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L.1.**SHIGA TOXIN ACTION IN TARGET ORGANS. STRATEGIES FOR PREVENTION OF HEMOLYTIC UREMIC SYNDROME**

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Gastrointestinal infection with STEC/EHEC strains causes diarrhea and hemorrhagic colitis, and in addition, Hemolytic Uremic Syndrome (HUS) defined by a triad of hemolytic anemia, thrombocytopenia, and acute renal failure. HUS develops in 10%-15% of small children several days after bloody diarrhea and is the most common cause of acute renal failure in infants in Argentina. It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products or direct contact with cattle. Because STEC are non-invasive, it is generally accepted that Shiga toxin (Stx) must be absorbed from the intestine to cause HUS by different mechanisms that contribute to the intestinal pathology. Renal damage has been strongly associated with Stx, which binds to the globotriaosylceramide (Gb3) receptor on the plasma membrane of epithelial and endothelial cells causing cellular death. We have shown that kidney lesions similar to those described in humans may be obtained in rats treated with Stx2 and that the kidney was protected by previous treatment with inhibitors of Gb3 synthesis. Furthermore, we have shown that Stx2 interrupted pregnancy in rats and we speculate that STEC infections during pregnancy could be detrimental to human fetuses.

L.2.**ATTENUATED MUTANT OF *Mycobacterium bovis* AS EXPERIMENTAL VACCINE AGAINST BOVINE TUBERCULOSIS**

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Introduction: *Mycobacterium bovis* is the causative agent of animal or bovine tuberculosis. This mycobacteria is highly related to *M. tuberculosis*, the Koch bacilli. *M. bovis* can cause tuberculosis in many animals including humans. Currently, there are no vaccines against bovine tuberculosis.

Objective: to test *M. bovis* mutant obtained by homologous recombination as experimental anti TB vaccine in cattle.

Results: A *M. bovis* strain, deleted in the *yrbE2B* and *mceA2* genes (*M. bovis* Δ mce2) was tested as an experimental vaccines in cattle that was subsequently challenged with virulent *M. bovis*. Groups of 5/6 calves 3,5 month-old were vaccinated and challenged 9 weeks later with a virulent strain of *M. bovis* by intratracheal route. Also a non-vaccinated group and a group vaccinated with BCG were included as controls. Along the assay samples were taken to measure IFN γ by an IGRA test, cytometry and cytokine responses of PPD restimulated PBMC. IGRA test showed IFN γ values similar to that in pre-vaccination except for the animals vaccinated with *M. bovis* Δ mce2, where an increase was observed at 30 dpv. The animals were euthanised and necropsied 18 weeks after challenge. The group vaccinated with *M. bovis* Δ mce2 was the one with lowest total score of lesions in lungs and lymphnodes. Histopathology of lungs and lymphnodes showed in the *M. bovis* Δ mce2 and BCG vaccinated groups a marked reduction of the severity of histopathological lesions. A positive reaction to tuberculin intradermal test after vaccination was observed in animals vaccinated with *M. bovis* Δ mce2 but not with BCG or *M. bovis* Δ lprG. In contrast, after challenge, non vaccinated animals gave induration diameters higher than non vaccinated controls. **Conclusions:** In summary, *M. bovis* Δ mce2 is a promising vaccine candidate to control *M. bovis* pathogenesis in cattle.

L.3.**INFLUENCE OF THE CUMULUS AND GONADOTROPINS ON THE METABOLIC PROFILE OF PORCINE CUMULUS-OOCYTE COMPLEXES DURING *IN VITRO* MATURATION**

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The substrate availability during *in vitro* culture of cumulus oocyte complexes (COCs) strongly impacts on oocyte maturation. Several aspects of porcine COC metabolism during *in vitro* maturation remain to be elucidated. The aim of the work was to examine the influence of the cumulus and gonadotropins on the metabolic profile of porcine COCs during *in vitro* maturation. Immature COCs were assigned to morphological classes A₁ (with a dense cumulus), A₂ (with a translucent cumulus), B₁ (with the corona radiata), B₂ (with only some remaining cumulus cells) and matured with or without gonadotropins.

Glycolysis and ammonia production were higher in the A class COCs. Gonadotropins increased both, especially in the A₁ COCs ($P < 0.05$). The A class COCs had the highest initial protein contents and at the end of *in vitro* maturation. Furthermore, hormonal stimulation induced a similar increase in protein contents of both A classes ($P < 0.05$). The neutral lipid content and ROS levels were similar in the immature oocytes of the COCs of all classes. A reduction was seen in both these variables when maturation proceeded either in the presence or absence of gonadotropins.

The cumulus type surrounding the oocyte is related to the metabolism of carbohydrates and amino acids by the COC during *in vitro* maturation under gonadotropic stimulation. Oocyte lipolytic activity and ROS production appear to be independent of the surrounding cumulus and the presence of gonadotropins.

L.4.**SUNFLOWER IMIDAZOLINONE RESISTANCE: GENE EXPRESSION ANALYSIS AND BIOCHEMICAL SCREENING**

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Acetohydroxyacid synthase (AHAS) catalyzes the first reaction in branched chain amino acids biosynthesis. This enzyme is of substantial importance because it is the target of several herbicides, including all members of the sulfonylurea and imidazolinone (IMI) families. In sunflower, IMI resistance was introgressed from a wild population into elite inbred lines. *Imr1* and *Imr2* was proposed as the genetic base of IMI-resistance inheritance, where *Imr1* is an allelic variant of the *ahas1* gene coding for the catalytic subunit of AHAS. *Imr2* is a modifier gene but his effect remains unknown. The objectives of this work were to study the early response to imidazolinones at a whole-plant and biochemical levels, to evaluate the *ahas* expression pattern, and to determine the resistance mechanism related to *Imr2*. Genotypes differing in IMI resistance showed differential growth parameters and AHAS *in vivo* activity levels in response to IMI treatments. This allowed to discriminate among genotypes and have a possible use as a selection method for evaluating AHAS inhibitor resistance. Relative transcripts levels of *ahas* genes were measured through RT-qPCR in leaf and root tissues of control and imazapyr-treated plantlets. Levels of expression in these tissues were consistent with previously observed *in vivo* and *in vitro* AHAS activity. AHAS response to P-450 inhibitors in combination with IMI was also evaluated. The effect of inhibitors in resistant plantlets suggested that a detoxification, P-450-mediated mechanism might be implicated in herbicide resistance. This mechanism could be related to the modifier effect of *Imr2* gene in this species.

L.5.**ARTIFICIAL WETLANDS AND THEIR IMPORTANCE IN BIRD CONSERVATION***Echevarria AL.**Fundación Miguel Lillo, Miguel Lillo 251, (4000). Tucumán.**E-mail: adaechevarria@yahoo.com.ar*

Artificial wetlands are dynamic ecosystems due to changes in water level designed by man. Over time, reservoirs become wintering sites for migratory birds and breeding sites for resident birds, a new role not predicted in their construction. As an example, plovers and sandpipers fly through our country during spring and summer and use beaches, superficial and deeper waters as food supply sites. Studies of bird communities and their variations are useful in the determination of the role wetlands play in the conservation of biodiversity in the biomes they are located in. Bird communities of many artificial reservoirs from northwestern Argentina were studied during 2 decades. Censuses were made using line transects and fixed points in different seasons in order to determine important variations in community composition and abundance. The recorded species richness included: Embalse El Cadillal (145), La Angostura (78), El Tunal (100), Escaba (92), Figueroa (127), El Cero (83) y Cuchi Pozo (102). Resident and migratory (nearctic-neotropical, altitudinal and regional) species were identified. Some species were observed nidifying and new distribution records were reported. Threats to these bird communities were also detected. This work will allow, on one hand, to determine the value of conservation of artificial wetlands in view of the progressive loss of natural wetlands and, on other hand, to establish management guidelines to improve the conditions of current and future reservoirs.

1. MICROSTRUCTURE AND MICROHARDNESS OF HUMAN ANTERIOR DECIDUOUS TOOTH ENAMEL

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The hardness enamel is important since it has a direct relationship with abrasion resistance. The aim of this work was to relate the enamel types to microhardness in deciduous tooth. Six incisors and six canines crowns were embedded in resin, polished, etched with acid and observed under a Environmental Scanning Electron Microscope (ESEM). Micrographs were identified on the free faces. The microhardness was measured with Vickers penetrators, loads of 10g and time 10" in the cervical, medial and incisal third in the inner and outer enamel. Results showed that in the incisal and medial third of the free faces enamel was of the radial type in the outer zone with bands in the inner zone. Aprismatic and radial enamel was the only enamel type present in the cervical third of the free faces. Microhardness HV_{10} external enamel $\bar{x}=351.47\pm63.84$ Vk (n=12); inner enamel $\bar{x}=251.15\pm27.25$ Vk (n=12), $p < 0.0001$. The microhardness comparison between the cervical, medial and incisal group showed no significant differences. The outer and harder radial enamel type presents higher abrasion or wear resistance that combined with the inner enamel, either irregular or with bands, constitute a biomechanical adaptation.

2. MORPHOLOGICAL DESCRIPTION OF INITIAL SEGMENT AND EPIDIDYMAL CAPUT OF VISCACHA (*Lagostomus maximus maximus*) IN RELATION TO AGE

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Initial segment (IS) and caput (C) is the first portion of the epididymis and the first selective barrier for the exclusion of abnormal gametes along the male genital tract. The objective of this study was to evaluate the possible morphological changes of IS-C in animals at different ages and observed the reached sexual maturity. Epididymal tissue samples from viscachas at different ages (n = 10) captured in summer, were removed and processed by conventional optical microscopy. The age of the animals was determined by the weight of them and confirmed by testicular histology. Epididymal parameters were quantified. Luminal diameter and epithelial height showed an increase in mature animals, while the thickness of the lamina propria decreased. The number of principal, basal and apical cells varies with age of the animal, while halo cells remained constant. Only in older animals were observed narrow cells. These results suggest that the epididymis expresses structural changes and variations in the distribution of the different cell population, to facilitate a suitable microenvironment for the capacitation and sperm maturation.

3. ANDROGEN RECEPTOR AND GONADOTROPHINS IN CASTRATED ADULT MALE VISCACHA

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Androgens participate in the reproduction control thus binding to their intracellular receptors (AR) in the pituitary gonadotrophs. The aim of this work was to study the castration effects on AR, LH- and FSH-gonadotrophs in pars distalis (PD) and colocalization between gonadotrophins and AR. Eight adult male viscachas were divided in two groups, control and 6-week castrate animals. The pituitaries were observed by light microscopy. For immunohistochemistry the primary antibodies used were polyclonal anti-AR (N-20), monoclonal anti-LH and anti-FSH. The morphometric study: %ARn-ir, %ARc-ir, %LH-ir, %FSH-ir, %LH-ir/AR-ir and %FSH-ir/AR-ir were analyzed. %AR-ir cells were observed in the PD. The immunolabeling was mainly detected in the nuclei (ARn-ir), but in some cells were also observed cytoplasmic labeling (ARc-ir). ARn-ir was more intense and numerous at caudal end. LH- and FSH-gonadotrophs were mainly in ventral region and rostral end. In intact animal PD, the %LH-ir/AR-ir was 45-66% and %FSH-ir/AR-ir was 49-57%. In castrated, a significant decrease of %ARn-ir, %ARc-ir, %LH-ir, %FSH-ir and %FSH-ir/AR-ir were observed. In conclusion, gonadal androgens are directly related to the immunolabeling of AR, LH and FSH in pituitary PD. The colocalization of AR and FSH is the most affected by the lack of androgens, and provide evidence of the existence of gonadotroph subpopulations that might have different regulatory mechanisms of hormonal synthesis, storage and secretion.

4. LOCALIZATION OF ANDROGEN RECEPTORS IN PARS DISTALIS OF ADULT MALE VISCACHA DURING THE REPRODUCTIVE CYCLE

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Androgens are involved in the regulation of reproductive function. They bind to specific intracellular receptors, followed by shuttling of the activated androgen receptor (AR) to the cell nucleus, where it stimulates or represses gene transcription. The aim of this work was to study the expression of AR in pituitary PD of adult male viscachas by immunohistochemical techniques. These rodents were captured during the reproductive cycle: reproductive (n=4), gonadal regression (n=4) and gonadal recovery (n=4) periods. Pituitaries were processed for light microscopy and the AR was immunohistochemically identified using the antibody AR (N-20). Immunohistochemical results revealed that AR-ir was detected in the nuclei (ARn-ir) and/or cytoplasm (ARc-ir) of PD cells. However, the nuclear labeled was more intense in the caudal end during reproductive and gonadal regression periods. The higher nuclear immunolabeling was in the dorso-medial during gonadal recovery period. The cytoplasmic immunolabeling pattern was heterogeneous, some cells presented labeling in the nucleus periphery whereas other presented stippled cytoplasmic immunolabeling, during reproductive and gonadal regression periods. In the gonadal recovery period the cytoplasmic labeling was more intense and homogeneous in relation to the previous periods. These results demonstrated variations in the expression and localization of AR during the reproductive cycle, being probably more active during gonadal recovery of viscacha.

5. ACETYLCHOLINE IN COELIAC GANGLION MODIFIES OVARY REGRESSION IN DIESTROUS

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Acetylcholine (Ach) is the major sympathetic preganglionic neurotransmitter. Using the *ex vivo* coeliac ganglion (CG)-superior ovarian nerve (SON)-ovary system, the aims were to investigate whether Ach in CG modulates: 1) the ovarian release of Progesterone (P_4) and the expression of 3β -HSD and 20α -HSD, 2) the ovarian release of Noradrenaline (NA) and dopamine (DA); 3) the expression of bax and bcl2. The system was incubated in Krebs Ringer at 37°C , keeping CG and ovary connected by the SON, in separate compartments. Ach (10^{-6}M) was added in the ganglion compartment in the experimental group. Periodic extractions of the ovarian incubation liquid were carried out at 60 and 120 min and ovaries were isolated at 120 min. P_4 levels were determined by RIA and catecholamines by HPLC. 3β -HSD, 20α -HSD, bax and bcl2 transcripts levels were measured by RT-PCR. ANOVA 1 followed by Tukey test with a statistical significance of $p < 0.05$ was used. At the end of the incubation, Ach in CG decreased P_4 (120 min.: 0.079 ± 3.10^{-3} vs 0.06 ± 3.10^{-3}), NA (49 ± 1 vs 26 ± 1), and 20α -HSD (0.86 ± 0.03 vs 0.53 ± 0.03) with $p < 0.05$. While increased 3β -HSD (0.85 ± 0.002 vs 1.37 ± 0.002) and DA (26 ± 1 vs 56.5 ± 1) increased with $p < 0.05$ and diminished bax/bcl-2 ratio ($p < 0.05$). The results show the importance of the ganglionic neurons in controlling ovarian functions in DII modifying ovarian regression through the release of NA and DA.

6. ESTRADIOL STIMULATES OVARIAN GnRH LIBERATION THROUGH DIRECT AND INDIRECT EFFECT FROM COELIAC GANGLION

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Estradiol (E_2) is involved in the regulation of reproductive processes, acting on peripheral organs and sympathetic neurons associated with reproduction. The objective was to evaluate if E_2 added in coeliac ganglion (CG) and in ovary alone modulates the ovarian release of GnRH and steroidogenic hormones in DII. We used two experimental schemes: a)- the CG-superior ovarian nerve (SON)-ovary system with and without the presence of E_2 (10^{-8}M) in ganglion compartment and b)- incubations of ovary alone with and without the presence of E_2 (10^{-8}M). The systems in both cases were incubated in Krebs Ringer at 37°C , in specific cuvettes designed for this purpose. Periodic extractions of the ovarian incubation liquid were carried out at 60 and 120 min. A_2 , E_2 and GnRH and were measured by RIA. ANOVA 1 followed by Tukey test with a statistical significance of $p < 0.05$ was used. In CG-SON-ovary system, E_2 in CG increased ovarian GnRH (120 min.: 2.49 ± 0.1 vs 4.50 ± 0.3), E_2 (60 min 2.6 ± 0.2 vs 10.51 ± 2.4 ; 120 min 924.5 ± 90.3 vs 2812 ± 540) ($p < 0.001$). In incubations of ovary alone, E_2 increased GnRH (120 min.: 4.1 ± 0.9 vs 12.4 ± 1.2) while A_2 in both systems not present significant changes. We conclude that through an indirect effect on the ovary E_2 favors luteal survival and the liberation of GnRH, in association with neural modulation from the CG via the SON.

7. LACK OF TNFRp55 MODIFIES CIRCADIAN RHYTHMS IN THE OVARY IN DIESTRUS

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Previously, we demonstrated that progesterone and the mRNA of its synthetic enzyme, 3β -HSD, display circadian rhythmicity in the ovary. Clock, Cry1 and Per1 are key components of the cellular clock and TNF is a cytokine that can act as a circadian modulator. Our aim was to investigate whether also 3β -HSD protein and Star mRNA present circadian variation in the ovary and whether the absence of the TNFRp55 receptor affects the temporal expression of Per1, Clock and Cry1 genes, Star mRNA and 3β -HSD protein. Wild type and TNFRp55 $^{-/-}$ mice, were maintained on a 12h light: 12h dark cycle, at $24 \pm 2^\circ\text{C}$, with food and water *ad libitum*. Five days before the experiment, mice were kept under constant darkness conditions. Ovaries were isolated every 6 h during a 24h period. StAR, Clock, Per1 and Cry transcripts levels were determined by RT-PCR and 3β -HSD protein was measured by western blot. As expected, Per1 and Cry1 genes expression as well as 3β -HSD protein levels display a circadian oscillation in the ovary. We did not observe circadian rhythms of Clock and StAR expression in this tissue. TNFRp55 deficiency modifies the temporal expression of clock genes Per1 and Cry1 as well as the mesor of 3β -HSD protein rhythm. The TNFRp55 might mediate the modulation of clock genes expression and thus the circadian regulation of the 3β -HSD expression and progesterone 24h-oscillation in the ovary in diestrus.

8. CONTRIBUTION OF THE GANGLIONIC NITRIC OXIDE/NITRIC OXIDE SYNTHASE SYSTEM IN THE OVARIAN NITRIC OXIDE RELEASE

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It is known the presence of the nitric oxide/nitric oxide synthase (NO/NOS) system in coeliac ganglion. Using the *ex vivo* coeliac ganglion-superior ovarian nerve-ovary (CG-SON-O) system, has been shown that the stimulation of CG with Acetylcholine (Ach) increases NO release in the ovarian compartment. The aim of this work was to study, in the first rat proestrus, if the presence in CG of a NO donor and inhibitors of NOS, modifies the NO release, with and without Ach 10^{-6}M in GC. The system was incubated in Krebs-Ringer buffer at 37°C . NO donor, sodium nitroprusside (SNP) $100 \mu\text{M}$ and inhibitors of NOS: non-selective L-nitroarginine methyl ester (L-NAME) $100 \mu\text{M}$ and the selective of iNOS (inducible), aminoguanidine (AG) $400 \mu\text{M}$, were added in CG. Ovarian nitrites were determined by the Griess method (nmol/mg ovary) at 30', 60' and 120'. One-way ANOVA and Tukey test were used ($p < 0.05$). Respect of the control, SNP stimulated at all times (3 ± 0.2 vs 4.1 ± 0.3 ; 2.3 ± 0.2 vs 3.3 ± 0.1 ; 1.6 ± 0.1 vs 2.5 ± 0.3 , $p < 0.05$), L-NAME inhibited at 30' (3 ± 0.2 vs 1 ± 0.1 , $p < 0.01$) and AG inhibited at 120' (1.6 ± 0.1 vs 1 ± 0.1 , $p < 0.05$). Ach caused greater significance to both effects: SNP stimulated (46 ± 3 vs 125 ± 6 ; 40 ± 2 vs 118 ± 10 ; 19 ± 1 vs 80 ± 9 , $p < 0.001$), L-NAME and AG inhibited (46 ± 3 vs 14 ± 2 ; 40 ± 3 vs 14 ± 2 ; 19 ± 1 vs 7 ± 1 , $p < 0.001$), (46 ± 3 vs 24 ± 1 , $p < 0.01$; 40 ± 3 vs 10 ± 0.5 , $p < 0.001$; 19 ± 1 vs 12 ± 2 , $p < 0.05$), at all times. We concluded that there is a contribution of ganglionic NO in ovarian through SON, possibly by neural and endothelial NOS isoenzymes.

9. EFFECT OF PROGESTERONE ON LUTEAL REGRESSION THROUGH A NEURAL PATHWAY IN LATE PREGNANT RAT

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Previously, we demonstrated that Progesterone (P) added to the coeliac ganglion (CG), through the superior ovarian nerve (SON), is able to stimulate the ovarian P release. The object of this study was to analyze the effect of P, by SON, on the functional and structural luteal regression of 21 pregnant rats using the *ex vivo* CG-SON-ovary system.

The system was incubated in Krebs Ringer at 37°C, keeping CG and ovary connected by the SON, in separate compartments. P (10^{-5} M) was added in the ganglion compartment [(P)_g], Controls were not stimulated. Periodic extractions of the ovarian incubation liquid were carried out at 30, 60, 120 and 180 min. The release of ovarian P (ng/mg ovary) was determined by RIA to corroborate previous results. At 180 min, the luteal mRNA expression (relative units) of 3βHSD, 20αHSD, Fas, FasL and S16 (housekeeping gene) were analysed by RT-PCR. One way ANOVA followed by Tukey test were used (*p<0.05). Results (mean ±S.E.M) were: P Control at 30': 0.02±0.006, 60': 0.03±0.007, 120': 0.04±0.007 and 180': 0.04±0.009 vs [(P)_g] 30': 0.05±0.009, 60': 0.08±0.008, 120': 0.10±0.01, 180': 0.11±0.01(*); 20αHSD Control: 0.33±0.005 vs [(P)_g]: 0.28±0.014(*) and FasL Control: 0.71±0.03 vs [(P)_g]: 0.48±0.05 (*). The expression of 3βHSD and Fas were not modify. P would promote corpora lutea protection from regression through the peripheral neural pathway.

10. PERITUBULAR MYOID CELL SHAPE IN ADULT RAT TESTES

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Peritubularmyoid cells (PM) are flat polygonal cells that surround seminiferous tubules (ST) as a monolayer in rodents. These cells are covered by a monolayer of endothelial cells (EC) from lymphatic vessels. PM are smooth muscle cells, with α-actin filaments arranged in two independent layers, following orthogonal directions. Both, PM and EC, have caveolae on their plasma membrane. By fluorescence microscopy, PM shape in the adult rat is recognized by its actin cytoskeleton, but by scanning electron microscopy (SEM) their limits are not well defined because of they are hidden by EC, also flat polygonal cells with similar size. The aim of this work is to set the optimal conditions to remove EC from isolated ST to observe them by SEM. We tested different periods of time and collagenase concentrations. Control and digested ST were fixed with PFA 4% and treated with anti α actin-FITC to recognize PM, and anti-caveolin 1 Cy3 to identify both PM and EC caveolae by confocal microscopy. Then, with the optimal conditions, digested ST are fixed with GA 5% and processed to SEM. By confocal microscopy, PM from control ST showed α-actin and caveolin 1 fluorescence, while EC only showed caveolin 1 one. By this way, caveolin 1 fluorescence from PM overlapped with that from EC. We chose 0.5 mg/ml collagenase during 5 min at 37°C as optimal digestion condition, because in this way PM keep their morphology without alter their intercellular spaces and EC are removed, as caveolin 1 fluorescence appear not overlapped. By SEM, control ST are covered by EC, but digested ST show PM surface by the first time because EC were removed.

11. EFFECT OF HYPERCHOLESTEROLEMIA ON CELL PROLIFERATION DURING SPERMATOGENESIS IN RABBIT NEW ZELAND

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Hypercholesterolemia (HCh) is a risk factor for human health. Cholesterol (Ch) is a component of cell membranes and a protein that is involved in their metabolism is caveolin 1 (Cav1). The caveolins are a family of proteins found in many cell types and also in signaling, transport, and cell growth control and tumor suppressor. In this investigation, the objective was to study the expression of Cav1 and ERK 1,2 / pERK1, 2 in spermatogenesis in adult rabbits *new zeland* by indirect immunofluorescence, immunohistochemistry and western blot. Cav1 was associated with the progression of meiosis by its regulatory effect on the Ras/ MAPK function. Cav1 is dependent upon its association with membrane microdomains rich in Ch, which provide a link between cell membrane and germ cell meiosis progression. High cellular levels of Ch are essential for the expression of Cav1. Cav1 is not sufficient to initiate the Ras signaling, but is necessary for maintaining the same elevated during the second meiotic phase dependent Ch levels. The over expression of Cav1 by HCh affects cell proliferation by acting on the signaling pathway of the Ras/ MAPK, inhibit the progression to meiosis II and producing an arrest in spermatocytes 1°.

12. LOW LEVELS OF TESTOSTERONE AFFECT THE OLIGOMERIZATION AND SECRETION OF PROSAPOSIN IN RAT EPIDIDYMIS

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In mammals, sperm maturation is favored by the secretory activity of the epididymal epithelium. The lumen of the organ is particularly rich in lysosomal proteins. This striking phenomenon seems to be crucial for acquiring the fertilizing capacity. Prosaposin (PSAP) is the precursor (65 kDa) of the lysosomal proteins saposins related with the sphingolipid metabolism. Previous findings show that PSAP (70 kDa) may covalently aggregate into oligomers (130-250 kDa). Moreover, these oligomers can not be recognized by the specific receptor (sortilin) and they enter in a yet unknown secretion pathway. Based on the characteristics of epididymal secretion and considering the hormonal dependence of this organ, we here evaluated the degree of oligomerization of PSAP in the rat epididymal fluid of controls, castrated or castrated followed by testosterone replacement. Samples were analyzed by electrophoresis under reducing or non-reducing conditions followed by immunoblot. We observed, for the first time, the presence of PSAP oligomers (250 kDa) in the epididymal fluid of the three regions of the organ. In turn, it was demonstrated that castration induces a decrease in secretion of monomeric PSAP (70 kDa) and an increase of the oligomeric forms along the whole duct. These effects were reversed by hormone replacement, indicating that testosterone influences on the oligomerization of PSAP, and consequently affects the secretion of this lysosomal protein in the epididymal epithelium

13. POSSIBLE HORMONAL REGULATION OF THE TRAFFIC AND SECRETION OF PROTEASES IN BREAST CANCER CELLS

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Evidence have demonstrated that lysosomal proteases, as cathepsins, are secreted by tumoral cells, and they may play a role in tumor progression. In most cell types, these enzymes are transported to lysosomes via mannose-6-phosphate receptors (MPRs), sortilin or other alternative mechanisms. Two forms of MPRs have been described so far; the cation-dependent (CD-MPR) and the cation-independent (CI-MPR) MPR. The CD-MPR could participate additionally in the secretion of lysosomal enzymes. In some cell types, pro-cathepsin D (pro-CatD) interacts with prosaposin (PSAP), and both proteins can be transported together via the receptor sortilin (Sort). Here, we proposed to elucidate the mechanism by which cathepsin D (CatD) is secreted by breast cancer cells, and if it is regulated by hormones. We studied the expression of Cat D, PSAP, CD-MPR and Sort in two cell lines (MCF-7 and MDA-MB231), in the presence or absence of 17 β -estradiol and/or tamoxifen. After treatments, proteins from cell lysates were subjected to electrophoresis and analysed by immunoblot. It was observed that expression of CatD, CD-MPR and PSAP in MCF-7 is higher than in MDA-MB231 cells. In turn, estradiol induced an increase in the expression of CatD and CD-MPR in MCF-7 cells, and this effect was reversed by tamoxifen. From these preliminary results we suggest that expression of Cat D and its receptor, CD-MPR, is influenced by estradiol and the differences observed between both cell lines may be due to the presence of estradiol receptors in MCF-7 cells.

14. EFFECT OF HYPOTHYROIDISM ON MAMMARY FUNCTION AT THE END OF LACTATION IN THE RAT

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During lactation, prolactin (PRL) binding to its receptor (RPRL) results in activation of JAK/STAT pathway, transcription of milk proteins and inhibition of involution. Hypothyroidism (hypoT) doesn't affect circulating PRL during lactation but alters ovarian hormone profile and milk composition and secretion. To continue investigating the mechanism through which hypoT alters mammary function, we studied its effect on day 21 (L21) of lactation, on histologic markers of involution, PRL signaling mechanisms and nuclear receptors expression, determining by real time PCR the mammary expression of PRL (*prlr*), estrogen (*er α* and *β*), P₄ (*pr*) and thyroid (*tr α* and *β*) receptors, *stat5b* and *stat5a* (PRL signaling mediator), *socs1*, *socs3* and *cis* (PRL signaling suppressors), α lactalbumin (*lalba*) and β casein (*csn2*) (milk protein), *ncor1*, *rxr α* , *cd1* (nuclear receptors co-regulators), *bax* and *bcl2* (apoptotic markers). HypoT induced premature mammary involution, lowered the expression of *prlr*, *lalba*, *csn2* and increased that of *socs1*, *socs3* and *er β* without affecting the expression of the rest of the studied factors, including *bax* and *bcl2*. These results suggest that hypoT may favour premature mammary involution and the fall in milk protein synthesis by repressing PRL signaling.

15. PIOGLITAZONE REDUCES OXIDATIVE STRESS AND DECREASES ATHEROGENIC MARKERS IN MICE WITH METABOLIC SYNDROME

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The metabolic syndrome (MS) increases the risk of type 2 diabetes (T2DM) and cardiovascular diseases. Pioglitazone (PIO) is used to improve insulin sensitivity in patients with T2DM. We analyzed the effect of PIO on the oxidative stress, the expression of inflammatory and endothelial dysfunction markers and the development of atheroma plaque in apolipoprotein E-deficient mice (ApoE-KO). Mice were fed with fructose overload (FF, 10% w / v) for 8 weeks and treated with PIO the latest 4 weeks. Serum level of the lipid peroxidation product, malondialdehyde (MDA) was performed. Atheroma plaques in aortas were measured with Oil Red stain and aortic VCAM-1 and MMP9 expression were determined by western blot. PIO significantly inhibited FF-increased glycaemia, triglyceridemia, insulinemia and MDA levels. No changes in the development of the atheroma plaque stimulated with FF were found, but the expression of VCAM-1 and MMP-9 were significantly diminished by PIO. Our results show that PIO improves insulin sensitivity, diminishes oxidative damage and reduces the expression of pro-atherogenic proteins. These findings suggest that PIO could increase the stability of advanced atherosclerotic plaques in ApoE-KO mice with MS.

16. PON-1 AND SR-BI POLYMORPHISM ASSOCIATION IN TYPE 2 DIABETES MELLITUS

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High levels of High Density Lipoproteins (HDL) are inversely related to cardiovascular disease (CAD) by its atheroprotective role. In Type 2 Diabetes Mellitus (T2DM) subjects HDL-c levels are decreased. The enzyme Paraoxonase-1 (PON-1) has an anti-atherogenic effect and protects lipoproteins from the oxidative stress. PON-1 activity is reduced in T2DM patients. Scavenger Receptor class B Type I (SR-BI) participates in reverse cholesterol transport by mediating the selective uptake of free cholesterol. Several polymorphisms have been studied in PON-1 and SR-BI genes. Decreased PON-1 activity is associated with rs705379 (-107 C/T) polymorphism. In SR-BI, rs838896 (IVS 11, G/C) could be related with decreased receptor expression and HDL-c levels modification. The aim of this study was to determinate the association between these polymorphisms and T2DM. 52 DNA samples were analyzed (33 diabetics and 19 controls). Genotypes were identified by Tetra Primer ARMS-PCR, allelic and genotypic frequencies were calculated. Rs705379 genotypic frequencies in Co and Diab were C/C: 84% vs 51.5%, C/T: 15,7% vs 45,4%, T/T: 0% vs 3,03%. In rs 838896 were G/G: 47,3% vs 24,2%, G/C: 47,3% vs 54,5%, C/C: 5,2% vs 21,2%. HDL-c levels significantly decreased in diabetics with two or more mutations in these genes ($p < 0, 05$). Our data show that the presence of two or more mutations conferred increased susceptibility to develop CAD ($p < 0,016$) OR: 10,6; (CI 1,7-65,3).

17. TIGHT JUNCTION PROTEIN GENE EXPRESSION OF THE INTESTINES OF THE BRAZILIAN FREE-TAILED BAT (*Tadarida brasiliensis*)

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Recently it has been demonstrated that flying mammals primarily absorb water-soluble solutes in the intestines using the paracellular route [across tight junctions (TJ) between adjacent enterocytes], while non-flying mammals mainly use the transcellular route (across cell membrane using transporter proteins). The aim of this study is to present a preliminary comparison of the gene expression of TJ proteins between the insectivore bat *Tadarida brasiliensis* and previously reported data from mice by Holmes and collaborators (2006). Claudins 1, 2, 4, 5, 12, 15, occludin, ZO-1 and JAM mRNAs were quantified by RT-qPCR using Eef1a1 and β -actin as reference genes from three sections of the intestine (proximal, medial and distal to the pyloric valve) of the bat. The comparison of the TJ protein RNAs profiles between bats and mice was performed to explore a potential molecular basis for the observed difference in paracellular absorption. Higher values of claudins 1 and 5 were found in all sections of the intestine of the bat, though these proteins are postulated to diminish leakiness through TJ. On the contrary, claudin 2, which forms pores, was increased in bats in the midgut, compared with mice, giving molecular support to the observed higher paracellular absorption in bats compared to mice.

18. EFFECT OF OLIVE OIL INTAKE ON CHOLESTEROL METABOLISM: ANALYZING HEPATOCYTES FROM HYPERCHOLESTEROLEMIC RABBITS

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Olive oil (OO), the principal fat of Mediterranean Diet, is known to be nef it hypercholesterolemia (HC), a recognized risk factor of cardiovascular disease. Hypercholesterolemic rabbits (HCR) obtained by fat-enriched diet (14% v/w) carry several disorders. Were port the study of hepatic tissue – the organ that regulates serum cholesterol – from male New Zealand HCR protected by the addition OO (7% v/w) obtained from specific olive varieties to diet. Under optic and electronic microscopy, hepatocytes from HCR showed big vesicles of lipids without a loss of organelles, with normal nucleus. These cellular defects decrease considerably with virgin OO incorporation to diet. The molecular mechanism involved in the intracellular pathway of cholesterol biosynthesis and metabolism so showed alterations in HC diet that were reversed by OO supplementation. Specifically, a transcription factor, SREBP, changed its expression (RNAm and protein) depending on the type of fat consumed, showing a putative molecular mechanism for OO protection.

19. Hsp70 INVOLVEMENT IN Nox4/p22^{phox} REGULATION AND CYTOSKELETAL INTEGRITY AS AN EFFECT OF LOSARTAN IN VSMCs

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Nox4 NAD(P)H oxidase subunit is expressed in VSMCs, but its regulation mechanism remains unclear. The chaperone Hsp70 regulates a diverse set of signaling pathways via their interactions with proteins. We study the Hsp70 involvement in the regulation of Nox4/p22^{phox} and cytoskeletal modulation in Losartan treated VSMCs primary cultures from SHR and WKY mesenteric arcades. Losartan 100 μ mol/L (**L**) 45/90min and Angiotensin II 100 μ mol/L 15min (**AII**) in the absence or presence of Losartan (**L+AII**) were used. Hsp70, Nox4, p22, α -Actin and Vinculin were analyzed by WB, IP, IFI and confocal microscopy. To validate results, we transfected VSMCs with p-SIREN RetroQ shHsp72 vector to knock-down Hsp72 or empty vector (ev). Membrane translocation of Hsp70 after 90min of (**L**) exposure showed interaction with decreased Nox4/p22. Decreased Vinculin and α -Actin disorganization and colocalization with Hsp70 were demonstrated in SHR (**L**). Hsp72 knockdown resulted in higher Nox4/p22 expression and increased NAD(P)H oxidase activity in SHR (**L+AII**) related to (**L+AII**) non transfected. Actin polymerization, stress fiber thickening and persistence of Vinculin were demonstrated in Hsp72 silenced VSMC SHR (**L+AII**). Our results suggest the involvement of Hsp70 in the regulation of the expression and activity of Nox4/p22^{phox}, and in the cytoskeleton modulation by reducing oxidative stress associated with actin cytoskeleton disruption within Losartan effect.

20. BREAST CANCER: MAST CELLS PARTICIPATION IN TUMORAL DEVELOPMENT

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It has been proposed that an inflammatory process can be a significant factor in the development of cancer. Mast cells can facilitate and inhibit tumoral development.

This study was designed to increase the knowledge of mast cells participation in breast cancer.

Cancer cells (IBH-4 and IBH-6) were injected in female mice (N:NIH(S)-nu). They were treated with 0.5 mg of estradiol. Animals were sacrificed, tumoral volume calculated and samples processed for histological study, mast cells were quantified by microscopy. In IBH-4 tumors, intratumoral mast cells increased with tumoral size, while peritumoral mast cells decreased. In IBH-6 tumors, mast cells population had not correlation with tumoral size. In conclusion, mast cells number in IBH-4 tumors, correlate with tumoral size and development. It was proved an inverse relation between intratumoral and peritumoral mast cells. This information suggests an active participation in carcinogenesis.

21.

COMPARATIVE ROOT ANATOMY IN LEGUMES

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Wood anatomy has been recognised as a useful approach to identifying legume timbers. Variability in the anatomical features of the stem secondary xylem has been studied in detail whereas only few studies have been carried out -to date- on the secondary structure of roots. The aims of the present study were i) to analyse and compare the anatomical structure of roots from *Geoffroea decorticans*, *Robinia pseudoacacia*, and *Gleditsia triacanthos* (Leguminosae); b) to identify root characteristics with systematic and phylogenetic value. Cross- and longitudinal sections of main roots were stained with safranin-fast green. Histochemical reactions were carried out on fresh material sections. To study the wood structure of roots, Carlquist (2001) criteria were followed. *Gleditsia triacanthos* showed root cross sections with aliform-confluent paratracheal parenchyma, absence of crystals, cortex with a continuous sclereid band, uni- to multiseriate rays, scarce starch in the cortex. On the other hand, *Robinia pseudoacacia* showed marginal parenchyma, uni- to multiseriate rays, cortex with sieve tube members of larger diameters than the other species analysed, presence of septate fibres with crystals, presence of tyloses in vessels. Finally, *Geoffroea decorticans* showed paratracheal parenchyma, confluent at some points, uniseriate rays, presence of crystals in septate axial parenchyma. Porosity, vessel grouping, and pore type were similar in the three species studied. Morphological variables, like axial parenchyma distribution, ray width, crystals and sclereids, taken into account in this study demonstrate to be consistent and, together with other characteristics, they should be included in systematic studies.

22.

GEN TCF7L2 HAPLOTYPES ANALYSIS IN TYPE 2 DIABETIC PATIENTS

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Transcription factor 7-like 2 (TCF7L2) is one of the genes that show a strong association with susceptibility to develop Type 2 Diabetes Mellitus (T2DM). The gen presents many polymorphisms. In this work we studied the simple nucleotides polymorphisms (SNPs) rs12255372 (G/T), rs7903146 (C/T). The aim was to determine the genotypic frequencies and to analyze the haplotypes of rs12255372 and rs7903146 polymorphisms of TCF7L2 in diabetic and non diabetic patients. 49 DNA samples were analyzed, 27 were diabetic and 22 non diabetic (Co). The genotypes were analyzed by Tetra Primer ARMS-PCR. Allelic and genotypic frequencies were determined and 4 inheritance models were proposed: dominant, codominant, recessive and superdominant, by using χ^2 in the InStat and Snpstat statistic programs. The genotypic frequencies for rs7903146 were: Co 55% CC, 41% CT and 5% TT; T2DM: 26% CC, 56% CT and 19% TT; for rs 12255372 : Co 50% GG, 45% GT and 5% TT; DMT2: 19% GG, 70% GT and 11% TT. Dominant model: OR=3.43 (IC 95% (1.03-11.41)) p< 0.04 for rs7903146; OR=4.40 (IC 95% (1.22-15.84)) p<0.02 for rs12255372. According to the genotypic frequency we could suggest that the presence of T allele increases the susceptibility to develop T2DM. The inheritance model that is more suited to both polymorphisms is the dominant model.

23.

ORAL CANCER. RISK FACTORS

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The etiology of the mouth cancer is not known. Nevertheless it is known of the existence of factors of risk that they might operate as agent carcinogens. The aim was to describe, with base in the scientific evidence, certain factors: viral infection, protooncogenes and p53. There were studied 5 cases of file of the Laboratory of Surgical Pathology of the FOLP, with previous diagnosis histopathological of carcinomas to scaly cells. Of them, the distribution for location was: Floor of mouth (2); Buccal mucosa (1); Language (1) and Lip (1). The degrees of differentiation corresponded to differentiation and semidiferenciación: degree 1 (2); degree 2 (2) and degree 1-2 (1). Molecular technologies were in use: a) Chain reaction of the polymerase (PCR), for the detection and genotyping of the Human Papilloma Virus (HPV) and Herpes Simplex Virus type 2 (HSV2); b) PCR with locus of reference, to study the amplification of the protooncogen c-myc and c) Technology specific PCR-alelo, for the polymorphism in the codon 72 of the protein p53. We obtained as results that the HPV type 16 was detected in 4 of the cases and the genotype 6 in the remaining one. Only in one of the cases it was detected HSV2. In an alone instance it amplified the protooncogen c-myc. The polymorphism of the protein p53 was: Arg/Pro (4) and Arg/Arg (1). From the description of the series it arises that the studied cases of mouth cancer associated strongly to the type of high risk (HPV16) and weakly to the HSV2. With regard to the protooncogen c-myc it was not significant and the polymorphism p53 Arg/Pro prevailed. Though her n it was reduced the results they approach previous findings.

24.

ORALSQUAMOUS CARCINOMA. HISTOLOGIC GRADES/FORECAST

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Squamous cell carcinoma accounts for 90% of malignant neoplasms of buccal mucosa. The degree of differentiation of squamous cell carcinoma of buccal mucosa are strongly associated with treatment and prognosis of this disease. In order to detect the degree of differentiation of squamous cell carcinoma was revised foot-age Surgical Pathology Laboratory of FOLP, from paraffin-embedded samples and stained with hematoxylin-eosin. Diagnoses were made by optical microscopy. Based on an= 68, the specimens were subdivided according to location and degree of differentiation (according to WHO): M1-gum (n=26), M2-lip inf. (n=14); M3 tongue (n=13), M4 (N=buccal mucosa (n=6) and M5 floor of mouth (n=9). We obtained results in 28/68 specimens showed differentiation fitting into grade 1 WHO; were semidiferenciados 23/68, the combined 1-2 in 10/68.'s indifferenciados oranaplasia in 6/68 and the combination 2-3 on 1/68. Except for the floor of the mouth, in the series analyzed, in other areas of oral mucosa predominated differentiated and semidiferenciados pictures. Anaplasia was detected in the floor of the mouth, gums and lip. The predominance of differentiation, united insignificant frequency, with locations accessible to observation motivates consider these traces and other evidence-based early diagnosis to emphasize that not only allow survival, quality of life but also healing. Precisely in this last item is essential to clinical diagnosis, histopathologic interpretation and understanding of the biological behavior of oral squamous cell carcinoma.

25. SERPIN 1F: DETECTION ON RAT TISSUES BY RT-PCR
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In many mammals' species during their epididymal journey, sperm associated by their heads when they reach the Cauda, but not in the proximal regions of this organ. These associations, called Rosettes were previously characterized in Mouse and Rat. This suggests a maturational process, probably due to the milieu that contains several proteins secreted by the caudal epithelium.

The physiological meaning of this behavior remains unclear. By chromatographic techniques we previously isolate and identified SERPIN 1F, a member of the Serpins family (Serin proteases inhibitors). This protein described by the first time in rat was involved in the sperm re association during *in vitro* assays. We isolate RNA from different epididymal regions and by RT-PCR the presence of the Serpin 1F mRNA was observed in the samples analyzed.

26. THYROID HORMONE REGULATES BREAST CANCER CELL MOVEMENT VIA SRC/FAK/PI3K
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Thyroid hormones play a fundamental role in diverse process including cellular movement. Cell migration requires the integration of events that induce changes in cell structure towards the direction of migration. These actions are driven by actin remodelling and are stabilized by the development of adhesion sites to extracellular matrix via transmembrane receptors linked to the actin cytoskeleton. Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase that facilitates cell migration and invasion via the control of the turnover of focal adhesion complexes. In this work we demonstrated that the thyroid hormone triiodothyronine (T3) regulates actin remodelling and cell movement on breast cancer cells (T47-D) through the recruitment of FAK. T3 control FAK phosphorylation and translocation at sites where focal adhesion complexes are assembled. This process is triggered via rapid signaling to c-Src, phosphatidylinositol 3-OH kinase (PI3K) and FAK. In addition, we used T47-D cells treated with T3 to induce a cellular model of hyperthyroidism and hypothyroidism. We found that in cellular hyperthyroidism model the expression of Src, FAK and PI3K remained constant respect to control, but in the hypothyroidism model Src, FAK and PI3K were significantly reduced. In conclusion, these results suggest a novel role for the thyroid hormone T3 as an important modulator of cellular migration, providing a starting point for the development of novel therapeutic strategies for the treatment of breast cancer.

27. FAT INTAKE PROMOTES CHANGES IN SPERMATOGENESIS
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The intracellular cholesterol regulation depends on a balance between: produced *de novo* and incorporated by specific receptors. A dietary imbalance –unknown at testicular level- may trigger apoptosis and proliferation arrest. Detection in the structure of testicular tissue alterations in spermatogenesis and / or spermiogenesis, lipid accumulation and apoptosis under different diets were performed. Adult male rabbits (New Zealand) were used feeding with specific diets: control group (commercial diet, Gepsa®), hypercholesterolemic (HC = commercial diet plus bovine grease) and protected group with olive oil (OO = commercial diet plus virgin olive oil).

The number of apoptotic cells (detected by TUNEL techniques), decreased proliferation (differential cell counted) and structural abnormalities (transmission electron microscopy) were evident in the HC while in AO reversed.

The mechanism underline at the cellular/testicular tissue level is not defined yet but structural evidence showed the Golgi-Acrosomal complex disorganized accompanied by an altered manchette. These abnormalities could trigger apoptosis and/or inhibit cell divisions.

28. PIOGLITAZONE PREVENTS OXIDATIVE DAMAGE AND INHIBITS VISFATIN EXPRESSION IN AORTIC PERIVASCULAR ADIPOSE TISSUE
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Perivascular adipose tissue (PVAT) has been shown to secrete a wide variety of adipokines, including visfatin and produces oxygen radicals via NADPH oxidase system. Thus, PVAT provides an important site of control of vascular (dys)function in obesity and type 2 diabetes (T2DM). Pioglitazone (PIO) is an insulin sensitizer which is currently approved for treatment of T2DM. We analyzed the effect of PIO on the oxidative stress, the expression of p22 and Nox4, NADPH oxidase complex subunits, and visfatin gene expression in apolipoprotein E-deficient mice (ApoE-KO). Mice were fed with fructose overload (FF, 10% w / v) for 8 weeks and treated with PIO the latest 4 weeks. We analyzed plasma biochemical variables and lipid peroxidation determined by thiobarbituric acid reactive species (TBARS). Visfatin, p22 and Nox4 gene expression were determined in ApoE-KO PVAT. Glycaemia, insulinemia, triglyceridemia and TBARS increased significantly in the FF group and PIO prevented such increases. Visfatin and FF-induced p22 and Nox4 expression were significantly diminished by PIO in aortic PVAT. Our results show that in ApoE-KO mice, PIO improves insulin sensitivity, diminishes oxidative damage provoked by FF treatment and induced suppression of visfatin, a pro-inflammatory adipokine.

29. EFFECT OF OLIVE OIL INTAKE ON CHOLESTEROL METABOLISM: ANALYZING TESTIS FROM HYPERCHOLESTEROLEMIC RABBITS

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Hypercholesterolemia (HC) is a metabolic disorder that can be secondary and contribute to many diseases, most notably cardiovascular disease. Recently, it has also been associated with poor semen quality that may lead to male infertility. We have previously found that hypercholesterolemic rabbits (HCR) have poor semen quality, and OO supplementation improved sperm physiology and semen parameters affected by high saturated-fat consumption. Our interest resides in studying at molecular level the intracellular pathway of cholesterol metabolism in testis, in order to find out the cause that ultimately led to altered semen parameters. HCR were developed by diet containing 0.05% cholesterol (chol), and were protected by addition of 7% OO. We found changes in the expression of enzymes related to cholesterol biosynthesis in HCR that were partially reverted by OO. Our data demonstrate that OO improves semen quality in our rabbit model of HC, and suggest that the mechanisms accounting for this protective effect likely involve complex alterations in cellular metabolism beyond simply a reduction in cholesterol.

30. CHARACTERIZATION OF CDXA GENE ALONG SMALL INTESTINES OF *Taeniopygia guttata*

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CdxA is a transcriptional factor specifically expressed in the small intestine. It is considered to play a pivotal role in intestinal differentiation and maturation by regulating specific genes. Gene expression of this factor has not been characterized in passerine birds, such as zebra finches. Therefore, the goal of this study is to characterize CdxA mRNA in the intestine of granivorous zebra finches (*Taeniopygia guttata*). Different pairs of primers were designed and tested for CdxA gene, based on the most conserved regions among RNA sequences of related species. Intestinal tissue samples were extracted from zebra finches following surgical procedures. The intestines were divided into three portions: proximal, medial and distal. Total RNA was obtained using Trizol reagent and integrity was evaluated by agarose gels 1%, stained with Gel Red. DNaseI treatment was performed to avoid genomic DNA contamination. Reverse transcription reactions were performed using MMVL from 10 µg of total RNA. Dilutions of cDNA were tested to carry out PCRs. Levels of mRNA were semi-quantified from agarose gels (2%) with β-actin as reference gene using an appropriate software. CdxA gene is expressed along the entire small intestine of *T. guttata*.

31. MODULATION OF GONADOTROPHINS ON BREAST CANCER CELL MOVEMENT VIA FAK AND MOESIN

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Breast cancer (BC) is the most frequent malignancy in Western countries. Most BC are hormone dependent and are affected by therapies that reduce these hormones levels or interfere with their receptors. Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are synthesized and secreted by the pituitary gonadotrope in response to pulsatile GnRH (Gonadotrophin-Releasing Hormone). The effects of gonadotrophins LH and FSH are poorly investigated in BC movement. Actin remodeling and cell movement are fundamental for breast cancer function and are controlled by the actin-binding protein moesin and focal adhesion kinase FAK. We studied the extra-gonadal actions of FSH and LH on moesin and FAK activation, with a specific focus on cellular movement, more specifically, on the migration and invasion in T47-D BC cells. Our findings show that LH and FSH trigger a rapid actin rearrangement, with the formation of cortical actin complexes, pseudopodia and membrane ruffles. Both gonadotrophins trigger a rapid moesin and FAK activation via a non-genomic signaling cascade involving Gα13 protein, ROCK-2 to moesin and Gαi/β, c-Src to FAK. In conclusion, LH and FSH regulate BC cell movement by rapidly signaling to moesin and FAK via the actin cytoskeleton. These findings provide new information on the biological actions of gonadotrophins on BC cells as well as to open the path to new approaches to prevent or treat breast cancer disease in ageing, through the modulation of gonadotrophin actions.

32. FLORAL BIOLOGY OF TWO POPULATIONS OF *Adesmia bicolor* (LEGUMINOSAE)

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Adesmia bicolor is a native legume with forage potential. One characteristic that should be studied in the preliminary evaluation of the forage potential of this species is the mode of reproduction. In line with this, it is also important to study the morphometric characteristics of floral verticils in different populations of each species. The aim of this work is to compare the floral morphology of two populations of *A. bicolor*. Flowers from Pampa de la Invernada (San Luis) and Villa Rumipal (Córdoba) were collected and morphologically described. The variables analysed were: length and width of keels, standards and wings, as well as length of sepals, gynaecia, and stamens. The results were statistically evaluated by descriptive analysis, ANOVA, Mean Comparison (LSD Fisher) and Principal Component Analysis. Qualitative data of floral pieces and racemes are similar between both populations and coincide with Burkart (1987) description of the species. On the other hand, quantitative data show important differences between populations. Sepals, gynaecia, stamens, keels, standards and wings 2 of Pampa de la Invernada individuals are longer than Villa Rumipal ones. The only exception is wing 1 length, which is shorter. In conclusion, this study proves that morphometric floral characteristics can be used to identify both populations of *A. bicolor*. Furthermore, the high correlations between petal and sepal length, each wing length and width, and standard length and width.

33. COMPARATIVE STUDY OF ATYPICAL SIGNAL TRANSDUCTION OF AT₂ RECEPTORS

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Studies of the signaling pathway of Ang II AT₂ receptors appears controversial and cell-type specific. In the present study we compared the signaling pathways of Ang II AT₂ receptors in two models: HeLa cells expressing AT₂ receptors and membrane preparations from PND15 rat hindbrain. Previous studies in PND15 rat hindbrain evidenced the presence of FAK and SHP-1 in AT₂ immunocomplexes following exposure to Ang II. Association of AT₂-FAK-cSrc-RhoA was observed in HeLa-AT₂ cells by immunoprecipitation and western blot, maximal after 5 min in stimulation with Ang II (10⁻⁷M). FAK cleavage was observed in response to Ang II stimulation. In PND15 rat hindbrain preparations we observed phosphorylation of Tyr^{576/7} FAK but not Tyr³⁹⁷ FAK, induced by Ang II. Besides, ERK phosphorylation was induced by Ang II and blocked by Na₃VO₄ (PTPase inhibitor) or PP2, the c-Src inhibitor in hindbrain. In summary, two different models, a cell line over-expressing AT₂ receptors and an *ex vivo* preparation, share a similar signaling pathway involving FAK, RhoA, c-Src and ERK in response to Ang II, an atypical transduction pathway for a GPCR family member.

34. ANTIBODIES ELICITED WITH PROTEINS OF *Larrea divaricata* Cav. IN THE CELLULAR RESPONSE AGAINST *Pseudomonas aeruginosa*

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Larrea divaricata is a plant widely used in folk medicine. It was observed that antibodies elicited with plant proteins (PLD) cross-react with *Pseudomonas aeruginosa* proteins (PPA). Also, respiratory burst studies suggest that these antibodies promote bacteria opsonophagocytosis by murine macrophages (MØ). In this work we aimed to evaluate whether phagocytosis of bacteria opsonized with anti-PLD sera has any effect in MØ activation state. Rockland mice were immunized with PLD or PPA. Then, we evaluated IgA and IgG titers in sera against PPA by ELISA and compared Igs' specificities by Western blot. A significant reactivity against PPA was observed ($p \leq 0.05$). Next, MØ were incubated with bacteria opsonized with anti-PLD and anti-PPA (MOI 1:10). After this we determined Nitric Oxide (NO) production with Griess reagent, Arginase-1 activity and cell apoptosis and necrosis by flow cytometry. An increase in NO production and a decrease in arginase-1 activity were observed in every tested condition, compared with MØ alone. No significant differences were observed between anti-PLD and anti-PPA. Apoptosis and necrosis were no different between the analyzed groups. Our results indicate that phagocytosis of bacteria opsonized with anti-PLD or anti-PPA does not affect MØ activation, which is pro-inflammatory. Future studies are needed to fully elucidate the involvement of PLD as immunomodulatory mediators in the innate response against *P. aeruginosa*.

35. ANTIGEN SIMILARITY BETWEEN DIFFERENT PROTEIC FRACTIONS OF *Larrea divaricata* Cav. AND *Pseudomonas aeruginosa* CELLULAR PROTEINS

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Larrea divaricata Cav. commonly known as jarilla is a plant with a documented folk use to treat different illnesses. *Pseudomonas aeruginosa* is an opportunistic pathogen of clinical relevance. The aim of this study was to evaluate the humoral response of different fractions of *L. divaricata* crude extract (JPCE) against cellular antigens of *P. aeruginosa* obtained by sonication (BP). JPCE were concentrated and partially purified by using membrane concentrators with 10, 30, 50 and 100 kDa cut off, obtaining four fractions of proteins (R10, R30, R50 and R100). JPCE and BP were analyzed by SDS-PAGE to define proteic similarities. Mice of Rockland strain were immunized s.c. with R10, R30, R50 and R100. Levels of IgG were evaluated against BP by ELISA and Dot Blot tests. In order to confirm the cross-reactivity between BP and fractions of JPCE the inhibition ELISA test was performed. Non-immune sera were used as negative control. The serum dilution was previously determined by titration assays. A 33% of proteic similarity between JPCE and BP was observed. The antibodies anti-R10, R30, R50 and R100 showed cross-reactivity between BP antigens. Anti-R30 and R50 showed higher inhibitions percent (85% R30 and 89% R50). Dot blot showed that R50 and R30 have the major signals. These findings suggest that molecular mass protein greater than 50 kDa of *L. divaricata* have antigenic similarity to *P. aeruginosa*.

36. CYTOKINE PRODUCTION IN MESENTERIC LYMPH NODES INDUCED BY *Yersinia enterocolitica* INFECTION: ROLE OF YOPH

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Yersinia enterocolitica (*Ye*) is an enteropathogenic bacterium that invades Peyer patches (PP) and may disseminate to mesenteric lymph nodes (MLN), spleen (S), lung and liver. YopH is a tyrosine phosphatase secreted by *Ye* that inhibits the phagocytosis and lymphocyte activation. The role of YopH during *in vivo* infection has yet to be completely elucidated. IFN- γ plays a central role in the immune response against *Ye*. The aim was to compare IFN- γ production in MLN after oral infection with *Ye* WA-314 (wild-type) or *Ye* Δ yopH (YopH deficient). Moreover, we analyzed the production of IL-17, a cytokine associated with mucosal immune response. C57BL/6 mice were orally infected with 1–5 10^8 colony forming units (CFU) of *Ye*. The CFU in PP, MLN and S were determined 5 days after infection. Intracellular cytokine production was analyzed in cells from MLN by flow cytometry. We detected lower CFU in PP and MLN after *Ye* Δ yopH infection ($p < 0.05$). The median fluorescence intensity (MFI) of IFN- γ^+ cells in MLN was higher after *Ye* WA-314 infection ($p < 0.05$). No differences were detected in IL-17⁺ cells. We conclude that YopH increases *in vivo* IFN- γ production in MLN, but this cytokine may not compensate the YopH-mediated immune evasion.

37.

Larrea divaricata AND Candida albicans CROSS-REACTION, ITS USE ON OPSONIZATION AND NEUTRALIZATION

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Larrea divaricata Cav.(Ld) is a plant with a documented folk use. There is a cross-reaction between proteins of Ld crude extracts (JPCE) and *Candida albicans* antigens (PTC). The aim of this study was to observe how antibodies against JPCE act on the opsonophagocytosis (OP) of *C. albicans* and the neutralization of the effect on nitrogen oxide (NO) production exerted by PTC on murine peritoneal macrophages (MØ). Rockland mice were injected s.c. with JPCE. IgG levels were obtained by ELISA test. ELISA inhibition test was made to confirm cross-reaction. Non-immune sera were used as negative control. *C. albicans* was marked with CFSE, opsonized with anti-JPCE sera and incubated with MØ for phagocytosis (MOI 1:3). OP was followed by flow cytometry (FC). NO was tested from the supernatants of MØ incubated with LPS and different concentrations of PTC after 24hs. Neutralization was performed incubating PTC with anti-JPCE (1:1). PTC were able to inhibit ELISA signal in a >50. FC showed a significant increase on phagocytosis when *C. albicans* was opsonized with anti-JPCE. NO production was reduced by PTC. Anti-JPCE serum was not able to neutralize the effect of PTC. These results suggest that proteins from JPCE have antigen similarities with proteins of *C. albicans*, which are related to the innate immune response. These findings could lead the use of JPCE as an immunomodulator on the first line of defense against *C. albicans*.

38.

HEMOCYTE SPHEROIDAL AGGREGATES IN THE KIDNEY AND LUNG OF THE APPLE-SNAIL (*Pomacea canaliculata*) AFTER AN IMMUNE CHALLENGE

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Hemocyte aggregation has been shown in decapod crustaceans as a response to bacterial injection. We have also observed the formation of spheroidal aggregates in hemocyte primary cultures of *P. canaliculata*. We here report the formation of spheroidal hemocyte aggregates in the lung and kidney of this snail 96 h after injection of a yeast cell suspension into the visceral hump. Normally, the kidney contains interepithelial hemocyte islets, but also three-dimensional spheroids are formed in response to yeast injection. The lung does not normally show either hemocyte islets or aggregations, but small to medium-sized hemocyte spheroids were observed within large vessels after yeast injection, and some spheroids got stuck in the smaller vessels and also grew into the surrounding connective tissue. We have hypothesized that both the kidney and the lung may represent distinct immune barriers against natural pathogens in *P. canaliculata*. Spheroid formation may be a key feature of the response of this snail to immune challenges. Further investigations will be needed to shed light into the differential role of the kidney and lung as immune barriers.

39.

IMMUNOMODULATORY EFFECT OF GALECTIN-1 IN A MODEL OF INFECTION WITH *Yersinia enterocolitica*

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Yersinia enterocolitica (*Ye*) causes gastrointestinal infection. IFN- γ , a Th1 cytokine, is essential for the control of *Ye* infection. Th17 cytokines control pathogen dissemination from the mucosa. Galectin-1 (Gal-1), a β -galactoside-binding protein, limits Th1 and Th17 responses. The participation of Gal-1 in bacterial infection is unknown. We investigated the role of endogenous Gal-1 in the development of immunity against *Ye*. C57BL/6 wild-type (WT) and Gal-1 knockout (*Lgals1^{-/-}*) mice were orally infected with 1–5 10^8 colony forming units (CFU) of *Ye* O:8 WA-314. Survival and weight of the mice, and bacterial load in Peyer's patches (PP), mesenteric lymph nodes (MLN) and spleen were determined 5 days after infection. Gal-1 expression in the organs was studied by Western blot. IL-17 and IFN- γ levels were measured in homogenates of the organs by ELISA. We found that the infection induces Gal-1 expression in spleen. Although the weight was not significantly different from WT mice, higher survival rate (100 % vs 75%) was observed in *Lgals1^{-/-}* mice after infection. Augmented IL-17 levels were detected in PP, MLN and S of *Lgals1^{-/-}* mice ($p < 0.05$). However, no significant differences in IFN- γ levels were found. Bacterial load decreased in PP of *Lgals1^{-/-}* mice ($p < 0.05$). We conclude that Gal-1 through IL-17 plays an important role in regulating the immune response against *Ye*.

40.

GROWTH AND BIOFILM PRODUCTION BY NON-O1/NON-O139 *Vibrio cholerae*

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Non-O1/non-O139 *Vibrio cholerae* is a versatile bacterium that exists in diverse environments, such as rivers and lakes. Factors involved in the survival of *V. cholerae* in these environments are unclear. The aim of this study was to evaluate the growth and biofilm production by non-O1/non-O139 *V. cholerae*. Culture medium (CM) g/l: proteose peptone 30, yeast extract 5, trypticase 5, glucose 2, pH 7.6. The experiments were performed adding 150 μ l CM by well, in sterile 96-well microplate. A quantity of 10 μ l of overnight bacterial culture was added and incubated at 37°C, with agitation at 120 rpm. The negative control contained CM only. The inoculum and negative controls were processed by sixfold in five different microplates. Every 24 h biomass (OD_{600nm}) and biofilm formation were monitored in a plate reader. To estimate the biofilm formed, the content of the each plate was washed with sterile PBS. The remaining attached bacteria were fixed with methanol, stained with crystal violet and removing the dye with PBS. The adhered cells were resuspended in 33 % (v/v) glacial acetic acid, and the OD_{550nm} was measured in a plate reader. Higher biomass was obtained at 72 h, (OD_{600nm} 1,550) and increased production of biofilm was observed after 96 h (OD_{550nm} 2,385). These results demonstrated that the cell density controls the production of biofilm and suggests that biofilm-mediated attachment to abiotic surfaces may be important for non-O1/non-O139 *V. cholerae* survival in the environment.

41. CHEMICAL COMPOSITION AND REPELLENT ACTIVITY OF *Senecio pogonias* CABRERAS AND *Senecio oreophyton* J. REMY ESSENTIAL OIL, FROM SAN JUAN, ARGENTINA

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Chagas disease is a major public health concern in Argentina with estimates near three millions infected people. Essential oils (EO) from two *Senecio* species (Asteraceae) were assayed *in vitro* for their repellence activity against five instar nymphs of *T. infestans*, the vector of Chagas disease. Fresh aerial parts collected in San Juan Province were subjected to hydro distillation for 2 h in a modified Clevenger apparatus. The composition of the essential oils was examined by GC and GC-MS. Twenty nine components were identified in the EO from *S. pogonias* representing 97.6% of the oil and 30 components in *S. oreophyton* oil representing 97.3% of the oil. Main compounds were α -pinene, α -phelandrene and α -pinene and *p*-mentha-1(7), 8-diene respectively. Repellency on *T. infestans* nymphs was determined according to a standard method. Based on the percentage of repellency (RP) in a 72-h treatment, the most repellent EO was *S. pogonias* (60-80% RP) and *S. oreophyton* (40-60% RP). According to repellency classification scale oils are Class IV and III respectively.

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42. THE *cagA* STATUS OF *Helicobacter pylori* ISOLATES FROM CELIAC PATIENTS IN SAN LUIS, ARGENTINA

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Helicobacter pylori, colonizes the human gastric mucosa of about 50% of the world population and synthesizes virulence factors such as urease, vacuolating toxin or cytotoxin-associated antigen (CagA) that increase the risk of peptic ulcer and gastric carcinoma.

Celiac disease (CD) is a T-cell-mediated disorder of the small bowel triggered by gluten in susceptible subjects; damage occurs in both the intestine and the gastric mucosa. In this study, we evaluated the *H. pylori cagA* status in celiac patients from San Luis. Fifty four patients were included here, being 15 of them (27.8%) CD positive based on histological study and endomysial or transglutaminase antibodies. DNA was extracted from antral gastric biopsy using the QIAamp kit. *H. pylori* status was assessed by positive urease and PCR. The *cagA* gene amplification was performed by PCR. In total, 46 patients were *H. pylori* positives (85.2%) with a prevalence of 60.9% *cagA* positive strains. Among celiac patients high prevalence (86.7%; $p \leq 0.005$) of *H. pylori* was observed. The *cagA* status in celiac and no celiac patients was 69.2 and 61.3% respectively. The prevalence of *H. pylori cagA*-positive strains varies by geographic regions and has been associated with infection complications. Presence of these virulent strains may enhance the pathological CD processes.

43. EFFECT OF VEGETABLE EXTRACTS ON ABILITY OF *Listeria* STRAINS TO FORM BIOFILM (SLIME)

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The objectives of the present work were: a) to study the capacity of *Listeria* strains isolated from seafood and *L. monocytogenes* CLIP 74910 to form slime onto polystyrene plates, and b) to investigate the effect of extracts of *Baccharis sagittalis* and *Porophyllum lanceolatum* on ability of *Listeria* to form biofilm. Briefly, each well was filled with 150 μ L of TSB and 50 μ L of 10^7 CFU/mL of each bacterial inoculum. After 48 h incubation in aerobiosis at 37°C, the contents were aspirated and the plates were washed 3 times with PBS and stained with violet crystal 1% for 30 min. The adhered cells were resuspended in alcohol 96% and read at 490 nm in ELISA reader. *S. aureus* ATCC 35556 (positive control) and *S. epidermidis* ATCC 12228 (negative control) were included. The determinations were assayed in quadruplicate. The OD values were considered: slime producers (OD ≥ 0.23), weakly slime producers (0.16 \leq OD ≤ 0.22) and not slime producers (OD ≤ 0.15). On the other hand, in each well were placed 100 μ L of TSB, 50 μ L of 10^7 CFU/mL of bacterial inoculum and 50 μ L of acetone extract of *B. sagittalis* (12.5 mg/mL and 8 mg/mL) or ethyl acetate extract of *P. lanceolatum* (8 and 4 mg/mL) and was continued as described above. Only one strain of *Listeria* isolated from mussels was characterized as weak production of slime (OD = 0.18). Adding *B. sagittalis* extracts reduced production of slime (40-60%). The addition of *Porophyllum* showed no effect on the production of slime.

44. DETECTION OF CARRYING *Streptococcus agalactiae* (GBS) IN PREGNANT WOMEN

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Streptococcus agalactiae (GBS) form part of the normal human microbiota gastrointestinal tract, respiratory and urogenital. Cause of sepsis, pneumonia and neonatal meningitis and serious infections in pregnant women. The objective was to detect carriers GBS in pregnant women at 35-37 weeks of gestation to prevent and reduce the incidence of neonatal infection and puerperal complications. The knowing prevalence GBS in pregnant women at the health district of Godoy Cruz, for reduce perinatal morbidity and mortality. We had analyzed 493 samples of vaginal and perianal swabs of patients treated in different Godoy Cruz CPHC (Center Primary Health Care), from 2008 to 2011. These were seeded in selective broth 'I' odd-Hewitt, supplemented with nalidixic acid and colistin or incubated with gentamicin at 37°C for 18 to 24 hours, and then passed to Columbia Agar Base sheep blood. We studied all beta-hemolytic colonies or nonhemolytic suspicious. The presumptive identification was performed by biochemical tests. The confirmation was performed using specific serology GBS was isolated in 10 of the pregnant women studied. (Prevalence: 2.03%). The positivity rate found is low, by a low number of pregnant women accessing the study, but this force to insist in search routine. The bearing detection of mother is essential when, taking therapeutic measures that result in the reduction of infection in the infant and perinatal morbidity and mortality.

45. DIFFERENT PROPERTIES OF BOTULINUM NEUROTOXIN (NTBo) TYPE A BETWEEN STRAINS OBTAINED FROM SOIL AND CASES OF INFANT BOTULISM OF MENDOZA

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Infant botulism (BL) is a wide world distributed; pathology caused by *Clostridium botulinum* (*Cb*) type A (toxiinfection). The soil (Su) is the main reservoir of the bacterial and probably the main source of infection in infants. The neurotoxin (NTBo) obtained from type A *Cb* is a complex of \approx 900 kDa (exclusive to this serotype). We have previously found morphological differences between colonies of strains obtained from diverse sources, and compared to prototype A Hall. The aim of this work was to compare NTBo from strains obtained from soil and patients with BL. To assess this, five strains were selected from Su, five from BL and three were from the prototype A Hall. Toxicogenicity of strains was controlled by IP inoculation in white mice in CCM subculture (34°C, 96 h). NTBo generated under anaerobic conditions (34°C, 96 h) was rescued from the medium, centrifuged and the supernatants were purified by salting. Toxicogenicity and the DL50/ml were estimated (Reed and Muench). The specific activity (AE = DL50/mg protein) of the toxins was determined after measuring protein concentration, and also was evaluated their hemagglutinating activity (HA). Moreover, purified NTBo was analysed by electrophoresis in gels of polyacrylamide (SDS-PAGE) under non-denaturing conditions. Results: We observed that AE varied significantly ($p = 0.001$) between NTBo of Su and BL strains (Su: 3.93 ± 0.59 ; BL: 1.73 ± 0.44). In addition, both toxins exhibited apparent Mr of 300 kDa, in concordance with the absence of HA, unlike the prototype strain (900 kDa), which showed a high HA. We concluded that NTBo from BL patients is molecularly similar to that from soil strain, although with different AE. This could indicate that either both strains are different or they are similar although the toxin may be processed somewhere in the patient.

46. BIOFILM FORMATION BY *Listeria monocytogenes* AT DIFFERENT NUTRIENT LEVELS

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Listeria monocytogenes is a bacterium widespread in nature capable of causing serious foodborne human disease. It has the ability of adhering and forming biofilm on different surfaces. The aim of this work was to determine the influence of growth medium on biofilm formation. Strain: *L. monocytogenes* CLIP 74902. Culture medium: trypticase soy broth (TSB) and enriched medium (EM) (g/l): proteose peptone 30, yeast extract 5, trypticase 5, glucose 2, pH 7.6. The experiments were performed by sixfold, adding 150 μ l of each medium undiluted and 1/10 diluted respectively, in 96 well microplate. Aliquots of 10 μ l of overnight culture were added into each well, incubated at 37°C under aerobic conditions during 5 days. The negative control wells contained broth only. To estimate the biofilm formed, the content of the each plate was washed with sterile PBS. The remaining attached bacteria were fixed with methanol, stained with crystal violet and removing the dye with PBS. The adhered cells were resolubilized with 33% (v/v) glacial acetic acid, and the optical density at 550 nm was measured in a plate reader. Statistical analysis of the results showed that *L. monocytogenes* was strongly biofilm producer in EM, moderately producer in diluted EM, whereas it was poorly producer in TSB and no biofilm produced in diluted TSB. *L. monocytogenes* had capacity for biofilm formation on plastic surfaces and its production was markedly affected by the nutritional environment composition and the concentration of its components.

47. SLIME PRODUCTION OF *Staphylococcus aureus* ISOLATED FROM FOODS

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Biofilms constitute a protected mode of growth which enables the survival of the bacteria in a hostile environment. The aim of this study was to evaluate the production of slime from 11 strains of *Staphylococcus aureus* isolated from 40 samples of cakes. The microplate method was used by placing 150 μ l of trypticase soy broth with 1% glucose plus 50 μ l of the bacterial inoculum (10^8 cfu/ml). After 48h of incubation at 37°C, the contents were aspirated and the plates were washed and dried. The wells were stained with crystal violet's 1% for 30 min, washed and air dried. The adhered content was resuspended in 200 μ l of 96% ethanol and read in the ELISA reader at 490 nm. The determinations were performed in triplicate. A scale was used to assess the ability to produce slime (-): OD < 0.500; (+): OD 0.500-1.500, (++) OD > 1.500. Moreover, strains were streaked onto Congo red agar plates and after incubation, a four-color reference scale was used: black and bordeaux almost black as slime-producing strains, and bordeaux and red as non slime-producing strains. *S. aureus* ATCC 35556 and *Staphylococcus epidermidis* ATCC 12228 were included as positive and negative controls, respectively. There was high concordance between the methods tested for the production of slime. Four strains of *S. aureus* were positive and 11 strains were negative by both methods assayed. Three showed slime production (+) and only one resulted (++) . The ability of bacteria to adhere to surfaces in contact with food leads to serious health problems and economic loss due to the decomposition of food.

48. ANTIBACTERIAL ACTIVITY OF EXTRACTS OF *Mulinum echegarayii* AGAINST STRAINS OF *Staphylococcus aureus*

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Mulinum echegarayii (Hieron) is a plant distributed in the Argentina Patagonian steppe. The antibacterial activity of three extracts of *M. echegarayii* was assayed against strains of *Staphylococcus aureus* ATCC 35556, *S. aureus* ATCC 25923, and three strains obtained from food using microwell dilution assay method. These extracts were prepared using as solvents ethyl acetate and *n*-hexane on flash chromatography. Suspensions of 10^6 UFC/ml strains were used. Extracts were dissolved in DMSO and tested in a concentration ranging from 8000 to 500 μ g/ml. 2,3,5-triphenyl-tetrazolium chloride was used as visual indicator of bacterial growth. Extract *M. echegarayii* 10% ethyl acetate/*n*-hexane showed inhibitory activity against *S. aureus* ATCC 35556 (MIC 2000 μ g/ml) and *S. aureus* ATCC 25923 (MIC 4000 μ g/ml); two strains of *S. aureus* isolated from food were inhibited at doses of 8000 μ g/ml, and one at 4000 μ g/ml. *M. echegarayii* 30% ethyl acetate/*n*-hexane extract showed inhibitory activity against *S. aureus* ATCC 35556 and all the strains of *S. aureus* isolated from food at doses of 2000 μ g/ml and against *S. aureus* ATCC 25923 (MIC 1000 μ g/ml). *M. echegarayii* *n*-hexane extract showed MIC 1000 μ g/ml against all strains of *S. aureus* assayed. This study could contribute to discovery of new compounds with antibacterial properties from natural sources.

49. arsC GENE EXPRESSION OF *Nostoc minutum* IN THE PRESENCE OF ARSENIC(V)

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Arsenic (As) contamination is a global growing environmental problem mainly due to leaching from geological formations and anthropogenic activities. Microorganisms have evolved As defense mechanisms being the most common the presence of the As resistance operon (*ars*). The *arsC* gene encodes an arsenate reductase involved in arsenate detoxification. In the present study, the presence and expression of *arsC* gene in *Nostoc minutum* was studied. Batch cultures were produced in Watanabe medium added with 1000 mg/l As(V) and incubated at 30°C under continuous illumination for 16 days. Growth was evaluated by OD and dry weight determinations. The *arsC* gene, together with the *sdhA* gene, as housekeeping gene, were analyzed. For RNA extraction, 7 and 14 days biomass were treated with TRIzol reagent. *N. minutum* specific growth rate and final biomass values were higher in 1000 mg/l As (V) supplemented cultures than the control (1,60 g/l and 0,98 g/l respectively). The *arsC* gene expression showed a 1.4 fold increase in relation to the control after 14 days in the presence of As(V). The low increase observed in *arsC* gene expression during the process of metal detoxification could be due to the minor toxicity of As(V) with respect to As(III). This is a preliminary work and future studies are needed in order to assess the role of *arsC* gene in As resistance.

50. STOOL PARASITES OF THE PIGEONS IN SAN LUIS CITY AND ITS SURROUNDING

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The presence of pigeons in streets, buildings, plazas and promenades in many cities of the world, is not an exception in San Luis city. There is a marked increase in the number of individuals of the species *Columba livia*, which could represent a potential risk to the population's health, since the dove is the vector of a long list of pathogens and parasites, ectoparasites, fungi, bacteria and virus. The aim was to determine the percentage of stool pigeons infected with parasites and to identify the parasites observed in 31 samples of pigeons' stool collected from different areas of the city of San Luis and its surrounding. Stool samples were preserved in formaline saline, and then subjected to concentration techniques such as Telean, Willis, Sheather, and stain techniques such as Kinyoun, Didier trichrome, Quick-Hot Gram-Chromotrope-2R. Also, direct observation with lugol was carried out. A 48.6% of stool samples was parasitized. The following parasites were found: *Cryptosporidium spp.* 22.6%, *Capillaria spp* 12.9%, *Ascaris spp* 9.7%, *Eimeria spp* 6.5%, *Trichostrongylus spp* 3.2%, and *Heterakis spp.* 3.2%. Regarding the techniques, the recovery of parasitic structures in Willis (77.8%) and Sheather (42.9%) were significantly lower than the others studied. These birds are of an important health risk since many of the proven pathogens can be transmitted to humans and cause serious diseases.

51. GENETIC CHARACTERIZATION OF *Escherichia coli* ISOLATED FROM SURFACE AND GROUNDWATER BY USING DNA FINGERPRINTING

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Repetitive element anchored PCR was used to evaluate the genetic profiles of *Escherichia coli* isolated from water in El Barreal watershed, Cordoba. This is an agroecosystem which develops livestock (mainly cattle and pigs). The water supply is sustained by aquifer and stream itself. Water samples were collected from 44 locations (8 from surface, and 36 from groundwater) throughout the watershed for the isolation of *E. coli*. BOX-PCR was used to show the diversity of *E. coli* populations and its relationship with land use. Fingerprints were clustered by using the Jaccard coefficient evaluated by the unweighted-pair group method (UPGMA, SPSS v.11.5). Twenty one strains were identified from samples obtained from fifteen sites. Cluster analysis revealed a dominant group (76% of isolates) which showed highest similarity (80 and 100%). In contrast, the remaining isolates were most genetically diverse throughout the watershed. The relationship between genetic profiles, hydrodynamic and land use could predict a strong impact of livestock in aquifer and stream. The results of the present study demonstrate the importance of using *E. coli* as a fecal indicator bacterium in this watershed and highlight the need to differentiate environmental sources of *E. coli* from fecal sources in water quality monitoring.

52. ISOLATION OF *Cryptococcus spp.* FROM ENVIRONMENTAL SAMPLES OF SAN LUIS CITY AND SURROUNDING AREAS

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The genus *Cryptococcus* includes encapsulated yeast that causes disease in humans, primarily HIV patients. This fungus had high impact on the onset of AIDS. It has been isolated from natural sources in different regions of the world. To isolate *Cryptococcus* from natural source (pigeons' stool and trees) different growth medias were used. 184 swabs samples were collected from pigeons' stool and trees from different areas of San Luis city and surrounding areas, which were then planted and grown at 28°C for 48 hours in a culture medium containing caffeic acid. Bright colonies with brown pigment were reisolated in differential medium containing glycine -canavanina- bromothymol blue (GCBT). The testing of the ink china and the planting on malt extract broth were also performed with the suspected colonies. We obtained 17 positive samples for the genus studied, which eight belong to the species *C. neoformans* and 9 *C. gatti*. (7 isolated from feces of pigeons and 2 from the trees) These results are in agreement with previous studies performed in Argentina and San Luis city. The percentage of positivity obtained (9.2%) is epidemiologically important due to the relationship of the microorganism with the immunocompromised population.

53.

XYLANASE GENES IDENTIFICATION FROM STRAINS OF *Pseudobutyrvibrio xylanivorans**Grilli D^{1,4}, Kopečný J², Mrázek J², Cerón M³, Schnittger L³, Cravero S³, Sosa Escudero M⁴, Arenas GN⁴.*¹UMaza; ²Academy of the Sciences of the Czech Republic; ³INTA Castelar; ⁴UNCuyo. E-mail: dieogrilli@yahoo.com.ar

Genes encoding glycosyl hydrolase family 11 (GH 11) xylanases and multiple xylanases have been identified from *Pseudobutyrvibrio xylanivorans*. However, relatively little is known about the diversity and distribution of the GH 10 xylanase genes in strains of *P. xylanivorans*. The xylanase and associated activities of *P. xylanivorans* have been characterized in greatest detail from the type strain, Mz5. In present study, we evaluated degradation and utilization of xylan by wild-type strain of *P. xylanivorans* isolated from rumen of Creole goats. *P. xylanivorans* 2 cultures were able to utilize up to 53% of the total pentose present in birchwoodxylan (BWx), after 12 h, but could utilize up to 62% of a water-soluble fraction prepared from BWx. In addition, we propose to identify GH 10 xylanase genes in strain 2 and Mz5 of *P. xylanivorans*. This is the first report of successful PCR amplification using primers for GH 10 xylanase-encoding genes in *P. xylanivorans*. Strain 2 contained xylanase gene clearly related to xynA family of *Butyrvibrio*-related strains. The xylanase gene sequence of *P. xylanivorans* Mz5 was designated to the xynB family. These genes were phylogenetically distanced, derived from a common ancestor but evolved independently, adapting to particular environmental conditions for two different host species.

54.

SYNERGISTIC EFFECTS BETWEEN ESSENTIAL OILS OF ARGENTINEAN ANDEAN SPECIES AND CONVENTIONAL ANTIFUNGAL AGENTS*Lima B¹, Agüero MB¹, Rodriguez MV², Zacchino S², Tapia A¹, Feresin GE¹.*¹Instituto de Biotecnología-Instituto de Ciencias Básicas, UNSJ, San Juan; ²Área Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Suipacha 531, CP 2000, Rosario. E-mail: blima@unsj.edu.ar

The increased fungal resistance to classical drugs, their toxicity, and the costs involved justify the search for new approaches. The new classes of drugs, or different molecular targets, drug combinations might be considered a strategy for therapy.

From those new approaches, the essential oils (EOs) are one of the most promising groups of natural compounds for usage in the prevention and treatment of fungal infections. The antifungal activity was determined by the microdilution technique according to CLSI. The effect of the EOs, in combination with commercial drug (itraconazole, fluconazole and terbinafine) was evaluated through the checkerboard method. The EO of *A. cryptantha* in combination with fluconazole showed a synergistic effect against *C. albicans* (FICI≤0.5). The combination of EOs with terbinafine showed an additivism effect (0.5<FICI≤4) against dermatophytes evaluated. The EOs from San Juan province could be an alternative to reduce the development of drug resistance, or to decrease the dose of antibiotics used.

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55.

ANTIBACTERIAL ACTIVITY OF SESQUITERPENES FROM *Azorella cryptantha* FROM SAN JUAN, ARGENTINA*Lima B^{1*}, Sánchez M^{2*}, Agüero MB¹, Tapia A¹, Palermo JA², Feresin GE¹.*^{*}equal contribution by both authors. ¹Instituto de Biotecnología-Instituto de Ciencias Básicas, UNSJ, San Juan, ²UMYMFOR-Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires. E-mail: blima@unsj.edu.ar /msanchez@qo.fcen.uba.ar

Azorella cryptantha (Clos) Reiche, Apiaceae), n.v. 'yerba del soldado', is used in traditional medicine of San Juan (Argentina) to treat problems caused for food-borne illnesses associated with enterobacterium (digestive troubles). Herein we report the antibacterial properties of *A. cryptantha* according to CLSI methods. The extracts and fractions showed strong antimicrobial activity, against *Escherichia coli* ATCC 25922, *Escherichia coli*-LM1, *Escherichia coli*-LM2, *Salmonella enteritidis*-MI, with MICs values between 31.2 and 125 µg/ml. Diarrhoea caused by *Escherichia coli* infection is an emergent problem in both the developing and developed world, and is responsible for high rates of mortality in newborns. The bioassay-guided fractionation of the extracts led to the isolation for the first time of the sesquiterpenes chrysothol, stachytriol, 1β, 10α, 4β, 5α-diepoxi-7β-germacran-6β-ol, and mulinol (MICs= 50 µg/ml) as the compounds responsible for antimicrobial activity, supporting the traditional use of *A. cryptantha* to treat digestive disorders.

Acknowledgement: CICITCA-UNSJ, UBA, PICT-2010-0608-ANPCyT, CONICET (BL, MS, MBA, JAP, and GEF)

56.

ESSENTIAL OIL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF WILD POPULATIONS OF *Artemisia mendozana* (ASTERACEAE)*Lima B¹, López SB¹, Haddad M², Zygadlo JA³, Feresin G¹, Caballero D⁴, Tapia A¹, Suero E¹.*¹IBT-ICB, UNSJ, Argentina, ²Dpto de Dendrocronología e Historia Ambiental, Inst Arg de Nivología, Glaciología y Cass Ambientales (IANIGLA); ³IMBIV, FCEFYN, UNC, Argentina; ⁴Lab Hosp "Dr. Marcial Quiroga". San Juan. ⁴Serv Nac de Chagas. Córdoba. E-mail: esuero@unsj.edu.ar

Most of the antimicrobial activity in EOs is found in its compounds oxygenated terpenoids (e.g., alcohols and phenolic terpenes), while some components hydrocarbons also exhibit antimicrobial effects. Some studies have demonstrated that whole EOs usually have higher antibacterial activity than the mixtures of their major components, suggesting that the minor components are critical to the synergistic activity. The essential oils of the wild populations of *A. mendozana*, were examined by GC/MS, and their chemical compositions were compared. Moreover, antibacterial activity was determined by the microdilution technique according to CLSI, using reference and clinical isolate strain of *E. coli*. The chemical composition was similar for all populations of *A. mendozana*, in different ratios. The main compounds were Camphene, *Alpha*-thujone and Camphor and the minor constituents: artemiseole and artemisia alcohol. The antibacterial analysis showed that all oils presented an interesting activity against *E. coli* ATCC and clinical isolate (value MIC of 1500 and 2000 µg/ml). These results are support the use of Essential oils due that its main components have been widely recognized as bactericide compounds.

57.

Tagetes filifolia Lag ESSENTIAL OIL. CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITYLima B¹, López SB¹, Zygadlo JA², Caballero D³, Petenatti E⁴, Del Vitto LA⁴, Tapia A¹, Suero E¹.¹Inst. de Biotecnología- UNSJ. ²IMBIV, FCFEYn, UNC.³Laboratorio. Hospital "Dr. Marcial Quiroga". San Juan. ⁴Herbarium/Proj. 22/Q-016 SPU-ME, Ej. de los Andes 950, D5700HHW San Luis, Argentina. E-mail: esuero@unsj.edu.ar

The essential oils (EOs) are an effective alternative for treatment human infections produced for bacteria. The bacterium *E. coli* cause persistent infantile diarrhoea and is a major healthcare problem in developing countries. Moreover, the aspiration pneumonia *Klebsiella* produces different types of infections, such as pneumonia, bloodstream infections, wound or surgical site infection, and meningitis. The antibacterial effect and the chemical composition of EO of *Tagetes filifolia* Lag. (Asteraceae, Helenieae) collected in San Luis province, Argentina was reported. The chemical characterization was carried out by GC-MS and its antibacterial activity was determined by microdilution method according to CLSI against bacteria of reference and clinical isolates. Fourteen components, representing 100% of the oil, were identified. Main compounds were *trans*-anethole (59.5%) and estragole (40.2%). The EO of *Tagetes filifolia* was active against of *K. pneumoniae* and *E. coli* with values MICs between 375 and 2000 µg/ml, respectively. The results indicated that these EOs are promising to treat bacterial infections.

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58.

ANTIFUNGAL SUSCEPTIBILITY OF *Candida* STRAINS ISOLATED FROM INDIGENOUS POPULATIONS OF LAVALLE, MENDOZA

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Candida, a fungus of the oral cavity of many people, is able to develop disease when there are predisposing causes, being one of the opportunistic agents in human infections. Microbiological quality of the water resources available to the huarpes descendants of Lavalle, is poor and one risk factor for health. We set out to determine, carrying and susceptibility of *Candida* in this risk group. We worked with seven strains: *Candida tropicalis* (A, B, C strains), *Candida albicans* (D, F, G strains) and *Candida sp* (E strain), isolated from huarpes descendants, who live in poor sanitary conditions. The identification probes was carried out by morphological and biochemical tests. The method used for antifungigram is the diffusion agar Sensitabs Neo-Rosco tablets.

In all strains tested, all were sensitive to ketoconazole, fluconazole, itraconazole, nystatin and amphotericin. Strains B, E and G were fluorocytosine resistant; strains A, C and F showed intermediate susceptibility; whereas the D strain was susceptible to the same antifungal. These results are of great epidemiological value. We will work to increase the number of strains for study and thus for contribute to improving the health status of the population huarpe of the NE from Mendoza.

59.

Azorella trifurcata ESSENTIAL OIL. CHEMICAL COMPOSITION, ANTI-INSECT AND ANTIBACTERIAL ACTIVITIESLópez SB¹, Aragón LM¹, Lima B¹, Zygadlo JA², Caballero D³, Stariolo R³, Tapia A¹, Suero E¹.¹IBT-ICB, UNSJ, Argentina. ²IMBIV, FCFEYn, UNC, Argentina.³Laboratorio. Hospital "Dr. Marcial Quiroga". San Juan. ⁴Servicio Nacional de Chagas. Córdoba. E-mail: esuero@unsj.edu.ar

Essential oils (EOs) have been recognized for their antimicrobial, insecticide and antioxidant properties. The EO of *Azorella trifurcata* Gaertn. Pers, a native species from San Juan province, were obtained by hydrodistillation in a Clevenger-type apparatus and characterized by GC/FID and GC/MS. We report for first time, the repellent and antibacterial properties of the EO of *A. trifurcata*, along with its chemical composition. The antibacterial activity was assessed against bacteria of reference and clinical isolates, by microdilution method according to CLSI. Repellent activity on *T. infestans* nymphs was determined according to a standard method. Twenty-seven components were identified which represent 98.2% of the oil. The major components were spathulenol (38.2%), limonene (9.8%), myrtenyl acetate (8.4%) and α -guaiene (7.5%). The EO showed an interesting antibacterial effect with values de MICs between 500 to 2000 µg/ml. On the other hand, *A. trifurcata* essential oil showed a repellent activity against *T. infestans* of (60-80% RP) equivalent to IV class. The results indicated that EO of *A. trifurcata* is promising as repellent of triatomines and to treat bacterial infections.

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60.

USING NATIVE MICROORGANISMS FOR ZINC RECOVERY FROM GUALCAMAYO ORE.Marchevsky N^{1,2}, Bernardelli C², Donati E².¹Departamento de Minería. FCFMyN. UNSL. Chacabuco 917. (5700) San Luis. ²CINDEFI (CCT La Plata-CONICET, UNLP). Facultad de Ciencias Exactas (UNLP), Calle 50 y 115, (1900) La Plata. E-mail: nmarchevsky@unsl.edu.ar

Biomining, the use of micro-organisms to recover precious and base metals from mineral ores and concentrates, has developed into a successful and expanding area of biotechnology. These processes employ microbial consortia that are dominated by acidophilic, autotrophic iron- or sulfur-oxidizing prokaryotes. A native consortium was artificially prepared using microorganisms from different samples taken in an acid mine drainage of La Esperanza Mine (La Carolina, Province of San Luis). This consortium was used in the bioleaching of Gualcamayo ore (Province of San Juan). The experiments were conducted in flasks containing mineral finely ground at a pulp density of 2% (w/v). Basal salt medium at pH 1.8 was used with or without Fe(II) 1 g/L, powder sulphur 0.67% w/v and/or yeast extract 0.02% w/v. Samples were withdrawn at regular intervals for measuring pH and Eh. Fe(II) concentration was measured using ortofenantroline spectrophotometric method. Iron and zinc concentrations were determined by atomic absorption spectrophotometry. At 27 days, the culture in basal medium with and without iron achieved a maximum zinc recovery closed to 100% while the sterile controls reached less than 80% in all cases.

61. IDENTIFICATION AND CHARACTERIZATION OF ACIDOPHILIC STRAINS ISOLATED FROM AN ACID MINE DRAINAGE (LA ESPERANZA MINE, LA CAROLINA, SAN LUIS)

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Acid Mine Drainage (AMD) is a consequence of mining activity; it results from bacterial and chemical oxidation of sulfide minerals in waste rock and tailings. AMD is characterized by low pH values, and elevated sulfate, iron and heavy metal concentrations that cause severe damage to the environment. The detection of acidophile microorganisms involved in AMD generation could allow the further development of strategies for the remediation of this important pollution problem. Samples containing water and sediment from the AMD of La Esperanza Mine (La Carolina, San Luis) were taken and enriched in selective liquid medium for bacteria. The isolation was also conducted onto solid medium specifically formulated for the cultivation of iron and/or sulfur-oxidizing acidophiles. Physiological experiments were made using ferrous or sulfur as sole energy (9K and 0K medium) in Erlenmeyer flasks. Molecular techniques (FISH and 16S rRNA gene restriction enzyme) were applied to confirm the identity of the isolates. The main strains identified were *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans*.

62. EFFECT OF REGIONAL PLANT EXTRACTS ON GROWTH AND SLIME PRODUCTION OF METHICILLIN-RESISTANT *Staphylococcus aureus*

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Approximately 60% of human infections are reported to be a result of biofilm formation on human mucosa. The use of plants against skin disease is a common practice in Argentine popular medicine. The objective of the present study was to test the antibacterial activity and the effect on slime production of both aqueous and ethanolic extracts obtained from *Prosopis torquata*, *Acacia cavens* and *Acacia furcatispina* on methicillin resistant *Staphylococcus aureus* (MRSA) strains. Microbiological assay: the minimum inhibitory concentration (MIC) of the extracts against MRSA, was determined by broth microdilution test using 96-well microplates. Serial dilutions in base two (8000 to 125 µg/ml) of different extracts were assayed. The test was performed in triplicate. The plates were incubated at 37°C 24 h, and read visually. The spectrophotometric micromethod and congo red agar technique were used for detecting slime production. The results showed antibacterial activity of all extracts against *S. aureus*. The range of MIC values was 250 to 4000 µg/ml. The aqueous extract of *P. torquata* was the most active (MIC 250 µg/ml). We detected a negative effect of plant extracts on the production of slime. This result may suggest that topical application of these plants on wounds may be useful as a prophylactic for biofilm-related infections. The antibacterial activity found can be attributed to flavonoids or saponins present in these plants.

63. INFLUENCE OF TEMPERATURE ON FREEZE- DRYING OF THE MIXTURE OF BIOCONTROL AGENTS

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The bacterium *Rahnella aquatilis* (BNM 0523) (*R.a*) and yeast *Cryptococcus laurentii* (BNM 0525) (*C.l*) are effective biological control agents (BCAs) against phytopathogenic molds alone and in mixture. Commercialization of BCAs requires developing a formulation that conserves the viability and a high concentration of cells. The objectives were: (a) evaluate viability of cells in mixture bacteria-yeast at different temperatures of freezing and lyophilization, (b) apply the scanning electron microscopy (SEM) to detect the BCAs in the formulation matrix. Fresh cells of BCAs were obtained in YG broth (yeast extract 5 g.L⁻¹; glucose 10 g.L⁻¹) and harvested at exponential phase. Samples with the same concentration cells were freezing at -20°C or -70°C, before to lyophilization, using SMYG as cryoprotectant (skimmed non fat milk 10%; yeast extract 0.5%; glucose 1%). Viability of BCAs was expressed as percentage of surviving cells compared with the initial count. The lyophilized formulations were observed by SEM. The viability obtained for *R.a*. in mixture frozen to -20°C/-70°C was 61%/98% and *C.l* 53%/89% respectively. In lyophilized formulation the results were 58%/81% for *R.a* and 36%/62% for *C.l* (p<0.05). The SEM micrograph of the lyophilized formulations shows the porous surface in both temperatures and the integrity of the cells in the cryoprotectant material. The microbial mixture lyophilized in SMYG quickly frozen at -70°C could be useful to achieve an adequate BCAs formulation.

64. ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF *Hieronimiella* SPECIES FROM ARGENTINA

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Amaryllidaceae family is well known for their pharmacologically active alkaloids. An important approach to treat Alzheimer's disease is directed to the inhibition of Acetylcholinesterase enzyme (AChE). In an attempt to find new sources of AChE inhibitors, three *Hieronimiella* spp. (Amaryllidaceae) collected in Tucumán, Salta y Jujuy province, have been tested for their AChE inhibitory activity by microplate assay according Ellman et al. In addition the alkaloids composition was identified by GC-MS comparing their MS data and RI (Kovats retention index). The alkaloids extract of *Hieronimiella aurea*, *H. clidanthoides* and *H. marginata* showed a strong inhibition with IC₅₀ values between 4.5- 11 µg/ml, as reference compound galanthamine was used. The GC-MS alkaloids profile was dominated by lycorine, haemanthamine/crinamine, tazettine, hamayne/11-OH-vitattine, as well as the recognized AChE inhibitors galanthamine, chlidanthine and sanguinine, which has a hydroxyl group at C9 instead of a methoxyl group, is around 10 times more active than galanthamine. These plants species can be considered a new renewable source of AChE inhibitors (galanthamine and other alkaloids of biological interest). We are grateful to (PICTO2009-0116), UNSJ, Generalitat de Catalunya (2009-SGR-1060). J.O. and GEF to CONICET.

65. BIOFILM FORMATION BY *Escherichia coli* O157: H7 AND ITS RELATION TO THE PRODUCTION OF EXOPOLYSACCHARIDE AND CURLI

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Escherichia coli O157: H7 is widely known as an agent of hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura in children and is associated with the consumption of undercooked ground beef among other foods. Cross contamination may occur in food processing plants and during subsequent handling and preparation, resulting in a wide variety of foods implicated in outbreaks caused by these strains. It produces exopolysaccharide and curli, which can provide a physical barrier to protect cells against environmental stress and are involved in cell adhesion. The ability of biofilm formation by 6 strains isolated from HUS cases were evaluated with the method of Danese et al. modified. We studied the formation of biofilm in four culture medium being three of them based in vegetables (lettuce, spinach and soy) and enriched media at different pH. Readings were performed at DO 540nm. The results suggest that the increased production of biofilm is achieved at pH from 6 to 7, the pH: 3 was the worst. Higher values were obtained by optical density vegetable broths in nutrient broths. It will continue to assess its environmental strains curli production and exopolysaccharides (biofilm) as important vehicles of pathogen transfer to various foodstuffs.

66. DETECTION OF *cagA* AND *iceA* GENOTYPES OF *Helicobacter pylori* IN ORAL CAVITY

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Helicobacter pylori, is a bacteria commonly found in the stomach and is the major cause of gastritis, duodenal ulcers and gastric carcinoma. *H. pylori* strains that have *cagA*-positive and *iceA*1/2 genotypes are associated with an intense inflammatory response, which leads to the development of more severe gastric pathologies. The oral cavity has been proposed as a reservoir of *H. pylori* infection and as a source of reinfection following successful eradication from the stomach. The aim of the study was to determine the prevalence of *H. pylori* in relation to *cagA* and *iceA* genotypes in the oral cavity of 12 individuals. Diagnosis of infection using specie-specific antigen primer and detection of *cagA*, *iceA*1 and *iceA*2 virulence genes was performed from 2-3 mL of saliva samples by PCR. In this study DNA from *H. pylori* was detected in 66% of saliva samples. The prevalence of the *cagA*, *iceA*1 and *iceA*2 genes was 25, 12.5 and 50%, respectively. One case presented all virulence genes, indicating colonization with more than one *H. pylori* genotype. The detection of *H. pylori* virulent genotypes is important because it is presumed that severe gastric diseases are seen more often in patients who have been chronically infected with *H. pylori* isolates that bear both the *cagA* and *iceA*1/2 genes. Thus, the transient colonization of oral cavity with *H. pylori* virulent strains, could lead to the spread these microorganisms by direct transmission from person to person.

67. ENZYMES OF *Botrytis cinerea* AND ITS RELATION TO PATHOGENICITY

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To penetrate fruits *Botrytis cinerea* produces enzymes of attack and detoxification as polygalacturonase (PG) and laccase (LC) respectively. We determine the activities of enzymes PG and LC in culture supernatants of *B. cinerea* and then relate them to the pathogenicity in Red Delicious apples. *B. cinerea* (BNM 0527 or BNM 0528) were grown in apple broth, 7 days at 28°C and 140 rpm. Cultures supernatants were used to determine the enzymes units (EU). Biomass was determined as dry weigh (g.biomass.d.w). PG activity was measured by release of reducing group in a reaction mixture containing supernatant and 0.5% polygalacturonic acid and LC activity by rate of oxidation of 2,6 dimethoxyphenol (DMP). The reaction mixture consisted in supernatant and solution sodium malonate-2,6-DMP. Enzymes activities were expressed as EU/g.biomass.d.w. The pathogenicity of *B. cinerea* was expressed as the rot areas (mm²) in apples, after 7 days of storage at 28°C. The activities of PG and LC for strain BNM 0527 were (72.6 EU/g.biomass.d.w and 22.6 EU/g.biomass.d.w, respectively). The activities of PG and LC for BNM 0528 were 25.0 EU/g. biomass.d.w and 6.7 UE/g.biomass.d.w ($p=0.05$). The rot areas were greater when apples was inoculated with *B. cinerea* BNM 0527 (3006 mm²) in compared with BNM 0528 (1609 mm²) ($p=0.05$). Results indicate a high correlation between increased production of extracellular enzymes and pathogenicity in apples for the *B. cinerea* BNM 0527.

68. TERNARY COMBINATIONS SYNERGISM AGAINST *Staphylococcus aureus* ATCC 43 300

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The spread of methicillin-resistant *Staphylococcus aureus* (MRSA) is a major concern in hospitals. Therefore, there is a growing need to evaluate the activity of new antimicrobial agents that can be potentially used for the treatment of staphylococcal infections. In order to reduce the use of high concentrations of antibiotic, the bacteriostatic action of four ternary combinations flavonoid dihydroxylated/oxacillin (commercial antimicrobial) (Ox) / rutin (Ru) was determined. The compounds employed were: 2',3-dihydroxy-chalcone (I); 2',4-dihydroxychalcone (II); 2',4'-dihydroxychalcone (III) and 3,3'-hydroxyflavonol (IV); the antibiotic Ox and Ru. To quantitatively determine the sensitivity of *S. aureus* ATCC 43 300 against ternary combinations dihydroxylated flavonoid/Ox (2µg/mL)/Ru (20µg/mL), a kinetic-turbidimetric method was used. Specific growth rates were calculated from the respective growth curves, and using a previously proposed action mechanism minimum inhibitory concentrations were calculated in µg/mL: MIC_{I/Ox/Ru} = 22.9; MIC_{II/Ox/Ru} = 29.8; MIC_{III/Ox/Ru} = 34.8; MIC_{IV/Ox/Ru} = 64.9. The results indicate that ternary combinations were effective with MIC values lower than 35.0 µg/mL for chalcones/Ox/Ru, while for the combination hydroxyflavonol/Ox/Ru the MIC value was markedly superior (64.9 µg/mL). These results encourage us to continue investigating the effect of different combinations of flavonoids to increase their antibacterial activity against pathogenic microorganisms, such as *S. aureus* MRSA.

69.

Cryptococcus neoformans: EPIDEMIOLOGY IN MENDOZA CITY. INITIAL STAGES

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Cryptococcosis is an opportunistic fungal, severe infection, caused by *Cryptococcus neoformans*, which usually affects patients with marked involvement of cellular immunity, such as people infected with human immunodeficiency virus (HIV). The main reservoir in nature is dried pigeon droppings. This considered zoonotic disease has broken between the potentially fatal opportunistic infections reemerging. The background distribution and frequency of isolates of *C. neoformans* and *C. gatti* in Argentina are scarce. Therefore, the main objective of this project is to study the presence and geographical distribution of the *Cryptococcus neoformans* species complex associated with pigeon feces in the city of Mendoza. Samples were standardized stool pigeons in various public places, squares. They were seeded in culture media and selective and differential biochemical tests carried identification and susceptibility. We hope to test the colonization of urban pigeons by *Cryptococcus neoformans* in our city. We provide data on the frequency of isolation of different serotypes and their respective reservoirs. Therefore contribute to the epidemiological knowledge of this mycosis in our environment, in order to implement proper and timely preventive measures.

70.

PROGRAMMED CELL DEATH AND OXIDATIVE STRESS PARAMETERS IN UNDIFFERENTIATED PLANT CELLS USED IN BIOCATALYSISAnello A¹, Mascotti M¹, Orden A¹, Zirulnik F², Molina A².¹INTEQUI-CONICET, ²Área Química Biológica; FQB y F, UNSL, San Luis. E-mail: abi_la_88@hotmail.com

Undifferentiated plant cells cultures are useful for biocatalysis. Xenobiotics substrates may alter the reaction yield and molecular or cellular metabolism in these processes. These alterations can be estimated by measuring antioxidant defense and oxidative cellular damage. Biocatalysis conditions may induce programmed cell death (PCD). In this work we evaluated the PCD induction and effects of xenobiotics substrates on oxidative stress parameters in biocatalytic cell cultures. Submerged cultures of undifferentiated *Tessaria absinthioides* cells were treated with acetophenone (AP). PCD induction was determined by DNA fragmentation (*DNA laddering*). Superoxide dismutase (SOD) activity, reduced glutathione (GSH) and lipid peroxidation levels were quantified. PCD was not induced with AP treatments lower than 10 mM, while higher concentrations than 20 mM induced it. However in both cases, GSH level was decreased compared to a control, and it was not enough to trigger PCD independently of other signals. SOD activity was reduced with 10 mM and was not detectable at 20 mM AP. Protein content was reduced in 20 mM AP, probably by specific proteases activation during PCD. Lipid peroxidation decreased independently of the PCD process. The biocatalytic ability of the system with lower concentrations than necessary to induce PCD was completely lost, and this mechanism should not seem to depend of installed oxidative stress condition.

71.

REDOXIMORPHIC FEATURES IN SAN LUIS WETLANDS

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When you produce anaerobic conditions in the soil, appear redoximorphic features. The objective of this study was to determine the morphopedologic characteristic of wetland soil. The work area is located to among the 33° 37' S and 65° 25' W, presenting a height of 505 msnm and covering some 200 has. The soils were characterized by means of *in situ* determinations (depth to the phreatic and redox potential) jointly with the morphological characteristics. Each physiognomic type presents different morphologies of soils, and as it progresses from the halophyte mound (highest sector) toward the saline beach (lowest area), the surface salinity increases and decreases the depth of the phreatic level. The last concept and the redox potential indicate significant differences between the physiognomy types for all months. In the halophyte bushes high the redoximorphic features are present in form of recovered pores of iron in the depth where begins to have influence the phreatic level. In the patches of halophyte bushes low are present these same features but more superficially product of a higher water table. In the halophyte prairies there are areas with iron depletions below horizon Ap. The saline beaches presented an Ap horizon with iron depletions. We conclude that the redoximorphic features, morphopedologic characteristic and studied parameters explain the scarce development of the soils of this sector that corresponds with the distribution of the vegetation.

72.

MICRONUTRIENT CONTENTS IN SELECTED NATIVE GRASSES FROM CORRIENTES PROVINCE

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Although micronutrients are required in relatively small quantities, they play important metabolic roles in the plant physiology, and deficiencies can lead to severe depression in crop productivity. Apart from deficiencies which limit crop growth, concentrations and bioavailability of micronutrients in food/feed stuffs may be lower than nutritionally desirable for livestock or humans. In this context, the aim of this work is to determine the concentration of 4 mineral micronutrients (Cu, Fe, Mn and Zn) in aerial parts of mixture forage, collected from the Corrientes Province region. Twenty samples were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Microwave-assisted acid digestion of samples was used to eliminate the organic matter. The means of total content in the raw material were: Cu (2.3 µg g⁻¹), Fe (45.3 µg g⁻¹), Mn (172.4 µg g⁻¹) and Zn (20.8 µg g⁻¹). The results revealed that the mixture forage analyzed were poor in the contents of various essential elements (from the nutritional quality point of view). These results suggest the need for mineral supplementation for livestock production.

73. SOYBEAN PROGENIES EVALUATION WITH INDUSTRIAL QUALITY ATTRIBUTES

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Soybean breeding programs have historically prioritized grain yield (Y) and they have not done enough selection pressure on oil (O) and protein (P) contents. So, Argentina's soybean has high oil content but its flour has the lower South America's protein content. In order to evaluate the soybean's industrial quality and select the most promising progenies in 2010/11, in the Department of Agricultural Sciences at the UNSL, 13 soybean progenies F₅ selected for quality attributes were sown in a randomized block design with two replications. Phenological characters were recorded, and plant height (PH), seed weight (SW), Y, O and P were determined. An analysis was performed of variance and estimated Pearson's coefficients correlation. The ANOVA showed significant differences ($p < 0.05$) between the progenies for all traits, and thus indicates that there is potential variability which allows an effective selection for productivity and quality characters. Y was positively correlated with O (0.29) and negatively with P (-0.44). Between P and O, the correlation was negative (-0.54). Two high Y and P progenies, three high Y and O progenies, and one high Y, O and P progenies were selected.

74. DETERMINATION OF MATURITY GROUP IN SOYBEAN GERMLASM WITH NUTRITIONAL QUALITY

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Argentine Soybean varieties meet yield and adaptation requirements, but the protein content is lower than the industry requires. The maturity group (MG) influences oil content (OC) and protein content (PC), so higher MG has more PC and less OC. The objective was to rate, according to MG, soybean advanced lines with high PC. In 2011/12 at the UNSL, "Preliminary C" tests were conducted with twelve F₆ soybean progenies selected by PC and three control varieties of the III long, IV short and V medium MG. The variable days for flowering and maturity were analyzed, and the cycle length was determined. To group genotypes based on phenological variables, we performed a cluster analysis using Euclidean distance as proximity measure. The results of hierarchical clustering were visualized on a dendrogram where four clusters were determined. Control varieties of III long and IV short MG with nine genotypes were located in the first group. The second group was formed by two genotypes, the third with one genotype, both without any control variety. In the fourth group the V long MG control variety was only located. Out of the twelve evaluated soybean lines, nine would correspond to the III long and IV short MG and the rest to a higher than V medium.

75. EFFECTS OF BORON ON *Medicago sativa* AND ITS SIMBIOT *Sinorhizobium meliloti*: APPLICATIONS ON PHYTOREMEDIATION

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Alfalfa needs high amounts of boron for his development. This property could be used to restore soils contaminated with boron. The aim of present work is to analyze the effects of boron over alfalfa and his simbiot *Sinorhizobium meliloti*. *S. meliloti* was grown in solid culture media supplemented with increasing concentration of boron. It was found that *S. meliloti* grows to concentrations of 20 ppm of boron. On the other hand, alfalfa seedlings were grown in greenhouse using perlite as substrate and supply with 0; 0.6; 5; 10; 20 and 30 ppm of boron. After 11 weeks of growth, dry-weight of aerial part increased significantly in boron treatments. Therefore, based on the obtained results we can conclude that both the bacterium and alfalfa are tolerant to high concentrations of boron.

76. BIOFERTILIZATION OF CORN WITH EDAPHIC CYANOBACTERIA OF SAN LUIS PROVINCE

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Agricultural soils in the province of San Luis and surroundings are poor in organic carbon, nitrogen and structural stability, limiting the sustainability of these ecosystems. Inoculation with cyanobacteria in poor soils improves the before mentioned parameters. The objective of this work was to evaluate the answer of the soil with cyanobacteria biofertilization on height and dry matter of a crop of corn field. Soil samples were collected from natural grassland EEA INTA San Luis and they were isolated species of agronomic interest. Algal biomass was obtained with a concentration of 1.10^6 cells/ml. Three treatments were formulated with different concentrations of cyanobacteria: 100% (T1), 50% (T2) and 25% (T3) which were applied in two stages: one on corn seeds before planting and the other at 35 days on the ground. Plant height was measured periodically through the entire cycle in the growing culture. When it was concluded, dry matter of the plant was determined. The height of plants obtained from all treatments were significantly superior to the control, starting from days 65 after sowing; while in the dry biomass no significant difference were observed.

77.

THE INOCULATION WITH *Pseudomonas fluorescens*, CONFERS TOLERANCE TO WATER AND SALT STRESS IN *Arabidopsis thaliana* PLANTS*Cohen AC, Salvadores C, Bottini R, Piccoli PN.**IBAM-CONICET/FCA-UNCuyo, M5528AHB Chacras de Coria, Mendoza. E-mail: acohen@fca.uncu.edu.ar*

Pseudomonas sp. is a well-studied group of bacteria that promotes plant growth (PGPR). This group has been studied for increasing agricultural production and as biocontrol agents against plant diseases. In a previous study *P. fluorescens* was isolated from roots of *Vitis vinifera* L. (cv. Malbec) and it was determined that *P. fluorescens* produces the plant hormone abscisic acid (ABA) in chemical-defined media.

The aim of this study was to evaluate the effect of inoculation of *P. fluorescens* in *A. thaliana* plants, well-watered (W), submitted to drought conditions (D) or salt stress (S). Experiments were carried out in chamber growth with 12 h light at 22°C on pot. Seven days-old *A. thaliana* seedlings were inoculated with *P. fluorescens* in PBS or PBS (control). At 30 days, the different treatments (W, D and S) were applied. After 25 days, morphological-physiological modifications produced in inoculated plants were measured. *P. fluorescens* stimulated leaf area and the fresh and dry weight of aerial part in all treatments. Also, inoculation improved seed yield and decreased stomatal conductance in plants submitted to drought and salt stress. The D+I and S+I plants reached wilting point later than D and S, so alleviating stress. This opens new alternatives for a strategy against drought and salt stress.

78.

BIOACTIVITY OF CRUDE EXTRACTS OF FIVE PLANTS OF THE SEMIARID PAMPAS AGAINST *Tribolium castaneum* HERBST*Cora Jofré F¹, Peralta MP¹, Manzano S¹, Pattacini SH¹, Martínez O², Bellozas Reinhard M¹, Scoles GE¹.**¹FCEyN-UNLPam, Av. Uruguay 151, CP6300 -Santa Rosa, LP, Argentina. ²Facultad de Agronomía, Ruta 35 - Km. 334 - C.P. L6300 Santa Rosa, La Pampa. E-mail: florenciacorajofre@hotmail.com*

Repellent effect of chloroform-ethanolic and hexanic extracts from aerial parts of *Ambrosia tenuifolia*, *Baccharis articulata*, *Urtica urens*, *Clematis montevidensis* and *Euphorbia dentata* were evaluated against *Tribolium castaneum* larvae and adults.

Repellency in larvae was evaluated by bioassays on treated diet with extracts (dose 0.04%). Repellent activity was evaluated at twenty four hours in larvae. Repellency test, in adults were performed using filter-paper circles cut in halves. The extracts, were applied on each half at a concentration of 0.31 mg.cm⁻². Percentual repellency (PR) was determined for each extracts.

The chloroform-ethanolic extract of *B. articulata* induced larvae repellency when the extracts were mixed in the diet followed by the hexanic extract of *A. tenuifolia*. Six extracts showed attractancy against *T. castaneum* larvae. A moderate repellent effect of *B. articulata* and *Urtica urens* extracts and hexane extract of *A. tenuifolia* on *T. castaneum* adults was observed; the hexane extract of *C. montevidensis* was the one with stronger repellency (class IV).

79.

ANTINUTRIENT DETERMINATION IN SEED FLOUR OF DIFFERENT AMARANTH SPECIES*Daguerre A, Lucero López RV, Escudero NL.**Facultad de Qca Bqca y Fcia. UNSL. 5700-San Luis. E-mail: adaguerre@unsl.edu.ar*

Amaranths have become an alternative food source of high nutritional value for humans, but antinutritional factors can affect their quality. Considering that work is being done with new regional varieties and lines of amaranth, the purpose of this study was to detect antinutrients in some of these varieties. The amaranths studied were *Amaranthus cruentus* var. *candil*, *Amaranthus hypochondriacus* var. *dorado*, *Amaranthus hypochondriacus* var. *antorcha*, *Amaranthus hypochondriacus* x *Amaranthus cruentus* and *Amaranthus cruentus*. Determinations were performed for oxalates (AOAC Official Method, 1998), obtaining 2.61 – 5.54 mg of oxalic acid/100g of sample; nitrates (Cataldo, DA y col. 1975), which ranged between 73.73 and 275.06 mg%; saponins (WHO/PHARM/92559, 1992), which gave a foaming index <100, lectins (Do Prado, VC *et al.*, 1980; Das Gupta, VR; Boroff, DA, 1968), which presented agglutination in dilutions between 1/64 and 1/128, and phytic acid (Rucci, AO; Bertoni, MH, 1974), obtaining concentrations of 48.29 to 61.63 mg of phosphorous/100g of sample. Since the values obtained for the antinutrients studied are within the allowed or acceptable ranges and thus do not represent a health hazard, the varieties studied can be considered apt for human consumption, provided further studies are carried out for assessment of other antinutrients.

80.

OXIDATIVE STRESS PARAMETERS MONITORING IN CADMIUM TREATED *Glycinemax* L. PLANTS*Felici CE^{1,2}, Molina AS², Arce S¹, Gil AR¹, Acosta G¹, Zirulnik F², Gómez R¹.**¹INQUISAL, CCT CONICET San Luis. ²Area Química Biológica, FQByF, UNSL. E-mail: emilianofelici@gmail.com*

Heavy metals induce different types of stress in plants. The monitoring of essential elements concentration, the analysis of chlorophyll level and the study of ionic permeability are good indicators of oxidative damage in the plant homeostasis.

The aim of this work was the study of the parameters that reveal the oxidative stress caused for Cd exposure.

Soybean seeds were germinated and developed under controlled conditions. On the 4th day of germination were placed in hydroponic conditions with Hoagland nutrient solution. On the 10th day, were exposed to Cd (40 µM) during 4, 6, 24, 72 h and 6 days. The seedlings were harvested and analyzed after microwave assisted digestion. The concentration of Cd, Cu, Fe, Mg, Zn and Mn in soybean roots and leaves was obtained by ICP-MS (Inductively coupled plasma-mass spectrometry). High levels of these elements were found in Cd treated plants. Chlorophyll level and ionic permeability were determined. At 24 h a reduction in the chlorophyll level was observed, indicating chloroplast damage. This parameter is related with a significant decrease in Mg uptake at leaf level. Ionic permeability was modified in Cd treated plants. Cu, Fe, Zn and Mn increased due to Cd exposure, it could be related with the antioxidant enzymes which use these elements as cofactors (CuZnSOD, FeSOD, MnSOD). In this model, Cd exposure alters the normal physiological status of soybean plants.

81. HPLC STANDARDIZATION OF THE "VALERIANS" OF CUYO PHYTOMEDICINE, ARGENTINA

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Valeriana officinalis L. and other species of this genus are frequently used in phytomedicine because of their sedative properties on the CNS. British Herbal Pharmacopoeia encodes *Valeriana officinalis* and proposes an HPLC technique for the assay of valerenic acid (including valerenic acid, acetoxyvalerenic acid, hydroxyvalerenic acid and valerenal). In this work, we used the same method in order to quantify and verify their validity both in rhizomes/roots and leaves from two regional species of *Valeriana*: *V. polybotrya* (Griseb.) Hock and *V. ferax* (Griseb.) Hock. To obtain the chromatograms we used a standard solution of valerenic acid 0.1% in methanol and methanol dilutions for each species, analyzing in a Gilson HPLC with DAD detector (column C-18, 5µm, 4.6 x 250mm) and methanol: phosphoric acid 0.5% (80:20) as mobile phase. Rhizomes/roots of regional species show similar valerenic acid concentration to those of *V. officinalis*. On the other hand, the valerenic acid concentrations were higher in rhizomes than in leaves for the three species investigated. Through analysis of the characteristic peaks it is possible to conclude that the encoded method is an excellent tool in the quality control and standardization of crude drugs and phytomedicines elaborated with these plants.

82. EFFECT OF *Amaranthus hypochondriacus* ON GLUTATHIONE METABOLISM IN RATS INTOXICATED WITH ETHANOL

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Oxidative stress has a significant impact on the pathogenesis of alcohol-related diseases and glutathione is a major line of defense against it. The present study investigated the effect of seed of *Amaranthus hypochondriacus* (*Ah*) on the content of total glutathione and reduced glutathione and the enzymatic activity, through kinetic assays, of glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glutathione-S-transferase (GST) in the liver of male *Wistar* rat treated with ethanol. The animals were divided into four groups of six subjects each; two of those groups were fed diet AIN-93M containing casein as protein source, and the other two with AIN-93M containing *Ah*. One of each protein group received 20% ethanol in the drinking water, being: *AhC* (*Ah* control) and CC (casein control), *AhE* (*Ah* ethanol) and CE (casein ethanol). The experiment was performed for 4 weeks. The value of reduced glutathione in the group *CAh* was increased relative to CC and to *EAh* ($p < 0.05$), and the GSH/GSSG index in *CAh* group was higher compared with the other groups. Furthermore, activities of GPx tended to reduce, approximately 16%, during ethanol administration. The other parameters remained unchanged. These results suggest a potential protective effect of *Ah* flour versus casein in control diets.

83. POLYPHENOS AND FREE RADICAL SCAVENGERS OF *Punica granatum* L. VAR WONDERFULL CULTIVARES FROM SAN JUAN, ARGENTINE

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The pomegranate (*Punica granatum* L.) fruit has been extensively used in folk medicine and the pomegranate juice is consumed widely for its possible health benefits. The aim of this study was to evaluate the total phenolics (TP), flavonoids (F) and anthocyanins (A) content, as well as antioxidant activity of juices and most of *Punica granatum* L. of the cultivars from Sarmiento (S) and 25 de Mayo (M) districts. In addition the juice was characterized by HPLC/MS/MS. The highest values TP content was found in juice from S (>1200 mg GAE/100mL), F (>200 mg QE/100mL), and the A levels (>100 mg/L). The juice showed a stronger DPPH scavenging activity (>90% at 1500 µg/mL), and the total soluble solids (TSS) (evaluated as juice quality) ranged from 18.40 to 19.80 °Brix. These results indicate the potential that would have regular consumption of pomegranate juice cultivars of San Juan for the maintenance of health and prevention of diseases associated with oxidative stress. *We are grateful to UNSJ, ANPCYT (PICT 2008-0554) and CONICET. (LL. GEF, WD, BV).*

84. STRESS PARAMETERS ANALYSIS IN COTTON PLANTS BY CHEMOMETRIC

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Stress parameters (antioxidant enzymes activity and lipid peroxidation) in cotton plants were analyzed by chemometric. Plant were grown in hydroponic culture and added with increasing dose of silicon (Si). Enzymatic activity of catalase, guaiacol peroxidase, ascorbate peroxidase and the lipid peroxidation were determined in leaves and roots. Data were analyzed by chemometric methods - principal component analysis (PCA), cluster analysis (CA) and linear discriminant analysis (LDA)-. The results showed that silicon affects the activity of GPOX, APX and CAT in roots and leaves, but lipid peroxidation was not affected in comparison to a control (i.e. without Si). Furthermore, chemometric analysis showed that the enzymatic activities in leaves and roots depend to silicon concentration; however, application of Si upper to 100 mg L⁻¹ in hydroponic solution produced a unique grouping in PCA, CA and LDA. As conclusion, these results could indicate that the metabolism it is stabilized beyond 100 mg L⁻¹ of Si and upper concentrations of Si did not induce change in plants.

85.

FTIR PROFILES OF *Valeriana officinalis*, *V. polybotrya* AND *V. ferax* DRUGS FROM ARGENTINEAN MARKET*Popovich M¹, Gette M¹, Cami G², Petenatti M¹, Petenatti E¹, Del Vitto L¹.*¹Proj. 22/Q-016; ²Química Inorgánica-FQByF-UNSL San Luis.

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Roots and rhizomes of *Valeriana officinalis* L. (Valerianaceae) are official drugs in many Pharmacopoeias, because of their sedatives properties. Aerial parts are used with the same object in folk medicine. Two Argentinean native species: *V. polybotrya* (Griseb.) Hock and *V. ferax* (Griseb.) Hock can replace to *V. officinalis* and require standards for their characterization. We have recently shown that FTIR spectroscopy can help establish additional parameters for quality control of medicinal herbs. Therefore, FTIR spectroscopic studies were carried out in extracts (methanolic, ME, and aqueous lyophilized, AE) from rhizomes and aerial parts of *Valeriana* species. The extracts were tableting with BrK and then measured in a Protégé Nicolet 460 FTIR spectrometer. Noteworthy differences were obtained among the vibrational spectral modes in EA and EM, mainly in the region comprised between 1,750 and 1,500 cm⁻¹. So, the vibrational modes are well defined in EM (because of the carbonyl groups), while in EA are poorly defined and are displaced towards lower frequencies. It is noteworthy that in EA from *V. officinalis* leaves showed a larger area and centered at 1,620 cm⁻¹ with respect to EA from rhizomes/roots. These results could be relevant in the quality control both of plant drugs and phytotherapies that contains these products.

86.

EFFECT OF SOYBEAN MEAL ON ANTIOXIDANT ENZYMES IN RATS FED HIGH-CALORIE DIET*Razzeto GS, Lucero López VR, Escudero NL, Giménez MS.*

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High calorie diets induce the generation of reactive oxygen species that cause damage to macromolecules. Soy has antioxidants that play a key role in free radical scavenging. The aim of this study was to evaluate the activity and gene expression through kinetic assays and RT-PCR of: CAT (catalase), SOD (superoxide dismutase), GPx (glutathione peroxidase) y GR (glutathione reductase) in the liver of rats fed high-calorie diets with different protein sources. The animals were divided into two groups: control, fed AIN-93 diet, and experimental, fed AIN-93 diet high-calorie (34.15% sucrose, 42% of calories from fat) for 9 weeks. After that, each group was divided into two: one using casein and the other one using soybean as protein source, respectively. Animals were fed for 45 days and then sacrificed. Thus, the groups being: CC (control casein), CS (control soybean), HC (high-calorie casein) and HS (high-calorie soybean).

In the CS group, SOD, GPx, GR and CAT activity were increased in relation to CC (P<0,001) and (P<0,01) respectively. SOD activity increased in HS compared with HC (P<0,05) while GPx decreased (P<0,001). The gene expression was not significantly different between groups.

The bioactive components of soybean meal in normal diet would modulate antioxidant systems. This favorable behavior in high-calorie diet is observed only in the SOD activity, which would indicate that the protective effect is higher when soybean diet is normocaloric.

87.

ANTIOXIDATIVE RESPONSE IN MICORRIZAL PLANTS OF *Digitaria eriantha* UNDER ABIOTIC STRESS*Rodríguez Rivera M¹, Gutiérrez M, Sosa L, Ruiz Lozano JM^{2,3}, Pedranzani HE^{1,2}.*¹Fisiol. Veg. FICES.UNSL Argentina. ²Lab. de Microbiología de Suelos. CSIC. Granada. España. ³Lab. Fisiol. Veg. Fac. Qca; BioQca. y Fcia. UNSL. E-mail: hepedra@fices.unsl.edu.ar

The aim of this work was to study the response of different biochemistry parameter in mycorrhizal (AM) and non mycorrhizal (non AM) plants under abiotic stress. Seedling of *D. eriantha* cv. Sudafricana inoculated with *Glomus intrarradices* and non AM plants were subjected to different conditions: 1) 23°C and soil at field capacity (no stress), 2) 23°C and 6% water for one week (dry), 3) 4°C for 72h (cold) and, 4) irrigated with 200mM NaCl for two weeks (salinity). The hydrogen peroxide concentration (H₂O₂) in shoots, the lipid peroxidation expressed as equivalents of malondialdehyde (MDA), content of Ascorbate (ASC) and Ascorbate peroxidase activity (APX), in shoots and roots was measured. H₂O₂ increased in plants non AM under all stress while in plants AM decreases 33% in drought and 42% in salinity and cold treatment. MDA in shoots of plants non AM didn't showed significant differences among the different treatments while in root increased significantly (≥35%). Drought enhanced MDA in shoots and roots of AM plants by 30%. The ASC contents were similar in all treatments (63nmol g⁻¹ DW) in both roots and shoots, APX activity only increased (in AM plants) with drought while in plants no AM this increase were significant under cold stress.

88.

ANTIDOTE-EVALUATION ON THE EFFECT OF HERBICIDES IN *Tetrachne Dregei* Nees, AND *Antephora Pubescens* Schreb*Terenti O^{1,2}, Riscosa D², Allocati JP^{1,2}.*¹Instituto Nacional de Tecnología Agropecuaria. ²Universidad Nacional de San Luis. E-mail: oaterenti@sanluis.inta.gov.ar

Tetrachne Dregei Nees and *Antephora Pubescens* Schreb are promisors' sub-tropical grass forage resources in a diffusion process over San Luis Province, Argentina. Since they have initial slow growth capacity, both have serious implantation problems in the presence of unfavorable weeds. On other related species like sorghum or maize, this problem is solved by applying a grass herbicide to the soil previous treatment of seeds with Fluxofenim (Syngenta), an antidote (safener) to avoid phytotoxicity caused by the herbicides. The aim of this study was to evaluate the protective effect and possible phytotoxicity of Fluxofenim in seeds of *Antephora dregei pubescens* and *Tetrachne Dregei* Nees in presence of the herbicides S-Metolachlor and Acetochlor. The results show that the grass herbicides does not cause any phytotoxicity to the seeds of *Antephora dregei pubescens*, with or without previous treatment with the antidote fluxofenim, which also shows to have non toxicogenic activity on this grass. On the other hand, experiments on *Tetrachne Dregei* Nees demonstrate high phytotoxicity of the herbicides with or without the previous treatment with the safener. In conclusion, the pre-emergence herbicides studied are not recommended for *Tetrachne Dregei* Nees, but S-metolachlor and acetochlor are viable herbicides for pre-emergence treatments in *Antephora pubescens*.

89. DIETARY FIBER IN NEW REGIONAL VARIETIES OF AMARANTH

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Amaranth is a plant species that was frequently used in America before the Spanish conquest. Then, this crop was no longer consumed and amaranth was forgotten. At present, the seed is studied as food due to its high protein content. In previous presentations, proximal chemical composition and excellent nutritional quality were reported for the varieties studied. Epidemiological and clinical studies demonstrated that consumption of dietary fiber and whole grain intake is inversely related to obesity, type II diabetes, cancer and cardiovascular disease. The purpose of this work was to study the fiber content. Flour seeds of *Amaranthus cruentus* var. *candil* (Acc), *Amaranthus hypochondriacus* var. *dorado* (Ahd), advanced lines of *Amaranthus hypochondriacus* x *Amaranthus cruentus* H17a (AH17a) and *Amaranthus cruentus* G6/17a (AcG6/17a) were used for this study. Soluble (SDF), insoluble (IDF) and total dietary fiber (TDF) were determined by Prosky et al. 1988; Lee and Prosky 1992 (AOAC98529/99143). The obtained results ranged between 15.91-17.80% of TDF, 3.72-5.21% of SDF, 11.98-13.13% of IDF. It was verified that IDF contents represented the greatest part of the total dietary fibre of the amaranths studied. The recommended daily fiber intake ranges from 25 to 36 g per day, of which 6 g must be soluble. It is suggested that these amaranths are an interesting contribution to the human diet.

90. ANTIOXIDANT COMPOUNDS OF PROPOLIS FROM *Larrea nitida* Cav.

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The last decades have witnessed a growing interest by consumers, the food industry, and researchers, in the ways food may help to maintain human health. The main goal of this work was evaluate the antioxidant capacity (DPPH, FRAP and Folin-Ciocalteu assays) of extracts and main compounds from propolis associated to *Larrea nitida* Cav. Furthermore, their botanical origin by analysis of chemical profiles from both (propolis and *L. nitida*) by HPLCMS-ESI-MS was established. Petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) propolis extracts showed a high phenolic content (19.91±1.98–62.51±4.86) and flavonoids (14.07±0.46–36.64±5.43), and a potent DPPH free radical scavengers >90% at 10 µg/ml. DPPH assay-guided of the active extracts were isolated: **1**: methyl nordihydroguaiaretic acid; **2**: nordihydroguaiaretic acid; **3**: 4-[4-(4-hydroxy-phenyl)-2,3-dimethyl-butyl]-benzene-1,2-diol; **4**: meso-(rel7S,8S,7'R,8'R)-3,4,3',4'-tetrahydro-7,7'-epoxilignano **5**: (7S,8S,7'S,8'S)-3,3',4'-trihydroxy-4-methoxy-7,7'-epoxilignano, witch showed a stronger antioxidant activity (DPPH, EC₅₀ 1-15.5 µg/mL). These results support that propolis associated to *L. nitida* is a valuable natural product with potential to improve human health.

We are grateful to UNSJ, ANPCYT (PICT 2008-0554) and CONICET (FMP, AMB, BV, FGE and WD).

91. PHYTOALEXINS ISOLATED AND IDENTIFICATED IN PLANTS OF *Vitis vinifera* WITH “DECAY VINE DISEASE”

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Diseases affecting the wood of the *Vitis vinifera* bring on necrosis of the wood, progressive decline and death of plants affecting longevity and their productivity. Diseases affecting the wood of the *Vitis vinifera* bring on necrosis of the wood, progressive decline and death of plants affecting longevity and their productivity. The moderately polar extracts of leaves (healthy and diseased plants) and wood (diseased plants) were obtained. These were positive for flavonoids and polyphenols and alkaloids negative. Through CC was isolated a secondary metabolite, present only in diseased plant extracts which was purified by PLC. At chloroform extract, sweep was performed spectral showing a absorption at λ_{MAX} to 350 nm. The spectrum of the ¹H RMN (400MHz, CD₃OD), showed signals at δ) 7.5 (s), 7.3 (s), 6.58 (s), 5,4 (d), 4.0 (d), 3.68 (dd), 2.37 (dd). The multiplicity of signals in the section of the δ 0.5 a 1.5 ppm reveal that the isolated compound is not pure, for that reason was decided to perform a separation by HPLC (isocratic). The extract was dissolved in the ACN and was separated using as stationary phase Kromasil 100-5-C8, 4.6*250 mm. and a mobile phase of the ACN:H₂O, 40:60, with a flow of 0.35 ml/min at 30°C± 2 . The chromatogram confirms than in the diseased plant extract (leaf / stem) are present two phytoalexins that are not present in the healthy plant extract. Rt: 16.13 min (55.68%) and 22.05 min (24 972%) with polyphenolic structures.

92. MORPHOGENESIS AT TILLER LEVEL IN *Digitaria eriantha* Borcosqui AA¹, Privitello MJL¹, Bacha EF^{1,2}, García EV¹.

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Digitaria eriantha (DE), perennial spring-summer grass, is an important forage resource for cattle breeding systems of semi-arid environment of the San Luis province (Argentina). In a plot located in the experimental field of Agronomy-UNSL, after a clean cut (CL: 09/09/2011) 36 plants were identified to determine morphogenetic variables during accumulated growth. One tiller for plant was marked, on which every ten days were recorded the appearance of leaves, final leaf length (LFF) and leaf senescence. Leaf appearance rate (TAH), maximum number of green leaves per tiller (NH), leaf life span (VMF) and leaf elongation rate (TEF) were determined. VMF was considered as the days elapsed between the appearance of a leaf and the beginning of its senescence, and as TAH the time interval (days) between the occurrence of two successive leaves. TEF was calculated as the quotient between the LFF (mm) and the time elapsed since the appearance of the leaf. In the phenological phase of tiller elongation, 68 days after CL, NH was 5 ± 0.2; LFF reached 350 ± 17 mm and TEF 5 ± 0.24 mm/day. TEF decreased with the advancing of phenological stages. The first leaf senescence (VMF) occurred at 82 days from CL, registering a TAH of 16 ± 2 days. For the environmental conditions of this study, DE behaved as specie with low TAH, slow leaf senescence and foliar recharge.

93.

DUNGS GRADIENT AND FORAGE ACCESIBILITY IN LOW WOODS REGION

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In the region of “Bosque bajo de algarrobo (*Prosopis flexuosa*), arbustal de jarilla (*Larrea divaricata*) y chañar (*Geoffroea decorticans*)” of San Luis province, the deposition gradient of breeding cows dung and its characteristics was determined, according to the distance to the water point, in a natural vegetation paddock. Into productive establishment, located 23 km southwest of San Luis, the study was conducted on 700 ha regularly shaped paddock with only artificial water point. Stratified sampling was performed, by defining three sectors at 450 (N), 1700 (M) and 3350 m (F) from water point (33°26'3.8"S lat, 66°29'47.9"W long), and 4 square sampling sites of 25 m side were randomly installed in each one. 7, 6 and 2 feces were registered in N, M and F sampling sites, respectively. The feces were dark brown colour on the surface, and its consistency ranged from firm and thick, round and high, to tough and dry into slices or consistent rings piled in small groups, with very marked furrows. These features indicate high fiber intake, low crude protein and digestibility. The variation of the number of feces indicated a forage utilization gradient according to the distance to water point. In this community, access to forage is compromised by the density of trees and low shrubs, while animals move away from the water point.

94.

EFFECT OF DENSITY AND LACTOSILO® ON SORGHUM MICROSILAGE

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The effect of density and lactosilo® addition on sorghum microsilage was evaluated, using pH and organoleptic characteristics as indicators. In 1010 cm³ plastic jar with screw cap was ensiled whole plant sorghum (Silage King of Pannar®) with 35% MS (soft dough) chipped fine (<30mm). Three equivalent densities were applied: 250 kg/m³ (D250), 400 kg/m³ (D400) and 600 kg/m³ (D600). Lactosilo® was added, implementing treatments with (L) and without additive (WL). 40 days after preparation, pH was measured on water extract and organoleptic characteristics were recorded by scales: 1-olive green, 2-light green to light brown, 3-brown, 4-dark brown, and odour: 1-fruity soft, 2-sweetened-tobacco, 3-stale, unpleasant, 4-fruity and 5-vinegary. The pH data set adjusted to normal distribution (p>0.10). Multivariate analysis by Cluster method (Ward) and dendrogram indicated two groups. The first, composed of D250 L and WL (pH 3.87, odour 5 and colour 1) and the second for the other treatments (pH 3.75, odour 4 and colour 2). With this technique, the pH values remained within the correct range of a fermentation indicator (3.8 to 4.2). The addition of lactosilo® not explain the change on pH or sensory characteristics. Considering the set of variables (quantitative and qualitative), with densities greater than 250 kg/m³ of chipped forage, ensures the correct fermentation process.

95.

pH VARIATION IN SORGHUM MICRO SILAGE

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The variation of pH of Sorghum (Silage King Pannar®) microsilage (whole plant shortly chipped, in 1010 cm³ plastic jar with screw cap) was evaluated by 18 treatments: cutting moments (CM): M1 “early” (22.5% DM, milk stage), M2 “recommended” (35% DM, soft dough) and M3 “late” (> 40% DM, hard dough); addition of lactosilo® (AD): with (L) and without lactosilo® (WL) and different opening moments since ensilaged (OM): 2, 12 and 40 days. Factorial ANOVA and average contrast (Bonferroni, p<0.05) were done. The main factors (CM, AD and OM) and double and triple interactions, explained variations in pH (p<0.05). Four groups were identified by contrast pH averages analysis. Two extremes: M1 (L and WL) at 40 days and M3 (L and WL) at 2 days of opening (3.0 vs. 5.5). Another group with intermediate values within the optimum range (3.8 to 4.2), composed of: M2 (L and WL) at 10 and 40 days of opening, M1 (L and WL) at 10 and M1 L at 2 days opening. The rest of the treatments formed a fourth group, exceeding the higher limit of the optimal range (4.4 to 4.9). With this technique, cut in paste grain helps stabilization of the pH at 10 days of ensilaged (with or without lactosilo®). In early cuts, lactosilo® can reach the desired pH at 2 days of ensilaged. Belated cuts do not guarantee properly pH stabilization, even with the application of additives.

96.

PRODUCTION OF GOAT CHEESE HYDROLYZED. EXPERIMENTAL DESIGN AND ASSESSMENT OF THE DEGREE OF HYDROLYSIS

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The enzymatic hydrolysis of the proteins is an attractive way to improve nutritional, sensory and functional properties of foods. In enzymatic hydrolysis processes must be considered numerous variables (pH, temperature, hydrolysis time, amount of water added, enzyme:substrate ratio) and is necessary to measure functional parameters accurately and with trials economy. The aim of this work was the enzyme hydrolysis of goat cheese to improve their functional properties and increase their applications as flavoring additive. We performed an experimental statistical design of 5 factors at 2 levels. Hydrolysis degree was measured as released soluble proteins (NT) of the cheese to the supernatant by Kjeldahl method, and as free amino nitrogen (AN) of soluble proteins, by method of phenol-modified hypochlorite. Maximum degree of hydrolysis was obtained with 13 gof buffer / gof protein, pH 8.65°C, 40min, and 0.22 mg of papain / gof casein. Hydrolysis degree of goat cheese proteins was favored by high pH and enzyme: substrate ratio, and low amount of buffer added. This work highlights the interactions between the variables and operational commitment situations when new flavor additives with improved functional properties are developed.

97.

THE EFFECT OF GELLING AGENT ON THE DEVELOPMENT OF MAIZE (*Zea Mays L.*) IMMATURE EMBRYOS *IN VITRO**Allocati JP^{1,2}, Manazza F¹, Terenti O^{1,2}, Pedranzani H¹.**¹Instituto Nacional de Tecnología Agropecuaria. ²Universidad Nacional de San Luis. E-mail: jallocati@gmail.com*

Efficient methods for *in vitro* calli generation, propagation, regeneration and transformation of plants are of pivotal importance to both basic and applied research in biotechnology. Plant tissue culture techniques often require optimization for cost reducing, and efficiency improvement. As maize is the World's third most important cereal crop, it is also one of the most difficult-to-handle plants in tissue culture which severely limits genetic engineering approaches. We report here on the importance of the type of the *in vitro* culture gelling agent (GE) for T940C Hi-II genotype. In order to determine best culture conditions, corn immature embryos were isolated from greenhouse-grown ears at 11 days after pollination and placed in dark on N6 media, containing (a) 8g/L Agar (SIGMA) or (b) 2,5g/L Phytigel™ as (GE). The media was supplemented with vitamins, 30g/L sucrose, 2.9g/L proline and 2mg/L 2,4-dichloro phenoxyacetic acid. The frequency of callus induction was 40% using Agar and 70% using Phytigel™. The treatment (a) give rise to yellower color calli, sometimes with large morphological structures. On the other hand, the treatment (b), which provides an economical alternative to agar, give rise to a more vigorous callus lines, friable, soft white callus coated by immature somatic embryos growing synchronously. In our cultures it has been shown that the nature of the gelling agent is crucial for the correct development of friable callus.

98.

METHOD FOR EXTRACTION OF MAIZE (*Zea Mays L.*) EMBRYOS*Allocati JP^{1,2}, Manazza F¹, Terenti O^{1,2}, Pedranzani H¹.**¹Instituto Nacional de Tecnología Agropecuaria. ²Universidad Nacional de San Luis. E-mail: jallocati@gmail.com*

The preparation of embryogenic tissue for corn propagation, regeneration and transformation is time consuming. It is also labor intensive, especially as it involves manual excision of desired explant tissue. In corn *in vitro* culture, the manual removal of immature embryos (1-2 mm) from individual excised kernels is a common means for isolating tissue useful for experiments, and it could take 1-2 hours per ear. This procedure is not only laborious; it may damage the embryos and is fraught with ergonomic issues. The aim of this study is to provide a simple method for the isolation of immature embryos. It consists on the following: First insert a straight nosed forceps into the tip end of an entire dehusked ear, sterilized it with ethanol 80% 5 min. Disinfect it for 15 min in a solution of 5,25% hypochlorite in water + 2 drops of surfactant Tween 20. Rinse the ears in generous amounts of sterilized water. Do not excise any seed. Cut off rounded, waxy surface kernel crowns (the top 1-2 mm) with a sharp scalpel blade. Then excise the embryos by inserting the narrow end of a sharpened spatula between the endosperm and pericarp at the basipetal side of the kernel popping the endosperm out of the seed coat. This exposes the untouched embryo, coaxed onto the spatula tip. The excision process described here takes about 30 min. per ear and offers a fast tool to improve *in vitro* culture; the embryos can be propagated and regenerated into plants.

99.

THERMODYNAMIC ANALYSIS OF THE AFFINITY BINDING BETWEEN IMMOBILIZED CIBACRON BLUE F3GA AND LYSOZYME*Ferraris P, Aguilar C, Pérez Padilla A, Rodríguez J.**Área Biol Mol. FQByF. U.N de San Luis. Argentina. E-mail: ferraris@unsl.edu.ar*

Affinity adsorbent microparticles for protein separation were prepared. Yeast cells were modified by chemicals and the Cibacron Blue (CB) ligand molecule was immobilized to the wall cell by covalent bond. The adsorbent was characterized by determination of the affinity equilibrium constant (K_a) by means of adsorption isotherms using Lysozyme (Lys) as target protein. The interaction forces (non-covalent) between ligand molecules and proteins may include electrostatic interactions, multiple hydrogen bonds, van der Waals interactions, hydrophobic and steric contacts within the protein-binding site. The thermodynamic parameters of the binding reaction allow evaluating the binding mode. These parameters were calculated from the van't Hoff plots based on the temperature dependence of the equilibrium constant for the Lys-CB binding: $\ln K = -\Delta H/RT + \Delta S/R$ (where T is the absolute temperature and R is the gas constant). In the temperature range studied (7°C, 15°C, 22°C and 37°C), the values of enthalpy (ΔH) and entropy (ΔS) change were obtained from the slope and intercept. Value for free energy change (ΔG) was negative and values for ΔH and ΔS were positive. These results indicate that the process is spontaneous, endothermic and entropy driven. Then, the hydrophobic interaction might be the major contributing force in the Lys-CB binding.

100.

SOMATIC EMBRYOGENESIS IN *Gomphrena pulchella* Mart.*Garraza R, Verdes P.**PROICO 50307, FICES, UNSL. (5730) V. Mercedes (San Luis). E-mail: peverdes@fices.unsl.edu.ar*

Somatic embryogenesis has emerged as a new way of propagation the applied plant breeding and germplasm conservation. Considering the potential of this tool biotechnology and lack of background on its use in propagation and breeding in native species with ornamental value in the Province of San Luis, this paper describes the protocol of somatic embryogenesis in *G. pulchella* Mart. The protocols describe aspects related to the early stages of embryogenesis: induction of somatic embryos and somatic embryo development. The action of 2,4-dichloro phenoxyacetic acid (2,4-D), naphthaleneacetic acid (NAA) and kinetin (K) were evaluated in the induction of embryogenesis from leaves and stems of micropropagated plants. The culture conditions were temperature of $22 \pm 2^\circ\text{C}$, with 16 h photoperiod ($116 \text{ mol. m}^{-2} \text{ s}^{-1}$). The development of embryogenic calli were obtained in the culture medium with the basic formulation of Murashige Skoog (1962) to 50% in presence of 2,4-D (3 mg.L^{-1}). The differentiation of somatic embryos was achieved in the nutrient medium supplemented with the combination of NAA (0.5 mg.L^{-1}) and K (0.5 mg.L^{-1}). In the same medium developed embryos. It provides a new mode of propagation in the domestication of *G. pulchella* as ornamental potential native resource.

101. COMPOSITION, SEMIOCHEMICAL ACTIVITY AND TOXICITY OF THE ESSENTIAL OIL FROM *Capparis atamisquea* ON *Ceratitis capitata* AND *Triatoma infestans*

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Botanical pesticides are gaining increasing attention as an alternative to synthetic insecticides. *Capparis atamisquea* (Capparaceae) is popularly known as “matagusano”. Only the antifeedant and repellent properties of their crude extracts have been reported against insects. In addition, the essential oil (EO) composition of this species is unknown. We evaluated the composition of EO from *C. atamisquea* by GC-MS and RMN analysis. Nine compounds were identified in the oil amounting 99.9%. The main component was hexanenitrile (85.1%) and heptanenitrile (7.3%). Toxic activity on adults of *C. capitata* was tested on both sexes. At 72 h post-treatment of topical bioassay 40% of mortality on males and 46% mortality on females was achieved at 100 µg/fly. The oil showed repellent properties on females of *C. capitata* while it was not significant on males. On the contrary, the EO showed an attractant activity on fifth instar nymphs of *T. infestans*.

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102. BIOTECHNOLOGICAL CHARACTERIZATION OF *Bacillus* sp. CELLULOLYTIC ACTIVITY, FROM MONTE CENTRAL DESERT SOILS, ARGENTINA

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Arid environment conditions are unfavorable for massive microbial growth because of their fluctuations in soil water content and available organic matter. The bacterial community composition is governed by interactions between microorganisms, thus adapted species are selected. In this matter, to isolate bacterial species adapted to desert specific environment conditions (pH, temperature) could be useful for biotechnological applications. The **Main Objective** of this research was to quantify and to characterize cellulolytic activity of *Bacillus* sp. isolations from Monte Central desert soil. Cellulolytic activity of fifty four bacterial isolations was characterized. Five of them showed the highest values and they were characterized at different pH cultivation conditions (5.2; 7.0 and 8.5). Samples were taken at 24 and 72 cultivation time hours. Enzymatic activity was quantified at 50° and 65°C. From these results, it could be inferred that species or strains could be differentiated according to their enzymatic levels. The highest values of cellulolytic activity were produced by BB127 and BBs6 at pH 7: 16,49±0,26 UI/mL and 18,02±0,23 UI/mL, respectively; measured at 65°C. Enzymatic activities were mostly high temperature tolerant. Cellulolytic activities maximum values were determined at similar isolation sample pH soils (neutral). Bacterial isolations BB127 and BBs6 could be neutrophilic, tolerant to high temperatures and basic pH.

103. EXPLORATORY SCREENING (S)-(-)-PERILLYL ALCOHOL AS INHIBITOR LIGNINOLYTIC FUNGAL GROWTH IN GRAPEWINE

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The grapevine diseases caused by ligninolytic fungi are internationally considered as great harm to the sustainability of wine-growing heritage. The fungicides traditionally used, have shown low efficiency, and high toxicity for man and environment, demonstrating the importance of control of disease with biofungicides. Woodchips of symptomatic grapevine plants (variety Imperial seedless) disinfected with NaOCl in APGA solid medium with the addition of 100mg/ml streptomycin sulfate, 50mg/ml of chlortetracycline and 5mg/ml of dicloran in the dark for 15-day at 28°C were cultivated. Fifteen strains were isolated. By SEM of the wood cankers was confirmed the ligninolytic character of fungi, (presence of mycelia and tylosas in xylem / phloem). The inhibitory activity of (S)-(-)-perillyl alcohol, through diffusion in agar, with wells of 4 mm in diameter (MDP) with 15µl of (S)-(-)-perillyl alcohol (dissolved in MeOH) to concentration of 0.5, 1 and 1.5µg /ml using H₂O and MeOH as witness, were evaluated. It was sown superficially by grooves for a lawn of growth the 0.1ml of inoculum to turbidity 0.5 McFarland scale, with incorporation simultaneously of (S)-(-)-perillyl alcohol at sowing, and to 48 hours of crop development. It was incubated at 28°C, and was readed at 24, 48 and 72 hours. They are presented inhibition rings (average 3.5 cm -0.5µg/ ml) of the growth for all fungi isolated (24, 48 y 72 hs.) and at all concentrations tested for the first procedure. While for the second method, morphological changes (development/culture medium) in a ring of 2.5 cm in diameter were observed. These results confirm that the (S) - (-)-perillyl alcohol, could be a potential source biofungicide.

104. IDENTIFICATION OF CAROTENOID PIGMENTS IN *Bacillus licheniformis* ISOLATED FROM ROOTS OF *Vitis vinifera*

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Carotenoids are the most widespread naturally occurring yellow, orange and red pigments produced by bacteria, fungi, algae and higher plants. Several species of bacteria accumulate high concentrations of carotenoids. They are of interest due to their antioxidant, UV protecting and natural food colorant properties. In a previous study *B. licheniformis* was isolated from roots of *Vitis vinifera* L. (cv. Malbec) and promoted the growth when it was inoculated in *Vitis vinifera* plants cultivated *in vitro* (Salomon *et al.*, 2010). The aim of this study was to identify carotenoids produced by *B. licheniformis* in LB medium.

B. licheniformis was cultivated aerobically for 3-10 days at 30°C and 120 rpm in orbital shaker in dark. Cells were centrifuged at 7000 rpm at 4°C for 15 min. The upper layer was discarded and residual cells were extracted with different solvents (MeOH, CH₃COCH₃, MeOH/CH₃COCH₃ 7:3, v/v) overnight at 4°C. Suspensions were sonicated 10 min and centrifuged. After that, the clear coloured organics phases were transferred into a clear tube. Finally, the carotenoids levels in the supernatant were quantified through absorbance between 300-600 nm. The best solvent was MeOH/CH₃COCH₃ and production increased from 3 to 10 days of culture with *B. licheniformis*. The highest peak was found at 474 nm corresponding to lycopene.

105.
USE OF THE ORGANIC FRACTION OF VEGETABLE RESIDUES BY MICROBIAL ACTION

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The natural or processed vegetable foods provide organic waste important chemicals for soil fertility. Given that environmental conservation is more effective when you get a direct return, immediate and concrete. The objective of this work was to value degradable organic matter and related agro-industrial waste in a biological fertilizer, obtained by microbial activity. Substrates were used consisting of poplar sawdust with remains different fruits and vegetables that were planted with crushed inoculum, consisting of natural microbial populations, mesophilic, aerobic. This inoculum was prepared from preinoculum with 10% inoculum and 90% organic material, incubated at 28°C for 24-48 hours, with pH and moisture control. Microorganisms analyzed in specific media were atmospheric nitrogen fixers, and cellulolytic amonificatores. The results showed that with a 60% humidity for 20 days was obtained a good degradation of organic matter from an initial pH of 7, increased to pH 8 and to stabilize at pH 6.7. Microbiological analysis determined an increase of nitrogen-fixing microorganisms and amonificatores, after cellulolytic. Conclude that under strict control of the inoculum, temperature, pH and moisture get good organic matter degradation and microbial activity necessary for soils that have lost the availability of essential chemicals.

106.
BIOACCUMULATING PLANTS: MONITORING OF HEAVY METALS IN LEAVES AND ROOTS OF WHEAT AND SUNFLOWER USING A FAST AND INDIRECT DETECTION BY OPEN-TUBULAR CAPILLARY ELECTROCHROMATOGRAPHY

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Human activity such as industrial development, urbanization and intensive agriculture, has led to extensive environmental problems. Since heavy metals are mobile and easily absorbed by plants in the environment, they are transmitted to the human body through nourishment. Metal-accumulating plants, such *Triticum* species and *Helianthus annuus* L., can accumulate high concentrations of heavy metals in both leaves and roots from polluted soil and waters. For the determination of these elements we used CZE with indirect detection. This technique require the dissolution of the sample, with this aim three digestion procedures were applied. The best results were obtained in batch using a mixture of HNO₃+HCl. The detection was performed at 214 nm; the voltage was set at 25 kV. Samples were pressure-injected at the cathodic side at 0.5 psi for 10 seconds. The background electrolyte (BGE) used consisted of 6 mM imidazole, pH 4.0 adjusted with 1 M acetic acid. The proposed method allows the rapid determination of heavy metals in natural products. It was recently demonstrated that MW-assisted acid treatment with H₂O₂ could be used satisfactorily for sample clean-up technique prior to CE determination of various metals species. This methods have a great potential to be applied in phytoremediation in which the use of green plants help to remove contaminants from soil and groundwater.

107.
MICROPROPAGATION OF *Oenothera affinis* Cambess.

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Oenothera affinis L. ornamental attributes have given for their showy inflorescences, which make it suitable to be part of flower beds. Production of selected specimens of natural populations of a limited number of seeds can be achieved by *in vitro* propagation offers great potential to support traditional breeding methods, multiplying agámicamente new varieties or cultivars. The aim of this study was to establish a protocol for the stages of introduction and multiplication *in vitro*. To assess the *in vitro* multiplication rates binodal segments were cultured (0.5 to 1 cm in length) on Murashige and Skoog media (1962) and nutrient levels differential growth regulators. The culture conditions were temperature of 22 ± 2°C, with 16 h photoperiod (116 mol. m⁻². s⁻¹). Behavior was observed statistically significant among treatments. It highlights the development of roots in the control treatment (without growth regulators), while the axillary bud sprouting was achieved in the treatment with kinetin (0.5 mg. L⁻¹). The combination of naphthaleneacetic acid (0,5 mg. L⁻¹) and kinetin (0.5 mg. L⁻¹) induced callus formation at the base of the stems, but no roots or shoots developed from lateral buds. It continues with step acclimatization for completed micropropagation.

108.
***Oenothera affinis* Cambess: DIRECT SOMATIC EMBRYOGENESIS**

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In the province of San Luis there is a significant number of native species attractive for their flowers, which undoubtedly have adaptive advantages when designing gardens for low water consumption. However, the use of our genetic resources for the development of ornamental plants is still underexploited. This paper reports on protocols multiplication through direct morphogenesis in explants of *O. affinis*. Behavior was assessed in cultured stems segments Murashige and Skoog nutrient medium at different concentrations of 2,4-Diclofenoxiacético (2,4-D). The nutrient medium was supplemented with agar (6 g.L⁻¹) and sucrose (30 g.L⁻¹). The sterilization of the nutrient media was autoclaved at 121°C for 20 minutes. The explants were extracted from micropropagated plants. Within ten days of culture was adventitious shoots directly generated in explants cultured in nutrient medium with 2,4-D (2 mg.L⁻¹). The average number was 6 adventitious shoots per explant. While somatic embryos were generated directly in the explants when the concentration of 2,4-D was 8 mg.L⁻¹. High rates of induction (84%) and regeneration of somatic embryos were obtained from the stems. Significant progress on regulatory requirements for growth in the direct formation of new organs for multiplication of *O. affinis* was reported.

109.
STUDY OF WATER SORPTION ISOTHERMS OF WHOLE AND SKIMMED MILK POWDER

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The knowledge of the influence of fat content of the milk powder at relative humidity range in conditions of its packing, storage and use, is necessary to verify the product safety conditions. The objective of this research is to obtain water sorption isotherms by plotting the moisture content vs. the water activity, a_w . The dew point technique by AQUALAB equipment (3TE Decagon) is used to measure a_w of the sample. The moisture content is gravimetrically determined. The drying of the restored samples is carried out in a drying chamber whose temperature is controlled. The dry sample is humidified by water vapour sorption during increasing times in a closed chamber containing a vapour generating device. The moisture content is referred to the dry base.

Experimental dates fit the Guggenheim-Anderson-de Boer (GAB) model. The whole milk powder has a moisture content equilibrium point lower than the skimmed milk powder measured at the same water activity. It is considered that the fat content of the whole milk hinders, in some extent, the water adsorption of the sample. In the case of the desorption isotherms no difference between the two types of curves is observed.

110.
FAMILIAL HYPOPHOSPHATEMIC RICKETS: ITS RELATION WITH ALKALINE PHOSPHATASE. A CASE REPORT PEDIATRIC PATIENT IN SAN LUIS

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Familial hypophosphatemic rickets (FHR) is a X-linked disorder associated with a mutation in the PHEX gene. It can cause bone deformity including short stature and *genu varum* (bow leggedness). It is biochemically characterized by hypophosphatemia due to renal phosphate wasting. Here, we describe a clinical case of FHR in a patient of 20 months of age, the treatment response and its relation with total alkaline phosphatase levels (AP). The patient was seen at the Nephrology Service of San Luis Hospital (Argentina), in November 2011. He presented clinical features of rickets, severe malnutrition, polyuria and polydipsia, delay maturational patterns. The laboratory assays showed a severe loss of renal phosphate and hypercalciuria, with hypophosphatemia (3.9 mg/dl) and hypocalcemia and a high AP levels (2343 U/I). The treatment was phosphorus syrup (30cc/day), calcitriol (0.25 mg/day), bicarbonate (1 stamp/48 h) and enalapril (1.25 mg/day). A good response to a treatment for seven months, was observed. The AP levels, used as indicator of FHR evolution, was decreased (870 U/I), the body weight was increased, and serum calcium (7.8 mg/dl) and phosphorus (5.6 mg/dl) were normalized. The early diagnosis and treatment of this rare disease, are essential to prevent bone sequelae of rickets.

111.
RELATIONSHIP BETWEEN BODY MASS INDEX AND HEALTH HABITS IN WOMAN OF SAN LUIS-ARGENTINA

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The most common indicators used to make a diagnosis that discriminates normal nutritional status of abnormal conditions (eg. overweight, obesity, malnutrition) are both the Body Mass Index (BMI), as common indicator of obesity, and the waist/hip ratio (IC/C) as indicator of cardiovascular risk. In order to assess the relationship of BMI and IC/C in relation to dietary habits, physical activity and substance use, we applied a semi-structured interview with health data in general and different habits of a sample of 82 women (18-40 years old). Sample was compared with normal values of BMI and IC/C. Women interviewed were distributed as follows, BMI: 20.73% <, 63.41% normal, and 15.85% >; IC/C: 21.25% <, 68.75% normal, and 10% >. About alimentary habits: 66% made the four meals. Healthy habits: 36.84% play sports, 63.16%, no play sports, and 26.82%, no answer. Substance use: Analgesics: 63.41%; Alcohol: 64.63%; Coffee: 57.31; Cigarettes: 30.48%; Marijuana: 3.65%, and Cocaine 2.43%. We conclude that the main influence on BMI and IC/C was the quality and quantity of caloric intake but not the life habits.

112.
PREVALENCE AND INCIDENCE OF CLINICAL MASTITIS IN ARGENTINA DAIRY HERDS: A PILOT STUDY

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The frequency of clinical mastitis (CM) among dairy herds in Argentina is a growing concern. Cause of that, a study was conducted to obtain preliminary estimates of clinical mastitis prevalence and incidence before a large survey is implemented. A cross sectional convenience sample of 2,535 cows from 19 herds was the study population. The randomly selected cows that showed milk abnormalities were considered a mastitis case. Herd prevalence was estimated the day of the visit. The cows recruited that showed no clinical signs were followed during 15 days in order to get incidence estimates. Herd median prevalence, incidence and incidence density of mastitis were, 1.69 (q1:0, q3:4.2), 3.6 (q1:2, q3:4.6) and 0.25 (q1:0.14, q3:4.2), respectively. At cow level, prevalence was 2.5% (95%CI 1.9-3.1), cumulative incidence was 3.9% (95%CI 4.5-3.8) and incidence density was 2.7 (95%CI 2.2-3.3) new cases per thousand milking cow-days. At cow level, prevalence was twice higher for cows with days in milk (DIM) <90 days and 1.5 higher for cows in comparison with heifers. In addition, cows showed an incidence of CM four times higher than heifers. These findings revealed the extent the potential losses due to clinical mastitis year around. Incidence estimates suggest that all cows in the herd may experiment at least one episode of CM. The study provided data and a framework to design a nationwide clinical mastitis survey.

113. OVERWEIGHT, OBESITY AND RISKS OF CARDIOVASCULAR COMPLICATIONS AND TYPE 2 DIABETES IN NATIONAL UNIVERSITY SAN LUIS STUDENT'S

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The prevalence of overweight is increasing among young people significantly in recent years. The aim of this study was to evaluate the prevalence of overweight and obesity in students of the National University of San Luis and associated risks. The study was performed in 32 students from the University of San Luis: 70% female and 30% male. The mean age was 21.5 ± 3.9 years (19-32). Anthropometric parameters were estimated: weight, height and BMI. The percentage of body fat (% BF) was calculated with the equation of Deurenberg. The BMI data was interpreted using the SEEDO criteria (2007) and the percentage values were used the cutoffs of Bray et al. (1998). The subjects were classified: 11% overweight, 5.5% obese and 2.8% underweight. The percentage BF was 85.7% normal weight and 14.3% obese. Considering waist to hip ratio and the waist circumference, 7.14% subjects reported risk of complications associated with obesity. Considering BMI and waist circumference 13.7% of college students presented a risk of complications, 3.44% high risk and moderate 10.3%. In conclusion, the prevalence of overweight was higher than other data published, as well as the percentage of students with risk of complications obesity associated. These results confirm the need to implement programs to promote health in this population.

114. STUDY OF THE PREVALENCE OF PATIENTS WITH HYPOTHYROIDISM FROM TWO LABORATORIES OF SAN LUIS CITY

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Hypothyroidism is the thyroid hormone deficit in target tissues, regardless of the cause. Currently, the determination of stimulating thyroid hormone (TSH) is used as primary strategy for the diagnosis of the thyroid dysfunction. The thyroid hormones (TH), free thyroxine (FT4) and total thyroxine (T4T), complement the diagnosis. The aim of this study was to determine the prevalence of patients (P) with hypothyroidism that went to the laboratory in 2011, to analyze the requirements of TH, and to discriminate hypothyroidism frequency by sex and age. After fasting, whole blood was taken. Serum TSH was measured by immunoradiometric method (IRMA). FT4 and T4T were determined by radioimmunoassay (RIA). 631 P were studied: 128 P (20.3%) had $TSH > 4.0 \mu U / ml$. 35 P (27.3%) were evaluated only for TSH: 27 women (77.1%) and 8 men (22.9%). In 67 P (52.3%) TSH and FT4 were determined: 56 women (83.6%) and 11 men (16.4%). TSH, FT4 and T4T were determined only in one woman (0.8%). The mean age of P was 38.8 years old. The remaining 503 P, euthyroid or treated hypothyroid had normal values of TH. 20.3% of studied P had hypothyroidism, and most of them (65.6%) were adult women, in coincidence with the published data.

115. TOXIC PLANTS OF THE PROVINCE OF SAN LUIS (ARGENTINA) FOR DOGS AND CATS

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The poisonings by plants represents about 10-15% of the total of poisonings that concern to the pets. The records of veterinary problems are of, approximately, 70% in dogs and 25% in cats. With the aim to contribute to the knowledge of the toxic plants to dogs and cats in San Luis' province, a register of the same ones was realized in urban and rural areas. More than 50 toxic plants that are frequent in the province (cultivated or spontaneous) were registered. In parallel, surveys were realized in veterinary local clinics on poisonings by plants. The species most registered like causative of poisonings was the paradise (*Melia azedarach*) and the most affected population was that of dog puppies. The symptoms changed from vomits and diarrheas up to death. In the work the symptoms are registered and the toxic components of the plants and the treatment adapted for every case of poisoning are indicated. One of the principal reasons for these poisonings is the ignorance of the owners of the pets on the existence of toxic plants. Added to this, the shortage of specific bibliography, particularly to local level, prevents the correct diagnosis of these affections in San Luis' province, making necessary the production and diffusion of knowledge related in this subject matter of the Veterinary Medicine.

116. ANALYSIS OF VARIABLES IN PATIENT WITH CYSTIC FIBROSIS

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We studied the population of 106 children / adolescents of the Program for Cystic Fibrosis and therapy Oxygen of the Children Hospital Humberto Notti. We determined the survival rate in four cohorts, using the nonparametric method of Kaplan-Meier, taking the diagnosis as zero time. We considered all patients from 1975 to the years 1999 to 2002, 2005 and 2008 respectively and the cumulative probability for each case. We performed chi-square test (X²) to compare the survival curves of the intervals until 1999 and until 2008. In the group from 1975 to 1999, for patients 17 years, we found that the survival rate values 0.45, about 45% survived to that age, whereas in the group from 1975 to 2008 survived about 80% for the same age. Was obtained for the X² test: 39.46 experimental and the theoretical value of 18.31, for $\alpha = 0.001$ and 10 degrees of freedom, so show statistically significant difference. Data were analyzed to compare medians of survival: in 1985, 50% of patients remained alive with four years or more, while in 2008, 50% survived 12 or more years.

117. INFECTIOUS MONONUCLEOSIS IN CHILDREN IN PRIMARY HEALTH CARE HOSPITAL OF SAN LUIS

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Infectious mononucleosis (IM) is an acute illness caused by Epstein-Barr virus (EBV, a member of the herpesvirus family) that occurs often in young adults. The incidence in children is very low. The classic symptoms include fever, pharyngitis, and lymphadenopathy. Enlargement of the spleen and inflammation of the liver may also occur. The infection can be spread by saliva, and the incubation period for IM is four to eight weeks. We reports four cases of atypical IM in children (2-5 years old) that were diagnoses in the Hospital del Oeste, San Luis, in 2011. These patients showed any of typical symptoms of IM. Their blood samples were subjected to automated hematologic analysis (ADVIA 60). Serological test (Paul-Bunnell-Davidsohn) revealed EBV infection in all patients, as well as leukocytosis, high percentage of lymphocytes, and atypical lymphocytes. Furthermore, serological testing were negative for cytomegalovirus and virus hepatitis B. Results evidence a crucial role of the laboratory in the diagnosis of an atypical and infrequent IM in children.

118. MENSTRUAL CYCLE AND THE RELATIONSHIP WITH HIGHER BRAIN FUNCTIONS

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During the menstrual cycle mood swings occur and they would impact in both psychical and higher brain functions (HBF). In order to evaluate HBF during the menstrual cycle a menstrual history questionnaire, Luria Neuropsychological Battery DNA test, and test mental rotation (MRT) were applied in women (n = 100, range = 18-40 years old). In menstrual history: 71% had regular cycles and 29% irregular; Painful menstruation: 23%, Painless: 23%, not always pain 41% and 13% rarely, with different areas of commitment (family and sexual, more than work and academic area). Follicular and luteal phase showed no differences in MRT (Wilcoxon for independent samples, mean \pm SE = 4.10 \pm 3.10, 2.88 \pm 2.63, respectively, P = 0.0498). The other variables (Luria DNA) did not differ significantly. Most of the HBF was not influenced by the luteal phase in female menstrual cycle. In contrast, in follicular phase women showed better performance in the MRT than the luteal phase. We also analyzed the variables with a correlation analysis between HBF and cycle phases. A considerable number of function tested (~50%) was different in the follicular phase than the luteal phase. This indicates that these functions (HBF) are sensitive to the hormonal changes in women menstrual cycle.

119. PREVALENCE OF HYPERTENSION AND CARDIOVASCULAR RISK FACTORS IN NURSING STUDENTS

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The study was designed to evaluate the prevalence of hypertension and the presence of cardiovascular (CV) risk factors in nursing students. The questionnaire was approved by Ethics Committee and the data were statistically analyzed. 140 students (18.5% men, 81.5% women) were tested with measuring rods, scales, and equipment for taking aneroid blood pressure. Mean age: 24.7 \pm 6.5 years (18-49), weight: 64.6 \pm 15.6 kg (42.5-130), height: 1.62 \pm 0.08 m (1.45-1.87), BMI: 24.3 \pm 4.6 (17-44.9), waist circumference: 84.0 \pm 15.0 (53-130), mean systolic and diastolic blood pressure: 107.2 \pm 10.7 and 68.5 \pm 10.4. Students with HTA 26.4% (20.7% women, 5.7% men, 13.5% treatment), without HTA 60.7%, unknown 12.8%. DBT: 25.7%, DLP: 2.1%, hyperuricemia: 4.2%, internment for breast pain: 7.8%, IAM: 7.1%, IC: 7.8%, ACV: 5%, coronary disease: 2.8%. Nicotinism 35.7%; alcoholic drinks frequently 2.8%, occasionally 78.5%, never 17.8%; physical activity frequently 18.5%, occasionally 80.5%, never 20.7%, diet fatty acid frequently 27.8%, occasionally 70.1%, never 2.1%, family history CV disease: 41.5% and 98.5% know about risk factors. It is advisable to identify risk factors early in life, in order to prevent the subsequent development of HTA in adulthood and its future complications. This study suggests the broadening of bases for bio-clinical examination to other sectors of our community in order to more effectively motivate students, parents, and teachers in healthy habits for CV disease prevention.

120. -493 G/T POLYMORPHISM OF THE MTP GENE AND ITS ROLE IN HEPATIC DISORDERS IN SUBJECTS WITH TYPE 2 DIABETES MELLITUS

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Microsomal Triglyceride Transfer Protein (MTP) is necessary for the assembly and secretion of VLDL, when the protein is not functional, a non-alcoholic steatohepatitis (NASH) occurs. Polymorphisms in the promoter region of the MTP gene have been associated with central obesity, elevated liver enzyme, development atherosclerosis and cardiovascular diseases. We assessed the association between the polymorphism of MTP gene (-493 G/T) and the biological features of NASH in Type 2 diabetic patients and normal subjects. DNA from 36 subjects (Co and Diab) were isolated by QIAmp DNA blood mini kit (Qiagen). The polymorphism was made by Tetra Primers ARMS-PCR. Increased liver enzymes (GOT, GPT, γ -GT and Phosphatase) were used as markers of liver steatosis. There was a higher proportion of diabetic subjects with increased enzyme concentrations in GG genotype: Phosphatase 50% (p<0.0001), GOT 33%, GPT 50% and γ -GT 33% (p< 0.0006). In Co the enzyme concentration was not significantly different between GG and GT genotypes. The -493 G/T MTP gene polymorphism is associated with biological steatohepatitis markers in patients with type 2 diabetes. The G allele which is responsible for a decreased in MTP gene transcription is prone to increased the intrahepatic tryglicerides G content, conferring by this a genetic susceptibility for steatohepatitis.

121.
**MILK YIELD LOSSES DUE TO CLINICAL MASTITIS:
 PRIMIPAROUS COWS MODEL**

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Mastitis is the most costly disease in dairy cattle. Milk losses are recognized as the main effect of the disease. The aim of this research was to study the effect of clinical mastitis (CM) on milk production. A total of 1,990 primiparous cows housed in free stall barns were followed through lactation. Milk production was recorded on daily basis until the 40TH week of lactation. Only the effect of the first clinical episode was evaluated. Reproductive syndromes, metabolic disorders, displaced abomasums and lameness were also recorded. A repeated measures model was fitted regarding daily milk production averaged by week as dependent variable, and diseases as predictors. This model quantifies milk production losses before, during and after the onset of CM, adjusted by the 7 disease entities recorded. Compound symmetry was chosen as correlation structure. A total of 50,864 lactation weeks and 183 clinical cases were studied. Daily milk yield started to decline 2 wk before diagnosis. Milk yield dropped 4.2 lts/day the week that follow CM onset. Moreover, milk yield for cows with mastitis remained lower in comparison to non mastitic cows for 10 consecutive weeks after the CM onset. As previously reported, first lactation cows with and without CM did not show difference in performance before clinical episode.

122.
**MILK YIELD LOSSES DUE TO CLINICAL MASTITIS:
 MULTIPAROUS COWS MODEL**

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Mastitis is the most costly disease in dairy cattle. Milk losses are recognized as the main effect of the disease. The aim of this research was to study the effect of clinical mastitis (CM) on milk production. A total of 2,260 multiparous cows housed in free stall barns were followed through lactation. Milk production was recorded on daily basis until the 40TH week of lactation. Only the effect of the first clinical episode was evaluated. Reproductive syndromes, metabolic disorders, displaced abomasums and lameness were also recorded. A repeated measures model was fitted regarding daily milk production averaged by week as dependent variable, and diseases as predictors. This model quantifies milk production losses before, during and after the onset of CM, adjusted by the 7 disease entities recorded. Compound symmetry was chosen as correlation structure. A total of 65,774 lactation weeks and 520 clinical cases were studied. Major production loss of 4.7 lts/day was evidenced the week after onset of CM. In addition, milk yield for cows with mastitis remained lower than cows without mastitis during the following 10 week after the case occurrence. Previous research has found greater production losses due to CM using similar modeling approach. Further analysis for multiple mastitis case per cow and pathogen specific effect on production will be explored in the future.

123.
**COMPOSITE SOMATIC CELL COUNT VALIDITY AND
 PREDICTIVE VALUE TO IDENTIFY SUBCLINICAL
 MASTITIS AT DRY-OFF**

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High proportion of the dry-off treatments are targeting uninfected quarters. Besides, all cows are treated because no cost-effective test is available to identify those with and without subclinical mastitis (SM). This study was conducted to assess the validity of the test day composite Somatic Cell Count (SCC) to detect cows with SM at dry-off. A total of 296 mammary quarters from 81 cows at dry off was the study population. Each cows studied provided the quarters and composite milk samples. The quarter sample was cultured for major pathogen isolation (*Streptococcus spp*, *Staphylococcus aureus* y enterobacterias). Both quarters and composite sample were subjected to SCC. A quarter was considered with SM when a major pathogen was isolated and showed a SCC $\geq 250,000$ cell/ml. A cow was considered with SM if at least one quarter showed SM (parallel interpretation). Sensitivity and Specificity of the CSCC was assessed using ROC analysis and calculating predictive values considering threshold level between 150,000 and 350,000 cells/ml. Fifteen percents of the quarters and 41% of the cows showed SM. The overall validity was 68% (IC 95%, 56; 80). The positive and negative predictive values ranged between 50-55% and 63-67%, respectively. The findings suggest that composite SCC was able to identify between 63 and 67% of the cows with non SM.

124.
**IS HYPOTHYROIDISM POSSIBLE RISK FACTOR FOR
 HYPERTENSION?**

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Hypothyroidism is related to an increased risk of functional cardiovascular abnormalities, particularly for hypertension. To characterize the prevalence of hypothyroidism as a riskfactor for arterial hypertension 1023 individuals were recorded at a cardiologic center (2008- 2010). Data abstracted: age, sex, hypertension (HTA $>140/90$ mm Hg), and serum thyroid function tests: TSH and T₄. The investigation involved 698 patients (68.2%) with HTA (74.2% women), and 367 (35,8%) with known hypothyroidism (81.5% women). Ages ranged from 22 to 92 years (mean: 49.9 \pm 11.9) and mean weight 84.3 \pm 17.0 kg. Age was positively correlated with body weight ($r=+ 0.312$, $p<0.003$). TSH mean: 7.67 μ IU/ml (Men: 6.28 μ IU/ml Women: 7.92 μ IU/ml). The mean systolic (SBP) and diastolic blood pressure (DBP) were 160.8 \pm 25.4 and 98.8 \pm 13.9 mm Hg, respectively. Positive association TSH and SBP ($r:+0.155$, $p<0.042$) and age ($r=+0.267$, $p<0.0001$), highest age range 51-60 years. DBP was positively associated with age ($r=+0.200$, $p<0.0001$), highest age range 41-50 years. High prevalence of hypothyroidism in our hypertensive patients was detected. We found a positive association between TSH and SBP that could have long-term implications for cardiovascular health.

125.
DEVELOPMENT OF AN ORAL CANCER MODEL IN RATS
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Experimental models play an important role in biomedical sciences in elucidating the pathogenesis of different disorders that affect living organisms, including humans, and they are also useful for studying the molecular basis involved in any disease, developing diagnostic markers and evaluating the efficacy and safety of different treatment options, including potential drug therapies.

There are general factors and those that are specific to the oral environment, leading to the development and progression of neoplastic lesions of the oral cavity, accordingly, make this project whose main objective is to develop an experimental model of oral cancer in rats.

Materials and methods: We used 4-(4-nitroquinoline-1-oxide) to induce tumors in the oral mucosa in rats according to previously established protocols.

Results: The first clinically suspicious lesions developed at 3 months into the experiment. The tissue was removed and fixed samples were processed and examined by light microscopy. The samples showed patterns consistent with intraepithelial dysplasia and squamous cell carcinoma. We hope to develop an experimental model as a basis for further study of basic and clinical applications.

Project 06/K083, supported by SECTYP.

126.
A FIRST APPROACH TO DETERMINING LUTEINIZING HORMONE LEVELS TO STUDY REPRODUCTIVE STRATEGIES IN THE PICHÍ (*Zaedyus pichiy*), A THREATENED ARMADILLO
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The pichi *Zaedyus pichiy* is a threatened, heavily poached small armadillo native to Argentina and Chile. This seasonal breeder produces one yearly litter of 1–2 offspring. Based on previous studies, we hypothesized that pichis are induced ovulators. As a first step, we collected hypophyses of 4 dead pichis and confirmed through Western Blot and immunohistochemistry that a commercial polyclonal anti-luteinizing hormone antibody recognized pichi LH, which we then used to develop a heterologous RIA. We collected blood samples from captive females before and after pairing and quantified the concentration of LH. Hormone levels were similar pre- and post-mating, as well as in females that were not paired. This does not exclude the possibility that pichis are induced ovulators, but may be related to the timing of blood sampling. No data are available on the interval between mating and the timing of the pre-ovulatory LH peak in pichis, which in other species varies between 6 and 72 hours. Additional experiments with varying intervals between mating and blood extraction are being carried out.

127.
REGISTER OF *Octolasion cyaneum* IN SAN LUIS - ARGENTINA
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This work includes new records of species of earthworms in the province. Water, soil and worms were sampled from 14 sites of La Florida dam, in two climatic periods: summer and winter. The earthworms collection was performed by the method of excavation and manual collection in areas close to the shore. The excavated soil was transfer to the laboratory to proceed to isolate specimens and subsequent washing, anesthesia, fixation, and taxonomic classification. We studied external and internal morphological characters for taxonomic identification of all preserved specimens under a stereomicroscope. In the area of Club Nautico 2 *Octolasion cyaneum* presence were recorded. Knowledge of earthworm species that are present in our country is scattered and incomplete. The lack of data on both native species and metal contents, the possibility of use for diagnostic purposes (pollution) and use as bioremediation, gives importance and significance to these studies in the area and the region. In general, these studies are conducted with species associated with human activity.

128.
***Amyntas* FIRST DATA OF SAN LUIS- ARGENTINA**
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This work includes new records of species and a new place where species have been collected and cited for that province.

In the Dam of the La Florida, samples of water, soil and worms were taken in two well-defined climatic periods each year: summer and winter. The earthworms collection was performed by the method of excavation and manual collection. In the laboratory, we proceeded to isolate specimens and subsequent washing, anesthesia, fixation and taxonomic classification. For taxonomic determination only adults specimens were used. Their external characters at least 90% overlap in the key right defined.

In camping of the UNSL three specimens of *Amyntas corticis* and one of *Amyntas morrissi* were recorded.

Taxonomic and ecological knowledge of key organisms as earthworms are fundamental to implement monitoring and handling projects attempting to raise or maintain soil fertility.

129.

GENETIC CHARACTERIZATION OF *Varroa destructor* COLLECTED FROM *Apis mellifera* IN SAN LUIS ARGENTINA USING PCR REAL TIME HIGH RESOLUTION MELTING*Fernandez CA^{2*}, Eguaras MJ¹, Ponzi M², Medici S¹.*¹Laboratorio de Artrópodos, UNMDP; ²INTEQUI-CONICET.

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Although mitochondrial DNA mapping of *V. destructor* revealed the presence of several haplotypes, only two of them (Korean - AF106899- and Japanese -AF106897- haplotypes) were capable to infest *Apis mellifera* populations. Even though the Korean haplotype is the only one that has been reported in Argentina, these conclusions were based on mites sampled in apiaries from geographical areas of Argentina. The aim of this work was to study the genetic structure of *V. destructor* populations from 11 apiaries located in San Luis province in order to determine the presence of different haplotypes. Phoretic adult *Varroa mites* were collected from honey bee workers sampled from apiaries for DNA extraction. Samples of crude DNA were used to amplify CO-I gene via PCR Real Time using COXF and COXRa primers. Amplified fragments were analyzed by High Resolution Melting. Sequences share 3 clusters of similitude. One sample of each cluster was purified and sequenced. Sequences were compared using ClustalW in order to identify which *Varroa destructor* haplotype was present. All DNA sequences obtained from mite populations sampled in Argentina, share 98% of similitude with Korean Haplotype. Taking into account these results, we are able to conclude that Korean haplotype is cosmopolitan in San Luis.

130.

TELLURIUM (TE) CHRONIC EXPOSURE AFFECTS DEFENSIVE AND SURVIVAL BEHAVIORS IN PREPUBERAL RATS*Gaglio E¹, Ratti SG¹, Banzan AM¹, Ojeda MS², Alvarez EO¹.*¹Lab Neuropsicofarm Exp, FCM, UNCuyo e IMBECU-CONICET. Mza. ²Fac Bioqca. y Farmacia U N San Luis, Argentina. E-mail: pelipato@hotmail.com

Trace elements are present in low concentrations in soil and water of earth. Recently, it was found that some trace elements participate in enzymatic systems and regulation of genetic expression in the cell. Previously, our laboratory has shown that prepuberal rats, exposed to one single chronic dose of ZnTe, decreased significantly their defensive response to a stressful challenge. The objective of the present work was to extend these studies evaluating if this defensive behavior is affected following a dose-response relationship to trace elements. Pups from parents exposed to chronic administration in drinking water to 0.03, 0.3 and 3 µg/L of ZnTe were used. Four groups were formed: 1) Control rats (n=21); 2) 0.03 µg/L (n=12); 3) 0.3 µg/L (n=12), and 4) 3 µg/L ZnTe treated animals (n=10). Animals were exposed continuously from their conception up to 30 days of age of treatments. At this age, all animals were subjected to a 3 min forced swimming trials. Results show that treatment with ZnTe at all doses was effective to reduce swimming time in an average of 33.6± 3.3% less than control ($p < 0.01$). Immobilization (floating) was increased in average about 2.6± 0.1 times over control values ($p < 0.01$) in ZnTe treated rats.

No curve dose-response was found between increasing doses of ZnTe and defensive score in trace elements treated rats, suggesting that a minimum concentration is required to affect brain structures controlling defensive behaviors.

131.

BED BUGS, NEW RISK FACTORS*Godoy ME.*

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Cimex lectularius is an endemic parasitosis in expansion. It affects prisoners and few unsanitary housing. 2008 Increase insect extermination requests and you notice a change in the habitat and distribution of the parasite.

Alert the population *Cimex* is present, that have changed the risk factors, which any can suffer their attacks.

Recommend actions to mitigate its expansion. A retrospective observational study of the records of the Vector Control Unit and Wildlife Protection (VCUFP) from Godoy Cruz, took place.

Risk factors were analyzed before 2008 related to prison populations and dwellings with Unsatisfied Basic Needs (UBN). It was observed an increase in incidence of the parasitosis, a change in the type of housing, geographic distribution, the status of those affected and the factors that favor the spread of parasites. Epidemiology refers to risk the probability of suffering from or develop a morbid process.

Disinsection applications have increased tenfold in the past five years

132.

VARIETY AND USES OF FLORA OF LOMA BLANCA (SAN LUIS)*Gómez M, Corral A, Furlan Z, Bazán P, Bogino S.*

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The native forest area of the province of San Luis has been reduced due to the increase of agricultural activities. This causes the disappearance of native species. The aim of this work is to analyze the variety and abundance of native species, its current and possible uses, and its landscape value.

The concerned area corresponds to an area of rocky outcrops at the base of the hill "el Morro", in the province of San Luis. The floristic survey conducted during the past two years shows the presence of 39 families with 75 genus and 82 species. Family Asteraceae: with 11 genus and 12 species; Fabaceae with 8 genus and 13 species; Cactaceae and Verbenaceae with 5 genus. The surveyed species cover all the spectrum of uses: medicinal 30, edible 5, bee-keeping 10, aromatic 7, dyer 10 and ornamental 40; the latter being the most promising one. It is assumed that the rocky constitution of the soil serves as a shelter for the biodiversity, which comprises both rare species (*Pachygenium bonariensis*) and endemic ones (*Agalinis genistifolia*, *Senna acanthoclada*).

133.

ALTERNATIVE INOCULA IN DETERMINING DIGESTIBILITY*Guzmán L, Sager R.**FICES-UNSL. CONICET. E-mail: mlguzman@fices.unsl.edu.ar*

The necessity to take care of the well-being of the animals used for experimentation, added to the importance of the knowledge of the nutritious value of the feed for ruminants, induces the development of alternative biological methods to assess animal responses. The use of cannulated animals as donors of rumen liquor is indispensable, what brings numerous problems to feed them and maintain them. In search of alternative procedures, the effectiveness of fecal inocula was evaluated versus rumen inocula and its interaction with the diet of the donating animal, through the comparison of the coefficients of true *in vitro* digestibility, using the technique described by Goering and Van Soest (1979), adapted by Ankom TC, (DaisyII®). The nonparametric test of Mann Whitney showed highly significant differences among the diets, giving the highest digestibility results with rumen liquor from the alfalfa fed animal. The low correlation coefficient (R^2 : 0,456), was increased when analysis were stratified by diet, being R^2 : 0,566 for digitaria and R^2 : 0,726 for alfalfa that confirm the effect of diet on inocula. Similar results among analysis (VC 1,64 for alfalfa and 2,33 for digitaria), and the low standard deviation for alfalfa faeces inoculums (1,41 vs 1,21) show the potential use of faeces as inoculants, for *in vitro* digestibility procedures.

134.

FIRST TOXICITY ASSAYS OF POLYBROMINATED DIPHENYL ETHERS (PBDES) ON THE FRESH WATER CLAM *Pisidium chiquitanum**Lana N^{1,3}, Koch E^{2,3}, Altamirano J^{1,3}, Ciocco N^{2,3}.**¹IANIGLA, CONICET; ²IADIZA, CONICET; ³ICB, UNCUIYO.**E-mail: nciocco@mendoza-conicet.gob.ar*

PBDEs are used as flame retardants in commercial plastics. They are persistent organic pollutants with harmful effects on human health and environment. They can be found in sediments and biota of different trophic levels. It is known that bivalves can incorporate PBDEs and endure their toxic effects, which makes them potential bioindicators. The goal was to explore *P. chiquitanum*'s sensitivity to the congener BDE-209 in water and sediments. Clams were collected from the Uspallata stream (Mendoza) and kept in aerated aquaria without food (temperature $23 \pm 2^\circ\text{C}$; artificial light: 12 hr/day). Assays were conducted following the 203 OECD protocol. To explore sediment toxicity clams were exposed to three BDE-209 concentrations (10, 100 and 1000 ng g⁻¹), one solvent blank and one control (8 clams, 15g substrate and 30ml chlorine free water/aquaria). To evaluate water toxicity clams were exposed to three concentrations (12.5, 125 and 1250 ng ml⁻¹), one solvent blank and one control (8 clams, no substrate and 500 ml chlorine free water/aquaria). Live/dead clam records were taken at days 7, 14, 21, 28 and 36 for both assays. No diseased clams were recorded in the blank, control or treated group for all concentrations tested at both assays, though water and sediments are potential paths of BDE-209 uptake for *P. chiquitanum*. These preliminary results suggest that the clam is resistant to exposure to the congener and unsuited as bioindicator.

135.

OPTIMAL ENVIRONMENTAL CONDITIONS, INCREASE THE POLLUTION TOLERANCE, IN TWO SENSITIVE LICHEN SPECIES*Lijteroff R, Galvez I.**Laboratorio de Química Analítica Ambiental. UNSL. E-mail: rlijte@yahoo.com.ar*

Pollution and climate are the major factors affecting the distribution of lichens. Under different climatic conditions, it is difficult to discriminate the effect of air pollution of other environmental parameters in frequency and distribution of lichens. The aim of this study was to determine if optimum laboratory incubation increases contamination resistance, in two species of lichens. Sensitive species, *Usnea amblyoclada* and *Ramalina ecklonii*, were used in the experiments. Treatment: spraying for 21 days with a solution of 0.1 N sulfuric acid (5 parts) and 0.1 N nitric acid (1 part), pH: 4.6 (for simulated acid rain). Control: distilled water. The samples were placed in Petri dishes, and incubated in an oven at $20^\circ\text{C} \pm 0.1^\circ\text{C}$, photoperiod of 14 hours light and adequate humidity. Control variables: total chlorophyll, chlorophyll a and b, phaeophytin ratio, conductivity and phenological stage. Statistics: ANOVA, t test. In all cases the phenological state showed degradation. *Usnea amblyoclada*: no statistically significant difference, between pre-test and post-test repeated (day 4, 7, 14, 21) for control and treatment groups, in all analyzed variables ($P \leq 0.05$). *Ramalina ecklonii*: same result as above except with lower total chlorophyll concentration (mg / l) at study end ($P = 0.0111$). The results show that the effect of degradation on the photo systems, caused by acid rain (pH 4.6), could be mitigated when environmental conditions are optimal.

136.

POLYPHENOLS IN WILD POPULATIONS OF *Zuccagnia punctata* CAVFRUITS*Luna LC¹, Baroni V², Wunderlin DA², Feresin GE¹, Tapia A¹.**¹IBT, ICB, UNSJ, ²ISIDSA, UNC, Argentina.**E-mail: lluna@unsj.edu.ar*

The recently publicized therapeutic benefit of polyphenols as well as the natural source that contain them, resulting in an increasing for phyto-pharmaceutical and botanical supplement industries investigating fruits that have high content of poly phenols for purpose of formulating new commercial industries. The aim of the present work was to identify and quantified the main phenolics including flavonoids, chalcones and anthocyanins, from *Zuccagnia punctata* fruits, collected in Baughaceta (B), Hualilán (H) and Ullúm (U) in San Juan province, by means of HPLCMS-ESI-MS analysis. Petroleum ether (PE), dichloromethane (DCM) and acid methanol (MeOH-H⁺) fruits extracts from B, H, and U presented an important polyphenols content. The highest amount of 2',4'-dihydroxy-3-methoxychalcone was found in MeOH-H⁺ extract from B with values of 1274.95 mg/g. Whereas U contained de low amount 1045,20 mg/g. B, H and U populations containing 473.19; 851.96 and 898.61 mg/g of 2',4'-dihydroxychalcone respectively. Regarding to the flavonoids content, the 7-hydroxyflavanone in the fruits analyzed in this study ranged between 192.13-195.92 mg/g. In addition, galangin, erodictiol-*O*-hexoside and pinocembrin were detected in low concentration. These results suggest that *Z. punctata* fruits are a source of recognized antioxidant compounds for industrial.

137. WATER USE EFFICIENCY GAP ON DAIRY SYSTEMS IN THE SEMIARID REGION OF ARGENTINA

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The Argentina agricultural sector is suffering a rapid productive transformation associated with the agriculturization of land use, intensification and relocation of livestock based systems. Certain basins of the subhumid and semiarid region are attractive by low land prices and groundwater irrigation possibilities. In consequence, there is an increasing hydric demand scenario in these fragile areas. Focus on the Bioeconomy paradigm, water use efficiency of different intensification dairy systems of San Luis and La Pampa provinces have been determined and compared. Their hydric ecoefficiency in terms of milk production have been assessed using Life Cycle Assessment (LCA) methodology, including virtual water indicators. Livestock feeding, particularly supplements, is the main contributing factor to the water footprint of the system product. However, the minor indicators resulted from the supply of intensive systems, revealing that system productivity compensates water use insensitivity; in the case of San Luis milk industry, 1.025 liters of water per liter of raw milk, under the small scale intensive system, and 828 liters of water per liter of raw milk from the industrial dairy system of La Pampa. The ecoefficiency gap from the representative system of each basin (Modal) was 55% and 24%, respectively. Economic valuation of hydric ecoefficiency improves cost competitiveness of intensive and ecoefficient systems, including those which use irrigation.

138. ALGAL CRUSTS IN SALINAS DEL BEBEDERO (SAN LUIS, ARGENTINA)

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The biological soil crust (CBS) is a biotic community formed by the intimate association between soil particles, *Cyanophyte*, algae, mushrooms, lichens, and bryophytes liver. It is widely distributed in many soil types and provides an important contribution of carbon and nitrogen at the same, increasing its stability and protecting it from the erosive action. Infiltration is also modulated and the vascular plants and other organisms are directly affected. In this work the indigenous CBS, especially the formed by cyanobacteria in Salinas del Bebedero (San Luis), was studied, due to there are not records of the same. Soil samples were taken from different sites, where representative vegetable communities exist. They were cultivated in liquid medium Watanabe (1959) and were placed in growth chamber under controlled conditions of temperature (20°-30°C) and a photoperiod of 12 h in light and 12 h in darkness. At the present time, four species for the genus *Nostoc*, three the *Phormidium* genus, one to *Synechococcus*, *Oscillatoria*, *Dolioschosprium*, *Calothrix*, *Anabaenopsis*, *Scytonema*, *Aulosira* and *Nodularia* have been identified. All of these *Cyanophyte* are essential members of the crusts of arid and semi-arid environments. The importance of these results is that they are soil aggregators and most of them can fix Nitrogen.

139. SPATIAL PATTERNS OF SOIL MOISTURE AND NITRATE AS A FUNCTION OF DISTANCE FROM THE LIVESTOCK CORRALS IN THE MONTE DESERT

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In the Monte desert, livestock settlements generate environmental grazing gradients and represent an opportunity to study the effects of grazing pressures on spatial patterns of nutrients and water. In such context, we expected these spatial patterns be modified, both horizontally and vertically, as a function of distance from livestock corrals. We analyzed nitrate and water contents and salinity (electrical conductivity) on six linear transects (65 to 75 m along) corresponding to three livestock stations (LS) and three undisturbed control woodlands (CW). On each transect, we sampled soil profiles (up to 200 cm depth) every 5 m. In the initial, middle and final points of each transect, we collected soil samples from up to 400 cm depth. Transects in the LS began in a corral, whereas those in the CW began at the bottom of the valley. Nitrate and water contents in all depth intervals were greater at the LS than at the CW. Horizontal distribution patterns in LS showed a negative relation of water, nitrate, and conductivity with distance from the corrals. CW did not show a relation of these variables with distance from the valley bottom. This pattern was evident for surface soils. Conductivity was lower in the LS, although the topsoil of corrals had the highest values compared to the CW soils. Our results demonstrate that LS produce biogeochemical soil changes, causing a centripetal pattern of nitrate, water, and salt accumulation in the vicinity of the corrals.

140. EXOTIC FLORA OF POTRERO DE LOS FUNES (SAN LUIS, ARGENTINA)

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Biological invasions are a widespread and significant component of human-caused global environmental change. Potrero de los Funes, a mountain town of the central Argentina, is undergoing a progressive human impact, and for this reason, is particularly vulnerable to plant invasion. The aim of this study was to analyze exotic plant characteristics, plant communities invasion, and the contribution of exotic species to plant diversity in Potrero de los Funes (San Luis, Argentina). Fifty vegetation samples were taken, according to the methodology of the phytosociological school of Zurich-Montpellier, in an area of approximately 690 ha. We analyzed: status of the plants, geographical origin, families, habit of aliens, as well as the invasion in 12 plant communities of the area. We identified 422 taxa, of which 23% were exotic. This percentage is distributed among invasive plants (18%) and adventitious (5%). The largest proportion of exotic plants was of European origin and the main families were: Asteraceae, Poaceae and Lamiaceae. As for the habit of the plants, annual herbs predominated. The highest percent of invasion was found in the "abrojo grande" and "cardal" communities. The invasion was lowest at the "chilcal serrano" community, where 100% of the plants were native. Although the study area is dominated by native plants, a high proportion of exotic plants is present, as a result of increasing human alteration.

141.

CURRENT STATUS OF NATURAL VEGETATION IN THE AREA OF SAN FELIPE DOCKBASIN, SAN LUIS PROVINCE*Ocampo EN, Larrusse AS, Andrada NR.**Pastizales Naturales, Proyecto PROICO N° 51408, Facultad de Ingeniería y Ciencias Económico Sociales, Universidad Nacional de San Luis. E-mail: enocampo@fices.unsl.edu.ar*

In 1970 the phytogeographical regions of the province of San Luis, a study which took years of collection and identification of the species found, was published. Extending the boundaries of the agricultural frontier west ward provincial, determined the occupation of areas that were originally natural grassland, changing the diversity of vegetation in these phytogeographic regions. The aim of this study was to determine the current state of the natural vegetation in the catchment area of the basin of San Felipe Dam, before agricultural activity. We compared job information and research conducted in the years 1947 and 1954 for Water and Power, and in 1970 by INTA, with the assessment made in 2005 under the project of the UNSL 51,408. For this purpose, 9 sites were sampled, and considering kind of vegetation, density, coverage, bare soil in areas of natural vegetation, censuring lots adjacent cultivated or used for cattle, determining history of the cultivated species, type of work and climate data used. The species were include into three groups and results showed a decrease of 27% in Poaceae, 47% in broad-leaf and 50% intreesand shrubs.

142.

THE SPECIES OF *Lycium* L. (SOLANACEAE) FROM SAN LUIS, ARGENTINA*Scappini EG¹, Arana MD², Oggero AJ.**¹Fac. Ing., UNSL. ²Fac. Cs Exactas, Fco-Qcas y Naturales, UNRC, Córdoba. E-mail: egscappi@fices.unsl.edu.ar*

The Solanaceae includes, in Argentina, 33 genera and approximately 328 species, being one of the best represented in the provinces of the centre of Argentina. The tribe Lycieae Hunz. includes woody plants that occurs worldwide and has its natural range on all temperate and tropical continents, with centers of diversity in southern South America, southern Africa, and southwestern North America. According with current morphological and molecular studies, the tribe is monotypic, with the single genus *Lycium* L. (including the traditionally segregated *Grabowskia* Schltld. and *Phrodus* Miers), one of the best represented in San Luis. The aim of this work is to present a floristic survey of the species of *Lycium*, in province of San Luis. The analysis was done using collected material in different environments of the province, and vouchers of the herbaria VMA, VMSL, RCV, RIO, CORD and SI. Floristic treatments, geographical distribution and identification keys for the entities are presented. The genus is represented by eight species and 12 entities inhabiting the San Luis territory, all of them are shrubs that inhabit in semiarid and saline zones of the province. All the entities found are native and five of them are endemic from the center and western parts of Argentina. *Lycium chilense* var. *glaberrimum* is cited for the first time in the province.

143.

PALEOEDAPHOCLIMATIC INFERENCES IN A LOESSIC COLUMNAR SUCCESSION OF THE SAN LUIS MOUNTAINS, ARGENTINA*Strasser E¹, Chiesa J¹, Osterrieth M^{2,4}, Marchevsky E⁴, Perino E³**¹Dpto Geol; ³Área de Qca Anal; UNSL. 5700 San Luis. ²C. Geol. de Costas y Cuaternario. UNMdP. ⁴CONICET.**E-mail: eperino@unsl.edu.ar*

The Pancanta pedostratigraphic profile in the San Luis mountains (32°51'S; 66°07'W; height: 1617m) is representative of the loessic sedimentation at the pampas. The moisture regime of the soil is udic-mesic. The climate variables condition a positive water balance that generates reserves in the soil of 25 - 50mm for 10 months/year. The main objective is to infer the variations of the paleoedaphoclimatic conditions in a registry of about 12k years BP and compare them with the present ones. In 14 samples, 22 elements (majors, minors and trace) were quantitatively determined by wavelength dispersive X-ray fluorescence (XRF) techniques. The material was separated by specific weight of $\delta < 2.3 \text{g.cm}^{-3}$. The identification and optical microscopy semiquantification of silicophytoliths morphotypes was performed. Two paleoedaphic levels were recorded by C¹⁴ (conventional). Three levels with chemical alteration index (CAI = 63%) were determined for which a development under edaphoclimatic conditions, similar to the present, is inferred. At the sampling mean depth (-98cm), a radiocarbonic age of: 2910 ± 90 years C¹⁴ BP and CAI = 64%; a (-60cm) 1270 ± 50 años C¹⁴ BP and CAI = 67%, was determined. A certain correlation between total silicophytoliths concentration, paleoedaphic levels and Zn/Ni ratio, was observed, as meteorization products.

144.

RELATIONSHIP BETWEEN POLLEN, SPORES AND METEOROLOGICAL VARIABLES OF THE ATMOSPHERE IN THE CITY OF SAN LUIS*Vazquez ML, Daguerre A, Moglia MM, Crinó E.**Univ Nac San Luis, 5700 San Luis. E-mail: mmmoglia@unsl.edu.ar*

Meteorological variables affect the initial release of pollen and fungal spores and its dispersal once airborne. In this work we analyze the content of biological particles in the atmosphere of the city of San Luis, during two periods of continuous recording, and its relationship with temperature (T), precipitation (P), solar radiation (R) and relative humidity (H). Samples were collected with a Hirsttype volumetric sampler (Lanzoni, VPPS 2000) during the sampling periods (I): May 2010/August 2011 and (II): May 2011/August 2012. The behavior of bioaerosols showed intra and inter annual fluctuations. Differences between periods were analyzed with the Wilcoxon test. Significant difference was observed in the total content of biological particles (p=0.02). Registration was greater in (I), where (P) was higher and (T°) lower (p=0.03 and p=0.02, respectively). (R) and (H) did not differ significantly between periods (p=0.92 and p=0.38, respectively). The pattern of particles through the year showed a similar distribution in both periods, with yearly and late peaks. We conclude that the lower temperatures and greater precipitation of (I) compared to (II) allowed greater water availability, the main limiting factor for the development of plants and fungi in San Luis. These conditions would have favored the production of pollen and spores in (I).

145.

BIOLOGICAL ACTIVITY OF *Plantago major* IN RATS. MICROSCOPICAL STUDY

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Infusions of *Plantago major* (Pm) are used in folk medicine to treat gastric diseases. This study was performed to evaluate the gastric cytoprotective activity of the 10% infusion and methanolic extract obtained from the Pm in rats. Methanolic extract and the 10% infusion were tested in Wistar rats under standardized conditions and a strict protocol (method of Robert *et al.*, 1979). The substance used to produce injury was absolute ethanol. The removed stomachs were used for the microscopical study. The phytochemical analysis of Pm revealed the presence of possible substances responsible for the gastric cytoprotective action. After several chromatographic purifications, oleanolic acid, 5,7,3',4'-tetrahydroxy-6-methoxyflavone and 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone were isolated. A very important blood vessels and cells structure disorganization was found by electronic microscopy in the experimental injury group. Both methanolic extract and infusion evidenced a gastric mucose unaffected in structure. The electronic microscopy study shows the gastric cytoprotective activity of *Plantago major* against the absolute ethanol injury and may be due to the presence of different compounds described in the phytochemical profile.

146.

ASSESSMENT OF TOXIC ELEMENTS IN AMARANTH SEEDSAguilar EG¹, *Marchevsky EJ*¹, Escudero NL¹, Camiña JM^{2,4}¹FQByF, UNSL and ²INQUISAL-CONICET Chacabuco y Pedernera. 5700-San Luis. ³FCEN, UNLPam and ⁴INCITAP-CONICET, Av. Uruguay 151. 6300- Santa Rosa, La Pampa.

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In the recent years, amaranth (Amaranthaceae) has been proposed as new food due to its nutritional properties (i.e. aminoacid, protein, lipidic and carbohydrates contents). However, the determination of toxic elements has been less studied in amaranth seeds. For this reason, the concentration of As, Cr and Pb was determined in three species of amaranth seeds: *A. hypochondriacus*, *A. cruentus* and *A. dubius*, which were harvested in Argentina (provinces of La Pampa and San Luis). The determinations were carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES). Evaluation of accuracy was carried out by standard addition method. The results show that, for the three species, Cr and As are present in low concentration. However, the concentration of Pb was high, at least two times the maximum allowed value (10 µg g⁻¹) by the World Health Organization (WHO). As a conclusion, the high concentration of Pb present in amaranth seeds from Argentina, indicates that Pb determination should be considered in these amaranth species to avoid undesirable consequences for the human health in the population that consumes these seeds.

147.

CHEMOMETRIC CLASSIFICATION OF AMARANTH BY USING FATTY ACID COMPOSITIONAguilar EG¹, Cantarelli MA^{2,3}, *Marchevsky EJ*¹, Escudero NL¹, Camiña JM^{2,3}¹FQByF, UNSL and ⁴INQUISAL-CONICET Chacabuco y Pedernera. 5700-San Luis. ²FCEN, UNLPam and ³INCITAP-CONICET, Av. Uruguay 151. 6300- Santa Rosa, La Pampa.

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In this work, the composition of fatty acids was analyzed in order to obtain a chemometric model for the classification of amaranth varieties seeds: *A. cruentus* var. *candil* (*Acc*), *A. hypochondriacus* var. *dorado* (*Ahd*), *A. hypochondriacus* line *H17a* (*AH17a*) and *A. cruentus* line *G6/17a* (*AcG6/17a*). Chemometric model was performed by linear discriminant analysis (LDA). The results show that the discriminant model was able to classify correctly all the studied varieties, with 100% of correct classification in all cases, for training and prediction test set. On the other hand, the discriminant functions plot (function 1 vs function 2) showed similarities between *A. cruentus* var. *candil* and *A. cruentus* line *G6/17a*, while *A. hypochondriacus* var. *dorado* and *A. hypochondriacus* line *H17a* showed the most important differences with the others ones. As conclusion, LDA in conjunction to fatty acid composition, were able to distinguish between varieties of amaranth, which can be useful as a non expensive method to classify amaranth seeds.

148.

MULTIVARIATE ANALYSIS OF MAJOR, MINOR AND TOXIC ELEMENTS IN *Sechium edule*Albarracín GJ¹, Cantarelli MA^{2,3}, Escudero NL¹, *Marchevsky EJ*¹, Camiña JM^{2,3}¹FQByF, UNSL and ⁴INQUISAL-CONICET Chacabuco y Pedernera. 5700-San Luis. ²FCEN, UNLPam and ³INCITAP-CONICET, Av. Uruguay 151. 6300- Santa Rosa, La Pampa.

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Multivariate data analysis was performed on *Sechium edule*, using as variables the concentration of the follow elements: Ag, Al, As, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hg, K, La, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, S, Sb, Se, Sn, Sr, Ta, Te, Ti, Tl, V, Zn and Zr, analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) on a total of 15 replicates *Sechium* fruits, including whole fruit (Fr), pulp (Pu) and seed (Se). Principal Components Analysis (PCA) was carried out, in order to find an optimal model of classification and find the most relevant variables for the analysis of the fruit (which were the concentration of Ca, Cd, Fe, Mg, P y S); after that, the same variables were used for Clusters Analysis (CA) and Linear Discriminant Analysis (LDA). Thus, it was possible to make a proper classification of the three components of the fruit: Fr, Pu and Se by the three multivariate methods. As conclusion, multivariate methods were able to classify different parts of *Sechium edule*.

149. CARDIAC ELECTROPHYSIOLOGICAL CHANGES LINKED TO VITAMIN D RECEPTORS DEFICIENCY

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Chronic kidney disease is highly prevalent and is associated with cardiovascular disease. Paricalcitol protects from some renal and cardiovascular complication. However, its electrophysiological effect during myocardial ischemia-reperfusion is currently unknown. Aims: Investigate in the ON model, potential structural and functional changes of the heart linked to vitamin D deficiency. Methods: Adult rats (n=10) were unilaterally obstructed at the ureteropelvic level. An inductor of vitamin D receptor (VDR) was administered for 15 days IP (30ng/Kg/day). We evaluated histological, molecular and biochemical parameters, as well, cardiac electrophysiological activity with ischemia-reperfusion protocol. Results: we found changes in action potential duration (APD) at 90% (* P<0.05 vs. C, +P<0.05 vs. O). The analysis reported arrhythmias ventricular tachycardia (VT) and ventricular fibrillation (VF). Arrhythmias quantified as: 0 - sinus rhythm, 1- premature ventricular complex, 2- saved, without sustained VT 3, 4- sustained VT or VF (>30 seconds). * P<0.05 vs. C, +P<0.05 vs. O. Of interest was the finding that appears to prolong Pari ischemia DPA in both controls and clogged. Also, a significant bradycardia was established ischemic phase clogged with Pari, reversing completely during reperfusion. We conclude that the reduction of VDR expression in ON hearts could be related to the increase arrhythmogenesis. The recovery induced by paricalcitol could protect against ventricular fibrillation by the lengthening of the action potential. Further studies will be necessary to elucidate these interesting kidney-heart interactions.

150. CADMIUM EXPOSURE ON RAT CEREBELLUM EFFECT OF DIFFERENT DIETARY PROTEIN

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Cadmium (Cd) is a toxic element and an important environmental contaminant. We studied its effect on the lipid profile and antioxidant enzymes of rat cerebellum under different protein diets. Four lots of female Wistar rats were used: 2 lots received casein (Cas) and 2 lots soybean (Soy) as protein source. Within each group, 1 lot received regular water (control-Co) and the other, 15 ppm of Cd in the drinking water for 60 days. Lipid extraction was made with isopropanol:hexane 3:2 and lipid fractions were separated by TLC. We measured total cholesterol (TC), triglycerides (TG) and phospholipids (PL). Proteins were measured by Lowry. Total RNA was isolated with Trizol and cDNA was obtained. Catalase (CAT), Superoxide Dismutase (SOD) and NF-E2 related nuclear factor (Nrf-2) were determined by PCR. Beta actin was used as internal control.

TC showed an increase in the Cd groups (p<0.05) with no differences among the different diets. TG increased in Cas Cd and decreased in Soy Cd (p<0.05). PL also showed an increase in Cas Cd and a decrease in the Soy Cd group (p<0.05). Nrf-2 decreased in both Cd groups and in the Soy Co group (p<0.05). SOD increased in Soy Cd group and CAT did not change its expression. We conclude that Cd induces several changes on the lipid profile and antioxidant system in cerebellum, and some of them are reversed by a soybean based diet.

151. FUNCTIONALIZED POLYENES WITH POTENTIAL JUVENOID ACTIVITY ON *Tenebrio molitor* L.

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Juvenoids are juvenile hormone analogues, environmentally safe insecticides, that possess significant biological activity towards different insect pest species. A new approach to insect pest control is the use of substances that affect moulting and metamorphosis by mimicking juvenile hormones (JH juvenile hormones agonist). They are also called third-generation insecticides.

The present research is aimed toward discovering the juvenoid properties of functionalized polyenes, obtained by chemical synthesis. The structures of the synthetic products and the relative stereochemistry were determined by a combination of high resolution MS, UV, ¹H NMR, ¹³C NMR spectroscopy. The biological activity of compounds was evaluated using *T. molitor* as an insect model. Fifth instars larvae of *T. molitor* were randomly selected. Two acetone solutions of each compound (60µg/µL and 120µg/µL) were prepared. Test solutions were topically applied to the ventral surface of the thoracic segments with a microsyringe (2µL/larvae). Controls were treated with the solvent alone. There were twenty five replicates of larvae for each individual compound tested. The insects were examined daily for 60 days. The duration of the pupal stage (days) was recorded as well as inhibition of molting imaginal. The results showed that two of the tested compounds affected the normal development of the larvae. These compounds induced different levels of inhibition of molting. Besides the growth inhibitory effects also showed some mortality.

152. SEARCH FOR NEW STRATEGIES FOR INSECT CONTROL

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Currently, chemical control strategies have evolved over the years, resulting in less aggressive chemical environment and greater specificity of action. Terpenoids have profound effects on insects. It is well known that structural characteristics of monoterpenes and sesquiterpenes can influence their insecticidal properties, their degree of penetration into the insect cuticle and the ability to move and interact with an active site. As part of a program for the study to bioinsecticides we begin the analysis of the activity of some monoterpenes and sesquiterpenes against *Spodoptera frugiperda* Smith and *Rachiplusia nu* Guen larvae, two sorghum pests. In laboratory tests, we investigated the antifeedant and toxic properties of limonene, camphene, terpineol, α-pinene, β-cariophyllene and mircene in the fourth larval stage of *S. frugiperda* and *R. nu*. The percentage of feeding inhibition (%FI) was calculated using choice test, however none of the compounds showed antifeedant activity. To assess toxicity, the compounds were topically applied to the ventral surface of the thoracic segments with a microsyringe (1µg/µL/larvae). Mortality was recorded at 24, 48 and 72 h. Percentage of insect mortality was corrected according to Abbott. Limonene showed that was the most toxic to *R. nu*, followed by α-terpineol. Furthermore, myrcene caused mortality in *S. frugiperda*. This data provide an important preliminary information, that should be fully explored for using in integrated pest management (IPM). More research is needed in order to understand the specific mechanism of action for each compound.

153.

RAT OVARY EXPOSED TO CADMIUM: EFFECT OF SOYBEANS AS PROTEIN SOURCE*Calderoni AM, Alvarez SM, Plateo Pignatari MG, Perez Diaz MF, Giménez MS.**Depto Bqca y Cs Biológicas. UNSL. IMIBIO-SL. CONICET. E-mail: amcal@unsl.edu.ar*

Cadmium (Cd) is a toxic element considered as an important environmental contaminant, and a major constituent of tobacco smoke. The aim was to study the effect of chronic exposure to cadmium on the lipid profile of rat ovary and the incidence of different diets: one casein based and one soybean based. Four lots of adult female Wistar rats were used: 2 lots received casein and 2 lots soybeans, as protein source in the diet. Within each protein group, 1 lot received water without Cd (control) and the other received 15 ppm of Cd (as CdCl_2) in the drinking water, for 60 days. Lipid extraction from ovaries was made with isopropanol: hexane 3:2. Lipids were separated by TLC using hexane:ethyl ether:acetic acid, 80:19:1. Total cholesterol (TC), triglycerides (TG) and phospholipid contents (PL) were measured. Proteins were determined by Lowry. The results are media \pm SEM. TC ($\mu\text{g}/\text{mg}$ protein): Casein Control: 5.979 ± 0.44 ; Casein Cd: 18.36 ± 1.62 ; Soy Control: 21.18 ± 3.17 and Soy Cd: 14.79 ± 0.07 ($p < 0.05$). TG ($\mu\text{g}/\text{mg}$ protein): Casein Control: 3.148 ± 0.062 ; Casein Cd: 2.98 ± 0.81 ; Soy Control: 5.234 ± 0.836 and Soy Cd: 5.971 ± 0.123 ($p < 0.05$). PL did not show significant differences among groups, but there was a trend showing an increase in the Soy Cd group. We can conclude that Cd modifies the lipid profile in ovary, suggesting an alteration on the membrane composition. In opposition to our hypothesis, Soybean protein did not decrease the cholesterol levels in the ovary.

154.

GENOTOXIC ASSESSMENT ON DESAGUADERO RIVER WATER (LAGOON COMPLEX GUANACACHE)*Cangiano MA, Moglia MM, Enriz RD.**FQByF UNSL. E-mail: macang@unsl.edu.ar*

The locality "La Represita" is part of the wetland "Lagunas de Guanacache" were nowadays live survivors of Huarpe Nation. To evaluate the water's characteristics of "Río Desaguadero" that crosses this region, genotoxic analysis were carried out using the *Allium cepa* test system. Water samples of dry (D) and wet (W) period were analyzed, May and September 2012, respectively. D study: One control treatment was made with distilled water and one experimental treatment with Río Desaguadero's water, for quadrupled. *A. cepa* bulbs were put in both treatments. After three days the control bulbs' roots grew 2.5 ± 0.5 cm while in the experimental group there was no root growth therefore, any genotoxic analysis could be performed. W study: The protocol was repeated. After three days the control bulbs' root had grown 2.0 ± 0.5 cm and the experimental bulbs' root had grown 1.0 ± 0.5 cm. These roots were put through fixation, coloration and squash. About 4000 cells were analyzed per treatment. The Mitotic Index (MI) and the spoilage produced to the genetic material were calculated. The cytogenetic analysis showed a MI = 12% in the control group and 3% in the experimental one. C-mitosis and sticky chromosomes were observed. These results would indicate a cytotoxic effect probably produced by the concentration of salt related with hydric seasonality.

155.

OLIVE OIL POLYPHENOLS ACTION IN MAST CELL MEDIATED INFLAMMATORY PROCESS*Cerrutti FA, Persia FA, Mariani ML, Penissi AB.**IHEM, Facultad de Ciencias Medicas, UNCuyo, 5500, Mendoza. E-mail: apenissi@fcm.uncu.edu.ar*

We have previously found that hydroxytyrosol (Ht) and oleuropein (Olp), two phenolic compounds purified from olive oil, inhibit 48/80-induced mast cell activation, acting thus as mast cell stabilizers in rat peritoneal purified mast cells. The present work was designed to examine the effects of Ht and Olp on live models of mast cell degranulation, in order to demonstrate their probable protective action in animal model of gastric ulcer.

Rats were orally intragastric administrated with: 1) Saline (negative and positive control) or 2) Ht or Olp or Sodium cromoglycate (Sc-commercial mast cell stabilizer), and injected intraperitoneally with: 1) Saline (negative control) or 2) 48/80. After been sacrificed, corpo-fundic stomach region was extracted and examined under stereomicroscope. Ulcerogenic index (UI) was assessed from a scoring system designed by Marazzi, Uberti and Turba. Statistical analysis: ANOVA-1/Tukey-Kramer.

Polyphenol groups presented significantly lower UI than 48/80 treated group and lower UI than Sc treated group.

The present study demonstrates that Ht and Olp inhibit mast cell degranulation in a live model and have a more efficient result as a gastric cytoprotective than Sc.

156.

INHALATION OF ENDOTOXIN AND INSULIN RESISTANCE IN OBESITY*Della-Vedova MC, Gomez N, Gomez-Mejiba SE.**Laboratorio de Medicina Experimental y Terapéutica. IMIBIO-SL-CONICET-UNSL, 5700 San Luis, Argentina.*

Activation of neutrophils in the lung microvasculature can be an important source of systemic inflammation, which can be worsened when obese patients inhale lipopolysaccharide (LPS) and thereby cause insulin resistance (IR). Myeloperoxidase (MPO) is a major protein in neutrophils and is the only enzyme that produces hypochlorous acid (HOCl), one of the strongest oxidants in biology. We hypothesized that the obese lung is more sensitive to MPO-driven oxidative stress caused by air pollutants, leading to more pro-inflammatory mediators in circulation and thereby IR. To test our hypothesis we fed mice for 20 weeks with a diet rich (obese) or normal (lean) in saturated fat. The obese lung was smaller and had more neutrophils than the lean lung. After 24 h of exposure to LPS by intratracheal instillation (ITI), the obese lung had more neutrophils, adhesion molecules, activity of MPO, chlorotyrosine (a marker protein oxidation by HOCl) and IR than obese mice non-exposed to LPS. Proinflammatory cytokines (TNF- α , IL-6 e IL-1) in serum and NF- κ B activation in the lung increased more in obese mice than in control mice. The ITI of either MPO inhibitors, scavengers of HOCl or DMPO before ITI of LPS ameliorated these effects by following different mechanisms. Homing/activation of neutrophils in the obese lung can be a therapeutic target to prevent IR in obese subjects exposed to endotoxin.

157.

PHARMACOGNOSTIC ANALYSIS OF *Aloysia gratissima* IN THE CENTRAL-WESTERN ARGENTINA*Dolab J, Fusco MR, Sosa A, Petenatti EM.**Pharmacognosy/Proj. 8702 and 22/Q016, FQByF UNSL, Ejército de los Andes 950, 5700, San Luis. E-mail: mffusco@unsl.edu.ar*

Aloysia gratissima (Gillies & Hook. ex Hook.) Troncoso (Verbenaceae), known as "usillo", "azahar del campo", "palo amarillo", "culén", or "cedrón del monte", is a northern and central Argentina native shrub, used mainly as a cardiogenic, sedative, carminative, diaphoretic and digestive drug in the Cuyo region, and flavor in the "mate tea". This study aimed to analyze the composition of the major fixed and volatile compounds. The aerial parts (leaves and flowers) were collected, dried at 45°C, and crushed. The aqueous extracts (infusions and decoctions) and ethanol, were made. Analytical march was performed on the ethanol extract to detected tannins, flavonoids, alkaloids, etc. In addition, fresh samples were submitted by hydrodistillation to analyze the composition of essential oils. AE analysis was performed in a gas chromatograph "Shimadzu" GC-MS QP2010 Plus model. We identified the presence of tannins, flavonoids and alkaloids. In AE was detected β -caryophyllene and isocaryophyllene (Tr = 20.24 and 20.78 min, respectively). Comparing the composition with bibliographic data, we observed absence of pulegone, high content in thujone and sabinene. Also important were the contents of sesquiterpene alcohols (viridiflorol and sphaulenol). It is carrying out the fractionation of the extracts for structural elucidation of fixed majority compounds.

158.

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF SEMICARBAZONES DERIVED FROM AROMATIC ALDEHYDES*Escobar E¹, Mattana C², Cifuentes DA¹, Laciari A², Tonn CE¹.**¹Área de Química Orgánica. INTEQUI. ²Área de Microbiología. UNSL. San Luis. Argentina. E-mail: cifuentes@unsl.edu.ar*

Semicarbazones (SCs) are a class of compounds that have shown great interests for their biological properties. A series of SCs, obtained from aromatic aldehydes with different stereoelectronic properties (R = 4-H, 4-NO₂, 4-Cl, 4-OCH₃), were synthesized using equimolar quantities of semicarbazide, AcONa en EtOH absolute. The reaction mixture was refluxed for 8 h. The products obtained were filtered, recrystallized and their structures were confirmed by spectral data (¹H and ¹³C RMN). Aldehydes and SCs were tested for their antioxidant and antibacterial properties. Antioxidant activity was evaluated by diphenylpicrylhydrazyl (DPPH) method. Also antibacterial properties were evaluated against *Staphylococcus aureus* ATCC 43300 (methicillin-resistant), *Staphylococcus aureus* ATCC 25923 (methicillin-sensitive), *Listeria monocytogenes* CLIP 74910, *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853. The antibacterial activity was assayed by micro-well dilution method in broth supplemented with 0.01% (w/v) of tetrazolium red as visual indicator of bacterial growth. Compounds were dissolved in DMSO and tested from 1000 to 125 μ g/mL. After 24h incubation at 37°C the antibacterial activity was defined as absence of red colour. SCs were more actives than the corresponding aldehyde. Selective antibacterial bioactivity was observed against *E. coli*, 4-Nitrobenzaldehyde SCs; against *S. aureus* 4-Methoxybenzaldehyde SCs and against *L. monocytogenes* 4-Chlorobenzaldehyde SCs.

159.

RATS EXPOSED TO CADMIUM IN DRINKING WATER. EVALUATION OF PRO- AND ANTI-APOPTOTIC MARKERS IN THE RAT HEART*Ferramola ML¹, Biaggio VS², Gómez NN², Ortega HH³, Anzulovich AC¹, Giménez MS¹.**¹IMIBIO-SL, CONICET-UNSL, San Luis; ²FQByF-UNSL, San Luis; ³FCV-UNL, Santa Fe.*

Cadmium has been recognized being able to induce apoptosis in cardiomyocytes *in vitro*. The aim of the present work was to assess the content of pro- and anti-apoptotic markers in the heart of Cd-intoxicated rats. We also evaluated the putative protection of giving a soy-based diet. Male Wistar rats, 8-10 weeks old, were separated into 6 groups and treated during 2 months, as it follows: groups (1), (3) and (5) were fed a casein-based diet; groups (2), (4) and (6) were fed a soy-based diet. Groups (1) and (2) received tap water; (3) and (4) received tap water+Cd²⁺ 15ppm; (5) and (6) tap water+Cd²⁺ 100ppm. Histological studies were performed by immunohistochemistry analyses, in order to evaluate the content of BCL-2, BAX and CASPASE-3 proteins, in the heart tissue. No significant differences were found among the studied groups. We conclude that exposure to Cd does not alter the amount of the pro- and anti-apoptotic proteins evaluated in the present work. No changes were observed in rats fed a soy-based diet. These results are in agreement with the lack of apoptosis previously observed by us with other assay.

160.

MITOCHONDRIAL CYTOPROTECTIVE EFFECTS OF PARICALCITOL-MEDIATED DURING OBSTRUCTIVE NEPHROPATHY*García IM, Altamirano L, Mazzei LJ, Manucha W.**Área de Fisiopatología, Depto. Patología, Ciencias Médicas, UN de Cuyo, Mendoza, Argentina. E-mail: wmanucha@yahoo.com.ar*

Recently, activators of vitamin D receptors (VDR) have demonstrated suppressant effects on the renin-angiotensin system, as well as anti-inflammatory and antifibrotic actions. This study aimed to evaluate the cytoprotective effects of paricalcitol, a VDR activator, at the mitochondrial level using the unilateral ureteral obstruction (UUO) model. Rats subjected to UUO and controls were treated daily with vehicle or paricalcitol. The control group underwent a sham surgery. The treatment was done for 15 days (30ng/kg). The following were determined: biochemical parameters, fibrosis/apoptosis, mitochondrial morphology, VDR, AT₁ receptor, and NADPH oxidase 4 expression, and NADPH oxidase activity (in total and in mitochondrial fractions from the renal cortex). VDR activation prevented fibrosis (20 \pm 5 vs. 60 \pm 10%) and the number of TUNEL-positive apoptotic cells (10 \pm 3 vs. 25 \pm 4) in UUO. Biochemical, histological, and molecular studies suggest mitochondrial injury. Electron microscopy revealed in UUO electronically luminous material in the nucleus. Some mitochondria were increased in size and contained dilated crests and larger than normal spaces in their interiors. These changes were not present with paricalcitol treatment. Additionally, high AT₁-receptor mRNA and NADPH activity was reverted in mitochondrial fractions from obstructed paricalcitol-treated animals (0.58 \pm 0.06 vs. 0.95 \pm 0.05 relative densitometry units and 9,000 \pm 800 vs. 15,000 \pm 1,000 relative fluorescence units/ μ g protein (-1) \cdot min(-1), respectively). These changes were consistent with an improvement in VDR expression (0.75 \pm 0.05 vs. 0.35 \pm 0.04 relative densitometry units). In conclusion, presents results suggest that VDR induction confers a protective effect and reveal, as well, a possible AT₁ receptor-dependent protective effect that occurs at the mitochondrial level.

161. PHARMACOBOTANICAL CHARACTERIZATION AND DIURETIC ACTIVITY OF “MOLLES” OF THE CUYO REGION, ARGENTINA

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In the central-western Argentina popular medicines two taxa of Anacardiaceae, *Lithraea molleoides* (Vell.) Engl. and *Schinus fasciculatus* (Griseb.) I.M. Johnston are used with the name “molles”. The first species is known as “molle” or “molle de beber” and is used as a diuretic and digestive. The second one is known as “molle” or “molle morado” and is used as an analgesic, antitussive and purgative. The aim of this study was to conduct pharmacobotanical characterization and determination of diuretic activity in both species. The aerial parts were collected in the Chaqueño-Serrano woodlands, preserved in aceto-alcoholic formalin or dried 45°C or diaphanized by measuring the micrographics parameters. The Lipschitz method (Lipschitz *et al.*, 1943) was applied to determine the diuretic activity. The differences between morphological and anatomical characters together with micrographics parameters contribute for an effective quality control of these species. Moreover, it validates the use in traditional medicine of *L. molleoides*, since this species showed a moderate diuretic activity with standard chemical parameters. The infusion 10% of *L. molleoides* showed diuretic activity between 60-105 min (Test *t* de Student: $p < 0.05$ vs. control), while *Schinus fasciculatus* had not diuretic effect at this concentration.

162. GASTROPROTECTION AND ANTIMICROBIAL ACTIVITY OF *Lithraea molleoides* AND METABOLITES AGAINST *Helicobacter pylori*

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Lithraea molleoides (Vell.) Engl. (Anacardiaceae), known popularly as “molle”, is used in folk medicine as digestive. In one study, the antibacterial activity of *L. molleoides* was assayed on ten clinical strains of *Helicobacter pylori* isolated from gastric biopsies, reference strain NCTC 11638, and evaluated by agar diffusion method in well, range 2.5 - 250 mg/ml. The MIC was determined by agar dilution method according to Clinical and Laboratory Standards Institute using dilutions of gallic, caffeic and ferulic acids, rutin, catechol or mannitol compounds isolated (500 to 0.125 µg/ml). Results indicate that *L. molleoides* extracts have antimicrobial activity against *H. pylori* strains and may be due to the presence of different compounds described in the phytochemical profile. In other study, necrotizing agents (0.6N HCl, acetylsalicylic acid 200 mg/kg or absolute ethanol) were employed as ulcerogenic agents in Wistar rats (Robert *et al.*). Aqueous and methanolic extracts and metabolites isolated of *L. molleoides* prevent the formation of gastric lesions ($p < 0.01$ and $p < 0.001$ vs. Control -Test *t* de Student). The results indicate that *L. molleoides* and metabolites prevent the formation of gastric lesions and have significant antimicrobial properties against *H. pylori*. *L. molleoides*. This could represent an useful tool in relieving digestive disorders even those associated with *H. pylori*.

163. *Artemisia douglasiana* Besser: GENOTOXIC EVALUATION

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Artemisia douglasiana Besser (*Ad*) (family Asteraceae) popularly known as “matico”, is used in folk medicine. This study was developed to evaluate the *Ad* genotoxicity: micronuclei frequency and toxicity potential on mice spermatogenic cells. Micronucleus test: albino mice (28-32g) (3 males and 3 females) were i.p. injected with distilled water (control group), *Ad* 10% and 25% and paracetamol 1g/kg (positive control) and were sacrificed at 24 or 48 h after dosing. The presence of micronucleus was evaluated in 2000 PCE for each animal. There were no differences between *Ad* treated groups (10%, 25%): 4.83 ± 0.88 ; 3.91 ± 0.15 vs control group (4.33 ± 0.30) neither 24 h. nor 48 h. *A.d.* (10%, 25%): 6.25 ± 0.86 ; 4.83 ± 0.38 , vs. control group: 4.16 ± 0.30 ; paracetamol produced a significant increase at 24h (13.83 ± 0.62) and 48h (15.00 ± 0.46) vs. controls ($p < 0.0001$). Sperm abnormality test: *Ad* lyophilized water infusion was administered orally, daily for 5 days, at doses 300 mg/kg and 500 mg/kg body weight to 10 males each selected at random. The animals were sacrificed by cervical dislocation after 35 first dose days. Morphological abnormalities of sperm head shape were assessed according to systematic criteria. The frequencies of sperm abnormalities for two different treatment were 1.76% and 1.82%. The negative control was 1.69%. Data showed that the frequencies of sperm abnormalities were not significantly affected by *Ad* and it has neither aneugenic nor clastogenic effects.

164. MINERAL PROFILE IN *Tripodanthus flagellaris* INFUSIONS

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Tripodanthus flagellaris (Cham. & Schlecht.) Tiegh. -Loranthaceae- is a hemiparasitic plant used in folk medicine for its putative hypotensive properties. This plant is normally consumed as an infusion. The concentrations of Cr, Cu, Fe, Mn and Zn elements in twenty two samples of medicinal herb, prepared as infusion, were determined by using inductively coupled plasma optical emission spectrometry (ICP-OES). The samples were digested with concentrated nitric acid and hydrogen peroxide in a microwave system. The aim of this work was to determine the nutritional value, in terms of these minor element contents, using recommended daily allowance data. Additionally, principal component analysis and cluster analysis were applied to the data matrix to evaluate analytical results. The decreasing sequence of the mean metal levels in the infusions was as follow: Fe > Mn > Zn > Cu > Cr. The average total contents (mg per liter of infusion) were 82.3 for Fe, 19.8 for Mn, 17.3 for Zn, 2.8 for Cu and 0.25 for Cr. These results are in agreement with values previously reported in the literature.

165.

SEASONAL AND GEOGRAPHICAL VARIATION IN THE CHEMICAL COMPOSITION OF *Baccharis salicifolia* RUIZ & PAVON, AND INSECTICIDAL ACTIVITY AGAINST *Triatoma infestans* KLUG

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Control populations of *T. infestans* were achieved through synthetic insecticides. Although these have shown high efficacy has been detected high levels of resistance. The possibility of using essential oils or their components deserves further attention. It has been shown that seasonal differences affects the chemical composition of the oils and therefore modifies its bioactivity. The aim of this work is to study the seasonal and geographical variation of the components of *B. salicifolia* and compare their insecticidal activity in fourth instar nymphs of *T. infestans*. The plant material was collected in Cruz de Piedra and Barrancas, San Luis, during the months of March and December. Essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS) and identify the components using the library provided with the instrument (Finnigan MAT GCQ) and reference spectra. Test solutions were topically applied to the ventral surface of the thoracic segments with a micro syringe (2 μ L/ nymph). Controls were treated with the solvent alone. Mortality was assessed every 24 hours until the 5th day. The chemical analysis showed that the composition of the oils was different in March and December in Cruz de Piedra as well as in Barrancas. *B. salicifolia* essential oil (Barrancas-December) showed toxic activity. The highest concentration of germacrene in this essential oil in these samples could be the cause of the higher mortality (66.66%). Based on these results, we consider it important to perform additional studies on the toxicological properties of this essential oil on this Chagas disease vector.

166.

LEAD CONTENTS IN AMARANTH FLOUR PRODUCED IN THE NORTHEAST REGION OF ARGENTINA

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The aim of this work was to study the levels of lead (Pb) in amaranth flour samples produced in the Northeast region of Argentina (Santa Fé, Chaco and Corrientes). The human toxicity of Pb is largely proved, in fact, it is able to bind to the SH-group of the proteins making enzymes inactive, or to replace other ions in metabolic functions with adverse effects on heme biosynthesis, kidney, nervous and cardiovascular systems. Numerous evidences indicate that the major exposure of Pb in food, to the general population, comes from fruits and grains. Twenty five samples were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Microwave-assisted acid digestion of samples was used to eliminate the organic matter. In order to assess the accuracy of the method, standard solutions were analyzed. The results of this study showed that the average concentrations detected, ranged from 4.2 to 16.8 mg Kg⁻¹ of Pb. Therefore, the amaranth flours collected from the Northeast region of Argentina, contained Pb within the safe limits prescribed by the WHO in 2007. However, the possibility of punctual contaminations, mainly principally caused by the indiscriminate use of phosphate fertilizers (that could bind heavy metals), should be considered. Therefore, we consider that it is very important to continue with the application of biomonitoring plans, in order to control the levels of this toxic element in foodstuff.

167.

PHARMACOGNOSTIC STUDY OF *Geoffroea decorticans* (GILL. EX HOOK. ET ARN.) AND MAYORITARY COMPOUNDS MONITORING BY CAPILLARY ELECTROPHORESIS

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Geoffroea decorticans, commoly known as “chañar” or “chañarillo” is a bush or tree widely spread in Argentina and used in popular medicine as emolient, balsamic, antitusive and expectorant. The aim of this paper is to analyze chañar bark, popularly used in decoctions and infusions for respiratory treatments. Samples were collected in San Francisco and Potrero de los Funes, of San Luis province. The pharmacognostic study revealed the presence of flavonoids and sterols through solvent extraction technique of increasing polarity. Screening was carried out by identification reactions after thin layer chromatography of the infusions and decoctions (FA VI Ed.) The solvent mixture ethanol:water (40:60) proved to be the most efficient/effective to obtain liquid extract. The electrophoretic analysis was applied to the separation and simultaneous determination of mayoritary compounds: oleanolic acid, stigmasterol and quercetine. The analysis time was less than 15 minutes. The use in folk medicine decorticans *Geoffroea* bark as an expectorant and anti-inflammatory airways will be justified by the presence of these components.

168.

EFFECT OF CADMIUM ON GLUTATHIONE METABOLISM AND PPAR EXPRESSION IN SMALL INTESTINE

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Cadmium (Cd) is an important environmental contaminant that induces oxidative damage in cells. Previous results from our laboratory indicate that Cd (oral via) produces lipoperoxidation and alters the expression of inflammation factors in small intestine (SI). Cd, thiobarbituric acid reactive substance and metalothioneine content, as well as TNF α , PPAR γ and Nrf2 expression were increased. Glutathione Reductase activity (GR) decreased in SI of rats treated with Cd. The objective of this work was determinate if Cd affects the glutathione metabolism and the expression of other PPARs. Wistar male rats (180g) were treated with Cd (15 ppm) in tap water during 2 months. GSH content and the activities of enzymes related to NADPH production were measured. The expression of mRNA PPAR α and β , and β -actin (internal control) were determined by RT-PCR. Tissue total RNA was isolated using TRIzol. The PCR products were analyzed on 2% agarose gels containing Gel Red. Band intensities of RT-PCR products were quantified using Image J software. GSH and Glucose-6-P-dehydrogenase showed a decrease, and Isocitrate dehydrogenase did not change. PPAR α and β expression increased in SI respect to control (P<0.05). Results indicate that 2 months of 15 ppm Cd intoxication alters the glutathione metabolism and triggeres the antiinflammatory response mediated for PPAR α and β , according to the data previously observed.

169.
PHARMACOGNOSTIC EVALUATION OF THREE NATIVE SPECIES OF FABACEAE

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The Fabaceae family is very important from the pharmacognostic point of view because many of its species are used in folk medicine. Three native species: *Acacia caven* (Molina) Molina var. *caven* “espinillo”, “aromo” or “tusca”, *Acacia gilliesii* Steud. (= *A. furcatispina* Burkart) “garabato”, and *Prosopis torquata* (Cav. ex Lag.) DC. “tintitaco” are used in folk medicine as an astringent and antiseptic agent. According to the three species that are attributed with medicinal properties, this study was carried out to isolate the components responsible for the therapeutic activity and validate their antibacterial activity. The aerial parts were collected of natural population of San Luis, dried at 45°C, crushed and the aqueous (infusions and decoctions) and ethanol extracts, were made. On the extract was performed a phytochemical screening, to detected the major compounds. Flavonoids are identified by TLC (against genuine standards, using as mobile phase: ethyl acetate: acetic acid: methyl ethyl ketone: water (5:1:3:1) and as revealing UV). We found quercetin and rutin in the three species. Furthermore, saponins, tannins and alkaloids have been detected. It is carrying out the fractionation of the extracts to isolate and elucidate of chemical structure of the compounds responsible for bioactivity and validate the antimicrobial therapeutic action.

170.
TRACE ELEMENTS IN CRUDE DROGUE AND INFUSIONS OF *Margyricarpus pinnatus*

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In this work samples of *Margyricarpus pinnatus* (Lam.)Kuntze (Fam: Rosaceae) as well as infusions prepared from them were investigated for their trace element composition. This herb is widely used in South America by its diuretic and carminative effects. Element concentrations in the medicinal herb and their infusions were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). The following trace elements were studied: Cd, Cu, Fe, Pb and Zn. Leaves and stems of the herb samples were digested in a microwave oven in order to determine the total contents. Infusions were prepared using distilled water at 85°C for 15 min. The decreasing sequence of the mean metal levels in the raw material of studied herb was as follows: Fe > Zn > Cu > Pb > Cd. The means of total content in raw material were: Fe (265 µg g⁻¹), Zn (45 µg g⁻¹), Cu (6.3 µg g⁻¹), Pb (1.2 µg g⁻¹), Cd (0.03 µg g⁻¹). The toxic elements Cd and Pb were found only in the whole overground herb at low concentrations, but not in the final infusions (below the limit of quantification). The extraction yield for the elements present in the infusions was calculated: Fe (18.5%), Zn (28.3%) and Cu (47.4%). Finally, the estimated daily intake was compared with current recommendations (WHO 2007). All products and its infusions were included within the upper tolerable limits for minerals, in trace elements such as toxic elements present at low levels.

171.
REVERSAL OF POST-EPIGENETIC CHANGES MODULATED BY ROSUVASTATIN IN NEONATAL HYDRONEPHROSIS

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Unilateral ureteric obstruction (UUO) in neonatal rodents can be used as a paradigm for in utero obstruction in humans and a platform for studying the potential of novel therapies for congenital obstructive nephropathy. The present study examined the effect of rosuvastatin (Ros) on key morphometric measures of renal injury and corresponding gene expression correlates following neonatal UUO in the rat. Neonatal rats subjected to UUO and controls were treated daily with vehicle or Ros for 14 days. Quantification of tubular dilatation, glomerular size and numbers and tubulointerstitial fibrotic area was performed and change validated by reference to appropriate renal gene expression correlates. UUO increased tubular diameter and interstitial fibrosis by 2.7- and 7-fold, respectively, in parallel with increases in renal transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α) mRNA levels. Glomerular number and size were reduced by 52 and 33%, respectively. Reductions in WT-1 mRNA and protein expression were noted following obstruction occurring in tandem with reduced mRNA levels for BMP-7 and E-cadherin. Ros attenuated tubular dilatation (33%) and interstitial fibrosis (72%) in association with the normalization of renal TGF-β(1) and TNF-α mRNA levels. Ros improved glomerular number and size (30 and 50%), and preserved mRNA and protein expression levels of WT-1 and normalized mRNA levels for BMP-7 and E-cadherin. Ros treatment attenuated all changes, most particularly the increase in interstitial fibrosis. Notably, Ros treatment was unable to completely salvage glomerular development. Together these data highlight the therapeutic potential and limitations of Ros in neonatal obstruction.

172.
TIOSEMICARBAZONES DERIVED FROM AROMATIC ALDEHYDES AS POTENTIAL ANTIBACTERIAL AND ANTIOXIDANT AGENTS

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During the last years Thiosemicarbazones (TSCs) have attracted considerable interest by their biological activities. A series of TSCs obtained from aromatic aldehydes, with different electronic groups (R= 4-H, 4-NO₂, 4-Cl, 4-OCH₃), were synthesized using equimolar quantities of thiosemicarbazide, AcOH 1% in MeOH. The reaction mixture was refluxed for 5 h. The products obtained were filtered, recrystallized and their structures were confirmed by spectral data (¹H and ¹³C RMN). Aldehydes and TSCs were evaluated for their antioxidant and antibacterial properties. The radical scavenging activity was evaluated using DPPH assay. Also antibacterial properties were evaluated against *Staphylococcus aureus* ATCC 43300 (methicillin-resistant), *Staphylococcus aureus* ATCC 25923 (methicillin-sensitive), *Listeria monocytogenes* CLIP 74910, *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853. The antibacterial activity was assayed by micro-well dilution method in broth supplemented with 0.01% (w/v) of tetrazolium red as visual indicator of bacterial growth. Compounds were dissolved in DMSO and tested from 1000 to 125 µg/mL. After 24h incubation at 37°C the antibacterial activity was defined as absence of red colour. TSCs were more actives than the corresponding aldehydes. Selective antibacterial bioactivity was observed. Against *S. aureus*, only 4-chlorobenzaldehyde TSCs was active. In addition, benzaldehyde TSCs and 4-Nitrobenzaldehyde TSCs showed antibacterial properties against gram negative bacteria.

173.

***Aristolochia argentina* GASTROPROTECTION: ROLE OF SULFHYDRYL GROUPS AND PROSTAGLANDINS**Paredes J¹, Fusco M², Sosa A², Wendel G¹, Pelzer L¹.¹Farmacología, ²Farmacognosia. FQByF, UNSL, San Luis. E-mail: jdparedes@unsl.edu.ar

Gastric cytoprotective activity and mechanism of *Aristolochia argentina* (familia Aristolochiaceae) were studied. Male Wistar rats (200-250g) were employed. A scanner examined the stomachs and the scanned image was analyzed by using a program developed by National Institute of Health. The role of sulfhydryl groups and prostaglandins were evaluated. Pretreatment with *A. argentina*, at the doses 125, 250 and 500 mg/kg, produced significant decrease in the intensity of gastric mucosal damages induced by the necrotizing agents (p<0.05 vs. HCl and NaOH; p<0.001 vs. HCl/ethanol). The inhibitory effect of *A. argentina* on ethanol-induced ulcerogenesis continued even after the inhibition of endogenous sulfhydryl following pretreatment with *N*-ethylmaleimide (p<0.001; inhibition 93,5%). On the basis of this evidence it can be speculated that *A. argentina* acts independently from this mechanism. Gastric damage induced by non-steroidal anti-inflammatory drugs (e.g. indomethacin) is due to a decrease in endogenous prostaglandins synthesis and an increase in acid secretion. *A. argentina*, also inhibited indomethacin-induced ulcerogenesis significantly (p<0.05). Present findings suggest that *A. argentina* gastric cytoprotection depends, at least in part, to a possible mechanism related with the modulation of endogenous prostaglandins.

174.

CADMIUM ON ANTIOXIDANT ENZYMES IN RATS BRAIN. EFFECT OF SOY BEAN AS DIETARY PROTEIN

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Cadmium toxicity has been associated to oxidative stress. In this study we investigate whether Cd induces oxidative stress in prefrontal cortex (PFC), parietal cortex (PC) and hippocampus (H), and the possible antioxidant protective effect of soybeans, as dietary protein. Four lots of adult female Wistar rats were used: 2 lots received casein and 2 lots soybeans, as protein source in the diet. Within each group, 1 lot received tap water (control), and the other 15 ppm of Cd (as Cl₂Cd) in the drinking water, for 60 days. Cd treatment in rats fed the casein diet decreases serum HDLc (p<0.05), without change in the paroxonase (PON-1) activity and thiobarbituric acid reactive substance (TBARS) levels, as indicator of lipoperoxidation, suggesting that Cd does not produce redox imbalance in serum. In casein groups, Cd did not modify TBARS and GPx activity in the three areas, but decreased CAT activity in PC (p<0.05). Cd treatment in soybean groups did not alter TBARS and CAT activity in all brain areas, but decreased GPx activity in PC and H (p<0.05). Soybeans increased TBARS content in all areas compared with casein. Unexpectedly, soybean induced lipoperoxidation in the brain areas, independently of Cd effect.

175.

THE IMPORTANCE OF NATURAL FRAMEWORK IN CYTOTOXICITY OF DIAMIDE DERIVATIVESReta GF¹, Teruel NA¹, García González C², Martín VS², González León L², Padrón JM², Tonn CE¹, Donadel OJ¹.¹INTEQUI-CONICET, FQByF, UNSL, San Luis, Argentina. ²IUBO "Antonio González", Universidad de La Laguna, España. E-mail: gfrete@gmail.com

Several diamides have shown a large number of bioactivities, including cytotoxic, antibacterial, antifungal, and anti-inflammatory effects. In this order we work in synthesize a group of cytotoxic compounds derivatives from natural products which ensure good levels of bioactivity keeping the specificity provided by natural framework. Diamides were synthesized by Ugi reaction using acetone, aniline or benzylamine and terbutylisocyanide. As source of carboxyl group was used a natural labdane. To assess the importance of the natural product reactions were repeated using cinnamic acid. The human solid tumor cell lines were maintained in 25 cm² culture flasks in RPMI 1640 supplemented with inactivated fetal calf serum and 2 mM L-glutamine in a 37°C, 5% CO₂, 95% humidified air incubator. Chemosensitivity tests were performed using the SRB assay of the NCI. Each agent was tested in triplicates at different dilutions in the range 1–100 µM. The results showing values lower than 5 µM for labdane derivatives and less than 100 µM for the cinnamic acid ones. This study demonstrates the importance of natural product and suggests that the development of diamide derivatives could be used for therapeutic application.

176.

TOXICOLOGICAL EVALUATION OF COLD AQUEOUS EXTRACT OF *Achyrocline satureioides*

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Achyrocline satureioides Lam. (DC) is used in traditional medicine. This species has several bioactive properties such as antiviral activity. Previously, we reported absence of genotoxicity by *Allium cepa* L test. However, the genetic toxicology requires more of one study to determine genotoxic effects of a substance in human. The aim of this study was to determine the genotoxic ability of cold aqueous extract (CAE) of *A. satureioides* by micronucleus test (MN) in mouse bone marrow and comet assay (CA). Comet assay was determined on Vero cells. Five concentrations of CAE (10, 25, 50, 250 and 500 µg/ml) were employed. Negative and positive cell controls were treated with MEM and Mitomicina C (0.011 mM), respectively. For MN assay 7 groups of Balb-C mice were employed. CAE (0.2 ml of 50, 100 and 250 mg kg⁻¹) was administrated by intraperitoneal injection. Negative and positive controls were inoculated with saline and cyclophosphamide (20 mg kg⁻¹), respectively.

No genotoxic activity was detected by means of MN assay, neither by CA at 50µg/ml of CAE or lower. This absence of genotoxicity, added to the relevant antiviral activity, could justify the use of CAE of *A. satureioides* in treatments of those pathologies; the results suggest its safe use in folk medicine.

177.

ASSESSMENT METHOD OF MODERATE TABLE ASTHMATIC PATIENTS WITHOUT BACTERIAL EXACERBATION TREATED WITH TWO DIFFERENT MEDICATIONS*Sagua MD, Elías PC, Ríos L, Lafi C, Telechea A.**Área Microbiología, FCMédicas. UNCuyo, INSARES, Mendoza.**E-mail: mdsagua@yahoo.com*

It was evaluated the use of monotherapy with inhaled ciclolesonide, compared with routine treatment consisting of a long beta-agonist as salmeterol more fluticasone propionate. Aims: To measure the inflammatory process by spirometry, impulse oscillometry and sputum cellularity, discard bacterial colonization and oropharyngeal colonization with fungi, and evaluate quality of life using a standard questionnaire. We selected 46 subjects with moderate asthma and identified: Group I (22 subjects) salmeterol-fluticasone and Group II (24 subjects) and ciclolesonide. The FEV1 showed significant improvement of patients after one year, corroborated this by decreasing of the total resistance of the airway R25 for both groups. The isolates were not associated with increased asthma exacerbations process. Colonization by *Candida* fungi was 45.7% and 54.3% respectively. The eosinophils in sputum were significantly decreased in all treated patients between the beginning and end of both groups. In quest showed satisfactory control of asthma and suggests the usefulness of indirect evaluation of the inflammatory process of the airways to compare two pharmacological treatments for asthmatic subject.

178.

 α -SMA IMMUNOLocalIZATION ON LUNG DEVELOPMENT AFTER PRENATAL TREATMENT WITH CAPTOPRIL AND ENALAPRIL*Sánchez SJ, Capelari DN, Ciuffo GM, Fuentes LB.**Universidad Nacional de San Luis. IMIBIO-SL, CONICET (San Luis). E-mail: lfuen@unsl.edu.ar*

The renin-angiotensin system (RAS) during fetal or neonatal stages has been involved in lung growth and differentiation. The aim was to evaluate the effect of angiotensin converting enzyme inhibition (ACE) in lung development alveolarization and septation. Wistar rats during late gestation (G13-G21) were administered subcutaneously with captopril or enalapril (2,85 mg/kg/day), and lungs from their offsprings analyzed at different postnatal ages (P0, P8, P15, P30). Histological and morphometric analyses, as well as α -smooth muscle actin (α -SMA) immunolocalization, were performed. ACE activity decreased significantly in P0 treated-pups ($P < 0.01$). Captopril treatment exhibited histomorphological changes with enlarged distal airway spaces at P8, P15 and P30 while enalapril only affected P0 lungs. α -SMA, was increased at the tips of developing secondary septa in captopril-treated lungs at P8 and P15 and enalapril-treated was observed at P15 evidencing an active elastogenesis. ACE caused alterations in developing tissue and thus the lung keeps its ability to recover once treatment was stopped. The damage observed suggests that RAS components are at least partially involved in lung development process among other local contributing factors.

179.

EFFECT OF STIMULATION/INHIBITION OF ANG II RECEPTORS DURING PREGNANCY ON MIDBRAIN DEVELOPMENT*Sánchez SJ, Arce ME, Ciuffo GM, Fuentes LB.**IMIBIO-SL CONICET- Universidad Nacional de San Luis. E-mail:**ssanchez@unsl.edu.ar*

Angiotensin II (Ang II) is an important regulator of growth processes. Two pharmacologically subtypes of Ang II receptor, AT₁ and AT₂, are differentially blocked by Losartan or PD123319, respectively. Expression of AT₂ receptors during fetal and early postnatal life implies an important role in cell differentiation, proliferation and apoptosis. The inferior colliculus (IC) is a relay station and a major integrative center within the central auditory system at the midbrain. The aim was to evaluate the effect of stimulation/inhibition of Ang II receptors during pregnancy on midbrain development. We performed a study of Ang II receptor localization by autoradiography. Specific binding at the IC increased with age (P0, P8 and P15), reaching a maxima at P15 ($p < 0.001$) with prevalence of AT₂ receptors, in coincidence with mRNA AT₂ receptor expression at this stage. Increased binding AT₂ was observed in the IC in treated animals respect to the controls in P0 and P8, this effect was more evident in Ang II treated group. Besides, IC appeared enlarged in Ang II and Losartan-treated rat versus control-group, suggesting increased cellular proliferation. The lack of receptor stimulation/inhibition during prenatal period by pharmacological blockade leads to changes in the developing midbrain, particularly, in IC. These observations suggest that both Ang II receptor subtypes are important regulators of neuronal development.

180.

IN VIVO AND IN VITRO EFFECT OF *Jodina rhombifolia* ON THE INTESTINE*Teves MR, Wendel GH, Pelzer LE.**Farmacología. Fac. Qca, Bqca y Fcia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700.**E-mail: maurote@unsl.edu.ar*

The leaves of *Jodina rhombifolia* (Hook. & Arn.) Reissek have a variety of uses in the Argentine traditional medicine. Based on their applications at intestinal level, we experienced the inhibitory activity both *in vivo* and *in vitro* of leaf extract of *Jodina rhombifolia* (EJR) at a dose of 220 mg/kg body weight. Inhibitory effect on intestine *in vivo*: oral administration the EJR reduced small intestine transit in mice vs. control ($p < 0.001$). Yohimbine (1mg/Kg) and verapamil (5 mg/Kg) did not influence *per se* small intestinal transit, but antagonized the effect of EJR ($p < 0.001$ and $p < 0.05$ respectively). This effect was not influenced by atropine (0.25 mg/Kg). The frequency of defecation and severity of diarrhea induced by castor oil in mice was significantly reduced vs. control ($p < 0.01$) prior administration of EJR. Furthermore, pretreatment with the EJR decreased the intestinal fluid accumulation induced by castor oil in rats vs. control ($p < 0.01$). Inhibitory effect on intestine *in vitro*: EJR was added to organ bath containing a segment of rabbit jejunum. It caused an inhibition of spontaneous contractions as well as a relaxation of the smooth muscle preparation. The present results suggest that EJR produces an inhibitory action on intestinal function, and may have some antispasmodic property. The antagonist effect of yohimbine and verapamil suggest a role for the α_2 adrenergic system and Ca²⁺ channels.

181.

PHARMACOGNOSTIC STUDIES OF *Buddleja cordobensis* IN THE CENTRAL ARGENTINA

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Buddleja cordobensis Griseb. (Buddlejaceae), known as “salvialora”, “salvia de la hora”, “s. blanca” or “palo blanco”, is a dioecious shrub, densely tomentose, endemic to the mountains of Cordoba and San Luis, San Juan and La Rioja (Argentina). Infusions are used as an astringent, vulnerary and at the time of parturition (hence its common name). In the aerial parts have been isolated iridoids (Pungitore *et al.*, 2004). This study analyzes the composition of the major compounds and characterizes the powder by qualitative micrographic parameters. The aerial parts (leaves and flowers) were collected, dried at 45°C, crushed, and the aqueous extracts (infusions and decoctions) and petroleum ether, were made. On the ether extract was performed analytical march, to detected tannins, flavonoids, alkaloids, etc. The powder shows abundant stellate trichomes, and simples, glandular trichomes and epidermis with numerous cruciferous stomata. We identified the presence of tannins, flavonoids and anthraquinones. It is carrying out the fractionation of the extracts for structural elucidation of majority compounds. This study contributes to the quality control of the drugs, mainly when it is finely crushed or powdered.

182.

CIRCADIAN RHYTHM OF BIOCHEMICAL PARAMETERS AND H/L RATIO IN *Passer domesticus*

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Vertebrate use different mechanisms to optimize their physiological functions that copes environmental changes. In mammals, it has been described circadian rhythms in biochemical parameters, however literature about passerine birds is scarce. Our objective was to determine if the profiles of biochemical parameters and leukocytes display circadian rhythm variations in *Passer domesticus*. To achieve our goal, thirty six birds were acclimated to laboratory conditions, 14L:10D photoperiod and water and food *ad libitum*. Because repeated blood sampling affects hematological parameters we established six independent groups of six birds for different times of the day (01:00; 06:00; 08:00; 12:00; 16:00; 20:00 hs). Blood samples were collected from brachial vein for smears and used to determine glucose, triglycerides, uric acid and total protein concentration in plasma. We used a multivariate ANOVA with Tukey post-hoc to compare biochemical parameters and an ANOVA repeated measures for H/L index. We found circadian variation in glucose, triglycerides, uric acid and H/L ratio; but not in total proteins. Plasma parameters increase during the afternoon and decrease at night. On the other hand, H/L index, which indicates stress, shows a peak at 6hs. These results are according to a natural overnight fasting rhythm that maintains the energy balance of the bird.

183.

AN IMPROVED TECHNIQUE FOR ISOLATION OF BRUSH BORDER VESICLES (BBMV) FROM AVIAN SMALL INTESTINE

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This study presents an improved method of brush border membrane (BBM) preparation from the avian small intestine using polyethylene glycol (PEG) precipitation. This technique is useful in physiology because small intestinal brush border membranes contain hydrolytic enzymes and nutrient transporters that are important for the digestion and absorption. Samples were obtained from adult house sparrows (*Passer domesticus*) with a body mass around 27 g. The small intestine was excised and divided in three portions (proximal, medial and distal to the pyloric valve), and the mucosa was scraped with a glass slide. A 2% homogenate of the mucosa was prepared in 2 mM Tris-HCl buffer (pH 7.1). A solution of PEG 3350 was added to obtain a final concentration of 7% and stirred for 15 min at 4°C. This was centrifuged at 7,500g for 15 min, the supernatant was respun at 12,000g for 15 min and finally supernatant was respun at 40,000g for 45 min. The pellet was washed twice with buffer and finally suspended in the same buffer. In the BBMVs were measured total protein content and tested the activity of marker enzymes, sucrase, maltase (for proximal-medial portion) and N-aminopeptidase (for distal portion). Isolated BBMVs were relatively pure as judged by an 8 to 20 folds enrichment of marker enzyme activities.

184.

EFFECTS OF CASTRATION ON CITOPROTECTIVE GENES AND APOPTOTIC MECHANISMS IN RAT LUNG

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There is strong evidence that oxidative stress plays a key role in the pathophysiology of several lung diseases. The presence of specific androgens and estrogen receptors in the lung implies that sex hormones play a physiological role in pulmonary function. The present study was designed to determine whether castration and androgen replacement result in changes in the lung histoarchitecture. Wistar male rats (200± 20 g) were separated in three groups: controls (Co), castrated (Ca), and castrated replaced with testosterone (Ca+T) and sacrificed 30 days after castrations. For light microscopy, the tissues were fixed and each antibody was assayed in at least three sections of each lung. ANOVA was used for statistical analysis. The results indicate that Hsp 27 immunostaining decreased in Ca group (p<0.001), while Hsp 70i (p<0.01), PTEN (p< 0.05), PCNA and Bcl-2 (p<0.05) positive staining, were increased in the same group, compared to control. In Ca + T group, the markers evaluated were increased significantly. Our results suggest gene association between androgens and variation in several citoprotective and pro and anti-apoptotic genes. Further studies are needed to confirm these associations in lung tissue and testosterone replacement after castration produces a partial recuperation.

185. OPPOSITE EFFECT OF HYPOXIA AND OUABAIN ON EPITHELIAL RESISTIVITY OF HUMAN COLON BIOPSIES

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Hypoxia induced *in vitro* in isolated mucosa samples causes a fast decrease in short-circuit current (Isc), transepithelial potential difference (Vte) and transepithelial resistivity (Rte). This work aimed at assessing whether the addition of the Na, K-ATPase inhibitor, ouabain (1 mM) before a hypoxic challenge or after it affects the epithelial response. With informed consent, samples were obtained from patients undergoing colonoscopy. Biopsy material was rinsed and mounted in an Ussing chamber for small samples (1 mm² window), gassed with O₂ and kept at 37°C. Isc and Vte were monitored and Rte was calculated. Analysis was performed by ANOVA followed by Tukey's HSD test. In 5 samples, a 2-min hypoxia was induced by switching the bubbling from O₂ to N₂ on both sides of the chamber, and then ouabain was added as oxygenation was reassumed, followed by a new cycle of hypoxia and reoxygenation. Hypoxia caused a reduction of Isc, Vte and Rte, while addition of ouabain further decreased Isc and Vte but increased Rte (all $p < 0.0001$). A new cycle of hypoxia and reoxygenation after ouabain did not significantly change Isc, Vte or Rte. In 4 samples, ouabain was added first, decreasing Isc and Vte (both $p < 0.0001$) while increasing Rte ($p = 0.0009$). A cycle of hypoxia and reoxygenation did not result in additional changes of Isc or Vte, which remained low, or Rte, which remained high. Thus, Na, K-ATPase blockade accentuates the effect of hypoxia on Isc and Vte, while overwhelming its effect on Rte. Rte depends on two parallel pathways, paracellular and transcellular. We suggest that hypoxia decreases the paracellular resistance while ouabain increases the transcellular resistance.

186. ASYMMETRY IN THE RESPONSE TO HYPOXIA INDUCED FROM EITHER SIDE OF HUMAN COLON BIOPSIES

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Previous work has shown that hypoxia induced from the serosal side causes a larger decrease of short-circuit current (Isc) than hypoxia from the luminal side of isolated mucosa samples of rat and human colonic epithelium. This work aimed at assessing whether this asymmetry also occurs in colonic biopsies, which inevitably have submucosal tissue attached to the epithelium. With informed consent, samples were obtained from patients undergoing colonoscopy. Biopsy material was rinsed and mounted in an Ussing chamber for small samples (1 mm² window), gassed with O₂ and kept at 37°C. Isc and transepithelial potential difference (Vte) were monitored, and transepithelial resistivity (Rte) was calculated, before and after induction of a 2-min hypoxia on either the mucosal or serosal side, followed by reoxygenation (n = 5 for each procedure). Data were analyzed by ANOVA and Dunnett's test. Values are mean ± SEM. Mucosal hypoxia induced a significant decrease in Isc from 32.0 ± 3.0 to 25.0 ± 2.2 μA/cm² ($p = 0.028$), Vte from 2.9 ± 0.4 to 2.0 ± 0.2 mV ($p = 0.007$) and Rte from 91.0 ± 4.2 to 80.0 ± 4.5 Ω.cm² ($p = 0.003$) with complete recovery on reoxygenation. Serosal hypoxia caused a larger decrease of Isc, from 32.0 ± 3.0 to 19.0 ± 1.7 μA/cm² ($p = 0.021$), Vte from 2.8 ± 0.2 to 1.3 ± 0.4 mV ($p = 0.0008$) and Rte from 88.0 ± 4.3 to 67.0 ± 2.3 Ω.cm² ($p = 0.0024$), with complete recovery of Isc on reoxygenation to 29.0 ± 8.0 μA/cm², but only partial recovery of Vte to 2.1 ± 0.3 mV and Rte to 72.4 ± 4.2 Ω.cm². Since previous work showed complete recovery in isolated mucosa samples, the diffusional barrier posed by remaining submucosal tissue may hinder complete recovery from a 2-min serosal hypoxia in biopsy samples.

187. ALKALINE AND ACID PHOSPHATASE ACTIVITY IN SOIL TREATED WITH MINING LEACHING SOLUTIONS

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Mine leaching involves the use of cyanide usually in gold, silver, and copper ores, which merits great attention because of the serious environmental and public safety impact. Nowadays, two compounds capable of complex gold have been proposed to be used in the leaching process. Those compounds are thiourea and thiocyanate, which are used mostly as moisture. Phosphatases (phosphoric monoester hydrolases) are required to mineralize organic P to release it as specie available for the plants. Microbial phosphatases are found to be more efficient in hydrolysis of organic P compounds than the phosphatases of plants. Among the hydrolases, acid phosphomonoesterase activity is the most frequently used enzyme for estimating changes in soil quality. These enzymes are classified as acid phosphatase (AcP) and alkaline phosphatase (AIP) according to their optimum pH. This enzyme is an important index of the quality and quantity of organic matter in the soil. The aim was to measure the changes in the activity of phosphatases exposed to different concentration of the cyanide, thiourea and tiocianate compounds at concentration using in a real mining process. Results were evaluated using χ^2 test. The AcP and AIP activities were higher in soils affected by thiourea compared with tiocianate. In soils treated with cyanide both enzyme activities suffer a significantly decrease. In all the studies a positive control were represented by a soil with any disturbance.

188. HEART RATE DURING THE ACUTE PHASE OF CHAGAS DISEASE IN A MODEL OF RATS IMMUNIZED WITH ACTINOMYCETES AND INFECTED WITH *Trypanosoma cruzi*

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Chagas disease (ChD) leads to arrhythmias. The heart rate (HR), which depends of autonomic nervous system (ANS), is one of the arrhythmias determinants. ANS's disturbances and autoimmunity have been observed in ChD. We describe the behavior of the HR during the acute stage of experimental ChD in rats immunized in early life with Actinomycetes. We studied four (4) groups of rats: GPs (inoculated with physiological saline (Ps)); GTt (infected with strain Tulahuen trypanomastigotes and inoculated with Ps); GRc (immunized with *Rodhococcus coprophilus* (Rc)) and GRcTt (immunized with Rc and infected with Tt). Electrocardiograms were recorded at 7, 14, 21 and 28 days post infection (dpi). HR was measured. The values were analyzed with a *t* test for unpaired samples. Throughout the acute phase (7, 14, 21 and 28 dpi): a) the HR is higher GTt than GPs, with significant difference at 7 dpi ($p \leq 0.05$); b) the HR is lower in GRc than GPs with significant difference at 7 dpi ($p \leq 0.05$) and at 14 dpi ($p \leq 0.01$); c) the HR of GRcTt has no significant difference respect to GPs. The increased HR in infected animals was consistent with reported in the literature. The decrease in HR in immunized animals might respond to an increase of cytokines and nitric oxide that negatively regulate β -adrenergic receptors.

189.

Pomacea canaliculata* DIGESTIVE PHYSIOLOGY: SYMBIOTIC MICROBIAL ORGANISMS THAT INHABIT THE DUCTS OF THE DIGESTIVE GLANDDellagnola FA, Vega IA.**Laboratorio de Fisiología (IHEM-CONICET), Fac. Cs. Médicas, UNCuyo. E-mail: dellagnolaf@gmail.com*

This current work is part of an investigation program dealing with the identification of an intracellular pigmented prokaryote that lives in specific cells of the digestive gland of the freshwater snail *Pomacea canaliculata*. In this context, we studied the bacterial composition (using the gene that codifies the 16S ribosomal RNA of ~1500 bp) that inhabit in the ducts of the digestive gland (which connect this gland and the stomach) to extend our knowledge of the scenario where this putative endosymbiosis is established. One hundred and five high quality clones were obtained and they represented 26 ribotypes (classified at lower taxonomical level) covering six bacterial phyla. The metabolic profile of these ribotypes is compatible with the herbivorous nature of this snail (cellulose-like polysaccharides degradation and nitrogen fixation) and its ability to tolerate heavy metals (metals reduction) in freshwater bodies.

190.

MACROPHAGE SECRETIONS INDUCE APOPTOSIS IN RAT POLYCYSTIC OVARY*Figuroa MF, Acosta M, Mohamed F, Oliveros L, Forneris M.**Dpto. Bioquímica y Ciencias Biológicas. Univ Nac de San Luis, 5700-San Luis. E-mail: mlflorenciafiguroa@hotmail.com*

We have shown that the androgen environment of rats with polycystic ovary (PCO), induces a higher release and mRNA expression of tumor necrosis factor- α from culture macrophages (M Φ). In this work, the effect of PCO M Φ secretions on ovarian apoptosis is studied. PCO was induced in Holtzman adult rats by 2 mg/rat (single i.m. injection) of estradiol valerate and the rats were sacrificed after 2 months. Spleen M Φ , from control (C) and PCO rats, were cultured (1×10^6 cells) for 24 h in RPMI medium. Their secretions were used to stimulate C and PCO ovaries for 3 h in a metabolic bath. M Φ secretions from PCO rats increased NF κ B and BAX/Bcl2 mRNA levels (RT-PCR) ($p < 0.01$) and the apoptotic nuclei number (TUNEL) in the PCO ovaries, compared to M Φ secretion from C rats. In addition, the ovarian prostaglandin E2 (PGE2) content (RIA) and nitrites release (Griess reaction) from those PCO ovaries were decreased ($p < 0.001$). These effects were not observed when C ovaries were stimulated with the PCO M Φ secretions. The high TNF α levels of PCO M Φ secretions could be associated with the ovarian apoptosis, which can be induced by the increase of NF κ B expression, and the decrease of PGE2 content. These findings, and the knowledge that androgens, which also have pro-apoptotic effects, are increased in PCO ovaries stimulated with PCO M Φ secretions, suggest that a complex regulation of ovarian function through a paracrine mechanism may exist in PCO ovaries.

191.

DIFFERENTIAL MODULATION BY NEUROPEPTIDES IN CELIAC GANGLION ON THE PROGESTERONE FROM POLYCYSTIC OVARY IN RAT*Garraza MH, Reta Moyano A, Figueroa MF, Oliveros L, Forneris M.**Lab Biol Reprod., Facultad de Qca. Bqca. y Farmacia. Univ. Nac. de San Luis. 5700-San Luis. E-mail: mhg@unsl.edu.ar*

Polycystic ovarian syndrome is a common and complex endocrine disorder which etiology is still unknown. We have shown in the ex vivo celiac ganglion-superior ovarian nerve-ovary system (CG-SON-O), that Vasoactive Intestinal Peptide (VIP) in CG decrease progesterone (P) release from ovary of rat at estrous. Here, we study whether VIP and Substance P (SP) in CG, modulate ovarian P release in CG-SON-O of rats with polycystic ovary (PCO) and, if nitric oxide is involved in ovarian response. PCO was induced in Holtzman virgin rats by estradiol valerate (2 mg/rat, single i.m. injection) and the experiment was performed after 2 months. Rats at estrous were used as control (C). CG and ovary, connected by the SON, were incubated in Krebs-Ringer buffer (basal values) in separated cuvettes. After addition of 50 ng/ml VIP or SP in CG, samples from the ovary compartment were taken at different times, up to 180 min, to measure P (by RIA) and nitrites (Griess reaction). VIP in CG decrease P from PCO ovary, similar to the decrease observed in C ovary, at all studied times ($p < 0.001$). SP in CG decreases P and increases nitrites from C ovary, but did not modify P and nitrites release from PCO ovary ($p < 0.001$). PCO ovary did not respond to the inhibitory effect on P induced by SP in CG. Results show the important role of peripheral nervous system in determining PCO.

192.

MOLECULAR CHAPERONE PROTECTION DURING HYPOMETABOLISM INDUCED BY ESTIVATION*Giraud-Billoud M, Vega IA, Rinaldi Tosi ME, Calderón ML, Castro-Vazquez A.**Laboratorio de Fisiología (IHEM-CONICET) y Departamento de Morfología y Fisiología (FCM-UNCuyo). Argentina. E-mail: mgiraudbilloud@gmail.com*

The invasive *Pomacea canaliculata* uses estivation as an adaptive strategy to survive prolonged periods of drought. Estivation involves a decrease in metabolic rate and requires defense mechanisms to cope with the harmful effects of reoxygenation that will occur during arousal from this state. We studied by Western Blot the expression of heat shock proteins (Hsc70, Hsp70 and Hsp90) in (1) active control snails, (2) snails after 45 days of estivation, and (3) aroused snails 20 min and (4) 24 h after water exposure, in both kidney and foot. Hsc70 and Hsp70 kidney levels were stable during the activity-estivation cycle and Hsp90 expression decrease during estivation and recovers in the early arousal. In the foot, the expression of Hsp70 and Hsp90 was high during the activity and estivation periods and diminished after arousal. Both families of Hsps may have an essential role during the activity-estivation cycle, since they may act as molecular chaperones during estivation, by protecting the existing proteome, and thus ensuring a long term metabolic stability. Additionally, Hsp90 may participate in the control of metabolic changes during arousal.

193. IMPLICATIONS OF AN ENDOSYMBIONT IN DIGESTIVE PHYSIOLOGY OF *Pomacea canaliculata* (CAENO-GASTROPODA, AMPULLARIIDAE)

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Pigmented C and K corpuscles are morphs of an endosymbiont akin to the Cyanobacteria that lives and reproduces within the digestive gland cells of *P. canaliculata* and it is later expelled in the feces. In previous studies we found an ubiquitous serine-protease of 30 kDa in the intestinal lumen of this snail, which is stored/secreted by the digestive gland. The aim of this work was to test the hypothesis that these symbionts can digest the proteins ingested in the diet. Gel zymography showed a 30 kDa protease in both morphological types of the endosymbiont which corresponds to that found in the gastric contents and digestive gland extracts. Furthermore, a study of *in situ* zymography using DQ-gelatin as substrate showed the total protease activity localized in the basal zone of the alveoli of the digestive gland, as well as in its endosymbionts. This activity was inhibited in presence of serine-protease inhibitor aprotinin, and not with non-serine-protease inhibitors. Together, our results suggest that the glandular protease of 30 kDa is originated in the endosymbionts and that they help to digest enzymatically the dietary proteins.

194. NEUROMODULIN EXPRESSION VARIES THROUGHOUT THE DAY IN THE HIPPOCAMPUS AND IS MODIFIED BY A VITAMIN A-FREE DIET

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Neuromodulin (GAP), the pre-synaptic PKC substrate, is involved in synaptic plasticity. Previously, we reported that Neurogranin (RC3), the pos-synaptic substrate of PKC displays a circadian rhythm in the hippocampus, which was modified by the vitamin A deficiency. Retinoic acid, a vitamin A derivative, regulates phenomena related to cognitive function by activating nuclear receptors, RARs and RXRs. Our objectives were to investigate whether GAP-43 also displays a circadian expression pattern in the rat hippocampus and to evaluate the effect of a vitamin A-depleted diet on GAP43 temporal patterns. Holtzman rats received a diet containing 4000 IU of vitamin A/Kg diet (Control), or the same diet devoided of vitamin A (VitaminA-deficient, VAD, group), during 3 months. Rats were maintained under constant darkness conditions during 10 days before the experiment. GAP-43 and ROR α mRNA levels were determined by RT-PCR and RAR α , RXR β , BMAL1 and PER1 proteins by immunoblotting, in hippocampus samples isolated every 4 h during a 24h period. Regulatory region of GAP-43 gene was scanned for clock- and retinoic acid-responsive sites. E-box, RXRF and RORE sites were found. GAP-43 expression displays an endogenously-controlled daily variation in the rat hippocampus, which was modified in the VAD group; probably, by altering the circadian patterns of key clock and/or retinoic acid receptors proteins.

195. ANALYSIS OF CARNITINE AND ACETYLCARNITINE IN SERUM SAMPLES, UNDERSTANDING THEIR ROLE IN THYROID DISORDERS

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Carnitine is a naturally occurring compound found in all mammalian species. One of the most important biological functions of this metabolite is related to the transport of fatty acids into the mitochondrial matrix for subsequent β -oxidation, a process that results in the esterification of L-carnitine to form several and chemical diverse acylcarnitine derivatives. Carnitine metabolism is closely related to a variety of metabolic disorders, which leads to the redistribution of carnitine and acylcarnitines.

The aim of this study was to determine the feasibility of using carnitine and acetylcarnitine as biomarkers based on the variation of their concentrations levels in biological fluids. Thus a fast, sensitive, selective and novel methodology for the baseline hydrophilic chromatographic resolution in less than three minutes and the μ M levels detection by tandem mass spectrometry of carnitine and acetylcarnitine in serum samples was optimized developed and validated. This method was applied to properly conditioned serum samples that, according to several clinical parameters, were classified as control (euthyroid) and anomalous (hyper and hypothyroids). Results suggested a statistically significant variation of carnitine and acetylcarnitine concentrations between the sample groups.

196. TEMPORAL PATTERNS OF ANTIOXIDANT ENZYME EXPRESSION AND ACTIVITY ARE MODIFIED IN THE HIPPOCAMPUS OF AGED RATS

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Hippocampus plays a key role in memory and learning and is especially susceptible to oxidative stress. Particularly, aging is associated to increased oxidative stress and altered antioxidant defenses. The objective of this study was to investigate whether endogenous rhythms of catalase (CAT) and glutathione peroxidase (GPx) expression and activity as well as GSH levels are modified in the hippocampus of aged rat. Holtzman rats from control (3-months old) and aged (22-months old) groups were maintained under 12h-dark:12h-dark conditions, during 10 days before the experiment. CAT and GPx mRNA and enzymatic activity were determined by RT-PCR and kinetic assays, respectively, in hippocampi isolated every 4 h during a 24h period. Regulatory regions of CAT and GPx genes were scanned for putative clock-responsive sites. GSH levels were measured by colorimetric assay. E-box (CANNTG) sites were found on regulatory regions of GPx and CAT genes. Antioxidant enzymes expression and activity vary significantly in a 24h period under constant darkness conditions, in the rat hippocampus. We also observed circadian variation of GSH levels. Aging phase shifts CAT and GPx mRNA and activity and modified GSH levels. Modifying 24h-patterns of antioxidant defenses would impair the circadian oscillation of cellular redox state and the endogenous clock activity, in the hippocampus of aged individuals.

197.
MACROPHAGE SECRETIONS MODULATE THE HORMONE RELEASE FROM OVIDUCT OF RATS WITH POLYCYSTIC OVARY

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The physiological mechanism regulating oviductal function, remains unclear and there are few studies in the polycystic ovarian condition (PCO). We have shown that pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF α), secreted from macrophage (M Φ), alter the steroidogenesis of PCO ovary. In this study, the effect of M Φ secretions on progesterone (P), estradiol (E2) and nitrite release from the oviduct is investigated. The PCO condition was induced in adult virgin rats by estradiol valerate (2 mg/rat, single injection), and rats were sacrificed after 2 months. Spleen M Φ from control (C) and PCO rats were cultured (1×10^6 cells) for 24 h in RPMI medium. Their secretions were used to stimulate C and PCO oviducts, for 3 h in a metabolic bath, to measure the release of P and E2 (by RIA), and nitrites (by Griess reaction). Basal values were obtained from oviducts incubated with medium alone. Basal values of P and nitrites from PCO oviducts were higher than C oviducts ($p < 0.05$). M Φ secretions from C rats did not modify P, E2 and nitrites release from PCO oviducts, compared with C oviducts. However, M Φ secretions from PCO rats decreased P and increased E2 from PCO oviducts, compared with C oviducts ($p < 0.001$). High E2 in the PCO oviduct could result in a protective effect against PCO condition, since E2 favours the gametes transport to the fertilization site.

198.
ADENOSINE ANTIARRHYTHMIC EFFECT IS UNAFFECTED BY ATP-SENSITIVE K⁺ CHANNEL BLOCKADE WITH GLIBENCLAMIDE

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Short-term adenosine treatment performed immediately after ischemia reduced reperfusion arrhythmias (RA) and shortened action potentials in isolated rat hearts. We tested whether adenosine effects could be mediated by ATP-sensitive K⁺ current (I_{KATP}) activation. After 10 min of regional ischemia, the hearts were divided in 4 groups: 1) control, reperfusion with Krebs-Henseleit solution during 10 min; 2) ADO, the initial 3 min perfused with the addition of 100 μ M adenosine; 3) GLI, the initial 3 min perfused with 10 μ M glibenclamide (I_{KATP} blocker); 4) ADO+GLI. We analyzed the incidence and severity of RA and its relationship with changes on epicardial action potentials. Almost all hearts developed ventricular fibrillation during the first minute of reperfusion without differences between groups. At the end of the 3-min treatment, the number of hearts that presented ventricular fibrillation were 10/12 in control, 5/11* in ADO, 8/10 in GLI, and 4/10* in ADO+GLI (* $p < 0.05$ vs control, by Fisher exact test). During reperfusion the action potential duration was 53.7 ± 5.2 ms in control and ADO reduced it to 36.6 ± 8.4 ms ($p < 0.05$ by ANOVA I). GLI and ADO+GLI did not show changes from control values (51.3 ± 4.8 ms and 44.3 ± 5.3 ms respectively). The antiarrhythmic effect of ADO was not affected by GLI, which indicates that ATP-sensitive K⁺ channel activation is not essential for the cardioprotection.

199.
ELEMENTAL PROFILES IN SYNOVIAL FLUID

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Trace element determination in blood is a routine protocol in clinical laboratories specialized in nutritional diagnosis and toxicology of chemical elements. Trace elements are the cofactors of most enzymes in the organism. They are found very less in the body, but deficiencies of them could cause serious problems. This paper proposes a simple and rapid method for the determination of trace elements in biological samples of clinical interest treated with formic acid. To this aim, inductively coupled plasma-mass spectrometry equipped with a high-efficiency and thermostated sample introduction system was used. Human synovial fluid (HSF) was treated with formic acid and subsequently heated at 90°C for one hour. The sample was performed using a concentric nebulizer and a cyclonic baffled spray chamber under refrigerated conditions at -5°C. Quantification was carried out using external calibration with and without addition of internal standard. The accuracy of the proposed method was verified by analyzing samples enriched, as well as comparing the results with samples treated in a conventional acid digestion with microwave. The results obtained for elements under study showed levels of concentration between 0.052 mg L⁻¹ (Ti) to 2040.7 mg L⁻¹ (Li). This methodology provides advantages over conventional elemental analysis seeking to obtain a high sensitivity, using a small sample volume and simple analytical procedures able to define a profile elemental marker of biological systems and specific diseases associated with the sample under study.

200.
COMPARING UTILITY OF CYSTATIN-C WITH DIFFERENT METHODS TO ESTIMATE RENAL FUNCTION

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Serum creatinine (SCr) is the most widely used parameter to assess renal function, some time is necessary measure 24 h creatinine clearance (CrCl), or estimate by Cockcroft-Gault (CG), MDRD or NKDEP (National Kidney Disease Education Program) formulas. Using Cystatin-C (CC) as new parameter of renal function could suppose an important improvement. The aim of the study was to compare the different methods from renal evaluation and establish the utility of cystatin-C. The study included 71 patients (38%M (men), 62%W (women)). Mean age 56.3 ± 18.6 (51.2 ± 22.3 M: 59.2 ± 15.2 W), weight: 77.6 ± 21.3 kg (86.2 ± 19.3 M: 72.0 ± 20.8 W), height: 1.6 ± 0.1 m (1.74 ± 0.05 M: 1.58 ± 0.07 W), BMI (body mass index): 27.8 ± 7.9 (27.1 ± 8.0 M: 28.5 ± 7.2 W). SCr: 1.40 ± 0.20 mg/dl (1.48 ± 0.27 M: 1.1 ± 0.14 W), CrCl: 87.8 ± 4.0 ml/min (90.1 ± 7.1 M: 86.3 ± 4.8 W), CG: 82.1 ± 4.7 (93.3 ± 9.0 M: 74.7 ± 4.8 W), MDRD: 80.8 ± 3.9 (82.77 ± 7.4 M: 79.6 ± 4.3 W) and NKDEP: 67.6 ± 3.1 mL/min/ 1.73 m² (65.9 ± 6.0 M: 68.7 ± 4.0 W), CC: 1.21 ± 0.7 mg/l (1.25 ± 0.7 M: 1.18 ± 0.8 W). Percentage of glomerular filtration rate (%): 16.9 (1.5 M: 15.4 W) SCr, 23.9 (2.8 M: 21.1 W) CrCl, 23.9 (7.0 M: 16.9 W) MDRD, 28.1 (7.0 M: 21.1 W) CG, 30.9 (14.1 M: 16.9 W) NKDEP and 33.8 (15.4 M: 18.3 W) CC. In conclusion, most of the urine collections could be avoided with the use of the formulas. CC is mainly detecting slight renal alteration becoming a promising alternative assay, that could reduce considerably hidden renal insufficiency (non detected by creatinine), although further studies are needed to confirm.

201. DAILY RHYTHMS OF CAT AND GPX ENZYMES ARE ENDOGENOUSLY DRIVEN IN THE HIPPOCAMPUS AND ARE MODIFIED BY A VITAMIN A-FREE DIET

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Alterations in the enzymatic antioxidant defense system lead to altered hippocampal synaptic plasticity and to a deficit in cognitive functions. Objectives of this study were: to investigate endogenous rhythms of catalase (CAT) and glutathione peroxidase (GPx) expression and activity, as well as RXR γ and CREB1 mRNA, in the rat hippocampus and, to evaluate to which extent the nutritional vitamin A deficiency (VAD) could affect temporal patterns. Holtzman rats from control and vitamin A-deficient groups received a diet containing 4000 IU of vitamin A/kg diet, or the same diet devoided of vitamin A, respectively, during 3 months. Rats were maintained under constant darkness conditions, during 10 days before the experiment. Circadian rhythms of CAT, GPx, RXR γ and CREB1 mRNA levels were determined by RT-PCR, in hippocampus samples isolated every 4 h during a 24h period. CAT and GPx enzymatic activities were determined by kinetic assays. Regulatory regions of antioxidant enzymes genes were scanned for clock, retinoic acid- and CREB1-responsive elements. E-box, RXRE and CRE sites were found in regulatory regions of GPx and CAT genes, which display a circadian expression in the rat hippocampus. VAD phase shifted CAT, GPx and RXR γ endogenous rhythms without affecting circadian expression of CREB1.

202. MODULATION OF ANTIOXIDANT ENZYMES EXPRESSION BY ANDROGENS IN LUNG TISSUE

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Lungs are very susceptible to injury mediated by free radicals and lipid peroxidation. This injury involves various adhesive and proinflammatory molecules. Also, androgens play a physiological role in pulmonary function. We tested the effect of castration on the prooxidant-antioxidant balance and lung histoarchitecture. Wistar male rats (200±20g) were separated in three groups: controls (Co), castrated (Ca), and castrated supplemented with testosterone (Ca+T) for five days. After 30 days rats were killed. Lungs were processed for microscopy and Image J analysis system was used. Bronchoalveolar lavages were made and an increase in protein content was found (p<0.05) while the nitrite level did not change. The expression of Androgen Receptor by RT-PCR showed an increase in Ca and a significant decrease in Ca+T. Castration significantly affected the antioxidant status of rat lung, evidenced by an enhanced lipid peroxidation (TBAR'S) (p<0.05). We found a significant increase in the expression of nrf-2 in Ca, even higher in Ca+T. CAT showed a tendency to decrease in Ca, and SOD a tendency to increase in Ca+T, but these results were not significant. Significant morphometric changes were observed in Ca (p<0.05). We can conclude that androgens play an important role in the prooxidant-antioxidant balance in lung and could be involved in several respiratory diseases.

203. EFFECT OF CORTICOSTERONE ON METABOLISM BALANCE IN *Passer domesticus*

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During fasting has been observed alterations in biochemical metabolites associated not only with food restriction, but also with variations of some hormones related with energy balance (i.e. insulin, glucagon, corticosterone). The objective was to determine the effect of corticosterone (CORT) on glucose (Glu) and triglycerides (TGL), as proxies of energy balance, and on uric acid (UA), total protein (TP) and heterophyl/lymphocyte index (H/L) as proxies of the physiological status of house sparrows. Two independent groups of 6 randomly chosen birds were exposed to either a high or low doses of CORT. In addition a basal and a DMSO (CORT vehicle) control groups were established. Blood samples of independent groups were collected at 0, 30 and 90 min to assay TGL, Glu, UA and TP. A multivariate ANOVA and a Tukey post-hoc test were performed to compare treatments. The high CORT group increased Glu level at 30 and 90 min, while effect on TGL was not homogeneous, the high dose showed an increased and the low dose exhibited a decreased TGL level at time=30 min. On the other hand, CORT increased UA level and H/L index, but no effect was apparent on TP levels. These findings support that CORT can be involved in the regulation of metabolism balance.

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204. HYPOTHALAMIC EXPRESSION OF TH AND PRL SIGNALING PATHWAY DURING LACTATION IN SPRAGUE-DAWLEY AND OFAhr/hr RATS

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The OFA hr/hr (OFA) strain, derived from Sprague-Dawley (SD) rats, has deficient lactation and high hypothalamic dopaminergic tone. During lactation, sustained hyperprolactinemia is due to suckling stimuli and decreased sensitivity to prolactin (PRL) negative feedback. We studied the regulation of signaling pathways involved in the activation of dopaminergic neurons during lactation in OFA and SD rats. Using real time quantitative reverse transcriptase-polymerase chain reaction, we measured changes in mRNA expression of tyrosine hydroxylase (TH, limiting enzyme for dopamine synthesis), long PRL receptor (PRLR), STAT5b (required for the negative-feedback action of PRL on hypothalamic dopaminergic neurons), SOCS-1, SOCS-3, CIS (proteins that disrupt downstream STAT translocation to the nucleus to suppress prolactin signaling) in medial basal hypothalamus (MBH) in OFA and SD rats during continuous breastfeeding (LC), and separated rats from their pups for 12 h (S/ss). The results were correlated with serum PRL levels measured by RIA. Removal the litter increased TH expression and decreased serum PRL in SD and OFA rats. PRLR expression decreased in separated SD rats. No change in STAT5b or SOCS1 was observed in either strain. Separation decreased CIS expression in both strains but SOCS-3 expression only in OFA rats. Suckling and PRL levels differentially regulate the expression of PRLR_L in both strains. Reduced expression of SOCS-3 in OFA rats during lactation suggests that the inhibitory signaling pathway of PRL in this strain is altered and may partly explain its lactation failure.

205.

OPTIMIZATION OF ANESTHESIA METHOD WITH CO₂

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In research laboratories, anesthesia is widely used for small animals. The most used administration ways are intramuscular or intraperitoneal injection, but these ways are not convenient for large periods of drug administration in chronic treatments, such as gastric administration by gastric tube.

In the present work, we optimized a method based on the CO₂ generation from chemical reaction between acetic acid and sodium bicarbonate for anesthesia of small rodents.

Wistar male rats were used in this study. We measured a chronic stress biomarker, corticosterone, by radioimmunoassay (RIA) in order to evaluate the chronic stress levels. We analyzed the histoarchitecture of airways after treatments. The protocol was carried out in animals anesthetized for fifteen days with CO₂ generated by our anesthesia method versus non-anesthetized animals (control group).

Statistics: ANOVA/Tukey-Kramer; $p \leq 0.05$ was considered statistically significant.

The analyses of the stress levels did not show differences compared to control group. The histology analyses did not show damage in airways of animals anesthetized with CO₂.

The present optimized CO₂-based method is recommended for anesthesia of small laboratory rodents because it neither increases the stress levels nor generates damage in the airways.

206.

PROGESTERONE AND IL-17 LEVELS VARY ON A CIRCADIAN BASIS IN THE MOUSE LIVER. EFFECT OF THE TNFRP55 DEFICIENCY

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Circadian rhythms in peripheral clocks are regulated by neuroimmunendocrine signals coming, directly or indirectly, from the master clock in the suprachiasmatic nucleus. TNF is a pleiotropic cytokine which exerts its biological function upon binding to its cognate membrane receptors TNFRp55 and TNFRp75. Many key metabolizing enzymes e.g steroidogenics ones, exhibit a circadian tissue-dependent expression profile. Our objective was to analyze the circadian variation of progesterone (Pg) and IL-17 levels as well as the temporal expression of three main enzymes of Pg metabolism (3 β -HSD, 20 α -HSD and Star), in the liver of C57BL/6, wild type and *TNFRp55*^{-/-} mice. Pg and IL-17 levels were measured by RIA and ELISA, respectively. Expression of hepatic 3 β -HSD, 20 α -HSD and Star was determined by RT-PCR. Levels of Pg and IL-17 oscillate circadianly in the mouse serum and liver. TNFRp55 deficiency affected the phase and the amplitude of Pg curves; however it did not modify those parameters of IL-17 rhythms. Unexpectedly, transcript levels of 3 β -HSD, 20 α -HSD and Star did not show circadian profiles along a 24 h period. Thus, we suggest the circadian variation of Pg levels could be the result of a circadian post-transcriptional and/or translational regulation of its metabolizing enzymes.

207.

PREFRONTAL CORTEX AND LATERALIZED BEHAVIORAL DECISIONS IN THE RAT

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Lateralization is one of the physiological functions representing a crucial advance in the evolution of the brain in higher organisms. Previous work has shown that the accumbens nuclei are lateralized in the rat. The objective of the present work was to evaluate if the prefrontal cortex (PF) can also be lateralized regarding the control of preferential decisions in two-choice environments, such as those previously tested in the rat. Adult male rats (90 day-old) were implanted bilaterally with guide cannulae into the PF for *in situ* microinjections. After 48 h, groups of animals were injected with saline (Control, n=10); lidocaine (Lid, 2 μ g/ μ l) into the left PF (n=14), right PF (n=26) or both PFs (n= 16). Five min afterwards, all groups of animals were tested in the Double Lateral Holeboard (DLHB) during 5 min as previously described. Results show that Lid administration into left, right or both PF cortices blocked in the same way the left-biased exploration of the animals treated. Preference in control rats (84.2 % animals exploring more the left wall than the contralateral in the LDHB, $p < 0.01$) was lost in the Lid-treated groups (61.9%, 55% and 50% for Lid left, Lid right, and Lid both, n.s.). Results suggested that PF does not appear to be lateralized but is clearly involved to modulate the biased exploration in the rat.

208.

NRF2 AND HSP70 SYNERGIC EFFECT IN OXIDATIVE STRESS MODULATION DURING HK-2 CELL MECHANICAL DEFORMATION

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Cellular mechanical deformation is characteristic of hydronephrosis during obstructive nephropathy. Cellular stretching induces NADPH oxidase activity (NOX-ac) and oxidative stress. The target was to evaluate mechanical deformation in human kidney cells (HK-2) at different periods of time and to assess cytoprotective effects of both Hsp70 and Nrf2 pathways. Oxidative stress was demonstrated by total antioxidant status (TAS) and NOX-ac assays, Hsp70 expression by Western-blot and GSTA2 induction by Nrf2, through RT-PCR. The increased NOX-ac and the fall off TAS contributed to oxidative stress after 1 and or 2 hours of mechanical deformation, ($p < 0.01$ - $p < 0.001$) and ($p < 0.05$ - $p < 0.01$) respectively. At the same time, Hsp70 had a three fold increase ($p < 0.001$), the chaperone overexpression providing cellular protection against cellular injury. However, Nrf2 and cytoprotective gene expression of GSTA2 did not significantly increase. After 4 and 6 hours, TAS recovered initial levels, a progressive decrease of NOX-ac and Hsp70 were shown, while increased expression of Nrf2 together with upregulation of GSTA2 ($p < 0.001$) were demonstrated. These results suggest that the early increase of Hsp70 and subsequent Nrf2 upregulation play a critical role in the protection against oxidative stress during cellular stretch.

209.**EFFECT OF KETAMINE ON EXOCYTIC AND ENDOCYTIC MACHINERY IN RAT NUCLEUS ACCUMBENS**

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Clathrin-mediated endocytosis (CME) is important in the central nervous system for maintenance of membranous homeostasis at nerve terminals. Ketamine (K) is a NMDA receptor blocker. We wondered if the drug affects the expression and cycle of coat proteins involved in CME in the rat nucleus accumbens (NA) and if it also induces alterations in exocytic machinery. Adult rats were treated with subanesthetic doses of K for 15 min, 3 h or 24 h. The NA were dissected, homogenized and centrifuged to obtain membranous and soluble fractions. The coat protein AP-2 was analyzed in both fractions by Western blot. We observed increased expression of AP-2 protein compared to non treated controls. In order to determine whether K affects exocytosis, the NA were subject to the superfusion method using [³H] dopamine. The release of [³H] dopamine was measured and referred to basal values of controls. It was observed that release of [³H] dopamine decreased from 15 min of K treatment. After 24 h, this effect was mitigated. These results suggest an acute effect of K on the endocytic/exocytic pathway in the nucleus accumbens.

210.**GINGIVAL MORPHOLOGICAL ALTERATION RELATED TO LEVELS OF SEX HORMONES IN SALIVA OF FIRST QUARTER PREGNANT TEENAGERS**

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The gingival changes during pregnancy are associated with deficiency or absence of oral hygiene and hormonal and vascular changes that cause an exaggerated inflammatory response. Our objective was to determine the morphological gingival alteration in first quarter pregnant teenagers compared to hormone levels in saliva. We studied a total of 30 pregnant women aged 14 to 19 years who were in the first quarter of pregnancy and who were instructed in hygiene, collected saliva and mouthwash prior to study by radioimmunoassay. Estradiol levels found ranged from 8 to 17 pg/ml and progesterone from 15 to 48 ng/ml. Clinically marked gingival hypertrophy was observed in the interdental papillae and marginal gingiva of maxillary molars, incisors and upper and lower canines. Bleeding tendency to scanning probe and discoloration were observed. For the examined sample, it can be concluded that the gingival morphology is modified in coincidence with increased salivary levels of estrogen and progesterone.

211.**MEASUREMENT OF SEX HORMONES IN SALIVA OF THIRD QUARTER PREGNANT TEENAGERS**

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During pregnancy there is an increase in sex hormone levels. The increase in gestational hormones influences the occurrence of gingivitis. Gingivitis is greater the younger the pregnant. In order to establish whether there is a correlation between the concentration of estrogens and progesterone in saliva and the severity of gingivitis in pregnant women, a study was conducted with a sample of 60 adolescent (14-19 year-old) pregnant women enrolled in the third quarter. Hormone levels were determined by radioimmunoassay in saliva. Samples were collected in polystyrene tubes. Unstimulated saliva was used. We used the Löe-Sillnes plaque index to record the amount of plaque present on the entry of the gingival sulcus and clinical assessment using a conventional periodontal probe. Estradiol levels were found in saliva to range from 110 to 270 pg/ml, and progesterone from 55 to 215 ng/ml. Thirty percent (18) of the adolescents had mild plaque and 70% (42) moderate plaque. For this sample, in the absence of irritants, gingival manifestations coincide with the increase in the levels of sex hormones in the salivary fluid.

212.**EFFECTS OF VITAMIN A DEFICIENCY ON APOPTOSIS AND CELL PROLIFERATION IN RAT MAMMARY GLAND**

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The mammary gland development is a complex program of cell proliferation and differentiation. We study if vitamin A deficiency alters the expression of apoptotic and proliferative proteins in mammary gland. Female Wistar rats at weaning were divided into six groups. One group was fed with a vitamin A-sufficient diet (A), another with a vitamin A-deficient diet (-A) for 3 and 6 months, and the third group with -A diet for 75 and 150 days, respectively, and after which they received A diet for 15 and 30 days, respectively. BAX, BCL-2 and PCNA proteins were detected by immunohistochemistry and apoptotic cells by TUNEL assay, and BAX and BCL2 mRNA by RT-PCR. In (-A) group, the number of apoptotic nuclei in glandular cells increased in relation to control groups (3 and 6 months). In (-A) group, the immunostaining of BAX, PCNA and BCL-2, and the expression of BAX and BCL-2 mRNA were increased in relation to the control group. Vitamin A deficiency induces apoptosis in mammary gland after 3 and 6 months. The proliferation marker (PCNA) increases in (-A) group and was reverted with the vitamin A-sufficient diet. Vitamin A deficiency provokes changes in apoptotic and cell proliferation processes of rat mammary gland.

A		Berdasco CV	183, 203	Cataldi A	L2
Abaca CR	109	Bernardelli C	60	Cattaneo N	73, 74
Abal A	1	Bernardis A	72	Cavicchia JC	125
Abud M	15	Bernasconi MV	188	Caviedes-Vidal E	17, 30, 182, 183, 203
Abud MA	145	Bertoluzzo SM	188	Cayado Gutierrez N	15
Acíar R	91, 103	Bettera S	51	Cayado N	25
Acosta A	176	Biaggio VS	159, 184	Cecati F	165
Acosta G	80	Bianco MV	L2	Cecho A	210, 211
Acosta M	190, 212	Bigi F	L2	Cejas J	15, 28
Actis E	126	Bilbao MP	152	Cejudo C	145
Adaro M	96	Biscaro AT	65	Centorbi HJ	40, 46
Agüero MB	54, 55, 90	Blanco F	L2	Ceñal JP	151
Aguilar C	99	Blarasin M	51	Cerda RA	71
Aguilar EG	89, 146, 147	Boarelli P	11, 18, 29	Cerón M	53
Aguilera C	12	Bocanegra V	19, 208	Cerrutti FA	20, 155
Aguilera Merlo C	2	Bogino S	132	Cerutti S	195
Albarracín GJ	148	Bologna S	73, 74	Cevallos C	168
Alcaráz L	62, 169	Bomben R	166	Chaves EM	2
Aliendro OE	40, 46	Bonatti C	115	Chediack JG	17, 30, 182, 183, 203
Allegretti PE	210, 211	Bonetto CC	123	Chiaravello A	48
Allocati JP	88, 97, 98, 137	Borcossqui A	94	Chiesa J	143
Altamirano J	134	Borcossqui AA	92	Chiofalo S	76, 138
Altamirano L	149, 160, 171	Bottini R	77, 104	Cid FD	182, 203
Alvarez EO	130, 207	Bravo MA	44	Cifuentes D	43
Alvarez G	L3	Breccia G	L4	Cifuentes DA	158, 172
Alvarez Rogel J	71	Brigada AM	127, 128	Cimenton NA	188
Álvarez SM	150, 153, 202	Bronzi D	5, 6	Ciminari ME	202
Álvarez-Olmedo DG	184	Brovarone R	110	Ciocco N	134
Ampuero A	69	Brun A	17	Ciuffo GM	33, 178, 179
Ampuero V	52	Brun J	75	Cohen AC	77, 104
Andrada NR	141	Buddle B	L2	Cora Jofré F	78
Anello A	70			Coralli M	83
Antón RI	127, 128	C		Corral A	132
Anzulovich AC	7, 159, 194, 196, 201, 206	Caballero D	56, 57, 59	Correa L	113
Aragón L	101	Caballero PA	45	Cravero S	53
Aragón LM	41, 59	Cabrera R	209	Crinó E	144
Arana MD	142	Cacciamani VE	19	Cruceño A	2
Aranibar JN	139	Calderón ML	192	Cruzado M	15
Arce L	151	Calderoni AM	150, 153	Cuello Carrión D	27
Arce ME	179	Calvente V	67	Cuello-Carrión FD	126
Arce S	80, 167	Calvo J	63	Cueto JA	38
Ardanaz C	165	Camí G	85	Curvale DA	127, 128
Arenas GN	53, 58, 69	Camiña JM	84, 146, 147, 148		
Arias JL	7, 206	Campo Verde Arboccó F	14, 205	D	
Arias RJ	182, 183	Canal A	L2	Daguerre A	79, 144
Arismendi Sosa AC	42, 66	Canale F	35, 37	Daneri C	5, 6
Arizmendi Sosa AC	162	Canale FP	34	Dave MN	36, 39, 114
Arnaez J	73, 74	Cangiano MA	154	Davicino RC	39
		Cannizzo B	15, 28	de Jong LIT	45
B		Cantarelli M	166	De la Vega M	7, 206
Bacha EF	92, 93	Cantarelli MA	147, 148	De Pauw C	167
Bacha F	94, 95	Cantú L	58	De Vicente L	119
Bannoud N	12, 13	Capelari DN	178	Debattista NB	68
Banzan AM	130, 207	Capodici N	111	Dejarbo S	58, 69
Barberis S	96	Caputo R	83	Del Vitto L	85, 164
Barbos a O A	71	Cargnelutti E	36	Del Vitto LA	57, 81, 161
Baroni V	83, 90, 136	Cariddi L	176	Delgado SM	8, 194, 196, 201, 206
Bartolomé C	69	Carosio C	76, 138	Dellagnola FA	189
Basconsuelo S	32	Carra GE	185, 186	Della-Vedova MC	156
Basconsuelo SC	21	Carreño NB	204	Delsouc MB	8
Bastias C	43	Carrera SE	187	Denita Juárez SP	42, 66
Bastida J	64	Carvelli L	12, 13, 209	Di Genaro MS	36, 39
Batista S	1	Casais M	5, 6, 7, 9, 206	Díaz MS	36, 39
Baudo J	24	Casas S	209	Diez E	149
Baudo JE	210, 211	Castagnolo B	65	Diez ER	198
Bazán P	132	Castell S	34, 35, 37	DiGenaro MS	206
Bellozas M	75	Castro C	15, 28	DiGenaro S	7
Bellozas Reinhard M	78	Castro NM	65	Dolab J	157
Benardon ME	19	Castro S	112	Domínguez G	210, 211
Benuzzi D	63, 67	Castro-Vazquez A	38, 192	Domínguez S	2

Dona R	111, 118	Garbaccio F	L2	Jakovcevic D	18, 29
Donadel OJ	175	Garbulsky M	93	Jofré Barud F	101
Donati E	60, 61	García A	111, 118	Jofré MA	167
Dorati P	23, 24	García Aseff S	163	Jofré MB	140
Durso G	1	García EV	92	Juez RA	58
E		García González C	175	Junqueras MJ	76, 138
Echenique DR	47, 48	García IM	149, 160, 171	K	
Echevarria AL	L5	García J	163	Karasov WH	17, 30
Eguaras MJ	129	García M	145	Kiriachek SG	84
Eliás PC	177	García P	75	Koch E	134
Eliçabe RJ	36	Garraza MH	191	Kopeèny J	53
Enríz RD	154	Garraza R	100	Kraus TA	21
Escobar E	158	Garro M	162		
Escobar F	176	Garro MF	161	L	
Escudero C	209	Gasquez J	106	Laciar A	43, 48, 62, 158, 172
Escudero NL	79, 82, 86, 89, 146, 147, 148	Gatica-Sosa CP	17, 30	Laciar AL	46, 47
Escudero S	74	Gavidia M	121, 122	Lacoste MG	196
Esquenoni SM	109	Gette M	85, 181	Lafi C	177
F		Gette MA	81	Lana N	134
Fabani MP	90	Ghersa F	9	Lapierre AV	195
Farias Altamirano LE	16	Gil AR	80	Larregle E	168
Fasulo V	111, 118	Gil J	109	Larriestra A	112, 121, 122
Felici CE	80	Gil L	163	Larriestra AJ	123
Felitti S	L4	Gil Lorenzo AF	19, 209	Larrusse AS	141
Ferder L	149	Gil M	L4	Lentini O	116
Feresin G	56	Gil RA	199	Licata L	1
Feresin GE	41, 54, 55, 64, 83, 90,	Giménez C	164, 166	Lijteroff R	135
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Fernández Belmonte MC	76, 138	Giordano M	110	Liza López M	101
Fernandez CA	129	Giraud-Billoud M	192	Lizabe M	65
Fernández G	67, 113	Godoy D	3, 4	Llompert G	1
Fernández Marinone G	17, 30	Godoy ME	131	Llompert J	1
Fernandez MR	168	Godoy MS	193	Lombardo D	51
Fernández RA	45	Golini RS	194	López GC	45
Fernández S	119	Gomez M	23, 24, 132	López LA	10
Ferramola ML	159	Gomez MR	167	Lopez Millán A	116
Ferrari C	212	Gomez N	156	Lopez MJ	117
Ferrari SG	49	Gómez NN	159, 184, 202	López ML	41
Ferraris P	99	Gómez R	80	López MT	28
Figueroa F	197	Gomez Zabala V	20	López S	101
Figueroa MF	190, 191	Gomez-Mejiba SE	156	López SB	41, 56, 57, 59
Filippa V	3, 4, 212	Gonzalez CJP	115	López Suárez F	58
Flamini MI	31	Gonzalez I	22, 113, 120	Losinno A	10
Floridia RA	50, 52, 114	González LE	50, 52	Lucero E	93, 95
Fontanella GH	188	González León L	175	Lucero Estrada CS	42
Forlin G	106	Grassano A	75	Lucero López RV	79
Forneris M	190, 191, 197	Gravisaco MJ	L2	Lucero López VR	82, 86
Forneris ML	110, 117	Grilli D	53	Lucero Postma D	197
Fornés M	11, 18, 27, 29	Grosso M	32	Lucero R	22
Fornés MW	25, 145	Grosso MA	21	Lucero RO	120
Foscolo M	125	Guerreiro A	165	Lucero V	73, 74
Fourcade G	110	Gutiérrez M	87	Luna L	83, 90
Frigerio C	51	Guzmán L	133	Luna LC	136
Fuentes L	119, 124, 200, 208	H		Lüthy IA	20
Fuentes LB	178, 179	Haddad M	56	M	
Funes A	18, 29	Hapon MB	14	Magallanes J	96
Furlan Z	132	Harrison R	95	Malossi E	12
Furlong O	72, 164, 170	Hidalgo M	164, 166	Malpassi RN	21
Fusco M	167, 173, 181	I		Manazza F	97, 98
Fusco MR	157, 169	Ibáñez JE	185, 186	Manazza JF	137
G		Ibáñez M	32	Manrique M	76, 138
Gaglio E	130	Ibarra C	L1	Manucha W	149, 160, 171, 208
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