mu$ 12,12); pH 6,26-8,22 ( $\mu$ 7,27); conductivity 96-721 m $\Omega$  ( $\mu$ 314,4); total hardness 5,6-32,5 °French ( $\mu$ 15,6); total ions 132-644 mg/L ( $\mu$ 353). In sites where lymnaeids were absent the following parameters were found: T° 1-27 °C ( $\mu$ 13,13); pH 6,29-8,8 ( $\mu$ 7,24); conductivity 726-4290 m $\Omega$  ( $\mu$ 1186); total hardness 33-125 °French ( $\mu$ 46,6); total ions 173-3640 mg/L ( $\mu$ 937,2). In the sites sampled of the province of Mendoza, lymnaeid snails dwell in waters which are soft, with low conductivity and ion concentration, being absent in waters which are hard, with high conductivity and ion concentration.

**94. IDENTIFICATION OF HUMAN PAPILLOMA VIRUS (HPV) IN ORAL SQUAMOUS CELL CARCINOMAS BY TWO DIFFERENT TECHNIQUES**

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Oral cancer is the result of an interaction of physical, chemical and microbial factors. Human papilloma virus (HPV) plays an important role. A probable differentiation was assessed between immunohistochemistry and PCR techniques identifying HPV in oral squamous cell carcinomas (SCC) and their viral types associated with low and high malignancy. Immunohistochemistry peroxidase-antiperoxidase (PAP), mouse monoclonal antibody against HPV types for 6,11 and 18 types (in block) and 18 (isolated), NOVO CASTRA (a kit lacking antibodies against HPV16 serotype epitope) were used to study 16 oral SCC samples. With PCR, oligonucleotides My09/My11 as exterior and Gp5/Gp6 as internal primers. with genotyping SSCP (single strand conformation polymorphism) were also performed. results with PAP: HPV+= 6/16= 37.5%; DNAHPV18+=2, and with PCR: HPV+=11/16= 68.8%; viral types: HPV16=6( 54.5%); HPV11=3 (273%); HPV6= 1 (9.1%) and coinfection with 16/18/= 1 (9%). The application of both techniques in our series of oral squamous cell carcinomas led us to support (as the international bibliography) the convenience of PCR as a sensible technique in this field, which also allows to discriminate between the different viral types of low and high risk. Immunohistochemistry is very useful for screening and does not require a high complexity laboratory. It should be noted that we did not use serotype HPV16.

*Technician: Silvia Del Viso.*

**95. DIFFERENTIAL INVASIVITY OF ALIEN PLANTS AT LOWLANDS AND UPLANDS OF THE RÍO SECO REGION (ARGENTINA)**

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Characteristics of alien plants related to the invasive behavior at lowlands and uplands of the Río Seco (San Luis, Argentina) were studied. Floristic data were obtained from detailed inventories of the taxa, paying special attention to exotic species. The relationships between invasive behavior and the different autoecological and other variables, were analyzed by cross-tabulation, using Chi square and Fisher's exact test. Variables related to invasive behavior at the habitat level were included in a logistic regression analyses. We found that 41% of the species in the total area were exotic. The best model for uplands ( $p < 0,001$ ) included: family, origin, flowering time, fruit time and germination time (79.8% of the non invasive species and 84.3% of the invader correctly classified). For lowlands, the best model ( $p < 0,002$ ) included origin and family (76% of the invaders correctly classified). The percent of non invasives correctly classified was low (56.2%). In summary, several environmental factors interact, at different scales, in the river region, originating appropriate conditions for the installation of alien plants. The knowledge of variables related to the invasive behavior at uplands and lowlands would allow the prediction of possible dangerous species on each environment. They should be not included in the planning of vegetated areas.

**96. EFFECT OF NICKEL CHLORIDE ON MOUSE SPERM CAPACITATION *IN VITRO***

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Capacitation is a process which sperm must undergo to acquire fertilization ability inside the female genital tract. Protein tyrosine phosphorylation (p-Y) was selected as a marker of sperm capacitation. This process *in vitro* depends on two main factors: culture media composition (presence of bovine serum albumine [BSA] and bicarbonate) and incubation time (90 minutes). NiCl<sub>2</sub>, a Ca<sup>2+</sup> channel blocker, was added to the capacitation media to evaluate its possible influence on mouse sperm p-Y. Concentrations from 0 to 0.9 mmol/L of NiCl<sub>2</sub>, either in the presence or absence of BSA, bicarbonate and different incubation periods were tested. Motility and vitality were also checked to avoid possible toxic effects on sperm. The optimal NiCl<sub>2</sub> concentration able to obtain proteins p-Y in mouse sperm in culture media lacking BSA and bicarbonate was 0.35 mM. On the other hand, in capacitating media, the presence of 0.35 mM of Cl<sub>2</sub>Ni leads to obtaining p-Y after 90 min (complete) or 30 min (incomplete) capacitation period. Under our working conditions, motility and vitality were not affected. These preliminary results suggest that the blockade of Ca<sup>2+</sup> channels by NiCl<sub>2</sub> promotes protein p-Y in the absence of BSA or bicarbonate in 30 min. The role of intracellular Ca<sup>2+</sup> during capacitation remains unclear.

**97. APOPTOSIS DURING THE FIRST WAVE OF SPERMATOGENESIS IN THE PUBERTAL RAT**

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During the first wave of spermatogenesis, frequent degenerated spermatocytes has been observed in the pubertal rat, when the blood testis barrier (BTB) has not yet been established. The aims of this work were to confirm if the process of cell death is actually apoptosis, by the TUNEL reaction (terminal deoxynucleotidyl transferase – mediated dUTP nick end labeling), and to identify these spermatocytes using transmission electron microscopy. Testes of Wistar rats from 15 to 17 days were used. TUNEL assay on paraffin embedded testicular sections was done using the color reaction of peroxidase with diaminobenzidine. In this period, only segments of the cords with the more advanced epithelium showed a positive reaction, as was indicated by nuclear diameter and chromatin arrangement in the spermatocytes. Additionally, samples were conventionally processed for electron microscopy and observed in a Zeiss 900 equipment. Cells in an apoptosis process were zygotene and paquitene spermatocytes, which could be identified by the presence of synaptonemal complexes into degenerated cellular debris. Thus, we can conclude that in some of the cords, when zygotene and paquitene spermatocytes appear with BTB still leaky, they degenerate by apoptosis.

**98. GERMINATION SEPARATED FROM *Lippia turbinata* GRISEB. (“POLEO”) UNDER TWO LABORATORY CONDITIONS**

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The “poleo” *Lippia turbinata* presents “secret benefits” in the field of the phytomedicine and the nourishing industry. Diverse studies demonstrate that the germinative power (PG) in poleo and other native species is relatively low. In these species the *in vitro* germination with special conditions and way of culture has had major success. These results might answer to the lack of substances of reserve for the embryo and/or to ecological factors that affect the germination. Essays were carried out by seeds (mericarpus) of poleo of 14 months of age. They were made to germinate in two different laboratory conditions: 1-conventional germination with the Standard Test and 2- *in vitro* germination, with means N6 and N6<sub>50</sub>%. The PG obtained in the essays was: 26.1% for the Standard Test; 31.25% and 44,08% in the *in vitro* germination with the means N6 and N6<sub>50</sub>% respectively. These results would indicate that the lack of reserves (related to the size) plays an important role in the viability and the germination of these seeds, which might imply the need of other organisms of the soil that help to their absorption of nutrients.

**99. THE CEREBRAL GANGLIA OF *Pomacea canaliculata* (CAENOGASTROPODA, AMPULLARIIDAE)**

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The cerebral ganglia are flattened paired structures located at the base of tentacles in the peripharyngeal sinus. Both ganglia are joined dorsally by a ribbon-like intercerebral commissure, and they are connected anteriorly with the buccal ganglia, and posteriorly with both the pedopleural ganglia and the visceral ganglia. Nerves of varying thickness, presumably composed of both afferent and efferent fibers, enter/leave the ganglionic borders, while a prominent tentacular nerve enters/leaves at the center of their dorsolateral aspect. The nerves originating on the ganglionic borders include the optic and otic nerves (on the posterior border) and the labial nerves (on the anterior border). Most neurons in the cerebral ganglia are located both in the ganglionic cortex and surrounding ependymal canaliculi that penetrate the ganglionic medulla. No hemolymphatic vessels penetrate the ganglia. Cortical neurons are either large polyhedral cells, with peripheral Nissl bodies and clear nucleolated nuclei, or small, elongated and heavily basophilic cells located mainly around the nerves origin. The ependymal canaliculi are surrounded by small polyhedral neurons, with nuclei close to the ependymal lumen. Also, some large pyriform neurons are seen either isolated or in small groups in the ganglionic medulla. Because of their neuronal composition and connections we consider the cerebral ganglia (together with the pleural ganglia) as the first order analyzer-integrator systems of incoming environmental information.

**100. LEPTIN, INSULIN RESISTANCE AND ATHEROGENIC RISK WITH THE BODY MASS INDEX IN APPARENTLY HEALTHY ADULT MEN AND WOMEN**

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Obesity is related to the metabolic syndrome and it is an important health problem. We aimed at evaluating the clinical and metabolic risk factors in 90 asymptomatic and apparently healthy men and women aged between 30-40 years old, using antropometric and biochemical serum data. The BMI was for overweight (OW): 25-29.9 and for obese (O) > 30 kg/m<sup>2</sup>. Homeostatic model assesment (HOMA) > 2.5 estimated the insulin resistance (IR). Leptin was measured by IRMA, insulin by chemiluminiscence, and glucose, lipids and lipoproteins by Wiener kits. In both sexes, 60% of O had insulin and leptin resistance, and 25% of OW had IR. Leptin showed sexual dimorfism. Compared to normal weight, the OW women showed an insulin - triglyceride/HDLc correlation (r= -0.7136, p<0.003) and in OW men an increase of LDLc and TG/HDLc, and a leptin - insulin correlation (r= 0.5226, p<0.01). O men with IR showed a lipid profile of atherogenic risk: increased cholesterol, LDLc, TG, TG/HDLc and cholesterol/HDLc, and a decreased HDLc. They also showed correlation of leptin with cholesterol/HDLc (r= 0.9078, p<0.002) and with TG/HDLc (r= 0.6934, p<0.05). Women and men with IR are still asymptomatic but present alterations of biochemical parameters, particularly the males, which are characteristic of the metabolic syndrome. The results suggest that TG/HDLc and cholesterol/HDLc ratios could predict IR in asymptomatic 30-40-year old subjects.

**101. ISOLATION OF MICROORGANISMS OF THE RHIZOSPHERE OF *Prosopis caldenia* BURK**

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With the purpose of studying the interaction among *Prosopis caldenia* (caldén) and microorganisms that promote the formation of roots *in vitro*, bacteria of the caldén rhizosphere were isolated. This objective is sustained in the application of metabolic basic principles that happening in the ground, among the different microorganism, microbial associations, interactions biotic-abiotic, direct interactions and insinuations between the microorganisms and the plants, the presence of bacteria in the area of the rhizosphere causes changes in the form and function of the roots. The modified Winogradsky and other modern methods were used. The search is guided to nitrogen-fixing bacteria and phosphorus bacteria. *Bacillus megaterium* has been isolated, with an accompanying of *Pseudomonas* and aerobic nitrogen-fixing bacteria, *Azotobacter*. In the future, these bacteria will be inoculated in caldén tissue culture.

**102. COMPARATIVE STUDY OF QUERCETIN TRANSDERMAL PERMEATION *IN VITRO***

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Dermal and transdermal distribution in skin is used for drug administration in systemic and localized treatment. Quercetin transdermal assays in carbopol gel (CG) through pig ear and abdomen skin were performed. Experiments were carried out using an automatic sampler Microtte with Franz cells. A spectrophotometric method was employed for quercetin quantification. Membrane thickness ( $\Delta X$ ), flux values ( $J_m$ ), and permeation (P) and diffusion (D) coefficients are reported:

Membrane	$\Delta X \times 10^2$ (cm)	$J_m \times 10^7$ (g/cm <sup>2</sup> .s)	$P \times 10^6$ (cm/s)	$D \times 10^7$ (cm <sup>2</sup> /s)
Pig ear skin	14.11	1.484	6.670	9.407
Pig abdomen skin	8.247	0.7482	3.363	2.773

Since  $J_m$ , P and D were higher for pig ear skin than for pig abdomen skin, the former zone is better suited for transdermal permeation of quercetin in carbopol gel.

**103. EVOLUTION OF THE WEIGHT AND SPIKELET QUALITY IN *Trichloris crinita* (Lag.) L.R. PARODI**

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*Trichloris crinita*, a native species of the Argentina semiarid region, used in cattle ranch, is of good palatability and quality of its defered. *T. crinita* is diminishing with processes of degradation range.. The present work evaluated parameters of ripeness of the reproductive parts of *T. crinita*. The essay was performed in INTA San Luis. Twenty one selected ears were cut in seven different dates (every 3-6 days). The state of development of the ears, at the beginning, was a full antesis with colour of green pole ear, with reproductive flower of colour white-yellowish structures. Weight of spikelet/ear, weigh of 1000 spikelets, and percentage of germination were measured. The percent germination was determined incubating spikelets in 10 x 14 cm plastic trays with germination paper in camera of cultiva a 30:10°C and 8:16 h (light-darkness), with a tray for ear. Up to 4 days since full antesis no increase in weight was found and germination was zero, indicating that the caryopsis or seed has not matured. From 7 days it was observed that spikelets increase in weight and germination was 35%. In the period of 13-19 days, the germination is maximal, as is the quality of seed. After this period dissemination or liberation of seed to the environment begins. Thus, the period of maximum quality and production of seed is attained between 13 and 19 days since full antesis.

**104. NOT SO BAD WEEDS OF VILLA MERCEDES, SAN LUIS (ARGENTINA)**

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The knowledge of agronomic engineering students of the Faculty of Engineering & Economic and Social Sciences (UNSL) about the medicinal properties of eight *Solanaceae* species that behave as very common weeds in Villa Mercedes region was surveyed. The aim was to assess the previous information and understanding of this group of students about the medicinal and properties of *Capsicum chacoense* "ají picante," *Cestrum parqui* "plaque," *Datura ferox* "chamico," *Physalis viscosa* "camambú," *Salpichroa origanifolia* "huevito de gallo," *Solanum eleagnifolium* "revienta caballo," *Solanum pseudocapsicum* "ají del monte," and *Solanum sysimbriifolium* "espina colorada". The students were asked about the species they recognized, which of them were considered weeds, which are toxic for human beings or animals. If any of these species was identified as a medicinal plant, they were asked which diseases this plant was used for. To date, with over 50 students surveyed, our results show that only 3 species are widely known as toxic weeds, but most students believe nonetheless that the cited species are medicinal plants. A low proportion of students opined that "chamico" is commonly used for digestive problems, but they do not use it. All of the surveyed students came from urban schools and showed a very poor non-academic previous information about the medicinal properties of these eight *Solanaceae* species.

**105. MORPHOLOGIC AND MICROGRAPHIC STUDIES OF ARGENTINIAN *Flaveria* (ASTERACEAE : HELENIEAE) DRUGS**

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The genus *Flaveria* Juss. comprises 2 species in Argentina: *F. haumanii* Dimitri & Orfila y *F. bidentis* (L.) Kuntze. Both are used in folk medicine because of their diuretic, digestive, emmenagogue and vermifuge properties. They are called by vernacular names "fique", "contrayerba", "balda" or "mata gusanos". This study was carried out because of their folk use in the region. The stems and leaves are collected in natural sites in wich both species coexist; were fixed and conserved in formol: acetic acid: alcohol (1:1:1), paraffin included, cutted and mounted in DPX. The species posses differential characters in the inflorescence, leaves shape and micrographic parameters. The diacritical characters are showed in the following Table.

Character	<i>Flaveria bidentis</i>	<i>Flaveria haumanii</i>
Inflorescence	compact glomerules	lax glomerules
Leaves shape	narrowly elliptic	linear
Stomata number (Ei)	14,36 ± 1.50	7,55 ± 0,51
Stomata number (Es)	5,56 ± 0,50	7,30 ± 0,48
Stomata index (Ei)	15.38 - 20	17.5 - 20
Stomata index (Es)	20 - 22.22	21.87 - 23.33
Palisade ratio	6,80 ± 1,50	8,60 ± 1,80
Vein-islets	11,66 ± 1,50	13,16 ± 2,65
Vein termination	10,80 ± 1,85	14,16 ± 1,45

**106. GENERA OF THE LICHEN FLORA OF PANCANTA VALLEY (PRINGLES DEPT., SAN LUIS PROVINCE, ARGENTINA)**

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As part of a study on the flora of San Luis, the identification of Lichen genera from Pancanta Valley, a rejuvenated penepplain along 200 Km at southwestern side of Pampasic Sierras from central San Luis, was carried out. The climate belongs to the mountain subhumid temperate type, with 600-700 mm of rainfall per year, and faulty hydric balance during the whole year. Grassy meadows, shrubby slopes and scarce copices of evergreen trees (mainly *Lithraea*) make up the current vegetation. The large amount of exposed rocks and the cortex of woody plants are excelent surfaces for lichenization. Thus the lichen communities are noteworthy in the landscape, especially on southeastern exposures. Thirty two genera were were identified, which belong to 19 families of Lichenized Ascomycotina, from ca. 250 *exsiccata* that are preserved in UNSL Herbarium. The major families are Parmeliaceae (with 6 genera), Teloschistaceae (4), Verrucariaceae (3) and Physciaceae (2), while the other ones are represented by one genus each. Surely, these records will be increased with the exploration of some less accessible areas and the pursuit of the taxonomic work.

**107. WATER BIODENITRIFICATION**

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A pilot system was designed and constructed to lower nitrate levels in water by the biodenitrification method, that is, the use of bacteria which utilize for their metabolism nitrate instead of oxygen when maintained in anoxic conditions. The denitrification process involves conversion of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , and this to  $\text{N}_2$ . An air pump was used for circulating water in the circuit, and natural aquarium stones furnished physical support for bacterial colonies to develop. A nitrate solution was placed in the device for 3 days, during which the oxygen present in the culture medium was completely utilized. Then denitrifying bacteria switched to an anaerobic condition. Sucrose was added to the solution as a carbon source, and water flow in the apparatus was started. Nitrate determination was done by IR spectrophotometry and nitrites were measured by a colorimetric method. A sample of 300 mL of  $\text{KNO}_3$  solution of an initial concentration of 120 ppm (77 ppm  $\text{NO}_3^-$ ) was treated during 19 days. Its concentration changed from 77 to 48 ppm  $\text{NO}_3^-$ . This sample was initially free of  $\text{NO}_2^-$ , which reached 3.6 ppm at the end. Thus, there was a conversion of  $\text{NO}_3^-$  to  $\text{N}_2$  of 25.4 ppm (33% of initial  $\text{NO}_3^-$ ). This method showed to be a good alternative for nitrate-contaminated water depuration. This simple and economic pilot system allowed reduction of the contaminant concentration to acceptable levels.

**108. MORPHOANATOMIC ALTERATIONS IN PLANT COMMUNITIES EXPOSED TO FIRE IN SOUTHWESTERN SIERRA OF SAN LUIS, ARGENTINA**

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The urban expansion of San Luis city and neighboring towns has brought serious environmental damages because of the increasing anthropic pressure that is performed on the whole area. The sierran and piedmont natural vegetation is frequently submitted to periodic fires, which cause soil denudation and strong hydric and eolic erosive processes. To evaluate the effects of periodic fires on plants, samples were obtained in burnt and non-burnt sites. The macroscopic morphological anomalies were recorded in the field. The histologic studies were made on fresh or fixed material, conserved in formaldehyde:acetic acid:alcohol (1:1:1), included in paraffin, cut and mounted in DPX. Histochemical tests were made using specific reagents. From the comparison of areas affected by recurrent fires with non-affected ones, the morphoanatomic changes in some species of the affected plant communities, probably as a consequence of fire, were recorded. Fasciation, vegetative proliferation and tannins storage have been the more evident changes detected. These parameters could be diagnostic elements in order to perform an evaluation of the environmental impact of fires in this region.

**109. ANTIBACTERIAL EFFECT OF AN HALOGENATED ANTISEPTIC IN COMMON USE IN HOSPITALS**

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Antimicrobial-resistant microorganisms are a major risk factor in nosocomial infections. As a contribution for an adequate use of germicides, the antibacterial activity of the currently most used halogenated antiseptic was evaluated in the Hospital Central. *Staphylococcus aureus* ATCC6538 as a reference bacterium, and *Pseudomonas aeruginosa* ATCC 27853 as a nosocomial strain, were exposed to the antiseptic according to the procedure indicated by AfNOR T72-150. The germicidal power was evaluated both in presence and absence of organic matter. A negative correlation was found between antiseptic concentration and counts of surviving bacteria. The antiseptic was more effective against the strain of *P. aeruginosa* than to the *S. aureus* strain. The presence of organic matter reduced the germicidal power of the antiseptic. The results underscore the need of validated procedures and continuous monitoring which allow optimal antiseptic use to avoid both toxic reactions and emergence of resistant bacterial strains.

**110. PHENOTYPIC CHARACTERIZATION ATTRIBUTABLE TO THE HSR GENE IN CHILDREN FROM LA RIOJA (ARGENTINA): EXPRESSION BY GENDER**

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The HSR gene (Hand Skill Relative) could be involved in right hand use, brain asymmetry, and cognitive mechanisms in humans. Previously, it was found that its phenotypic expression is modified in one of two regions of primary school children of La Rioja. This work evaluated whether HSR expression might be different according to children gender. As previously, La Rioja was divided into 2 regions (Region 1, n= 351, considered control, and Region 2, n= 253, problem). All children were evaluated for handedness (Edinburgh Inventory); brain asymmetry (Scalp hair-whorl direction), and writing tests. Each trait was analyzed by measuring the ratio of boys to girls ( $R_{MF}$ ), evaluating any deviation from the theoretical ratio of 1:1. Results showed that  $R_{MF}$  for children using the right hand was 0.96 for Region 1 and 1.06 for Region 2. For children not using the right hand,  $R_{MF} = 2.08$  for Region 1 ( $p < 0.05$ ) and 5.0 for Region 2 ( $p < 0.01$ ).  $R_{MF}$  for scalp hair-whorl clockwise direction was 0.95 for Region 1 and 1.26 for Region 2. For non-clockwise direction,  $R_{MF} = 2.08$  for Region 1 and 0.67 for Region 2 ( $p < 0.01$ ). A similar finding was observed for  $R_{MF}$  with writing disturbances. Results suggest that there exists a sexual dimorphism regarding expression of the HSR gene in Region 2.

**111. HANDEDNESS, BRAIN ASYMMETRY, AND WRITING CAPACITY IN CHILDREN FROM 2 REGIONS OF LA RIOJA (ARGENTINA): AN ASSOCIATION ANALYSIS**

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The HSR gene (Hand Skill Relative) putatively located in the chromosome region 2p12-q11, could control hand use, brain lateralization and some cognitive functions, such as writing. This work evaluated whether the phenotypic expression of the gene is modified in one risk region of La Rioja. As previously, La Rioja was divided into 2 regions (Region 1, n= 351, considered control, and Region 2, n= 253, problem). All children were evaluated for handedness (Edinburgh Inventory); brain asymmetry (Scalp hair-whorl direction), and writing tests. Phenotypic proportions were analyzed for (1) handedness and scalp hair-whorl direction; (2) brain asymmetry and writing capacity; (3) handedness and writing capacity. In Region 2, a lower proportion of right-hand users with a clockwise hair whorl direction was found compared with Region 1 (68.9% vs 88.7%;  $p < 0.01$ ); a larger proportion of writing disturbances was found in clockwise hair whorl children (46.6% vs 2.6%;  $p < 0.01$ ), and a greater proportion of writing disturbances was found in right-hand users (43.6% vs 5.1%;  $p < 0.01$ ). Results suggest that in Region 2 the expression attributable to HSR is altered, simultaneously affecting the three assessed traits.

**112. EFFECT OF "ENERGY DRINKS" ON DIFFERENT PHYSICO-CHEMICAL AND BIOCHEMICAL PARAMETERS IN RATS**

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Caffeine, taurine and vitamin-containing drinks have been in the European and American markets for about a decade, and they are widely consumed as so-called "energy drinks". The aim of this study was to investigate the effect of energy drink on several physico-chemical and biochemical parameters in serum, plasma and urine. Wistar rats weighting 200-250g were divided into two groups: experimental (E) and control (C) (n = 10), and deprived of water and food for 18 h prior to the experiments. Treated rats were administered by gavage a volume of 50mL/Kg of energy drink, while the control group received saline solution. The animals were placed in individual metabolism cages. Urine was collected for 6 h and blood was obtained by cardiac puncture under ether anesthesia. The plasma and serum was separated. Significant increases in proteins, glucose, pH, and density in urinalysis, and differences in qualitative characteristics and microscopic analysis were found. Pathologic ammonic-magnesium phosphate crystals were seen, which could be related to the urine alkalinity. No significant changes were observed in creatinine, calcium, sodium, potassium, and alkaline phosphatase in serum or plasma; however the transaminases showed slight increases. The energy drink produced significant modifications in several of the physicochemical and biochemical parameters studied.

**113.**  
**LEVELS OF ORGANOCHLORINES IN *Odonthestes bonariensis* DURING DIFFERENT REPRODUCTIVE STAGES IN LA FLORIDA (SAN LUIS, ARGENTINA)**

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La Florida (33°07'S-66°02'W) is an important water reservoir for the province of San Luis. Environmental pollutants, such as organochlorines (OCs) may affect aquatic wildlife. Recently, we reported the presence in La Florida of OCs in the water and fish. OCs are liposoluble and are accumulated in the fat. Several authors have demonstrated that, during the fish reproductive period, a mobilization and transference of the lipids of the body to eggs and sperm occurs. An interesting problem is whether spawning represents an important route to eliminate OCs. To test this idea we analyzed the OCs concentration in silversides (*O. bonariensis*) captured in La Florida at different reproductive stages: before, during and after the reproductive period. OCs were assayed by GC-ECD. We detected 16 OCs (ng/g lipid), divided in 4 family: 1) HCHs (A:1079,7-B:1184,8-D:747,7):  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  HCH isomers; 2) DDTs (A:3387,5-B:2408,3-D:2928,3): DDE, DDD, DDT and Methoxychlor; and cyclodiene groups 3a) CHLs (A:802,8-B:862,3-D:541,0): Chlordanes and Heptachlors; 3b) ALDs: (A:1207,9-B:1451,5-D:880,2). No significant differences among reproductive stages were detected (ANOVA,  $p > 0,05$ ). This fact may suggest that spawning does not represent an important route of elimination the OCs in the silverside.

Supported by CyT-UNSL 22/Q451 to ECV.

**114.**  
**EFFECT OF FREEZING METHOD AND PROTECTANTS ON VIABILITY OF THE BIOCONTROL BACTERIUM *Rahnella aquatilis***

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Biological control using microbial antagonists is an alternative to chemical methods of controlling pre- and post-harvest plant pathogens. To be of practical use, microbial agents must be formulated as products capable of being stored. Freezing is one of methods of preserving bacteria. In a previous work Calvo et al. showed the antagonistic activity of *Rahnella aquatilis* against the pathogens *Penicillium expansum* and *Botrytis cinerea*. The objective of this study was to compare the effect of freezing and the additions of a range of additives in combinations with powdered skimmed milk as freezing protectants for preserving the viability of *R. aquatilis*. Bacterial cells were produced in YGM broth. Dilutions of this suspension were plated in duplicate onto the surface of YGM media in Petri plates before and after freezing. The number of colony forming units per milliliter were counted (CFU/mL) and percentages of survival were estimated. Statistical significance was set at  $p=0.05$ . Freezing at  $-20^{\circ}\text{C}$  was a good method to preserve the viability of *R. aquatilis* using 10% skim milk (1.2% survival). Survival of *R. aquatilis* was increased by using appropriate protective media containing combinations of skim milk and others such as 10% galactose or glucose (4.0% and 3.5% survival) and 1% of  $\text{Ca}^{2+}$  (2.5% survival).

The effects of the freezing method with the use or not of protective agents on the viability of the biocontrol bacterium *Rahnella aquatilis* were studied.

**115.**  
**COMPARATIVE ANALYSIS OF THE FERTILIZATION ON THE GRASSLANDS IN TWO DIFFERENT MOMENTS**

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The mixed grasslands are characteristics in San Luis' province, with important species as *Poa ligularis*, *Piptochaetium napostaense*, *Schyzachiryum plumigerum*, *Chloris retusa*, and others of low or null importance like *Elyonurus muticus*, *Panicum urvilleanum* and *Stipa tenuissima*. Being dry matter production (DM) one of the limiting processes for the use of the grasslands in different production systems, two experiments were carried out of application with urea in two different moments, namely in early spring and in early autumn. These experiments were carried out in Nueva Escocia (San Luis, Argentina). Although the doses were different (the used fertilizer was urea) and also the applied techniques for the fertilization, the results indicated that there is a significant response in the production of DM. It is important to keep in mind which group of species benefits, since for the nitrogen application with the increment of DM production not all of them are used for livestock feeding.

**116.**  
**DRY MATTER PRODUCTION OF FERTILIZED TALL WHEATGRASS (*Elytrigia elongata*)**

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Characteristic of Tall wheatgrass are its capacity to establish itself in degraded soils and its adaptability to prevailing climatic conditions. For this reason, an experiment was carried out with three nitrogen levels, to evaluate the dry matter production (DM) of this pasture, which had never been fertilized. The levels were 0, 50 and 78 kg N/ha. The evaluation was carried out monthly from the end of spring ends until the end of the next winter. The results demonstrated that there are significant differences among treatments and among the monthly cuts. This would allow, for example, to increase the stocking rate of the systems in those where the Tall wheatgrass is used for the feeding of the livestock, keeping in mind the need of periods of rest adapted to avoid the death of the plants.

**117. IMPRINT OF EPIDERMIS, A SIMPLE METHOD FOR STOMATA OBSERVATION**

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Stomata are structures for gas exchange, found in the epidermis of all plants. In most plants they open at dawn and close in darkness, allowing the entrance of carbon dioxide, which is utilized for photosynthesis. This work tested a simple technique for stomata observation in *Vitis vinifera* epidermis. Sections of leaves fixed in FAA during a week were utilized. Imprints were obtained spreading a film of colorless enamel over the abaxial face and allowing the surface to dry for 10 min. Then they were removed with an histological tweezer and mounted on a slide. They were observed in a light microscope at 200x for counting the number of open stomata, partially open and closed in each sample. A stomatal conductance index was produced, considering 1 the conductance in a totally-open stoma; 0.5 the conductance in a partially open stoma, and 0 in a closed stoma. Thus  $SCI = (n^{\circ}Os * 1) + (n^{\circ}Poa * 0.5) + (n^{\circ}Ce * 0)$ , where SCI = stomatal conductance index,  $n^{\circ}Os$  = number of open stomata;  $n^{\circ}Poa$  = number of partially open stomata, and  $n^{\circ}Ce$  = number of close stomata. The values of this index were correlated with measurements of stoma conductance (Sc) obtained with a porometer before extracting the samples. The good correlation between SCI and Sc indicates that the observation of stomata in vine imprint is a simple and effective method to measure the degree of stomatal opening.

**118. EFFECT OF LOW TEMPERATURES ON GERMINATION OF THREE *Atriplex* SPECIES**

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Temperature is one abiotic factor regulating germination and establishment of seedlings. We evaluated the effect of low temperatures on germination of three species of fodder shrubs for arid and semi-arid zones: *Atriplex crenatifolia*, *A. argentina* y *A. nummularia*. Scarified seeds (n = 25) were planted on germination paper in Petri dishes in a random design with 4 repetitions. They were incubated in the dark during 30 days, at either 5°C or 25°C (control). The number of germinated seeds was recorded daily, and final percentage of germination (PG) and emergency speed rate were calculated. An ANOVA and DMS Fisher's test were applied for analysis. Significant differences were found between temperatures, species and the interaction species-temperatures. *A. argentina* germinated less at low temperatures (58%), whereas at 25°C the PG was 80%. *A. nummularia* showed a PG of 73% at 5°C vs 90% at 25°C. *A. crenatifolia* germinated the most, without a temperature difference, 96 and 98% at 5°C and 25°C respectively. The emergency speed rates for all species were significantly lower at 5°C. The results show that, even at low temperatures, these species show good germination rates, which makes them – and specially *A. crenatifolia* – apt to be planted in periods of low temperature.

**119. TREATMENT WITH RAS COMPETITORS DURING PREGNANCY AFFECTS ANGIOTENSIN II RECEPTOR EXPRESSION IN NEWBORN RATS**

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Angiotensin II (Ang II) is the main effector peptide of the renin – angiotensin system (RAS). Recently, Ang II receptors have been related to a potential role in growth and cellular proliferation. The aim of the present study was to analyze by RT-PCR the expression of Ang II receptors during hindbrain development in the offspring of pregnant rats treated with Ang II antagonist or ACE inhibitors. Treatment was performed during the last week of pregnancy with vehicle (control), Ang II, losartan (AT<sub>1</sub> antagonist) PD 123139 (AT<sub>2</sub> antagonist), or angiotensin-converting enzyme inhibitors (enalapril and captopril). Pups at first (PND0) and eighth (PND8) post-natal day were analyzed. For both developmental stages, PND0 and PND8, AT<sub>1</sub> receptor expression was lower in treated than in control animals. mRNA for AT<sub>2</sub> was detected both in PND0 and PND8. At PND8, AT<sub>2</sub> receptor expression was lower in animals treated with ACE inhibitors than in control animals. However, a higher expression was observed in animals treated with Ang II or its competitors compared with controls. These results correlate with previously found changes in the binding pattern observed by autoradiography. Altogether, present results suggest that treatment of mothers during late pregnancy affects AT<sub>1</sub> and AT<sub>2</sub> receptor expression in their offspring.

**120. β-CYCLODEXTRINE INFLUENCE ON MEBENDAZOLE POLYMORPHOUS A AND C SOLUBILITY**

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Mebendazole is a benzylimidazolic derived wide spectrum anti-helminthic, used for parasite infections. This drug has three polymorphous A, B, and C, hardly soluble in water. While polymorphous C (active) is a lesser component, polymorphous A (non active) is often found in commercial products. Taking in account that cyclodextrines can modify diverse drug physicochemical properties, it was tested whether cyclodextrines have a positive effect on mebendazole solubility (an important biopharmaceutical parameter) through complex formation. A 25°C thermostated stirrer during 72 h was used to favor complex formation and their solubility. In order to measure the recovered dissolved amount, a high performance Gilson liquid chromatograph equipped with a constant 25°C temperature oven, an UV detector by diode arrangement at 247 nm and mobile phase A system: Buffer phosphate 0.05M pH5.5, mobile phase B: methanol: acetonitrile (3:2), 50% A and 50% B, and as HPLC column 30cm x 4.6mm, 5 μm C18, were used. To assess results, a calibration curve was prepared for polymorphous A and polymorphous C in 0.1N HCl methanol solution. A significantly increased water solubility was found for both the polymorphous A and C in the presence of increasing amounts of β-cyclodextrine (β-CD), the increase being larger for polymorphous C than for polymorphous A.



**121. VALIDATION OF THE OSTEOPOROSIS SELF-ASSESSMENT TOOL (OST) IN POSTMENOPAUSAL WOMEN OF MENDOZA: A PILOT STUDY**

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The osteoporosis self-assessment tool (OST) score is calculated as body weight (kg) minus age (yr); the result is multiplied by 0.2 and rounded to the closest digit. The OST is a simple screening test for risk of low bone mass, developed in Asiatic women and later validated in European white women. In these women, low risk is indicated by a score of 2 or more, medium risk by scores from 1 to -3, and high risk by a score of -4 or less. Validation studies have not been performed in our region. Therefore, the OST score of 450 postmenopausal women, whose bone mineral density (BMD) had been measured by DXA (Lunar Prodigy) at the lumbar spine and hip, was calculated. Values found (mean  $\pm$  SEM): age, 61.3  $\pm$  0.4 yr; body weight, 66.8  $\pm$  0.5 kg; lumbar spine BMD, 1.040  $\pm$  0.008 g/cm<sup>2</sup>; femoral neck BMD, 0.833  $\pm$  0.006 g/cm<sup>2</sup>. Low BMD at either or both sites was found in 350 women (spine only = 35; femoral neck only = 88). All women with a score of -4 or less (n = 23), and most women (93%) with 1 to -3 had low BMD in at least one site. However, this was also the case for 56% of women (108/192) with scores of 2 or more. Present results suggest that the upper cut-off of the intermediate risk score is too low for the studied population.

**122. GLUTAMIC ACID AS A TRIGGER MOLECULE OF ACROSOMAL REACTION (AR) IN MOUSE SPERM: RECEPTORS AND PRESENCE AT THE FALLOPIAN TUBE**

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Glutamic acid (Glu) is an excitatory neurotransmitter acting at the central nervous system (CNS). Its receptors (GluRcs) have not been thoroughly described in sperms, but specific areas of the CNS present two types of GluRcs. Curiously, we have previously reported actions of Glu on sperm cells probably acting through both types of Rcs: ionotropic (NMDARc, AMPARc, Kainic AcidRc) activating ion channels, and metabotropic (mGluRc 1-8), which act by means of protein G. To detect both kind of receptors, immunoblots were performed using homogenates of mouse sperm and rat cerebral cortex and hippocampus. The presence of mGlu2/3Rc, mGlu5/1Rc and NMDAR-2CRc in both preparations and Re-NMDA-NR1 only in the CNS was established. The acrosomal reaction is carried out by sperm cell at the fertilization zone, the oviduct. This prompted us to check if Glu is uptaken and released by the Fallopian tube. We found <sup>3</sup>H-Glu uptake and release in the ampullary portion of the oviduct of female mice (where the fertilization process takes place). The results presented here suggest that Glu acts through receptors similar to those found in the CNS, and is present at the fertilization zone.

**123. INCIDENCE OF GRAPEVINE LEAFROLL ASSOCIATED VIRUS 2 (GLRAV-2) IN CABERNET SAUVIGNON VINEYARDS IN MENDOZA, ARGENTINA**

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Clones of Cabernet Sauvignon mattered of France, free of virus infected with GLRAV-2, they show decline and death from 3<sup>rd</sup> year of vegetation. Plants affected by GLRAV-2 show a potential total water pre sunrise and noon more negative than the healthy ones. GLRAV-2 is transmissible by sap to *Nicotiana benthamiana*. To confirm the etiology of the disease, incidence in vineyards and clones, to compare the hydric state of healthy and ill plants. Sampling to field, multiplication of ill and healthy ligneous material in camera and greenhouse, mechanical inoculation of extracts concentrated by ultra centrifugation on *Nicotiana benthamiana*. Leaves with winding were made a will by DAS - ELISA. *Nicotiana benthamiana* did not show symptoms. Tests of total water measurement were made potential pre sunrise and noon. The infection detected by clone was: 191 and 337/Gravesac with 3% and 7%, 191 and 341/101-14 with 3% and 10%, 337/3309 with 7%, 191 and 337/RGM with 3% and 169/1103P with 13%. In parcels at random 337/Gravesac with 83%, 191/RGM 75% and 341/101-14 with 50%. There is significant statistical total water difference potential between ill plants and healthy for 337/Gravesac in pre sunrise but does not to 191/RGM. The presence of GLRAV-2 in vineyards of Cabernet Sauvignon de Mendoza, Argentina is confirmed.

**124. STORAGE OF *Helicobacter pylori* BY CYANOBACTERIAL EXTRACT AT LOW TEMPERATURE**

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*Helicobacter pylori* is a microaerophilic, gram-negative spiral bacterium that is currently recognized as a pathogenic microorganism; it is associated with peptic ulcer, malignant lymphoma, and gastric cancer. There is continued interest in morphological, physiological and genetic studies in order to better characterize the prevalence of infection, as well as the natural history and transmission mode of the infection. One problem has been the long term storage of *H. pylori* strains. For this reason we evaluated the feasibility of using a cyanobacterial extract (CE) to conserve *H. pylori* at low temperature. Three *H. pylori* strains were stored in CE plus 20% glycerol (CE-20%) and broth tripticase soy plus 20% glycerol (BTS-20%, standard medium), at -80°C for six months. The recuperation index (IR = ufc/ml recovered / ufc/ml initial inoculum X 100) was used to evaluate the ability of the CE to maintain viability of *H. pylori* strains. Our results showed an important increase of recovered cells with the CE-20%. The RIs for CE-20% were 100%, 71.4% and 26.6%; while, the RIs for BTS-20% were 66.6%, 8.57% and 3.6%, respectively. We concluded that the CE can successfully replace, the standard storage medium for the conservation of *H. pylori* strains at low temperatures. Although we observed variations in the IR between the strains stored with CE-20% or BTS-20%, this can be attributed to different abilities of the strains to survive under these conditions.

125.

**MICROPROPAGATION OF *Saintpaulia ionantha* WEND**

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The african violet (*Saintpaulia ionantha* Wend.) is characterized by high *in vitro* regeneration capacity. It is a plant of ornamental value and high cost. The present work was done to analyze the production of adventitious shoots and roots *in vitro* starting from leaves, using standard culture medium with hormonal combination. This response was quantified in the different micropropagation stages. The following parameters were evaluated: number of buds and roots formed *in vitro*, and formation of callus. The appropriate sterilization method was determined and the highest multiplication rate, with significant differences, was achieved in Murashighe-Skoog medium with 4-naphthaleneacetic acid (0.1 mg/L). Determining the multiplication rate *in vitro* allows to specify the feasibility of its production in commercial scale.

126.

**PRODUCTION AND QUALITY OF *Bothriochloa bladhii* SEED WITH FERTILIZATION**Terenti O<sup>1,2</sup>, Bellezze P<sup>2</sup>, Nievas M<sup>2</sup>, Rossi R<sup>2</sup>.<sup>1</sup>EEA, SAN LUIS INTA; <sup>2</sup>FICES, Univ Nac San Luis, Villa Mercedes. E-mail: oaterenti@sanluis.inta.gov.ar

*Bothriochloa bladhii* cv. Bill Dhall. is a perennial poaceae, growing in spring and summer. A native to India, China and SW Asia, it was introduced to the U.S.A. and improved there. This work assessed the effect of different nitrogenous and phosphorated fertilizations in the production and quality of the seed. The test was carried out in EEA San Luis (INTA), in the typical Ustisament soil. The block design was random, with 3 repetitions and 8 treatments, combining different doses of urea as nitrogen source, and diammonium phosphate as a phosphate and nitrogen source. The germination was carried out in high impact polystyrene 400-mL trays, with caryopses. Seeds were incubated in germination chambers at 30°C and 8-h light, and at 20°C and 16 h darkness, counting on the 17th and 14th days. Seed were manually harvested and their yield and quality were determined. Statistical analysis was performed with SAS software. No important differences were observed in percent germination with a rank of 83 at 90%. The weight of seed was higher in both the control and with 50 kg/ha of urea and diammonium phosphate. The largest production of seeds and caryopses was seen in the control and in samples treated with 200 kg/ha of urea. Under present test conditions, fertilization is not necessary to improve seed yield or quality.

127.

**PRODUCTION AND QUALITY OF PERENNIAL FODDER MAIZE SEED WITH FERTILIZATION**Terenti O<sup>1,2</sup>, Llobel D<sup>2</sup>, Risio L<sup>2</sup>, Perlo V<sup>2</sup>.<sup>1</sup>EEA, SAN LUIS INTA; <sup>2</sup>FICES, Univ Nac San Luis, Villa Mercedes. E-mail: oaterenti@sanluis.inta.gov.ar

*Tripsacum dactyloides* "Perennial Fodder Maize" is a species of monoecious diclinous multicaules grassy perennials and reaches 2 and 3 meters in height. The inflorescences are fascicled endings over the rachis. The distal portion is provided with male ears and the basal part with female ears. This work evaluated the effect of different nitrogenous and phosphorated fertilizations in the production and quality of the seed. Thirteen growing fertilization treatments combined with urea and diammonium phosphate ones were performed in field with a random block design and 3 repetitions. The seed were manually harvested and their quality determined. The germination was carried out in high impact polystyrene 400-mL trays, with caryopses. Seeds were incubated in germination chambers at 30°C, 8-h light and at 20°C, 16 h darkness, with countings on every 7th day for 28 days. Germinating power and viability (with tetrazolium technique) were determined. Statistical analysis was performed with SAS software. The weight of 1,000 caryopses was 366 mg with 250 kg/ha of urea and 30 kg/ha of diammonium phosphate. The yield of the seed in the core was 360 kg/ha with a 10% germination and 90% dormancy (viability germination difference by tetrazolium). The biggest yields of 690 to 830 kg/ha were obtained in a rank from 100 to 250 kg/ha of urea with 30 kg/ha of diammonium phosphate, with no significant differences among them. Thus, good yields of quality seed are obtained with 100 kg/ha of urea and 30 kg/ha of diammonium phosphate.

128.

**EFFECT OF DIFFERENT SUBSTRATES ON THE GERMINATION OF *Tetrachne degrei* NEES**Terenti O<sup>1,2</sup>, Risio L<sup>2</sup>, Llobell D<sup>2</sup>.<sup>1</sup>EEA, SAN LUIS INTA; <sup>2</sup>FICES, Univ Nac San Luis, Villa Mercedes. E-mail: oaterenti@sanluis.inta.gov.ar

*Tetrachne degrei* Nees is a poaceae specie, perennial fodder, of summer growth, which sprouts again in September- October, growing until the end of March-April. It is native to South Africa; introduced to INTA three decades ago, which keeps good fodder quality during winter. This work evaluated the effect of different substrates over germination. The germination was carried out in high impact polystyrene 400-mL trays, with 50 caryopses per tray and 3 repetitions in a completely random design. Seeds were incubated in germination chambers at 30°C and 8 h light, and at 20°C and 16 h darkness, with countings every 7th day, for 28 days. The length of the leaves and roots, and fresh and dry weight of plantule were determined. Statistical analysis was performed with SAS software. Good sandy soil typical Ustortent was enriched in the treatment as follows: 1, 10% of womb component of cow (LV); 2, 20% LV; 3, 30% LV; 4, 40% LV; 5, 50% LV; 6, paper core; 7, sandy soil; 8, potassium nitrate soil (2 g/L); 9, Soil with eq. to 5,208 Tn/ha of stubble; 10, soil with eq. to 10.416 Tn/ha of stubble; 11,12,13,14,15 paper with 1 to 5 g of lemna and ground palea, respectively. The weight of one thousand caryopses was of 0.3351 ± 0.0265; the treatments with lemna and ground paleas did not inhibit germination. Main significant differences favored the potassium nitrate treatment and mixtures with womb components, in both germination and length of leaves and roots, with high correlation between fresh and dry weight of plantule.

**129.**  
**EFFECT OF MARINE ALGAE AND MYCORRHIZAS ON INTA IMPROVED *Digitaria eriantha* CV.**

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*Digitaria eriantha* cv. INTA Improved is a perennial species native to South Africa and made known by EEA- San Luis INTA since 1991. It adapts to the Argentine semiarid region with good behavior both in productivity and in drought resistance. It is of summer growth, sprouting again in October and displaying vegetative growth until the first frosts of April. It presents implantation problems due to its low initial growth rate. Both marine algae and mycorrhizas improve seed germination and play a role in a faster implantation and more efficient nutrient use. This work assessed the effect of marine algae and mycorrhizas on germination and initial growth of the plantule. The seed was placed in 10 x 14 cm plastic trays with germination paper in cultivation chamber at 30:20°C and 8:16 h (light-darkness) with 0.25 g per tray, with 4 repetitions in a random design. Four marine algae and 4 mycorrhizal concentrations and a control were tested. The test lasted 28 days with checks every 7 days. Statistical analysis was performed with SAS software. The height of the leaf plant was statistically higher with low concentrations of mycorrhizas, than with marine algae treatments. The number of germinated seeds and fresh and dry weight of plantule was significantly superior to control and marine algae treatments. Thus, mycorrhizas promote the germination and initial development of plantule.

**130.**  
**DENTAL PROSTHESES AND THEIR INCIDENCE IN SALIVARY pH**

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Oral rehabilitation contributes to re-establish a normal masticatory function. Prostheses are foreign bodies, which can influence the modification of the saliva medium. During 2003 and 2004, 120 patients were selected and divided into three categories, 40 for PPR, 40 for PPF and 40 for PC. In each study protocol accurate age, sex and occupation, kind of diet and any medication (type and dose) were recorded. Saliva samples were collected in sterile assay tubes, individualized for each patient. Determinations were simultaneously performed in their mouths with pH indicator strips and in the tubes with a pH meter. Records were carried out previous to installation, a week later, a month later and twelve months after installation. Results. In PPR, no change in pH values was found in 20 cobalt chrome prostheses; 14 out of the 20 which were made of acrylic presented a slight pH increase (0.2 to 0.4) which was sustained in later controls (6 patients were lost in follow up) Out of the 40 patients with PC, 31 completed the follow up; pH was increased between 0.2 and 0.6 units. No change was observed in patients with PPF. Most patients showed changes of habits; therefore we can conclude that for the present sample, dental prostheses did not have a significant influence in the pH values of the mouth medium.

**131.**  
**SPRAYED ABSICISIC ACID IMPROVES STOMATAL CONDUCTANCE BY INDUCING HALF-OPENING OF THE PORES IN WHEAT PLANTS**

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The plant hormone abscisic acid (ABA) controls stomatal closing, and it has also been reported as regulating the transport of assimilates to developing seeds or fruits. The purpose of this study was to evaluate the effect of ABA on physiological parameters and production in wheat plants grown under field conditions, especially in anthesis and at the mid and late grain filling stages. The experiment (from July to November 2004) included a completely randomized block design, with 6 replicates of the treatments: Control (foliar spray of water + 1% alcohol) and ABA (300 mg/L foliar spray of cis ± trans-ABA, 99% purity, Sigma-Aldrich). At different phenological stages, biomass production, stomatal conductance on flag leaf at midday (LI-1600 portable porometer, LiCor, USA), aperture by microscope observation (Zeiss Axiophot), water-soluble carbohydrate content (phenol-sulfuric-acid method) and yield components were measured. Exogenous ABA enhanced stomatal conductance and promoted a high percent of half-opened stomata, thus improving the balance between the loss of water by transpiration and the CO<sub>2</sub> uptake for photosynthesis. It also enhanced re-mobilization of shoot-stored carbon reserves towards the grains, thereby enhancing the grain yield.

**132.**  
**ACTIVITY OF *Eupatorium inulaefolium* ESSENTIAL OIL AGAINST *Listeria monocytogenes* AND *Salmonella enteritidis* ON SOFT CHEESE**

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*Listeria monocytogenes* and *Salmonella enteritidis* are important foodborne pathogens which have been isolated from milk, cheese, seafood and meat products. These foods have been identified as vehicles in outbreaks of listeriosis and salmonellosis. Essential oils have been used as preservatives due their antimicrobial properties. We assessed the effect of three concentrations of *Eupatorium inulaefolium* essential oil against *L. monocytogenes* and *S. enteritidis* in soft cheese. We used low-fat and full-fat cheese contaminated with 10 µL of an inoculum of about 10<sup>9</sup> CFU/mL of each bacterium. They were incubated at 4 °C for 14 days. The concentration of *L. monocytogenes* and *S. enteritidis* in both cheeses was determined at 0 h and 1, 2, 3, 4, 7, 10, and 14 days using the serial dilution and spread plate technique. Cheese composition has been shown to be an important factor in determining the effectiveness of the plant essential oil. In low-fat and full-fat cheese, essential oil did not show an inhibitory effect over *L. monocytogenes* and *S. enteritidis*, but there was not increase in the growth. Therefore, we assume that there was a bacteriostatic effect on both microorganisms during all incubation periods. This would be an important factor to prevent the overgrowth of *L. monocytogenes* contaminated food at refrigeration temperatures.

**133. ANDROSTENEDIONE LUTEOTROPHIC ACTION THROUGH THE NEURAL PATHWAY IS MODIFIED BY FLUTAMIDE IN COELIAC GANGLION**

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In the coeliac ganglion-superior ovarian nerve-ovary (CG-SON-Ovary) system, we have shown that at the beginning of the functional luteal regression (pregnancy day 19), androstenedione ( $A_2$ ) stimulates the release of luteal progesterone (P) through the neural pathway. Here we studied if the ganglionic action of  $A_2$  is mediated by androgen receptor and if that action is modified by ganglionic sympathetic stimulation. The CG-SON-Ovary system was incubated in Krebs Ringer-glucose-albumin (0.1 mg/mL) at 37 °C, keeping CG and ovary in separate cuvettes, connected by the SON. To the CG compartment  $A_2$  or NA ( $10^{-6}$  mol/L), the androgen receptor antagonist flutamide ( $10^{-4}$  mol/L) [ $(A_2)_g$ ,  $(NA)_g$ ,  $(Flu)_g$  respectively] and the combinations [ $(Flu+A_2)_g$ ,  $(NA+A_2)_g$ ,  $(NA+Flu)_g$ ,  $(A_2+Flu+NA)_g$ ] were added. Controls were not stimulated. Released P was measured (by RIA) in the ovary compartment at 30, 60 and 120 min of incubation time. Control P (ng/mg ovary; mean  $\pm$  SEM) was  $0.20 \pm 0.02$  at 30 min;  $0.22 \pm 0.03$  at 60 min; and  $0.22 \pm 0.03$  at 120 min.  $(Flu)_g$  did not modify P release. With  $(A_2)_g$  vs  $(Flu)_g$  and  $(NA+A_2)_g$  vs  $(A_2+Flu+NA)_g$ : P release was decreased at all times ( $p < 0.001$  and  $p < 0.025$ , respectively). Thus, flutamide has no effect *per se* in CG, but modifies the  $A_2$  effect and the  $(NA + A_2)$  interaction, suggesting an involvement of CG androgen receptors in the  $A_2$  effect on P release.

**134. CHROMIUM RETENTION BY BIOSORPTION WITH *Penicillium crustasum***

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Chromium (Cr) is one of the heavy metals, present in many wastes, which are dangerous for human health and environment, since they are not decomposed by microorganisms. Overexposure to Cr can affect nervous tissue and the circulatory system. Several countries allow no more than 50  $\mu$ g Cr per liter of water; therefore, a preconcentration must be done before a detection can be performed. Preconcentration procedures may use plant material, microorganisms or biomass. In the biosorption method, metallic ions are adsorbed on a microorganism surface through interactions with functional groups like carboxylates, hydrosulfates, phosphates and amino groups. In this way biological material, like fungi, bacteria and algae, can take heavy metals from aqueous solutions. Here we propose chromium preconcentration using the fungus *Penicillium crustasum*. We made studies of Cr retention with this fungus, using different conditions. The metal concentration was measured reading absorbance variation versus time. Preconcentration (pH, temperature, ionic strength, etc.) and desorption variables were studied. We conclude that best results can be obtained with slightly acid solutions, temperatures between 25° and 30°C and a high ionic strength. After the metallic ion was desorbed we observed an important retention of it. Therefore, this methodology can be used for flow injection chromium analysis.

**135. DIHYDROCOUMARIN AS A TARGET MOLECULE FOR BIOCATALYSIS**

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Looking for organic compounds with a novel structure and a potential antioxidative activity, we tested several microorganisms like phytopathogenic fungi, because of their reported ability to biotransform a wide variety of compounds, such as terpenes and steroids. This time we chose dihydrocoumarin (**1**) as a substrate, because of its structural relationship with many natural and synthetic compounds with biological activity, and because no data on its biotransformation was found in the literature. The biotransformation was carried out on a two-stage standard protocol. Biotransformation was achieved after 20 days at 25°C. Only one compound in very low yield was obtained, presumably an oxidized more polar one, which was isolated, purified and analyzed by spectroscopic methods. This compound was identified as 7-hydroxydihydrocoumarin (**2**). The biocatalysis of **1** was performed using the phytopathogenic fungus *Aspergillus terreus* from the Cátedra de Microbiología de Alimentos de la Facultad de Ciencias Exactas, Físicoquímicas y Naturales (Universidad Nacional de Río Cuarto). The hydroxylation of compound **1** using a fungus as mentioned proves that this is a good method, that could lead to the finding of new compounds with potential antioxidative activity. No other compounds were detected by TLC; therefore it seems that biotransformation of dihydrocoumarin (**1**) is a regioselective oxidation.

**136. SUSCEPTIBILITY OF *Yersinia enterocolitica* AND *Escherichia coli* O157:H7 TO CHEMICALS USED FOR FRESH VEGETABLE WASHING**

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Consumption of fresh vegetables has been linked to foodborne illness in humans. Detergents and organic acids included in washing water can be effective in the inactivation or removing of microbial pathogens from vegetable surfaces. Before washing of artificially contaminated vegetables with different chemicals was performed, the susceptibility of two *Yersinia enterocolitica* O:9 pYV+ and one *Escherichia coli* O157:H7 strains against benzalkonium chloride (BzCl), lactic acid (LA), sodium lauryl sulphate (SLS) and a domestic use detergent (D) was assayed. The broth macrodilution method was used. Each chemical stock solution was 1:2 serially diluted in 15 tubes containing 1 mL Mueller Hinton broth. Suspensions of  $1 \times 10^6$  cfu/mL of each strain were separately inoculated. Series were incubated during 24 h at 25°C for *Y. enterocolitica* and at 37°C for *E. coli* O157:H7. The following minimal inhibitory concentrations (MIC) were observed: 0.05 mg/mL BzCl, 0.175 to 0.35% LA, > 0.10 g/mL SLS and > 50% D. Results demonstrated that SLS and D were not effective for inhibition of assayed strains. The effect of 0.1 mg/mL BzCl and 0.2% LA in the washing water of fresh vegetables will be assayed in forthcoming studies.

**137. AMBIENT INTERACTION EDUCATION IN EGB3 AND POLYMODAL: A CHALLENGE**

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According to chemical science, the transformations with change of matter can have very slow reversibility, posing risks for the environmental balance. From this premise, the integration of environmental education in teaching in EGB3 and Polymodal levels appears as a requirement that incorporates the suggestions and recommendations of different international forums (UNESCO). However, the actual situation does not appear as positive as the suggestions. The "suggested transversality" is indeed a very complex process. This work relates to the application of a program of environmental education directed to teaching at EGB3 and Polymodal. Difficulties ensue in the integration of the theory with its application in actual cases, since the answers do not consider the ambient as a physical-chemistry-biological interrelation. Our challenge is to surpass this obstacle, for the relevance of the knowledge of environmental parameters being understood and suitably transmitted to the students, for its application in environmental preservation. Once we understand that mere information is not enough, it is necessary to start off with an integrating presentation which incorporates knowledge, emotion, reflection and values. On the contrary, attempting to solve the obligatory nature of the environmental education in a shallow way with the introduction of some "cosmetic adjustments" in the teaching traditional practice really does not solve anything.

**138. GERMINATION OF *Cercidium praecox* "BREA" (FABACEAE) AND ITS RESPONSE TO HYDRIC AND SALINE STRESS**

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We studied the presence of repose in seeds of "brea" of Dept. La Capital, San Luis; the response, during germination, to induced water and saline stress, and their post-stress germinative capacity. Seeds were placed to germinate according to the standard method in Petri dishes, in stove at  $26 \pm 2^\circ\text{C}$  with the following processing: I) Study of repose in the germination: a) control (seeds without processing); b) scarification with sandpaper, c) immersion in hot water during 12 h and d) immersion during 24 h. II) Response to stress: the boxes with the seeds were dampened with solutions of PEG PM6000 to simulate water potentials de -0.5 MPa; -1MPa; -1.5 MPa and -2 Mpa, and with solutions of NaCl (0.5, 1.0, 1.5, and 2 mol/L). III) Post-stress germinative capacity: to study their recovery, non-germinated seeds were washed with distilled water and transferred to germinate in optimal hydric conditions. In all tests, the germinated seed number was recorded and percent germination (PG) was calculated. Data were analyzed by ANOVA. The results show that repose of primary type exists (mechanical, by hard teguments) and that scarification with sandpaper is the method adequate to break it. The PG decreases with increasing hydric stress, with a very good post-stress response, while at all saline concentrations tested, germination was very low and recovery was null.

**139. ADRENERGIC MODULATION OF PRL RELEASE INDUCED BY NALOXONE AT THE END OF PREGNANCY. ROLE OF SEROTONIN AND DOPAMINE**

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At the end of pregnancy, progesterone (Pg) maintains a high dopaminergic (DA) tone and inhibits prolactin (PRL) release. Pg blockade and serotonin (5-HT) action facilitate PRL release, an effect enhanced by opioid antagonist naloxone (NAL). While opioids act in absence of Pg, 5-HT inhibits PRL secretion with high Pg levels. A fall of DA tone is necessary for the opioid system to act as a modulator-inhibitor of PRL secretion. Adrenergics participate in regulation of PRL induced by Pg decrease. Our aims were: a) to establish if lack of 5-HT action modifies DA tone, measured as medial basal hypothalamus (MBH) tyrosine hydroxylase (TH) expression, and to assess adrenergic system participation in the PRL increase induced by 5-HT blockade. Pregnant rats were sc injected on day 18 with pCPA (inhibitor of 5-HT synthesis), 200 mg/kg; and at 17.30 h of day 19 with ip  $\alpha_1$ - (prazosin) or  $\beta_1$ - (metoprolol) adrenergic antagonists (10 mg/kg, ip) and NAL (2 mg/kg). PRL and Pg were measured by RIA at 18 h. MBH were obtained from pCPA-treated rats for TH Western blot. pCPA treatment inhibited TH expression in MBH without modifying serum Pg. Prazosin and metoprolol prevented the PRL increase induced by pCPA. Thus, 1) the lower TH expression after PCPA treatment suggests a diminished MBH DA tone facilitating NAL stimulatory action on PRL release despite elevated Pg levels; 2) Adrenergic system through  $\alpha_1$ - and  $\beta_1$ - receptors regulates PRL secretion induced by blockade of 5-HT and opioid action.

**140. CHANGES OF  $\Delta^9$  DESATURASE OF *T. cruzi* PROVOKED BY KETOCONAZOLE**

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Our previous studies indicated that  $\Delta^9$  desaturase of *T. cruzi* can use phosphatidylcholine as substrate and modifies its activity under antifungal effect. Here we tested  $\Delta^9$  desaturase activity under the effect of ketoconazole, using palmitic acid as a substrate on different subcellular fractions of *T. cruzi*. The fractions were obtained by differential centrifugation of *T. cruzi* and used as enzymatic source. Ketoconazole (1  $\mu\text{mol/L}$ ) was added to the culture medium after 24 h of growth. Reaction mixture contained palmitic acid [ $1\text{-}^{14}\text{C}$ ] and all necessary cofactors.  $\text{AgNO}_3$  TLC plates were used to separate and identify the reaction substrates and products. Ketoconazole decreased both the number and the weight of *T. cruzi*. The highest  $\Delta^9$  desaturase activity was obtained with the precipitate fraction and with 5% PBS. However ketoconazole was able to reduce  $\Delta^9$  desaturase activity in approximately 75%, in 105,000 g precipitate when 10% PBS was used. Since we previously found a large decrease of  $\Delta^9$  desaturase activity using PC as substrate and 5% PBS we conclude that  $\Delta^9$  desaturase activity shows different behaviour depending on the substrate used and might represent several isozymes. A reduced  $\Delta^9$  desaturase activity can lead to drastic alterations in ionic content and osmotic balance; therefore the importance of desaturases as possible targets for drug treatment is emphasized.

## 141.

***Passiflora caerulea* VS. *P. foetida* DRUGS (PASSIFLORACEAE) IN CUYO REGION, ARGENTINA**

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Some species of *Passiflora* L. are used for medicinal, nutritional and ornamental purposes. There are 19 species in Argentina, and 2 grow in the Cuyo region, where they are marketed both as folk- and phytomedicines. *P. caerulea* L. is used as a sedative under the name "pasionaria". *P. foetida* L. is reputedly anti-inflammatory, pectoral and emmenagogue, and sold as "pasiflora hedionda". This study was carried out to define morphologic and micrographic characters of the drugs, in order to contribute to their quality control. The samples were collected in the region, fixed and conserved in formol:acetic acid:alcohol (1:1:1), included in paraffin, cutted and mounted in DPX. The main diacritical characters are showed in the following Table.

Character	<i>Passiflora caerulea</i>	<i>P. foetida</i>
Plant indument	subglabrous, eglandular	hirsute, viscosa
Floral bracts	entire, subglabrous	pinnate, hirsute
Extrafloral glands	sessilis to shortly stipitate	pedicellate
Berry	elliptic, orange	globose, whitish
Seed aril	reddish to purple	hialine
Stomata number (Es)	0	4.63 ± 0,92
Stomata index (Es)	0	3.89 – 6.97
Vein-islets	23.87 ± 3.6	6.15 ± 1.28
Vein termination.mm <sup>2</sup>	70.75 ± 5.9	7.50 ± 1.93

## 142.

**AQUATIC MICROCRUSTACEA AS BIOINDICATORS OF CHEMICAL CONTAMINATION OF WATER**

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Ecotoxicologic studies with Diazinon using *Artemia saline* (to verify developmental damage i.e. teratology) or *Daphnia magna* (treated with Metamidophos) to observe motility changes, were used *Artemia saline*/Diazinon. After determining the LD50 evaluated as mortality rate judged by immobilisation of the larvae, these values were used for the CE50 to which larvae were exposed for 24, 48 and 72 hs post-hatching. Morphological changes were recorded in the three initial stages of development. The best correlation dosis/response was found at 24 hs after exposure.

*A. saline* is very sensitive to the organophosphorate contaminant, those being a good ecotoxicological indicators of saline aquatic environment contaminated with pesticides.

*Daphnia magna*/Metamidophos. This organophosphoric pesticide was analyzed since it is widely used. Cultures of *D. magna* were set in the laboratory following Chilean norm 2083.of 1999. Later, cultures were done from one only genetic line and exposed to different concentrations of Metamidophos observing immotility at 12 and 24 hs after exposure. Significant results were found at 24 hs, demonstrating that this organism is a good bioindicator of fresh water chemical contamination

## 143.

**MALATHION“ EFFECTS ON *Eisenia foetida* (BIOCENTINEL) OOGENESIS**

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Great efforts have been done in Latinamerica to eliminate agricultural plagues using of organophosphates. Among them Malathion is widely employed. Only few studies have been done to explain its chronic effects, including those related to reproduction. The earthworm *Eisenia foetida* (Annelida, oligochaeta), is a good model of a biosentinel organism to evaluate the toxical risks of xenobiotics in terrestrial ecosystems.

The goal of this study is to analyze the histological damage of the ovariole of *E. foetida*, in control and exposed animals using commercial, Malathion 57%. Earthworms, with a well developed clitellum are treated with a single subletal dose of 1/10, 1/10, 1/3 and 2/3 of LD 50 (LD 50 = 880 mg/kg soil). Controls were wet with water. Groups of animals were examined 1, 5, 15 and 30 days post-exposure.

Histometrical results show that Malathion affects germ cells from the first day, with increase in nuclear diameter with the highest dose. At 15 days effects are seen in cytoplasm and nuclei. Follicular cells decrease in diameter at day 5 at high doses. By 30 days there is a recuperation even with high doses.

Therefore the ovariole seems a good sensor organ for detection of cytotoxic and cytogenetic damage. *Eisenia foetida* is a good biosentinel as it has been recognized in the literature.

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