



TUCUMAN BIOLOGY ASSOCIATION

(Asociación de Biología de Tucumán)

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of the Tucumán Biology Association*

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LECTURES

“Miguel Lillo” Lecture

A1

PHYSIOLOGICAL AND PATHOLOGICAL MECHANISMS INVOLVED IN ANGIOGENESIS AND OVARIAN FUNCTION

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Angiogenesis is the process of blood vessel formation in which new vessels sprout and mature from a pre-existing vasculature. Vessel formation is critical during tissue development in embryogenesis (vasculogenesis) and in several pathological conditions. However, in healthy tissues, physiological angiogenesis is mainly limited to the reproductive system. The ovarian vasculature particularly associated with the dominant structures (preovulatory follicle and corpus luteum) during the ovarian cycle is one of the few sites where nonpathological development and regression of blood vessels occurs in the adult. Recently, local factors such as VEGFA and angiopoietins, which act specifically on vascular endothelial or support cells (pericytes or smooth muscle) to control angiogenesis, were identified in the ovary of several species, including nonhuman primates and women. Recent reports indicate that VEGFA plays a pivotal role in the ovary, in particular regulating follicular and CL development. Studies performed in our laboratory established that inhibition of VEGFA and angiopoietin 1 (ANGPT1) action in rat ovaries by intrabursal administration of VEGFA-Trap or ANGPT1 antibody respectively, produces an imbalance in the ratio of antiapoptotic/proapoptotic proteins leading to atresia of a larger number of follicles. Besides, we have demonstrated that VEGFA prevents apoptosis and stimulates proliferation of granulosa and theca cells of antral follicles through a direct interaction of VEGFA with its KDR receptor localized in granulosa cells and also that the PI3K/AKT pathway is involved in this mechanism. Collectively, the data support the notion that these angiogenic factors have an important role in controlling follicular growth, ovulation and CL development. In recent years members of the Notch family were recently identified as novel factors involved in angiogenesis regulation. We demonstrated that Notch signalling has a critical role in the regulation of the survival of the corpus luteum and we described a crosstalk between Notch members and Progesterone in luteal cell function. In addition, we have shown that the Notch system acts as a survival pathway in a human cancer cell (KGN), and might be interacting with the PI3K/AKT pathway. It was described that abnormal production of angiogenic factors associated with ovarian dysfunction may have a causal role in polycystic ovarian syndrome (PCOS) and ovarian hyperstimulation syndrome (OHSS). Studies conducted in our laboratory have demonstrated alterations in angiogenic factors and its receptors in these pathologies. The comprehension of the precise ovarian role of angiogenic factors would be useful for the development of new therapeutic strategies based on anti-angiogenic agents.

Other lectures

A2

ALTERATIONS IN THE ANTIOXIDANT DEFENSE SYSTEM IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Diabetes Mellitus (DM) is a chronic metabolic disorder caused by deficient insulin secretion or action characterized and diagnosed by the presence of persistent hyperglycemia, whose main symptoms are polyuria, polydipsia and polyphagia. DM is one of the most prevalent diseases with social impact, not only because of its prevalence but also due to the impact of chronic complications. The rate of Type 2 Diabetes Mellitus (T2DM) varies around the world, in Argentina is estimated at about 7% of the adult population. In T2DM the antioxidant capacity is impaired, but the exact mechanism by which gene expression and activity of antioxidant enzymes are altered is unknown. Under normal physiological conditions, there is an

oxidant / antioxidant balance. The rupture of the oxidant / antioxidant balance generates a metabolic condition called Oxidative Stress. Hyperglycemia causes overproduction of free oxygen radicals leading to breakage of the redox balance, which brings an increase in protein oxidation and lipid peroxidation. While the causes leading to the onset of T2DM have not been fully elucidated, it is firmly established that the reactive oxygen species (ROS), derived from multiple sources, play a key role in insulin resistance by contributing to the dysfunction of the pancreatic β cells and promoting the development and progression of T2DM. Our research contributed, to some extent, to elucidate possible molecular mechanisms involved in the decrease of antioxidant defense in these patients. Consequently, we demonstrated that the activity of the antioxidant enzyme PON-1 is diminished and is positively related to plasma levels of HDL-c. Moreover, it was shown that the expression levels of the transcription factor Nrf2, essential for regulation of antioxidant enzymes, is decreased in diabetic patients, resulting in a lack of cell redox state by decreasing the expression of Nrf2 target genes.

A3

TAXONOMY OF THE GENUS *Rebutia* (CACTACEAE)

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For the purpose of delimiting and circumscribing the genus *Rebutia* and its species, and to contribute to the knowledge of the Cactaceae-Cactoideae, *Rebutia* was treated with a narrow criterion. This study was carried out on the basis of morphological, anatomical and palynological characters, reproductive system, environment, and geographical distribution pattern. Circumscriptions were complemented by existing nomenclatural types, original descriptions and illustrations. The taxonomic treatment of *Rebutia* recognizes 11 taxa for the genus, and *Aylosteria* is treated as a subgenus of it. Of the 11 taxa, 6 are placed under the subgenus *Aylosteria* and most are living in Bolivia. The remainder are endemic to Argentina and correspond to the subgenus *Rebutia* (except *Rebutia padcayensis* that occurs in both countries). Nine new synonyms are proposed, 4 lectotypes and 13 neotypes are established; 14 nomenclatural synonyms and 73 taxonomic synonyms are ratified, and 1 new species is recorded for Argentina. Entities of the subgenus *Aylosteria* possess areoles in apical position over the mamelon, no adaxial furrows, style attached to the floral tube for a length greater than 10 mm, open-type nectar chamber and pollen grains 6-8 pantocolpate; those of the subgenus *Rebutia* have subapical areoles, adaxial furrows are present, style fused with the floral tube for a length less than 10 mm, diffuse-type nectar chamber, tricolpate pollen grains. Analyzed anatomical characters are fairly uniform and do not allow separation into subgroups. Descriptions of species are given, as well as keys for taxa identification, illustrations, geographical distribution is mentioned as well as the risk status of each species. The taxonomic study presented herein is the basis for future phylogenetic, ecological and biogeographic work, which depends on the botanical knowledge of the taxa here treated.

SYMPOSIA

“BIOLOGICAL INTERACTIONS”

A4

PLANTS - FRUIT FLIES - PARASITOIDS: INTERACTIONS UNDER DIFFERENT LANDSCAPE CONDITIONS OF THE YUNGAS FROM TUCUMÁN

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Agricultural development and human activities have caused deforestation of native vegetation, producing landscape fragmentation, habitat loss, and invasion of non-native species. This involved a major environmental disturbance, resulting in a simplification of ecosystems. It is known that habitat structure plays an important role in the dynamics of pest species. A good example of this is the fruit fly (Diptera: Tephritidae) and its natural enemies (parasitoids). The distribution and abundance of host plants, the surrounding vegetation and the distribution of essential resources (food, habitat, oviposition substrates) strongly influence the behavior, distribution and abundance of these insects.

In Argentina *Ceratitis capitata* and *Anastrepha fraterculus* are two highly polyphagous tephritid species that cause significant annual damage to fruit production. In contrast, there are native species such as *Anastrepha schultzi*, which is oligophagous and of no economic importance, because its larvae develops in fruit of no commercial importance to the region. Studies on the interaction between fruit flies and their habitat are scarce, and the majority have been performed in agroecosystems while information on natural and urban environments is rare.

The aim of this study was to compare the abundance and dynamics of pest and non-pest tephritids and their natural enemies (parasitoids) on a mosaic of habitats differing in structure, diversity and history of disturbance on the western slopes of the Sierra de San Javier, Tucumán.

As a result we found that the degradation of the environment, and the introduction and spread of exotic host plant strongly affected distribution patterns, abundance and phenology of native and exotic tephritidae.

A5

AZOSPIRILLUM-STRAWBERRY: MODEL OF BENEFICIAL INTERACTION BETWEEN PLANT AND PLANT GROWTH-PROMOTING BACTERIA

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The plant growth-promoting bacteria (PGPB) are a group of different bacterial genera, including *Azospirillum*, able to increase plant growth and productivity. The PGPB fall into two groups: (i) those that promote plant growth through different mechanisms (e.g., nitrogen fixation, phosphate solubilization, production of hormones and siderophores, ACC-deaminase activity, etc.) that directly affect the plant's metabolism, enhancing root development (greater absorption of water and minerals), modifying the enzymatic activities of plants, or "helping" other beneficial microorganisms to act better on them; and (ii) PGPB capable of biological control, which promote plant growth by inhibiting or suppressing phytopathogens. These bacteria are promising in biotechnological approaches seeking to secure an adequate supply of nutrients to plants, and to reduce the negative environmental effects of fertilizers.

In this presentation our experience on the use of *Azospirillum* in strawberry (*Fragaria ananassa*) crop in Tucumán, Argentina, is shown as a model of beneficial interaction between bacteria and plants. Stages of isolation, microbiological and molecular characterization of strains, determination of their plant growth-promoting skills, chemotaxis toward root exudates,

plant colonization, structural and biochemical effects of the interaction, protection against anthracnose disease and evaluation of the interaction in controlled and field environments will be considered.

A6

MICROBIAL CONSORTIA WITH ARBUSCULAR MYCORRHIZAL FUNGI INDIGENOUS OF BUENOS AIRES PROVINCE: POTENTIAL PLANT GROWTH PROMOTERS OF AGRICULTURAL CROPS?

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Fertilization is a common practice for agricultural production in the Buenos Aires Province (Argentina). However, fertilizers applied in excess can depress diversity and abundance of soil microbial populations which could potentially be plant growth promoting. In the search for alternatives that would encourage agricultural production in a sustainable way, we are exploring how soil characteristics, result of agricultural management, affect arbuscular mycorrhizal fungi (AMF) indigenous of the region, which are known to increase nutrients uptake and growth of colonized root plants. Inoculation with microbial consortia (with AMF) indigenous of agricultural fields of Lobería that showed high mycorrhizal root colonization capacity, showed positive early (two weeks) growth responses in corn and tomato plants. Inoculation with microbial consortia (with AMF) indigenous from agricultural fields of Balcarce increased growth and zinc uptake of corn plants at V6. Combined inoculation of phosphorus (P) solubilizing from the agricultural fields under no tillage of Balcarce with native microbial consortia (with AMF) from pristine sites of Tandil also increased growth and P uptake of wheat plants in preanthesis. However, the highest plant growth increases were obtained after inoculation with the P solubilizing alone. Results suggest that more than the management; what allows to select microbial consortia with AMF potentially plant growth promoters are the geographical area, the soil characteristics and the mycotrophic ability. Single strand conformation polymorphism studies revealed high genetic diversity of the AMF belonging to the genus *Glomus* (= *Funneliformis*; Glomeromycota) in the analyzed consortia. The next step is to assess the potentiality and establishment of the consortia promoters with indigenous microbial populations.

“BIOLOGY AND BIOTECHNOLOGY OF ANIMAL REPRODUCTION”

A7

MECHANISMS OF SPERM ALTERATIONS IN HYPERCHOLESTEROLEMIA AND ITS REVERSAL BY VIRGIN OLIVE OIL (varietal arauco)

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A fat diet (FD) promotes hypercholesterolemia in adult male rabbits and consequently semen and sperm abnormalities (Saez et al., 2010). The decrease in sperm count, reduction in motile cells and morphological alterations were described as well as the reversion by the addition of Virgin Olive Oil (VOO) to diet (DO) (Saez et al., 2013). The teratozoospermia should be mostly attributable to wrong sperm assembling during spermatogenesis while the decreasing of sperm number would be related to an increase in apoptosis and a drop in seminiferous tubule efficiency. The first part of the hypothesis has been tested through studies of ultrastructure of the germinal series as well as the organization of microtubules, the Golgi apparatus and the nucleus with specific fluorescent markers and classical / confocal fluorescence microscopy observation. The other idea is tried by analyzing the cell types and distribution / frequency in the proliferative and differentiation compartments. Comparing both diets, FD and DO, it was demonstrated that under hypercholesterolemic environment the distribution of microtubules, sperm head shape, number of apoptotic nucleus were increased while adding VOO reverses these changes. In

greater detail, the insertion of the microtubules involved in acrosome development is abnormal and abnormal spermatocytes enter apoptosis. As a consequence, the total number of cells that complete spermatogenesis decreases dramatically. Analysis of more cases and using complementary and/or different methods could give us more data to support the hypothesis.

A8

ANOTHER VIEW OF REACTIVE OXYGEN SPECIES IN *IN VITRO* EMBRYO PRODUCTION

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In vitro cell culture conditions, like high oxygen tension and the loss of the physiological environment that controls redox state, contribute to produce oxidative stress. This situation is characterized by the increase in the production of reactive oxygen species, such as superoxide anion and hydrogen peroxide, which produce oxidative damage to biomolecules with the subsequent detrimental effect on cell function. It has been observed that under pro-oxidant culture conditions *in vitro* embryo production is affected due to the decrease in sperm motility and the fusogenic capacity of spermatozoon, oocyte maturation and early embryo development rates. Our studies in the bovine model have demonstrated that the addition of natural antioxidants, such as alpha-tocopherol (vitamin E) and ascorbic acid (vitamin C) prevent oxidative stress in gametes. Nevertheless, the fertilizing capacity of gametes decreases in the presence of vitamin E and/or vitamin C. It has been proposed that certain levels of reactive oxygen species would be necessary for the normal fertilizing process in the bovine. Reactive oxygen species would participate in events related to sperm capacitation and acrosome reaction, cytoplasmic oocyte maturation and early embryo formation in this species. Recently, it has been proposed that reactive oxygen species could perform an active role in cellular signaling mechanisms and that their high reactive capacity could be beneficial for biological systems at physiological levels.

A9

HOW HAS THE INCORPORATION OF REPRODUCTIVE BIOTECHNOLOGY IN DAIRY COWS HELPED US?

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Reproduction biotechnology includes all techniques from artificial insemination to cloning, or the combination of them that would enable to increase reproductive efficiency of animals, and in this case, of dairy cows. Production systems that use dairy cows which have been selected for high production of milk have a reduced fertility. This has happened in countries and regions that operate with different production systems, continuous calving herds which are mainly in the United Kingdom and the United States and in seasonally calving herds found mainly in Ireland, New Zealand and Australia. In USA a decrease has been reported in conception rates in dairy cows during the last 40 years. In fact, in 1950 the rate was 65% and in 2000 was less than 40%. Consequently, one of the biggest challenges for researchers of reproduction, nutrition and genetics is to obtain an understanding of the underlying factors that contribute to dairy cow low fertility and therefore develop strategies to improve it. Fertility is a multifactorial trait and its deterioration has been caused by a network of factors: genetic, environmental and management-related, while their complex interactions make it difficult to determine the exact reason for this decline.

ORAL COMMUNICATIONS

A10

EFFECT OF UROKINASE TYPE PLASMINOGEN ACTIVATOR IN CELL PROLIFERATION OF BOVINE OVIDUCT

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Plasminogen is a molecule that becomes an active enzyme, plasmin, important in extracellular remodeling processes. The urokinase type plasminogen activator (uPA) cleaves plasminogen generating plasmin and binds to its receptor, uPAR, which is anchored to the plasmatic membrane. This interaction localizes proteolytic activity in the vicinity of cells and activates intracellular signaling pathways. Previously we studied the localization and expression of uPAR in bovine oviduct. Bovine oviductal epithelial cell cultures induced with uPA present high levels of phosphorylated proteins of MAPK pathway and increase expression of transcriptional factors. The purpose of this work was to analyze if uPA addition to bovine oviductal epithelial cell cultures affects cell proliferation, by cyclin D1 expression. This protein is required for cell-cycle progression during G1. We obtained epithelial cells of diestrous bovine oviducts. Cells were cultured with 10nM of uPA. Some cultures were treated with 0.4µg/ml of anti-uPAR antibody prior to uPA addition. Total RNA was extracted, cDNA was synthesized and the expression was analyzed by Real Time RT-PCR. The relative proportions of mRNA levels were normalized to glyceraldehyde-3-phosphate-dehydrogenase. Cyclin D1 is expressed in high levels in cell cultures with uPA. Cultures treated with antibody and subsequent addition of uPA showed lower level of cyclin D1 expression than samples induced only with uPA. These results could help conclude that uPA regulates cell proliferation through its receptor in the bovine oviduct by intracellular signal pathways activation and stimulation of cyclin D1 expression. The uPA could be involved in changes in the oviduct favoring the microenvironment required for fertilization and early embryonic development.

A11

OMEGA-3 FATTY ACIDS AND ANHEDONIA IN A HORMONALLY INDUCED POSTPARTUM DEPRESSION MODEL

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Introduction: Postpartum depression (PPD) leads to serious consequences for both mother and newborn, epidemiological studies suggest that the deficit in omega-3 fatty acids is associated with a higher prevalence of PPD. Anhedonia is one of the major symptoms of depression that can be examined in animal models. **Objective:** To determine the role of omega-3 fatty acids in the manifestation of anhedonia in a hormonally induced DPP model. **Materials and methods:** Experimental study with female Wistar rats. After ovariectomy, the animals were randomly divided into four groups, placebo hormone (sesame oil) was administered to the groups I (control) and III (control+ω3); and pseudopregnancy regime (17-β-estradiol and progesterone for 16 days and progesterone in the next 8 days) to the groups II (pseudopregnant) and IV (pseudopregnant+ω3). Omega-3 fatty acids were supplemented to the groups III (control+ω3) and IV (pseudopregnant+ω3). Behavioral evaluation was performed from the third postpartum day (19h) using sucrose preference test; variables measured were: sucrose intake in the first hour and 24th hour, sucrose preference in the first hour and 24-hours. The groups were compared with Kruskal-Wallis test, with a significance of 5%. **Results:** Statistically significant differences between groups were found for 24-hours sucrose consumption (p=0.0174). 24-hours sucrose consumption was 2.3 times higher in pseudopregnant group than control (group I vs. II), and 4.3 times higher in pseudopregnant+ω3 group than control (group III vs. IV). The remaining comparisons had no statistically significant differences.

Discussion: The hormonal "withdrawal" in rats subjected to pseudopregnancy regime leads to depressogenic behavior establishment, however the results found in this study differ from those reported by other authors.

A12

INFLUENZA SURVEILLANCE: FOLLOWING THE VIRUS AFTER A PANDEMIC

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The global monitoring of influenza virus through surveillance is the mechanism that enables us to see the evolution of circulating virus. WHO coordinates an international network that provides information to members and suggests measures for prevention and control. Tucumán has participated since 1999 in the National Laboratory Network for surveillance of influenza virus. The goal is to make a descriptive study of the circulation of influenza A (IA) virus and predominant subtypes in the period 2009 until 2013. 19,098 respiratory samples of outpatient and inpatient of public and private health facilities were collected and processed for immunofluorescence (IF) for antigen detection. Positive samples by real time RT-PCR and IF to IA have been subtyped in H1 and H3 in our laboratory since 2011. In 2009 and 2010 the subtyping was made in the National Reference Laboratory. Of the 19,098 samples, 1,454 (7.6%) were positive for IA. In 2009, IA was 13.5% (100% H1pdm); 2010 IA was 4.9% and 100% H3. 2011 with 2.1% of IA and 2013 with 8.7%, had similar percentages of H3 and H1 (73.3% and 26.7% respectively). In 2012 with 10.2% of IA, H3 and H1 was 51.7% and 48.3%. The period during which more cases occurred was: 2009 and 2013 between epidemiological weeks 25-35, in 2011 and 2012 weeks 33-38 and 2010 weeks 40-45. Conclusion: 2009 was the year with the highest number of cases due to an IA pandemic virus emerging, for which the population was susceptible. In 2010 the predominance of H3 could be due to selective pressure exerted by the monovalent vaccine H1pdm supplied to the population. In 2011 and 2013 both subtypes co-circulated, with H3 dominance and in 2012 the proportions were similar. These data allow us to review and improve preparedness plans in front of emergency and also the ability to detect and monitor any new threat to public health.

A13

SYPHILIS IN PREGNANCY AND CONGENITAL SYPHILIS IN INSTITUTE OF MATERNITY, TUCUMÁN DURING 2011, 2012 AND 2013

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Syphilis is an infectious disease transmitted sexually, during pregnancy and by uncontrolled blood transfusion produced by the *Treponema pallidum*. The incidence and prevalence of congenital syphilis in Argentina show discrepancy between regions and provinces.

To determine the prevalence of gestational syphilis and incidence of congenital syphilis in the Maternity Institute "Ntra. Sra. de las Mercedes" in Tucumán and the clinical sociodemographic characteristics, 273 pregnant women suffering from gestational syphilis were analyzed. They had antenatal care and/or delivery in that hospital and their maternal and perinatal results were known during the years 2011, 2012 and 2013. Through a descriptive cross-sectional study, the prevalence was 1.64% per year and incidence by year was: 2011: 10%₀ (HF 7%₀, 120%₀); 2012: 10%₀ (HF 7%₀, 110%₀); 2013: 10%₀ (HF 8%, 120%). A lot of them came from central and eastern areas of the province. Each year more than 50% were in the age group of 20-34 years and most of the AG diagnosis was puerperium; in first control more prominent EG was 20 weeks and less. For CCRN we found that "Born alive with asymptomatic syphilis" is the most frequent group with 35% (2011), 38% (2012) and 45% (2013). In the three year period most frequent CCE was "latent syphilis" with 78%, 80% and 82% in the years 2011, 2012 and 2013 respectively (p=0.9771).

The variation of CCRN per year may be due to underreporting. The incidence of congenital syphilis is greater than that proposed by PAHO (0.5 cases per thousand live births).

A14

APPLICATION OF MccJ25(G12Y) IN FOOD BIOPRESERVATION

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Microcin J25(G12Y) is a microcin J25 derivated, with a great potential as food preserver since it is susceptible to digestion by chymotrypsin and is able to resist extreme pHs and high temperatures. Moreover, it has been demonstrated that MccJ25(G12Y) is inactivated by digestive enzymes present in rat intestinal contents and its administration has not shown a negative effect on coliform population of mouse intestinal flora. The goal of this work is to evaluate the potential of MccJ25 (G12Y) to preserve food. To this end, two specific objectives were raised:

1-Determine the *in vitro* antimicrobial activity of MccJ25(G12Y) in a collection of pathogen and food-spoiling strains. For this purpose, we determined the minimum inhibitory concentration (MIC) of MccJ25(G12Y) versus 51 strains, noting that MccJ25(G12Y) was active on 28.

2-Evaluate the antimicrobial activity and stability of MccJ25(G12Y) in yogurt. To study the antimicrobial activity of MccJ25(G12Y) in food; yogurt was inoculated with three MccJ25(G12Y) sensitive strains, *E. Coli O157: H7*, *Salmonella cholera* and *Enterobacter cloacae*, and treated with 0.06 mg/ml of microcin. The CFU of the pathogens was monitored daily, observing that MccJ25 (G12Y) significantly reduced the CFU/ml of pathogens after five days. To analyze the stability of MccJ25(G12Y), a sample of yogurt was prepared from milk previously inoculated with MccJ25 (G12Y). It was conserved at 4°C and aliquots were daily taken to evaluate the remaining microcin antimicrobial activity. During 15 days the microcin was stable and active in the food matrix.

The results obtained showed that microcin J25(G12Y) has a potential use in food biopreservation since, in addition to its promising features previously mentioned, it was active against a large number of pathogenic and food spoiling bacteria and preserved its antimicrobial activity in food intact.

A15

RESISTANCE PROFILES AND BIOFILM FORMATION BY EXTREMOPHILES ISOLATED FROM HIGH-ALTITUDE ANDEAN LAKES

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High-Altitude Andean Lakes (HAAL) in northwest Argentina are ecosystems exposed to a wide variety of extreme conditions mainly high UV radiation (UV) and high concentration of toxic elements such as arsenic (As). Nevertheless, extremophilic microbial communities thrive there, which have great biotechnological potential mainly as source for novel antibiotics and alternatives for bioremediation.

The aim of this work is to assess resistance profiles of *Acinetobacter* sp. Ver3, *Exiguobacterium* sp. S17 and *Exiguobacterium* sp. N39 towards arsenic and UV-B exposure. The biofilm formation in different culture conditions is also investigated. The resistance profile to UV was measured in LB agar media under increasing doses of UV-B while the tolerance to As was studied in LB liquid media at different concentrations of arsenite (AsIII) and arsenate (AsV). The production of biofilm was tested on different supports and culture media and quantified. The results were compared with strains from the DSMZ German Culture Collection; *A. johnsonii* DSM6963 and *E. arantiacum* DSM6208. All strains were selected from LIMLA-PROIMI-CONICET Microbial Collection.

Our results showed that HAAL bacteria are much more resistant than the control strains to both UV-B and arsenic. On the other hand, biofilm production was more significantly when the strains were cultured in LB media supplemented with glycerol. The present study demonstrated the ability of HAAL strains to endure physico-chemical stress and to develop a resistant phase (biofilm) in different experimental conditions. These results represent an experimental basis for its application in bioremediation processes.

A16

REPRODUCTIVE BIOLOGY OF *Tephrocactus alexanderi*, AN ENDEMIC CACTUS OF ARGENTINA

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Tephrocactus alexanderi (Britton & Rose) Backeb. is an endemic cactus of Argentina, with entomophile flowers, and its reproductive biology is unknown. This work investigates the flower longevity, the stigma receptivity, the breeding system, the flower visitors, and the reproductive success of this species in the Sierra de Mazán, province of La Rioja, Argentina. The floral longevity was studied in 20 flowers of different plants at intervals of 3 h. The stigma receptivity was evaluated with the hydrogen peroxide test. The breeding system was determined with a six-treatment assay (spontaneous self-pollination, hand self-pollination, geitonogamy, apomixis, xenogamy, and natural pollination). The reproductive success was studied in 20 plants monitored through the flowering and fruiting period. The number of flowers, ovules, fruits and seeds produced by plant was quantified. The flowers open between 10:00 and 12:00 AM and live on average 30.5 h. The stigma was receptive throughout the flower lifespan, except in a premature phase of the anthesis. In flower senescence, the stamens (without pollen) attach to the petals. The 60% of the flowers produced fruits. There was no fruit production by apomixis nor by self-pollination (natural, hand, and geitonogamy). The hand cross-pollination produced 20% of fruits and natural pollination produced 62%. The floral visitors were hymenoptera of the family Apidae, Halictidae and Andrenidae. The results suggest that *T. alexanderi* is self-incompatible and depends on cross-pollination. These cacti show a massive flowering in spring, producing numerous flowers, pink to white, visited by hymenoptera which could act as pollen vectors that promote outcrossing.

A17

ATMOSPHERIC DUST EFFECT ON PHOTOSYNTHESIS IN NATIVE AND EXOTIC SPECIES IN TUCUMÁN (ARGENTINA)

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In the autumn-winter period Tucumán suffers severe air pollution. It is related to industrial activity, biomass, urban and forest residues and fossil fuel burning, among others. Because of the topography, climate seasonality and wind direction, these particles accumulate on the Tucumán Capital forming dense and dark clouds called ABC (Atmospheric Brown Clouds) (González et al., 2014). Plants are continuously exposed to such pollution and it produces alterations to their physiologic functioning. The objective of this study was to assess the effects of dust accumulation on the maximum photosynthetic assimilation (A_{max}), stomatal conductance (g_s), leaf perspiration (E), internal CO₂ concentration and water use efficiency (WUE) in the leaves of 17 species which was measured in cleaned and dusted leaves. Portable gas analyzer (LICOR) was used. Results, the first on this subject in Tucumán, showed: period of autumn-winter pollution strongly correlated with satellite Aerosol Optical Depth data (AOD550) Aerosol Index (AI) and Total Solar Irradiance (TSI). Leaves with dust reduced significantly A_{max}, E and g_s. Maximum reductions were recorded in *M. germanica* (-43.7%), *Tabebuia chrysotricha* (-40.5%) and *Hibiscus rosasinensis* (-27.8%). The values of C_i and WUE were increased in dusted leaves. Pubescent leaves with prominent veins exhibited the greatest reduction in A_{max}. Also broad and rough and horizontally-arranged leaves accumulate more dust and were more affected. This study confirms that although plants can grow in environments with high air pollution, accumulation dust on the leaves causes changes in the photosynthetic assimilation of CO₂. Further studies are necessary to confirm if dust accumulation can affect development and productivity of the species.

A18

THE USE OF LEMON ESSENTIAL OIL TO CONTROL CITRUS GREEN MOLD (*Penicillium digitatum*) IN TUCUMAN PROVINCE

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Citrus Green mold caused by *Penicillium digitatum* Sacc is the most important postharvest disease of lemon in Tucuman. The use of lemon essential oil (LEO) is a promissory alternative to control this mold. The objective of this work was to evaluate LEO effectiveness at two doses to control citrus Green mold in lemon fruit.

Mature fruits were inoculated using a puncher previously dipped in a suspension of 1×10^6 spore/ml of isolated strain of packing house and incubated in chamber at 22°C. Subsequently the fruit were dipped during 30 seconds with the following treatments: T1 Control water; T2 Lemon essential oil 0.5%; T3 Lemon essential oil 1%. The experimental unit was composed of 10 fruits. A DCA design with tree replicates (30 fruits per treatment) was used. After 10 days stored at 6°C the sporulation grade (SG) was evaluated using a 5 grade (0-4) scale at 10 intervals. Disease Progress Area Curve (DAPAC) was constructed and ANOVA was used to compare mean values with the Fisher test (5%).

Both treatments with LEO were different ($p=0.0012$) from control (DAPAC 56.0), the best treatment control was T3 (28.7) presenting no difference with T2 (36.4). Although it is necessary to continue the comparison with fungicide, LEO is a promissory alternative to control citrus green mould caused by *P. digitatum*.

A19

STEM-END ROT (*Phomopsis citri*) CONTROL IN LEMON FRUIT USING ALTERNATIVE CHEMICAL CONTROL METHODS

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Stem-end rot (SER) caused by *Phomopsis citri* Faw, occurs at the beginning of the exportation campaign in Tucuman with rainy and humid weather. Its control is based on the application of synthesis fungicides in packing houses. The objective of this work was to compare different alternative chemical methods to control SER.

Mature lemon fruit were inoculated with a strain isolated from Burrucacú (Tucumán) and incubated in a chamber at 20°C during 24 hours. Then fruits were dipped during 30 seconds with the following treatments: T1 Control (water); T2 polyhexamethylnendiguaniehydrochloride 10 mL.L^{-1} ; T3 peracetic acid + hydrogen peroxide 10 mL.L^{-1} ; T4 Lemon essential oil 1%. The experimental unit was composed of 10 fruits. A DCA design with tree replicates (30 fruits per treatment) was used. Then the fruits were stored in chamber during 10 days at 5°C and 12 days at 20°C. SER incidence (%) was evaluated at three different times and Disease Curve (DAPAC) was constructed. ANOVA was used to compare mean values with the Fisher test (5%).

The best control was T2 (DAPAC 306.7) which presented difference with respect to the other treatments ($p=0.001$). T3 treatment (560.0) presented an acceptable control followed by T2 in efficacy, while T4 (840.0) was similar to the control (778.3) in SER levels. The Polyhexamethylnendiguanide hydrochloride at 10 mL.L^{-1} is suitable alternative chemical method to control SER.

POSTER PRESENTATIONS

A20

PHYTOPLANKTONIC COMPOSITION AND WATER QUALITY IN A NORTHWESTERN RIVER IN ARGENTINA

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The orographic conditions and climate of the Argentinian Northwestern allows the existence of numerous aquatic ecosystems. Tucumán has an extensive hydrographic network. Las Moras river is a tributary of Escaba Reservoir. The study area is located in the Yungas where the climate is temperate, with rainy summers. The aim of this research was to evaluate the temporal and seasonal variation of phytoplankton and its relationship with water quality at the mouth of the river. Seasonal samples were collected according to standard methodology from august/10 to march/12. Conductivity (EC), pH, dissolved oxygen (DO), biochemical oxygen demand (BOD₅), major ions, nitrogen and total phosphorus, species richness, abundance, biomass and algal diversity were analyzed. Water was characterized as calcium-sodium-bicarbonate alkaline and oxygenated. Total nitrogen and total phosphorus reached 10 mg/l (march/12) and 0.56 mg/l (march/11). Conductivity fluctuated between 89-130 µS/cm, the BOD₅ varied between <5-46 mg/l. We identified 25 phytoplanktonic species represented by: Bacillariophyceae (14), Chlorophyta (6), Cyanophyta (2) Euglenophyta (2) and Dinophyta (1). Biomass fluctuated between 23-1379 chlorophyll a/l. The highest algal abundance was recorded in march/11(1441 ind/ml). The specific diversity ranged from 0.12 (march/11) and 1.93 (august/10). Highly significant correlations were found between EC with nitrate and with OD, between pH and BOD₅, total phosphorus with biochemical oxygen demand, OD and nitrate. During the winters of 2010-11 the greatest richness and specific diversity was noted, because of the contribution of diatoms. The input of nutrients in the summer of both years, favored the development of *Ceratium hirundinella* which determined an increase in the density and biomass of the phytoplankton.

A21

DIVERSITY OF SEASONAL ODONATA (INSECTA) IN A STRETCH OF EL TALA RIVER

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The dragonflies are insects known as "dragonflies", "damselflies", "helicopters". The objective of this research was to determine the diversity of Odonata in a stretch of river Tala (Catamarca, Argentina) and seasonal variation in an annual cycle. Samples were seasonal. Larvae were collected combining manual sampling, wire strainers and red "D". The adults were collected with entomological net. Families reported were: Calopterygidae, Coenagrionidae, Gomphidae, Libellulidae and Aeshnidae. Of a total of 316 larvae, the relative abundance was: Coenagrionidae (62.34%), Calopterygidae (28.80%), Libellulidae (85.38%), Aeshnidae (2.85%) and Gomphidae (0.63%). Seasonal variation in absolute abundance was observed, with the fall (164) and winter (80), the most abundant seasons, followed by spring (39) and summer (33). In autumn and winter the faunal richness was higher (4 of the 5 families reported present). Regarding adults 30 individuals of four families (except Gomphidae) were collected. The relative abundances were Coenagrionidae (53.33%), Libellulidae (26.67%), Calopterygidae (13.33%) and Aeshnidae (6.67%). Season variation of absolute abundance was observed, which was at its highest in spring (15), decreasing in summer (9 adults) and fall (6). Adults were not collected in winter. Most faunal richness was reached in spring (found 3 of the 4 families reported). The findings could be related to the life cycle of these insects. These results represent the first contribution to the knowledge of the diversity of Odonata Tala River and its seasonal variation in an annual cycle.

A22

POLLEN MORPHOLOGY OF FOUR SPECIES OF THE GENUS *CITRUS* (RUTACEAE) GROWN IN SAN MIGUEL DE TUCUMAN (ARGENTINA)

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The *Citrus* genus belongs to the Aurantioideae subfamily (Rutaceae), and includes numerous species of agricultural and ornamental importance. This work aims to analyze the pollen morphology of 4 main species of the genus *Citrus* traditionally grown in the urban trees of the city of San Miguel de Tucumán. This study is part of the project "Flora polínica del NOA", which is a reference collection in order to facilitate the identification of pollen types present in the honey produced in the region. The following species were analyzed: *Citrus aurantium* L. ("Naranja agrio"), *Citrus sinensis* (L.) Osbeck ("Naranja dulce"), *Citrus jambhiri* Lush ("Limón rugoso") y *Citrus reshni* Hort ex Tan ("Mandarino cleopatra"). Samples were obtained from fresh material from collections made in city streets. The control sheets were deposited in the Laboratory of Palynology of the Fundación Miguel Lillo. The material was processed according to conventional techniques for acetolysis and natural pollen. Photos level Optical Microscopy (OM) were taken. The preparations were placed in the reference pollen library of the Fundación Miguel Lillo (PAL-TUC). Grains are isopolar and radially symmetric. The shape varies from oblate-esferoidal to prolate-esferoidal both in the natural as well as acetolized state. The grains are medium size, 30 to 45 µm on acetolyzed grain and 24 to 37 µm on natural ones. Scope is round, square or pentagonal according to the number of openings. They can vary from Tricolporate to pentacolporate, finding combinations of 3 and 4; 4 and 5 or 3, 4 and 5 in different proportions for each species. The colpi are short; lalongated endoaperture. Present exine 2 to 4 µm thick, sexine as thick as nexine; reticulate heterobrochate surface, brochi decreasing in size towards the poles. Variability is highlighted in the number of intraespecific and interspecific openings, no differences were observed in the other morphological characters suggesting that genus is stenopalynous.

A23

NEW RECORD *Pseudodoros clavatus* AND *Ocyptamus gastrostactus* (DIPTERA: SYRPHIDAE) FROM TUCUMAN, ARGENTINA

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Argentina is the world's largest lemon producer, with a production of 1.300.000 t at 43.277 ha., Tucumán being the leading producer and industrializing of this crop with 1.100.000 t at 35.000 ha. Major pests are aphids, as *Toxoptera citricidus* (Kirkaldy, 1907) being the most common and important vector of CTV. Primarily, the treatment for this plague is chemical control but market requirements promote alternative strategies within which is biological control (BC). Aphids have many natural enemies that keep their populations below the UDE. Among them are predators such as hoverflies, which are efficient pollinators in their adult stage and as immature are important as aphid control agents, with two known hoverflies marketed for control as *Sphaerophoria rueppellii* Weidemann, 1820 and *Episyrphus balteatus* (De Geer, 1776); therefore the aim of this study was to survey the species of hoverflies present in farms in Lules-Tucumán for possible BC strategies. This plant material from a lemon farm in Lules-Tucumán was collected. The samples consisted of outbreaks of lemon (*Citrus limon* L.) which had aphid colonies *T. citricidus* and syrphid larvae, which were conditioned in the laboratories of the Department of Agricultural Zoology FAZ-UNT until their development was completed. Once emerged adults were identified, two species were obtained: *Pseudodoros clavatus* (Fabricius, 1794) and *Ocyptamus gastrostactus* (Wiedemann, 1830) belonging to the Family: Syrphidae, subfamily: Syrphinae. It is concluded that this sampling of syrphid contributes to enhancing knowledge of the distribution of these species and new plant-herbivore-predator interactions. *P. clavatus* is cited for the first time in northern Argentina and *O. gastrostactus* is cited for the first time in Argentina

A24

**CONCENTRATION VARIATION OF NON ARBOREAL AND ARBOREAL POLLEN IN THE
ATMOSPHERE OF SAN MIGUEL DE TUCUMÁN**

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Aeropalynology is the study of pollen grains found in the atmosphere. The aim of this study was to analyze the variation of the concentration of non arboreal pollen (NAP) and arboreal pollen (AP) in the atmosphere of San Miguel de Tucumán (26 ° 49'0 "S, 65 ° 13 '0") from August to December 2007. Airborne pollen monitoring was performed with a Burkard spore trap located 20 m on the roof of the building of Fundación Miguel Lillo. The samples were prepared according to standard methods and 6 daily transects were analysed. During this period 6129.08 grains were recorded and 59 pollen types (PT) were identified, 40 of them correspond to AP, representing 94.42% of the total pollen grains and 19 to NAP providing the remainder. PT were classified into 3 groups according to their relative percentage. High representation (> 1%): *Broussonetia*, *Morus*, *Celtis*, *Platanus*, Myrtaceae (AP) and Urticaceae (NAP). These accounted for 91.35% of the record. The PT underrepresented (1-0.5%) were: *Fraxinus*, *Salix*, *Alnus* and Cupressaceae (AP) and for NAP Poaceae, *Ephedra* and Chenopodiaceae. They all contributed 4.51% to the pollen cloud. The trace PT (<0.5) contributed 2.66%. The representation of AP was 85% higher than NAP in the months of August, September and October, and 57% higher in November. The contribution of NAP was 10% higher than AP concentration in December. We observed a great richness of PT although few of them were dominant. Only 8 PT had more than 94% of the total number of pollen grains. There was a brief and intense concentration of AP since mid-August to mid-November. The wide prevalence of AP on NAP can be partly explained because of the flowering peak of native arboreal vegetation and urban trees that occurs in spring. However, these observations should be confirmed by carrying out long-term studies.

A25

**DATA-GATHERING AND IDENTIFICATION OF NEMATOFUNA ASSOCIATED WITH TOMATO
CROP IN TAPIA TRANCAS-TUCUMÁN**

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Tapia Trancas is located in north central Tucuman, it has semiarid climate with permanent hydric deficit. Secondary production of tomato is relevant. The production techniques used in this area are of low investment, such as irrigation mantle and conventional seedlings, which resulted in a passive dispersal of nematodes. The aim of this study was to survey and identify nematodes present in the tomato crop in the field of Tapia Trancas. One hectare culture was selected and soil samples from 10 points randomly extracted vol. 20x20x20 cm³. It was mixed in a bucket and a 2 liter laboratory subsample was screened, sieved, spread on a tray and soil was removed with a spoon until obtaining 100 cm³. Then by Cobb Decantation and Sieving Method and differential centrifugation on sucrose gradients the nematodes were collected. They were identified through slides and codes. This process was repeated 3 times to obtain averages. 100 g roots were also extracted, washed, cut in pieces of 10 cm and on petri dish gills dissected and identified as well. 12 genera were identified: *Tylenchus sp.* (0.38), *Tylenchorhynchus sp.* (4.07), *Pratylenchus sp.* (1.03), *Helicotylenchus sp.* (11.32), *Meloidogyne sp.* (6.66), *Criconemoides sp.* (0.72), *Aphelenchus sp.* (0.04), *Rhabditis sp.* (5.54), *Panagrolaimus sp.* (0.31), *Dorylaimus sp.* (3.06), *Xiphinema sp.* (0.02) and *Nacobbus sp.* (0.69) in soil. And 1 genus: *Meloidogyne sp.* (100) in roots. No predators found. A high percentage of the genera *Tylenchorhynchus* and *Helicotylenchus* were observed, the damage they cause is unknown. It is concluded that in the area of Tapia Trancas tomato crop has a rich and varied nemato fauna, where *Meloidogyne* and *Nacobbus* stand out for the injuries they cause. This information is important to implement crop management strategies.

A26

VASCULAR FLORA OF THE VALLEY ANTINACO-LOS COLORADOS (LA RIOJA-ARGENTINA)

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The term flora defines the set of plant species inhabiting a given area. Its knowledge is essential in research and to undertake actions related to their use, conservation and recovery. The present study describes the vascular flora (species, genera, families) growing naturally in the Antinaco-Los Colorados Valley, La Rioja Province, Argentina. The valley is a vast plain (≈155 km long. X 33 km wide) located between the Sierras de Velazco and Famatina, and is characterized by vegetation of the Monte ecoregion. During the years 2006-2014, 87 herborization trips were carried out through the Chilecito, Famatina and Independence regions in the north. The survey was conducted in four types of environments: typical steppe of *Larrea cuneifolia* ("jarilla") and *Bulnesia retama* ("retama"), riparian vegetation, halophyte steppe, and vegetation of route edges. In total 226 species corresponding to 165 genus and 54 families were recorded. The best represented genera were *Atriplex*, *Lycium*, *Prosopis*, *Solanum* and *Tillandsia*, all with 5 species. The 6 families with more species were Poaceae (30), Asteraceae (29), Fabaceae (20), Solanaceae (17), Chenopodiaceae (13), Cactaceae (11). The analysis of life forms reveals predominance of herbs (65%), followed in importance by shrubs (27%), trees (4) and vines (4%). Regarding the status, 98% were native species, 1% endemic species of Argentina and 1% introduced species. 73% of the registered species compose the natural ecosystems of the valley, and 23% are ruderal species growing on edges of routes. The flora recorded in this study represents 13.5% of all native species suitable for the Province of La Rioja. For the first time in the province 12 native species and three introduced are mentioned.

A27

EPIGEOUS GASTEROID FUNGI FROM CATAMARCA, ARGENTINA. I- *Agaricus aridicola* & *Montagnea arenaria*

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Agaricus aridicola Geml, Geiser & Royse ex Mateos, J. Morales, J. Muñoz, Rey & Tovar and *Montagnea arenaria* (DC.) Zeller (*Agaricomycetes*, *Agaricomycetidae*) have very similar macroscopic morphology. They are characterized by fruiting bodies with a well developed stalk; head conical to campanulate, in the margin are adhered plates or gussets on which the spores are borne. They differ as regards the presence of ring in *Agaricus aridicola*, the spore size and apical germ pore presence/absence. Both species share similar habitats, sandy or very dry soil and exposed sites. Until 2007 they were classified related to Gastermycetes Class under the name Agaricoid Gasteromycetes. Subsequent investigations by Hibbett y col. (2007) placed them in Agaricales Order.

Agaricus aridicola in Argentina was previously described in Catamarca, La Rioja, Mendoza and Salta.

Montagnea arenaria was previously described from Buenos Aires, Catamarca, Chubut, La Rioja, Mendoza, Neuquén, San Juan and Salta. The aim of this paper is to contribute to the knowledge of the Gasteroid Fungi diversity, distribution and ecology from Catamarca.

As a part of an integral study of mycobiota Catamarca province, seasonal field works were made in different phytogeographic regions where basidiomata was collected. The study and the descriptions were made on collected material, following the methodology and terminology by Domínguez de Toledo (1989) and Dios et al (2001). The specimens collected were deposited in the personal herbarium M.M.Dios (FACEN, UNCa.)

Although both species were already mentioned in the province, in this work, they were compared with *typus* material deposited in BAFC and LPS.

A28

FUNGAL COMMUNITY FROM LA MERCED DE ALLPATAUCA, DEPARTAMENTO FRAY MAMERTO ESQUIU, CATAMARCA, ARGENTINA. II- ASCOMYCOTA.

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La Merced de Allpatauca it is the only Wildlife refuge in Catamarca province and is part of the Wildlife Refuges Network of Argentina; it comprises 623 acres.

Plant species are characteristic of Chaco Serrano, as Horco Quebracho (*Schinopsis marginata*), Palo borracho (*Ceiba insignis*), Sacha membrillo (*Ruprechtia apetala*) and Sombra de Toro (*Jodina rhombifolia*), and of Chaco árido, as Quebracho blanco (*Aspidosperma quebracho-blanco*), Algarrobo blanco (*Prosopis chilensis*), Mistol (*Zyzphus mistol*) and Mistol del zorro (*Castela coccinea*).

Under the study of fungal component of plant communities of the arid Chaco and with the aim to contribute to the knowledge of fungal diversity associated with plant communities of the ecoregions of the province of Catamarca seasonal collections from Reserve La Merced de Allpatauca were studied and analyzed. The specimens collected were deposited in the personal herbarium M.M.Dios (FACEN, UNCa). The results presented in this research correspond to Ascomycota Phylum.

Species of the genera were identified: *Amerosporium*, *Bactrodesmiella*, *Cyanodiscus*, *Diatrypa*, *Didymosphaerea*, *Epicoccum*, *Hipoxylon*, *Mycocalicium*, *Nemania*, *Nitschkia*, *Rhytidhysterion*, *Sirodesmium*, *Sphaeropsis*, *Stictis*, *Xylaria*, and two samples that were classified as *aff. Cryptosporium* y *aff. Plenodomus*.

Many of the species are the first recorded for the province of Catamarca and northwest, such as *Mycocalicium americanum*, *Nemania serpens*, *Stictis radiata*, *Xylaria adscendens*, *X. coccophora*, *X. enteroleuca*, *X. gracillima*, *X. grammica* y *X. myosurus*.

A29

CHARACTERIZATION OF EPIDERMAL STRUCTURES FROM *Caiophora lateritia* (LOASACEAE)

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Caiophora C. Presl. (Loasaceae, Loasoideae) includes about 60 species occurring almost exclusively in the Andes mountains, 13 have been reported for Argentina (Ackermann & Weigend, 2007). *Caiophora lateritia* Klotzsch. found from Northwest Argentina to Bolivia, is a perennial, voluble, stinging herb with colored flowers. The aim of this study was to characterize the nature of leaf indument, stomata and epidermal models of this species. Conventional anatomical techniques for observation of epidermal structures in optical and scanning electron microscopy were performed.

C. lateritia leaves are hypostomatic. Adaxial epidermis consists of prismatic cells with sinuous undulate anticlinal walls, smooth cuticle. Abaxial epidermis consists of prismatic cells with strongly undulate anticlinal walls. Stomata are anomocytic anomocíticos 30.1 (± 9.2) x 30.0 (± 5.1) μm risen above epidermal surface, cuticle slightly striated.

Both epidermal present:

A- Eglanular Trichomes: 1- Conicall scabrid antrorse unicellular, bicellular rare; 2- Glochidiate erect unicellular with uncinat apex; 3- Escabrid-glochidiate, two-celled.

B- Glandular Trichomes: 1- Short capitate bicellular.

C- Emergencies: 1- Formed by a multicellular solid base with a stinging unicellular trichome, in some cases cystolitic with CaCO₃ deposits.

Trichomes and epidermal structures described in this paper are of taxonomic importance. In Loasaceae family they allow the identification of subfamilies, genera and species.

A30

AQUATIC MACROINVERTEBRATES AND WATER QUALITY IN A STRETCH OF RIVER AMBATO.

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The Ambato River runs along the west slope of the Sierras de Ambato-Manchao (Catamarca, Argentina). The objective was to evaluate the water quality of the Ambato River, along a longitudinal gradient using aquatic macroinvertebrates as bioindicators. Sampling was carried out in shallow waters. Four sampling stations were established considering a longitudinal gradient. At each station we obtained: altitude, latitude, longitude, height of the dry and wet bed, river depth and current velocity. Water physicochemical parameters were measured: temperature, total dissolved solids, dissolved oxygen, pH and electric conductivity. Macroinvertebrates were collected with sampler type "Surber" (0,09m² surface, mesh width 300µm). At each station three samples, integrated for analysis were obtained. Organisms were identified to the taxon family. As measures of biodiversity we analyzed: number faunal diversity; Shannon-Wiener index (H'); Simpson dominance index and Jaccard. The biotic indices were calculated: BMWP' (Biological Monitoring Working Party) adjusted for NOA; ASPT' (Average Score Per Taxon) e IBF (Family Biotic Index). 12,699 individuals representing 28 taxa were collected. H' was 3.11 and Simpson Dominance was 0.15. The BMWP' (140), ASPT' (5.38) and IBF (3.72), indices allow to assess water as very clean, with no impact and no apparent organic pollution, respectively. Variation between the four seasons in all metrics and indices obtained was observed, with the Station III, the most diverse, abundant and better water quality. It is concluded that the section of the Ambato River is in good condition, as evidenced by the values reached by the indices obtained, which are the first data available for the environment studied.

A31

USE OF SPACE BY A GROUP OF BROWN BROCKET DEER (*Mazama gouazoubira*, Fischer 1814), IN A GRASSLAND AREA IN CAPTIVITY

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The brown brocket deer (*Mazama gouazoubira*) is a cervid in the genus *Mazama*, which consists of 10 species of American deer in which the males have spike antlers. The brown brocket is found from S Brazil to central and N Argentina. Its activity period varies with habitat and season. Both sexes exhibit territorial behavior, which involves marking and defense. The aim of this study was to determine brown brocket deer preferences in space of use and increase knowledge about marking territory and sociality. The study was conducted in an open grassland enclosure of 8332.28 m² in the Reserva Experimental Horco Molle, Tucumán. This area was divided into 3 sections: Edge of forest, Isolated trees and Open grassland. The individuals observed were 4 adults, 1 juvenile and 1 fawn. Activity was recorded through Ad libitum and focal animal methods. The behaviors were classified into 2 categories: Individual and Interaction. The deer showed increased activity in the afternoon hours (17-20 hs). The sector most used was the forest edge; the breeding and female used more grassland, maybe for trophic requirements. 31 individual and 36 interaction behavior patterns were observed. Half of these involved the fawn, the mother, and the juvenile male and only one-third involved reciprocity. There is no reciprocity in allogrooming. Brown brocket deer were mainly observed alone; the number of observations in groups decreased as the number of individuals in a group increased. There were gender differences in activity periods observed in the grassland; females use it during the day and males were rarely seen. The study area corresponds to the core area of the female based on the increased use of latrines, presence of bedding areas and the birthplace of the fawn. No aggressive interactions were observed.

A32

ENVIRONMENTAL ENRICHMENT IN YAGUARUNDI (*Puma yaguarundi*), PUMA (*Puma concolor*) AND COATI (*Nassua nasua*) IN RESERVA EXPERIMENTAL HORCO MOLLE (REHM)

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Animals in captivity often show stereotyped behaviors. To reduce these and improve their welfare environmental enrichment techniques are applied to stimulate activities of interaction with the environment. The objectives of this project are to conduct behavioral assessments of animals to detect the presence of stereotyped behaviors and implement enrichment activities to improve the welfare of individuals. The REHM has several species housed in enclosures and we work with: jaguarundi (1 male and 1 female), coatis (2 female) and puma (1 female) housed in larger enclosures. We performed ethograms of species and evaluated their behavior before and after enrichment. Food, furniture and olfactory enrichment were used. Before enrichment the male jaguarundi performs much pacing particularly to visitors, this happens in one of the coatis and puma also. The puma spends much time resting. After enrichment a slight reduction in coati pacing was achieved, and an increase in the variety of behaviors. In yaguarundi the pacing was reduced as in puma. In both cats game increased and inactivity decreased. In jaguarundi the food enrichment was more successful; in coatis incorporating new elements and odors generated increased activity and in the case of the puma incorporating balls to play was very successful. This project provides results of interest both at the level of the animals and the visitors. Indeed, the welfare of captive animals is improved and visitors have more attention and pleasure to see animals in a state of higher psychological and physical health.

A33

SEASONAL VARIATION AND DEMOGRAPHY OF *Leprolochus birabeni* Mello-Leitão, 1942 (ARANEAE: ZODARIIDAE) IN ENVIRONMENTS OF CHACO SERRANO OF NORTHWESTERN ARGENTINA

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The Chaco Serrano is a highly disturbed ecoregion and one of the few with dry subtropical forests in the world, so its study and conservation are essential. Among arachnids, spiders are considered indicators of environmental quality because their communities are strongly influenced by the type of habitat. Zodariidae family is mainly present in tropical and subtropical areas. *L. birabeni* Mello-Leitão, 1942 is a myrmecophilic species distributed from North Brazil to Central region of Argentina, and it is very common in Chaco and Argentinian Monte. The aim of this study is to characterize the demography and the seasonal variation of *L. birabeni* in different environments of Chaco Serrano of Salta province. Seasonal samplings (2006-2007) were performed using pitfall traps in three environments: NA (native sites), NG (native sites affected by cattle and goats), and NC (native patches surrounded by crops). We collected 531 spiders with the juveniles reaching 65% of total abundance, followed by males (25%) and females (10%). Spring and summer had the highest abundance with 46.3% and 28.4%, respectively. *L. birabeni* showed the highest abundance in NA representing the 42.37% of total abundance, followed by the NG 32.96% and the NC 24.67%. Likewise, the number of juveniles and females decreased from NA>NG>NC, while the abundance of males was at its lowest in NG ($n_{NG}=27$), and was similar in NA and NC ($n_{NA}=53$, $n_{NC}=57$). These results suggest that seasonality influences the abundance of this species in the study area, in addition to the land use. These results provide basic information for future studies to test if this species will be useful as a possible indicator of the conservation status of Chaco Serrano.

A34

RELATIONSHIP BETWEEN PESTS AND BENEFICIAL ORGANISMS DIVERSITY IN TOBACCO CROPS WITH VARIABLES OF CROP HETEROGENEITY AND WINDBREAKS BARRIER IN SALTA PROVINCE (ARGENTINA).

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Tobacco is the main intensive crop of Valle de Lerma, and its agricultural pests are an economic, ecological and social problem that should be considered to reduce crop losses. Therefore, in this paper we want to evaluate which is the relationship between variables of habitat heterogeneity with the arthropod guilds in tobacco crops and barriers surrounding the fields. We selected 22 tobacco farms implanted in different dates. We took at least 10 samples of arthropods on vegetation with a G-Vac in both sites, tobacco crop (T) and the herbaceous layer of windbreaks (B). The number of samples per farm was calculated in relation with the land size. Sampling was conducted in three stages of the phenological cycle of the crop from November (2013) to April (2014). Here, we considered the results of the first sampling date. At each farm variables of the habitat heterogeneity related with the height, canopy, percentage of tobacco cover, of weeds in the herbaceous layer of the barrier and its stratification and composition (native vs introduced) was taken, among others. We related the environmental variables with the composition of guilds by a non-metric multidimensional scaling (NMS) to evaluate which of them explained the presence of natural enemies. We recorded 24,866 arthropods: 18,012 phytophagous ($N_T=7.059$ y $N_B=10.953$); 1,699 predators ($N_T=668$ y $N_B=1.031$), 1,530 parasitoids ($N_T=273$ y $N_B=1.257$) and 3,625 of "others" guild (pollinators, detritivores and omnivores) ($N_T=2.052$ y $N_B=1.537$). The farms were divided into two groups according to the percentage of implanted tobacco and the composition of barrier vegetation. The NMS showed that a low percentage of weeds in the tobacco crop, in addition with a presence of a barrier represented by an herbaceous layer up to 5 meters surrounding the complete tobacco field increases the abundance of natural enemies. Furthermore, barriers with a percentage of non-native vegetation also increase the presence of predators, while phytophagous and parasitoids are favored by native vegetation on the barriers.

A35

ANTHROPOLOGICAL AND EPISTEMOLOGICAL CONTEXT OF ECOEFFICIENTISM IN ENVIRONMENTAL SPECIALISTS IN SAN FERNANDO DEL VALLE DE CATAMARCA.

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Eco-efficientism is a dominant ideological trend concerning deep ecology and popular ecology in theoretical and methodological frameworks of environmental specialists. In this paper epistemological characteristics were addressed from specialists from Ministry of Environment of Catamarca from anthropological and environmental points of view. The objective was to determine anthropological - epistemological context of eco-efficientist vision. The methodology used was an ethnographic interview structured around topic axes as notions of "ecosystem", "impact", "environmental component", "tangibility", "intangibility", "scientific language", "methodology", "synergy", "feedback", "observation", "predictability" and "complexity". The sample comprised 15 specialists who work in Ministry of Environment, whose specialties are 90% biology and 10% engineering and geology. The results revealed that most experts do not have an integrated view of the environment as a system, there is no use of holistic vision, which is not consistent with an ecoefficientist instrumentality, the context of representations of the environment corresponds to a sum of disaggregated components. Finally, the absence of a systemic view does not lead to a view of recursive interconnection between environmental components, they assume linear logic. In conclusion, ecoefficientism contradicts ideological objectives of sustainability. These have to do with a holistic view of development, this perspective is absent as epistemological principle, thus there is no application except ecoefficientism's aspects of remediation or mitigation but not as sustainable design.

A36

CONTENT DEVELOPMENT OF GENETICS IN TEXTBOOKS

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Genetics has ethic and economic implications that make genetic studies relevant and interesting for biology teachers in early stages. To make this presentation, we have made a qualitative revision of contents. The categories of analysis have been determined according to historic and epistemological considerations. The statistic frequency in relation to the emerging concepts as well as the number of pages in which they were developed was quantified. As a group of researchers, we have been analysing textbooks for a long time and this is the main reason why we intend to investigate why genetics is superficially studied in most school textbooks since it is highly dealt with in our daily lives and scientific surroundings. We have analysed 24 different high school textbooks on the fields of biology and natural sciences. This includes editions from 1997 to 2010. Genetics was studied from a historic and didactic perspective as well as from other scientific contexts. From a historic perspective, we have considered the contributions of important scientists. In relation to the didactic point of view we have taken into consideration the transposition processes. Genetics deals systemically with these contents while biology has a disassociated relation with them. Some books consider historical data with limited considerations to this topic. To conclude, we can mention that our study has been carried out in a reduced number of pages with (average 7) a great amount of concepts (66). Most of the texts present contents in isolation without considering procedural aspects. DNA and RNA involved in genetic processes are superficially studied and they are not related to evolution processes.

A37

INTEGRATION OF BASIC HISTOLOGY AND CLINICAL KNOWLEDGE IN DENTISTRY

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In the project “Areas of knowledge integrated into the curriculum of the Faculty of Dentistry of the National University of La Plata”, Histology contents were identified in the syllabuses and reference material of clinical subjects. A methodology was designed to be applied in the analysis of dentistry textbooks at formal, content and pragmatic levels. The objectives were the following: that students demonstrate the relationship between basic and clinical knowledge by means of activities that bind pragmatic contents together; that students get motivated for group work, recognize the worth of both interdisciplinary approach and the use of the library. After detecting students had difficulty when using textbooks, we organized an informative literacy workshop. Afterwards, students made a comparative analysis on the topic of “tooth development” in textbooks from the reference material for the subjects Histology and Embryology II and Pediatric Dentistry. Results evidenced that students detected terminology problems, different criteria to define the tooth development stages: a morphological criterion in Histology and Embryology II textbooks, with mention of the genes acting in each stage and illustrations of histological sections; a physiopathology criterion in Pediatric Dentistry textbooks, with indication of those anomalies caused by the absence of genes and with images of clinical cases with pathologies. They emphasized the importance of Histology knowledge to solve clinical problems, the interrelation between both subjects and the purpose of their contents. We conclude: the task assigned was significant for students, it motivated teachers and students and improved the teaching-learning processes.

A38

DENTISTRY STUDENTS' ATTITUDES TOWARDS THE STUDENT-PATIENT RELATIONSHIP IN THE TEACHING-LEARNING PROCESS

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The student-patient relationship constitutes a major factor in successful odontological consultations. Nevertheless, its relevance in the teaching learning process is often disregarded by teachers and students when tending patients. Thus, the interest arose to analyze the attitudes of students of Clinical Practice on Prosthesis I, a subject at the School of Dentistry in U.N.T. (FOUNT), as regards the student-patient relationship in the teaching learning process in a clinical subject.

A descriptive cross-sectional study was conducted. An unstructured questionnaire was utilized on 65 students taking the aforementioned subject. The discourse stemming from the gathered data was analyzed, taking into account the following: what is uttered, by whom, how, why (T. van Dijk, 2000), later grouping these into categories of analysis. Results show that 95% of students consider the relationship they establish with their patient to be favorably influential in their learning, as attitudes of 'trust' or 'respect' on part of the patient are reflected, and in that way, learning becomes fulfilling. 91% maintain that communication with their next patient proves simpler since they have acquired more 'confidence', 'less fear', 'more security' and 'more calm'. 94% claim that this relationship grows throughout the school year, highlighting categories such as 'mutual trust', 'patient's confidence', 'student's confidence', 'frequency in the relationship'. Given the question 'Do you believe the School [of Dentistry] should put more stress on communication with patients during the students' training?', 76% answered positively. The results obtained allow us to conclude that the students-informants express favorable attitudes with respect to the fact that the student-patient relationship, developed in clinical practice subjects, constitutes an important factor which would improve the teaching learning process.

A39

FREQUENT CONCEPTUAL ERRORS IN STUDENTS OF SECONDARY SCHOOL OF SAN FERNANDO OF THE VALLEY OF CATAMARCA, RELATED TO THE TOPIC OF DIET

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Knowledge is linked to each person's history and the errors are constituent of one's own act of knowing. Understanding in an integrated way the topic of diet on the part of the students implies a complex didactic approach from multiple aspects: social, economic, cultural etc. Didactic research confirms that the evolution of the ideas of the students toward a systemic and coordinated conception of these multiple aspects is a slow process that carries certain difficulties that can be translated into conceptual errors. Objective: To detect and evaluate the frequent conceptual errors on the topic of diet in secondary school students.

Materials and methods: observational, interpretive, descriptive, cross study. Observation units: six teachers of 3^o year of the secondary level, public schools of Catamarca city, by means of interview were asked about more frequent conceptual errors in their students on the topic of diet.

Results: They confuse concepts diet and nutrition considering them synonyms, they relate a healthy diet with quantity and not quality of foods. Quick foods are preferred being considered tastier and more nutritious.

Conclusion: The conceptual errors can be associated to the knowledge capacities of the students or the complexity of the topic. Teaching can favor the understanding of a more integral and more reflexive perspective creating problematic situations, with inquiry tasks that facilitate a reflexive conceptual change.

Key words: Diet, Teaching, Educational, Students.

A40

PROTEINS WITH GST AND HEMERYTHRIN-LIKE DOMAINS IN VASCULAR PLANTS REVEAL NEW MECHANISM IN ENVIRONMENTAL STRESS RESPONSE.

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In the moss *Physcomitrella patens*, proteins with a Fe binding motif belonging to hemerythrin class (H) were recently described in Glutathione transferase (GST) family. The patterns of transcript accumulation in diverse treatments suggest a role for GSTHs in the mechanism of the stress response in non vascular plants. The aim of this study was the identification and characterization of proteins with GST and hemerythrin like (Hr) domain in land plants, and the reconstruction of its molecular evolution. The search in public data bases identified 31 proteins that were divided in two groups according to the analysis of the functional domains. In **Group I**, proteins with a Hr-like domain present a complete GST (**Ia**) or partial GST domain (**Ib**); and in **Group II** the proteins show a thioredoxin-like fold and a Hr-like domain. **Group Ia** proteins, with conserved residues for GSH and Fe binding, were detected in early land plants (*Sellaginella* and *Physcomitrella*), **group Ib**'s only in vascular plants, and **group II** in angiosperms. All these proteins could be involved in iron homeostasis and saline stress according to the dbEST. Phylogenetic analysis showed complex patterns of evolutionary divergence in these proteins in land plants. The characterization of these proteins not described at present, also suggests the involvement of Fe in environmental stress response.

A41

EFFECT OF SHIFT WORK ON DIABETES: PRELIMINARY STUDIES IN JUJUY PROVINCE

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There is evidence relating shift work with increased risk for obesity, diabetes and cardiovascular disease. An increasing number of diabetic patients are shift workers, although the potential deleterious effect on their health is still unknown. The aim of this work was to study the effect of shift work upon the metabolism of type 2 diabetic patients (DM2).

From a total of 410 DM2 patients in Jujuy province, 24 patients (33-73 years old) were identified as shift workers and another 27 patients were selected as control by matching them on gender, age, and years being sick. They were surveyed for chronotype (Horne-Östberg questionnaire) and total amount of daily sleep. Fasting plasma glucose, glycosylated hemoglobin A1c, triglycerides, total cholesterol (TC), low and high density lipoprotein values were extracted from medical charts collected during a 1-5 year period between 2009 and 2014. ANOVA was performed and mean test (Tukey) for a significance level of $p < 0.05$ and $p < 0.10$.

The results show that DM2 male shift workers slept 1.15hr less (6.44hs vs 7.59hs) and have higher TC levels (228mg/dl vs 204mg/dl) compared to male controls. When chronotype was considered, morning types DM2 shift workers had the highest levels of them all (257mg/dl). No statistical difference was found for other biochemical parameters or between females, probably due to the sample size. Working on shift affected the DM2 patient's metabolism as reflected in the increased level of TC; probably due to chronically sleeping and eating at abnormal circadian times. The morning type (with poor adaptation to night shift) showed the highest TC levels and, therefore, they are more susceptible to suffer a cardiac event.

A42

WNT/ β -CATENIN SIGNALS IN THE EFFECTS OF *Smallanthus sonchifolius* (YACON) ON ADIPOSE TISSUE OF OBESE RODENTS

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Adipocyte differentiation is regulated by transcription factors such as Peroxisome Proliferator-Activated Receptors (PPARs), which maintain mature adipocyte phenotype by regulating the expression of specific adipokines. Wnt growth factors regulate the maintenance and remodeling of adipose tissue, constituting an attractive target in developing therapies to combat obesity and its metabolic complications.

In the present study, the involvement of Wnt/ β -catenin in the anti-obesity properties of *Smallanthus sonchifolius* (yacon) roots were investigated in an experimental model of obesity induced by diet. Male Wistar rats were fed a standard-diet (SD) or high fat-diet (20% w/w)/fructose (10% w/w) (HFF), for 12 weeks. Then the rats were randomly assigned into the following groups: SD, SD+Y (340 mg yacon flour/kg bw), HFF and HFF+Y (340mg yacon flour/kg bw). Administration of yacon flour for 60 days significantly reduced body weight, food intake, feed efficiency and body mass index in the HFF+Y group compared to HFF. Serum triglycerides, glucose and insulin in HFF+Y animals were similar to SD and SD+Y animals. Yacon treatment significantly reduced the weight of perigonadal, perirenal and visceral adipose tissue. Histological study showed a reduction in the number and size of adipocytes from HFF+Y group compared to HFF. Serum levels of leptin and adiponectin were normalized in HFF+Y animals improving insulin sensitivity. Yacon treatment was associated with a marked decrease in Wnt3a expression, decrease in AKT phosphorylation and increase in PPAR γ expression in adipose tissue of HFF+Y respect to HFF animals. Our results show that the Wnt/ β -catenin signaling is involved in the anti-obesity effects yacon regulating PPAR γ gene expression.

A43

BENEFICIAL EFFECTS OF *Smallanthus sonchifolius* (YACON) ROOT IN DIABETIC INTESTINAL DYSFUNCTION

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Diabetes is associated with gastrointestinal complications characterized by nausea, vomiting, early satiety, bloating and abdominal discomfort or pain. It has been established that nutrition has an impact on the physiological and pathological functions of the gastrointestinal tract. In this work we investigate the effect of *Smallanthus sonchifolius* (yacon) roots as a dietary supplement in an experimental model of diabetes in rodents. Male Wistar rats were randomly assigned into the following groups: control, control supplemented with 340 mg flour yacon/kg bw (control+Y), diabetic (STZ) and diabetic supplemented with 340 mg of flour yacon/kg bw (STZ+Y). Diabetes was induced by an intraperitoneal injection of streptozotocin (50 mg/kg bw). Rats with blood glucose levels >350 mg/ml were selected as STZ group. The administration of yacon flour for 30 days did not modify the levels of glucose, insulin, total cholesterol and HDL-cholesterol in STZ+Y animals. However, the levels of triglycerides and LDL-cholesterol were significantly reduced in this group. Although body weight of STZ+Y animals did not change significantly compared to STZ group, the food intake decreased slightly. Treatment with yacon flour kept the size and weight of small intestine and colon of STZ+Y animals within normal values. Interestingly, the cecum showed marked hypertrophy in all groups of animals that consumed yacon. These changes were accompanied by an increased substance P and decreased Vasoactive Intestinal Peptide (VIP) expression. The histological analysis showed that yacon consumption prevents alterations in the size and number of enteric ganglia of STZ+Y animals. Our results indicate that treatment with yacon roots prevents the development of morphological and functional changes in the diabetic intestine.

A44

EFFECT OF LOW DOSES OF LITHIUM CHLORIDE ON ANGIOGENESIS

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Previous studies have shown that low doses of lithium chloride (LiCl) stimulate the *in vitro* proliferation and migration of endothelial cells, through the activation of the Wnt/beta-catenin pathway. The aim of the present study was to assess the *in vivo* effects of therapeutic and subtherapeutic doses of LiCl on angiogenesis, by using zebrafish (*Danio rerio*) as an animal model. Embryos 24 h post-fertilization were incubated for 48 h at 28.5°C in six well plates containing 5 mL of embryonic medium (control) or embryonic medium enriched with different concentrations of LiCl (0.1 mM, 0.2 mM, 0.5 mM and 1 mM). Three repetitions were performed with 30 embryos per treatment. The embryos were processed for subsequent analysis of the subintestinal vascular plexus by means of enzyme-histochemical identification of endogenous alkaline phosphatase. The results showed that the therapeutic (0.5 mM, 1 mM) and subtherapeutic (0.1 mM, 0.2 mM) doses of LiCl did not affect the normal embryonic development of zebrafish. We observed that only 0.2 mM LiCl had proangiogenic capacity *in vivo*, stimulating the neoformation of vessels in the subintestinal plexus. These findings may be relevant in regenerative medicine of vascularized tissues since LiCl would act as an inorganic angiogenic agent at a dose below the therapeutic one for humans.

A45

ORTOPANTOMOGRAPHY: A COMPARATIVE STUDY OF DENTAL AGE ACCORDING TO THE TABLES ON CARMEN NOLLA, DERMIJIAN Y HAAVIKKO

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Introduction: An indicator to determine more accurately the dental age has long been sought after. The models proposed by C. Nolla, Haavikko and Dermijian are the most used. Dermijian, assigns a numerical value to each tooth element, which in addition, relates to a conversion table that depends on sex. It's different from Carmen Nolla that places the stadium which already has a value assigned. Haavikko is based on estimating the dental age of four teeth with their corresponding face development. **Objective:** To determine the correlation between the methods of C. Nolla, Dermijian and Haavikko with chronological age of the child in Rx View. **Materials and Methods:** The study was cross-sectional. The sample consisted of 50 children who underwent Panoramic digital images. The variables were: sex, age range 5-7, stadiums evaluated radiographically with Carmen Nolla, Haavikko and Dermijian tables of tooth maturation. Statistical analysis of the data obtained allowed a descriptive analysis of variables. To compare the chronological age with the three methods, coupled Test "t" was used. To compare the differences between the methods we used ANOVA. We worked with a significance level of 5%.

Results: 60% of patients are female. Significant differences between chronological age with the 3 methods are observed. (Paired t test, p <0.0001). C. Nolla-Dermijian show no significant differences (ANOVA P <0.5282), but Haavikko does (ANOVA P <0.0001). **Conclusion:** The method C. Nolla- Dermijian, would be suitable for calculating tooth age with respect to Chronological Age, according to the results, while Haavikko is approximate.

A46

MECHANICAL PROTECTION OF POST EXTRACTION CLOT TO PREVENT PHYSIOLOGICAL BONE RESORPTION. PRELIMINARY STUDY.

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Introduction: Once a tooth is extracted a physiological alveolar bone resorption process takes place in all its sockets, mainly the vestibular wall, which may affect both treatment and cosmetic results of rehabilitated patient. Alveolar ridge preservation depends on a non traumatic surgical technique and post surgical care and control. At present, there are many techniques to

retrieve alveolar bone level maintaining both optimal levels of width and height. The aim of this work was to keep the post extraction bone volume by creating a technique to protect the post surgical socket. **Methods:** After the extraction, a Vacuum plaque, previously made, was used by the patient for 30 days to protect surgical zone from masticatory impact, protecting therefore the blood clot. An immediate post surgical and a 30 day control TAC Conebeam (Siemens equipment - Germany) was done, which allowed us to compare bone volume changes by measuring both vestibular and palatal's wall height and the middle of the socket, using a software (Galileans, Germany). **Results:** **Case 1** (tooth 17) vestibular wall (VW): initial: 12.01mm; three months: 12.65mm; palatal wall (PW) initial: 12.92mm; three months: 14.79mm; alveolar height (AH) initial: 11.83mm; three months: 10.63mm. **Case 2** (tooth 48): VR: initial: 9.72mm; three months: 10.51mm; lingual wall (LW) initial: 11.09mm; three months: 11.22m; AHinitial: 11.13mm; three months: 10.27mm. Tooth 38 (Control): VR: initial: 11.10mm; three months: 11.61mm; LW: initial: 11.63mm, three months 11.68mm; AH: initial: 12.05mm; three months: 10.97mm. **Conclusion:** From the measurements made, we can conclude that sockets' preservation using this technique minimizes bone loss allowing us to maintain adequate bone levels for rehabilitation.

A47

MORPHOLOGY OF MAXILLARY MOLARS. CONE-BEAM STUDY

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Knowledge of the internal anatomy is essential to decrease endodontic failure produced by preparation and incomplete obturation of root canal. The objective of this research was to study *ex vivo* the internal and external morphology of maxillary molars using Computed Tomography Cone Beam. Sixty extracted human maxillary molars were selected, first (N=30) and second (n=30). The teeth were scanned for an evaluation of root number and configuration, root canal number and configuration according to Vertucci classification; mesiobuccal (MB) and mesio palatal (MP) root canal distance and pulp chamber roof and furcation distance. A significant greater frequency of three separated roots in first molars (60%) with respect to the second ones (27%) was observed. Three melting canals (17%), four (3%) and two separated roots (3%) in second molars only (p=0,007) was found. A 40% frequency of two joint roots and one disjoint in the first molar and 50% in the second molar was observed. The most frequent joint was the three thirds of MV and DV roots (33.33%) in the two groups. 53,33% of the first molars and 33% of the second ones had a fourth root canal (MP); 46.66% had three root canals in first molars and 56.66% in the second molars. In the first molars, all with three roots, root canal shape in order of frequency was type VI in MV root canal and type I in DV y P. In second molars with three roots type I was for MV root, Type V for DV and type I for P. The average distance between MV y MP root canals in first molars was 1.90 mm and in second molars was 2.10mm, while between pulp chamber roof and the furcation zone was 2.49mm in the first molars and 2.30mm in the second ones. The Computed Tomography Cone Beam allows the visualization of the complex radicular anatomy of root canals in a volumetric form.

A48

INTEROBSERVER MATCHING IN SERIAL DIGITAL X-RAYS IN PATIENTS WITH PERIODONTAL DISEASE

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Introduction: The Serial Digital Rx is used in Periodontal Diagnosis to detect changes in the amount of remaining bone. **Objective:** To determine the interobserver agreement in viewing Digital Rx patients with periodontal disease serials (EP), by observing radiographic signs. **Material and methods:** The study evaluated 66 Rx Serial Digital 3 calibrated examiners who observed variables: age, gender, and other signs of the disease. This was classified according to the severity of alveolar bone in categories: mild, moderate and severe. Periodontal widening, bone height ≥ 2 mm insertion loss and bone loss. Other visible signs: occlusal trauma, pathology, Furcation lesion and radiographic technique. Each teacher performed the analysis of variables separately. Statistical tests and Fisher Kappa exact test were used. **Results:** Serial Digital Rx 66 was analyzed. 67% were female and 33% male, mean age 46 years. Images of the total studied: in 41% the way was severe, 31% moderate, 21% mild and 5% had no disease. In 86% of the mild form had periodontal widening, whereas the severe form only 2% of

cases. 81% (n = 63) had bone loss below 2 mm; vertical bone loss was found in 73% of cases and 92% had a horizontal loss. 32% had trauma, 25% had periodontal disease. Radiographic technique still adequate in 86% was also evaluated. Relating the bone height variables with EP (n = 63) no significant association (Fisher exact test) $p = 0.004$. Vertical Bone Loss periodontal disease (Fisher test, $p = 0.56$) Disease periodontal Horizontal Bone Loss (Fisher test, $p = 0.13$) (no significant association) $p > 0.05$. When analyzing the correlation between examiners, it is low, between 2 partial observers with the three variables. Conclusion: The agreement is subject to wide variation, being low, interobserver, there would be no visual training in the recognition of radiographic signs.

A49

DESCALCIFICATION AND FLEXURAL STRENGTH OF HUMAN DENTIN AFTER ENDODONTIC IRRIGATING SOLUTIONS

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Introduction. Endodontic irrigating solutions produce structural changes on dentin, affecting its mechanical properties. The **aim** of this study was to evaluate *ex vivo* the effect of endodontic irrigating solutions, using them alone and combined, on human dentin decalcification and flexural strength and to determine if there is a correlation between them. Materials and Methods. 78 middle third dentin segments were used, obtained from mandibular single-rooted recently extracted premolars: 18 dentin discs were used to determine the concentration of calcium ions by absorption spectrometry; and 60 dentin bars (1x1x10 mm each), to determine flexural strength using three point bend test. Each specimen was immersed for 5 min at 37°C in 1 ml of different solutions: distilled water (DW), 1% sodium hypochlorite (NaOCl), 1% citric acid (CA), 17% EDTA, 1% citric acid (CA) + 1% sodium hypochlorite (NaOCl) and 17% EDTA + 1% sodium hypochlorite (NaOCl). Results were analyzed using one-way ANOVA, Tukey test and correlation test. Results. EDTA and CA used alone or combined with NaOCl, extracted significantly most amount of calcium ions compared with DW or NaOCl ($p \leq 0.05$). NaOCl, used alone or consecutively with CA or EDTA, significantly decreased dentine flexural strength ($p \leq 0.05$). There was no significant difference between EDTA and the control group ($p \geq 0.05$). There was no correlation between flexural strength decrease and dentin decalcification. Conclusion. Reduction on dentin flexural strength would not be related to dentin decalcification.

A50

APICAL FORAMINA IN UPPER FIRST MOLAR. SCANNING ELECTRON MICROSCOPE STUDY. CONTRIBUTION TO MORPHOLOGICAL RESEARCH AND DENTAL ANATOMY AND ENDODONTIC AREA.

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Introduction: The apical third of the root is considered a critical area for its complex anatomy, with side ducts, fittings, side, isthmus, and apical deltas inter root canals. Bacteria, byproducts and endotoxins are located through foramina through which the periapical region is reached. The aim of this study was to determine the amount of apical foramina, its location according to the root canal face and distance to the apical end of the mesio-buccal root of the maxillary first permanent molar. Material and Methods: 34 mesio-buccal roots of maxillary first molars were used, were cut transversely 14 mm from the anatomic apex, after preparation were observed with Scanning electron microscope with a 35 X. Photographs were taken, which were digitized and transferred to a computer where the images were subjected to analysis SOPRO IMAGING Software Program. Results: showed the roots of 1-6 foramina per root, finding them at an average of 1.12 mm apical end distance, its most frequent location was the distal face 46%, followed with decreasing values of 25% mesial, 16% palatal and 14% vestibular. Conclusions: The highest percentage of foraminas was on distal face, and then with decreasing values the mesial, palatal and vestibular. The total number was 81 foraminas. Average distance of the anatomical apex foramen was 1.12 mm.

A51

PREEMERGENT HERBICIDES IN CHIA (*Salvia hispanica* L.) WEED CONTROL vs. PHYTOTOXICITY.

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Chia is a species grown by pre-Columbian cultures and actually its culture area is increasing in northwestern Argentina because of the rediscovery of its properties - fiber content and high concentration of essential fatty acids. There is not enough information about the use of pre-emergent herbicides for weed control, especially in the first stage of the cycle, in which the culture is not competitive with weeds. In order to evaluate the effectiveness of pre-emergent herbicides and its potential phytotoxicity to the crop, a trial was carried out in the experimental field of the Facultad de Agronomía y Zootecnia - Univ. Nacional de Tucumán. Herbicides: Flurochloridone, linuron, trifluralin, diflufenican, pendimethalin, s-metolachlor, sulfentrazone, imazethapyr and imazethapyr + commercial mixture Saflufenacil + imazetaphyr were used. Visual evaluations were performed at 7, 14 and 28 days after application. Preliminary results indicate that flurochloridone, diflufenican and linuron perform weed control close to 70% with 10 to 15% levels of phytotoxicity, whereas sulfentrazone, imazethapyr and imazethapyr + Saflufenacil mixture made weed control close to 80% but with high levels of phytotoxicity, increased to 50%. The need is proposed of studies about dose adjustment of herbicides that perform adequate weed control as well as crop phytotoxicity. **Keywords:** chia – preemergence herbicides – phytotoxicity.

A52

STUDY OF THE EFFECT OF PLANTING DATES ON PHYSIOLOGICAL CHARACTERISTICS OF CICA QUINOA BEANS GROWING IN HIGH VALLEY IN TUCUMÁN, ARGENTINA

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Quinoa is the seed of herbaceous plant, though it is considered a grain; its most important potential in Argentina is food for celiacs and diabetics. The effect of two planting dates in physiological characteristics of quinoa grain was studied, Cica in Tafi del Valle, Tucumán, at 2000 masl. Planting was done on 15/11 and 05/12/2011. The seeds were harvested in April and May 2012, respectively; then threshed and stored at 6°C chamber. In July 2014 they were determined at 100 seed Weight Starting Fresh (WsF), fresh finish Weight (FfW) at 24pm. with water absorption, germination power (GP%) and water absorption index (WAI)% = $FfW - WsF / FfW \times 100$ in seed samples in the two planting dates, with eight repetitions. The results showed in the 1st planting date: WsF 0.297g average; FfW 0.796g; IAA of 168.235% and 95% average PG. In the 2nd planting date of 0.260g PFi; Pff 0.563; WAI of 87.84% and 0% PG. These results indicate that in the 2nd planting date showed GP% grain stress due to the effect of early frosts in the grain's phenological time of milk stage that damaged the seed embryo and/or produced a state of immaturity in the embryo 12.46% significant reduction in WsF seed relative to the 1st planting date and grain lacking germination for later use as seed and/or food; this seed absorbed water which is a necessary condition, which demonstrated it is insufficient to produce germination. It is concluded that the optimum planting date of quinoa cv. Cica is recommended November at the agro ecological conditions of the assay site.

A53

EVALUATION OF QUINOA CULTIVARS IN AMAICHA DEL VALLE, TUCUMÁN

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Quinoa is a food grain that is widely cultivated in the Andes from Colombia to northern Argentina in the conditions of high mountains. There is little technical information about growing quinoa in Amaicha del Valle, Tucumán. The objective of the experience was to evaluate the agronomic requirements of the crop yields and cultivars cycle quinoa (*Chenopodium quinoa* Willd.), Cica and Inga Pirca in Amaicha del Valle ecosystem to 2000asl Tafi, Tucumán, between 2013/2014. The average

annual temperature is 15.6°C and annual rainfall 160.1 mm. A test with a statistical design of BCR, with 8 replications was used. Each plot consisted of four rows, 3 m long, spaced 0.50 meters between them. Sowing was done manually in November, with a density of 6-8 kg/ha in order to obtain a density of 15 plants per linear meter. One pre-sowing irrigation furrow was made. Weed control was performed by manual weeding. The duration of the complete cycle in each cultivar was recorded. Harvest was done manually and subsequent drying sheaves of grain threshing, and then cleaning and weighing of each plot. No differences ($P < 0.05$) for yield (kg/ha) between varieties (836.53 kg/ha and 1016.58 kg/ha to Inga Pirca and Cica respectively) were found; with respect to cycle, Cica had a mean value of 173 days (early) from emergence to harvest time, while Inga Pirca stages completed in a period of 180 days (middle), with significant differences between the two cultivars, with p value less than 0.0008 0.05 significance level. No presence of disease was detected in both cultivars. It was determined that quinoa has differentiated production relative to crop cycle according to the evaluated varieties with high yield potential and quality healthcare for low incidence of diseases under the agro ecosystem Amaicha del Valle, Tucumán.

A54

THE EFFECT OF (2R)-6-HYDROXY-TREMETONE ON WALNUT TREE-INFESTING INSECTS

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The culture of walnut tree is a major source of income for the small and medium-sized companies of La Rioja and Catamarca. Crop yields depend, among other factors, on the phytosanitary condition of plots. The use of synthetic pesticides lowers the quality of marketable products (in-shell and husked walnut) and produces serious environmental imbalance. Current research tends to seek natural bioactive products, of microbial or plant origin. *Plodia interpunctella* (Lep.) larvae constitute a major pest for stored nuts. Previous studies revealed that (2R)-6-hydroxy-tremetone, an isolated secondary metabolite of the *Asteraceae* family, is able to control various species of mites affecting olive trees.

The aim of this paper was to study the lethal and sub-lethal effect of low-dose (2R)-6-hydroxy-tremetone on *P. interpunctella* larvae fed on crushed nuts. Toxicity bioassay on *P. interpunctella* larvae were carried out, with low-dose tremetone-imbued diet (50, 100 and 200 mg/L). Morphological and quantitative death rate and weight observations were analyzed statistically according to Infostat v. 2013.

As regards larvae weight, significant differences emerged between those treated with (2R)-6-hydroxy-tremetone 200 mg/L (17.08 ± 3.81) and the control group (14.65 ± 7.68), according to the Shapiro-Wilks and Friedman tests. Death rate with 50 and 100 mg/L doses was 9% and 11% respectively. In both treatments abdomen and prolegs malformations were observed and color changes that resulted in death of insects at pupal stage.

These preliminary results show that (2R)-6-hydroxy-tremetone in low doses has sub-lethal effects on *P. interpunctella* larvae. Future histological studies will allow us to identify the target tissues and organs of this compound.

A55

EFFECTS OF AERIAL PARTS EXTRACTS OF *Ipomea cairica* (CONVOLVULACEAE) ON *Oryzaephylus surinamensis* (COLEOPTERA)

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We study *Ipomea cairica* (L.) Sweet, a weed commonly found in antropoficed environments. Previous studies revealed the presence of lignans, synthesized by *I. cairica* in defense against predators. Our aim was to investigate the effect of both flowers (EEF) and leaves (EEH) extract added to the natural diet of *O. surinamensis* beetle, a pest of stored nuts. 500 mg/L for each extract in two separate tests was added to 10 g of natural diet. To evaluate dietary preference five containers were used, a central container and four located around connected to the first. In the central container 40 unsexed adults of *O. surinamensis* were released; in two of them was placed 3 g of treated diet and the rest the same amount of control diet. The bioassay was assessed for 48 h. After this time, preference values were determined using $IP = (IDT\% - \% IDC) / (\% IDC +$

IDT); where: % ITD = % of insects in the treated diet; IDC = % insect in control diet. Results were estimated as follows -1.00 and -0.10 repellents; IP between -0.10 and +0.10 IP neutral substance and IP between +0.10 and +1.00 attractant substance. Mortality was assessed by a bioassay of forced intake of 20 adults per extract with their respective controls. In both tests the results were: IP: 0.77 for EEF (attractant) and EEH: -0.68 (repellent). EEF was more toxic (55% mortality) with respect to EEH (35%). From these results we concluded that EEF is attractant and toxic to *O. surinamensis*. Both products can be used as natural insecticides of low environmental impact.

A56

WEEDS PRESENT IN THE CHÍA CROPS IN THE PROVINCE OF TUCUMÁN, ARGENTINA.

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Chia (*Salvia hispanica* L.) is a crop of American origin belonging to the Family of *Lamiaceae*. Its seeds are known for their beneficial properties for the body due to its high content of omega 3 fat acids, antioxidants and dietary fiber that slow cellular aging and prevent cardiovascular disease. The crop was introduced in Tucumán in 2007 and its implementation is considered an interesting alternative of production in the NOA. One of the main problems of chia plant is the low competitive capacity of the crop against weeds during the early stages of development, and when competing for longer periods, yield is reduced up to 90%. The species is very sensitive to herbicides commonly used, which is why farmers must use mechanical control. There is a group of weeds that turn this control difficult, because they have similar characteristics to chia, mainly when they are in a vegetative state. This study's objective was to identify weeds present in the chia crop, differentiate and characterize the species that present exomorphological and reproductive structures similar to the cultivated species. Surveys were performed in fields located in three different agro-ecological zones of the province (campaigns 2013-2014). Weed species present in the culture were collected, and identified by using botanical keys. They were also described considering easily observed characters in the field.

Registered species were: *Spheralcea bonariensis*, *Conyza bonariensis*, *Amaranthus quitensis*, *Sonchus oleraceus*, *Solanum nigrum*, *Ageratum conyzoides*, *Physalis angulata*, *Chenopodium album*, *Medicago lupulina*, *Carduus toermerii*. Species with features similar to chia are: *Verbena bonariensis*, *Verbena hispid*, *Leonurus sibiricus* and *Glandularia peruviana*. An accurate identification of species will help producers to deal with serious drawbacks that come up when trying to control weed manually.

A57

SEED GERMINATION REQUIREMENTS OF THREE NATIVE SPECIES OF CHACO SERRANO

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In view of the continuing loss of native forests in the province of Córdoba, reforestation of degraded areas with native species is presented as a need to restore plant diversity. This encourages the study of germination requirements of these species, for successful seedling production. The aim of this study was to evaluate physical and physiological characteristics of seeds of two tree species "Orco Quebracho" *Schinopsis marginata* (sp1) and "Tala" *Celtis ehrenbergiana* (sp2) and a shrubby "Tramontana" *Ephedra triandra* (sp3) and requirements for its germination. Seeds of sp1 and sp2 were collected from "Valle de Punilla" (30°50'S-64°30'W.), while seeds of sp3 were obtained from "Valle de Calamuchita" (31°50'S-64°32'W). Collection dates: sp1- sp2: 05/14; sp3: 01/14. Moisture content and weight of 1000 was determined according to ISTA (2012). The germination trials were conducted between papers at 25°C with an 8/16 h (L/D) photoperiod (4 replicates x 25 seeds each). Three treatments were assessed on sp1: T1, control; T2, moist scarification; T3, removal of the wing. Two treatments were carried out on sp2: T1, control; T2, removing part of the fruit. Three treatments were performed on sp3: T1, control; T2, moist scarification; T3, mechanical scarification. Seeds of sp1 had a low germination percentage (<10%), with no significant differences between treatments. They showed high fungal contamination, which was attenuated in those previously soaked. However, this treatment did not improve germination. Seeds of sp2 reached a high germination rate (>

80%) and pre-germinative treatments did not increase that value. Germination rates of sp3 were 33% and 31% for treatments T1 and T3 respectively. The moist scarification increased this percentage to 50%.

A58

ESTIMATION OF SEED VIABILITY IN TWO GENOTYPES OF *Stevia rebaudiana* BERT.

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Stevia rebaudiana Bert is an herbaceous plant from Asteraceae family that has steviol glycoside of high sweetness power. It is a very important natural source of non-caloric sweets. It is used as an alternative to sugar substitutes. It has hermaphrodite flowers in apical or axillar capitulum. Some authors suggest the existence of variable levels of ecotypes with viable pollen. The fruit is an achene of wind-dispersion. Two types of achenes are found, black and light, being the black fertile and the light unfertile. One of the reasons of infertility could be the sporophyte self-incompatibility. Under mono-crop condition the great scale seed production would not be possible in this species which is why it is vegetatively multiplied or by micro-propagation. The objective of this work is to perform an estimation of the seed viability of two genotypes of *Stevia rebaudiana* Bert. The material came from Perico (Jujuy) and Cerro Azul (Misiones). The determination of viability was done with tetrazolium test to dark seeds because the light ones had no embryos. The viability results were of 62.5% (Jujuy) and 80% (Misiones). There are some reports of very low germination rate genotypes, these results are encouraging because they permitted to obtain plantlets for doing a further evaluation and selection of the more promissory genotypes.

A59

ADAPTABILITY OF INTA ORNAMENTAL VARIETIES OF THE NATIVE *GLANDULARIA* GENUS IN FAMAILLA (TUCUMÁN), ARGENTINA

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Glandularia is a native genus of Argentina with high ornamental potential. The Floriculture CNIA-INTA Institute (IT) has a breeding program on this genus. With the aim of evaluating the adaptability of national varieties obtained from IT, demonstratiojn plots were implanted in EEA Famaillá-INTA Tucumán. The national varieties 'Extrema Roja INTA', 'Natali Rosa INTA' and 'Alba INTA' were evaluated. The plant materials were prepared as rooted cuttings cultivated in plugs and then transplanted to N° 12 pots. Ten plants were planted on 17-07-2013 in the field, at double alternate rows distance 35cm and 70cm width, with drip irrigation and black plastic mulching. The characters evaluated were: plant cover, plant height, flower/plant number, plant diameter and flowering; and the data were recorded every 15 days till 07-03-2014. The three varieties reached full cover at mid-October. The flowering started for Alba and Extrema Roja varieties in August and for Natali Rosa in October. The varieties Extrema Roja and Natali Rosa had a prolonged flowering till 08-08-2014. The number of flower/plant was 80, 66 and 27 for Natali Rosa, Alba and Extrema Roja. The Alba, Natali Rosa and Extrema Roja varieties recorded 47, 41.5 and 55 cm height. The diameter plant was 140, 270 and 200cm respectively for Alba, Natali Rosa and Extrema Roja. These result indicated that the national varieties have a good performance for border use in Tucuman region.

A60

DETERMINATION OF THE EQUATION OF THE CURVE OF DISSIPATION OF IMAZALIL IN LEMON THROUGH THEORETICAL MODEL OF TWO AREAS

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The pesticide imazalil is used in the production of lemon in the stage of postharvest to combat fungi of different genres. Toxicological tests with imazalil carried out in animals showed symptoms such as lack of muscle coordination, shaking,

dermatitis and vomiting. The maximum limit value of residues of imazalil in lemon established by the Codex Alimentarius is 5 mg/kg and is the same limit used in Argentina. In this work the curve of dissipation of imazalil in lemon from Tucuman Province is determined experimentally, in simulated conditions of transportation. A mathematical model of two areas is proposed to determine the equation of the curve of dissipation. Imazalil was applied during the process of packing. The simulation of the conditions of transportation took place retaining the sample for 2 days at room temperature, then in cold storage chamber. Samples were taken 0, 1, 2, 7, 14, 21, 28 and 35 days after application and analyzed by HPLC. The mathematical model of two areas has an excellent fit of the curve of dissipation, with a correlation coefficient equal to 0.98. The lemon fruit presented a concentration of imazalil that does not exceed the maximum limit established in Argentina and by the Codex Alimentarius.

A61

STUDY OF THE CONDITIONS OF CHEMICAL POTATO PEELING IN THE PROCESS OF PRE-FRIED FROZEN POTATOES

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The Fruit-Horticultural Sector is mainly characterized by processes based on the seasonality of production, because of this, it presents vacancy months in their facilities and equipment, implying certain consequences. As an alternative solution to this problem, it was proposed to process potatoes and freeze them, in that period, since a potential export market was detected. In this sense, a Packaging company that process strawberries, blueberries and peaches was studied.

To make production of pre-fried frozen potatoes effective, it is necessary to define the operating conditions of processing taking into account the peculiarities and potato varieties produced in the NOA. From the different process stages, it was considered that the peeling of potatoes was the most important one, due to its contribution to the final quality of the frozen potatoes. An appropriate method of chemical peeling was adopted. The objective of this work was to study the operating conditions of this process: caustic soda concentration and temperature adjusted to the operating parameters of the peeling Packaging studied. Concentrations between 2% and 10% of caustic soda, and temperatures of 80, 85 and 90°C were used in the study, determining in each case the peeling time. From the data it was determined that in the working range of studied temperatures, there is no statistical difference in the peeling time; however statistical difference was observed in concentrations, where the amount of 10% is the one that achieves potato peeling in the residence time of the machine to be used in the manufacturing process. In conclusion it is possible to perform chemical peeling of potatoes with caustic soda at 10%, in the range of temperatures of 85-90°C at a residence time of 5 minutes.

A62

PHYSIOLOGICAL CHANGES IN LEMON FRUITS GROWING ON DIFFERENT CANOPY POSITIONS

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The canopy microclimate, especially solar radiation level and temperature, influences the appearance, quality and productivity of fruits, so canopy position seems to play a predominant role in biochemical parameters of both the skin and pulp of fruit. The aim of this study was to evaluate fruit size as well as contents of both photosynthetic pigments and UV-B absorbing compounds in flavedo of fruit grown inside and out of the plant canopy.

Good-sized lemons, located inside (shaded) and outside (sunny) canopy were selected as plant material. PAR radiation was recorded in both top and equatorial region of shaded and sunny fruits over a 42 days period. Photosynthetic pigments and UV-B absorbing compounds (CAUV-B) were spectrophotometrically measured in both solar-exposed (E) and nonsolar-exposed (NE) lemon flavedo.

Growing lemons (0-12-d) were green whereas 42-d old lemons were yellow. The equatorial perimeter of shaded fruits significantly increased between 0 and 42 days of experimental period. Total chlorophyll and carotenoid contents of both sunny and shaded lemons were higher in E than in NE, at 0 and 12 days, while at ending (42 days) strongly decreased. The

absorption spectra of lemons photosynthetic pigments were markedly different between 0 and 42 day. CAUV-B content was higher in sunny E lemons compared with shaded ones. Interestingly shaded lemons did not show significant differences between E and NE flavedo surfaces.

Total chlorophyll and carotenoid contents did not depend on the canopy region. Highest levels of CAUV-B in sunny lemons indicate an increased carbon flow and energy investment for solar radiation protection which could be associated with lower fruit development.

A63

FOLIAR ANATOMICAL CHANGES IN SOYBEAN PLANTS UNDER WATER STRESS

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The foliar characteristic could be decisive for plants survival under water deficit conditions. Through evolution, plants have responded to water stress by developing physiological, morphological and anatomical adaptations. The aim of this study was to identify anatomical changes in leaf of *Glycine max* varieties subjected to hydric shortage. We worked with control and stressed plants after 21 days. Leaves fixed in FAA (formalin, Acetic acid, ethyl alcohol) of 5 individuals per treatment (control and stressed) of A8000 (tolerant), Munasqa (tolerant) and TJ2049 (sensitive) were analyzed. Transparence and transverse cuts of leaflets and petioles were made. For staining different dyes were used. The observations were made with an optical microscope Leica DM500. Trichome density on both epidermis increased in Munasqa and A8000 stressed varieties, while TJ2049 remained similar to the control values. Stomatal density decreased in TJ2049; increased in Munasqa and unchanged in A8000. The leaflets' thickness decreased markedly in A8000 and mild in Munasqa variety, but in TJ2049 thickness increased. Changes in petioles at vascular bundles were observed. In Munasqa and A8000 varieties, beams size decreased and lignification increased, while in TJ2049 variety lignification was lower. The diameter of xylem vessels decreased in all varieties; however, the number of vessels was lower in A8000 and Munasqa. The results coincide with tolerance/sensitivity description for each variety. The most sensitive characters to stress conditions, indirectly evaluated using test coloring, were: pubescence, stomatal density and lignification vessels.

A64

EFFECT OF SALINE STRESS ON BARLEY PLANT COLONIZED WITH ENDOPHYTIC FUNGI FROM LAGUNA BRAVA (LA RIOJA-ARGENTINA)

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Laguna Brava is a natural freshwater body located in the La Rioja province (4200 m.a.s.l.). Plants of this arid/semiarid region are subjected to stress conditions such as extreme temperatures, salinity and high UV radiation. A strategy used by plants in this region is the beneficial association with soil fungi. Thus plants obtain benefits in relation to nutrient absorption and resistance to biotic and abiotic stresses. The aim of the present work was to determine the effect of salt stress on the growth and concentration of photosynthetic pigments in barley (*Hordeum vulgare*) seedlings colonized with endophytic fungi from a highland lake.

Barley seed were germinated in pots containing sterile soil inoculated with 3 endophytic fungi isolated from the roots of *Puccinellia parlatore*, collected in Laguna Brava lake. Pots were irrigated with 0 or 150mM NaCl, substrate salinity was measured by conductivity. Control plants were not inoculated. Plant emergence, leaf development, biomass and photosynthetic pigments were measured.

Strains used were identified as H1 sterile mycelium, H10 sterile mycelium and *Alternaria* sp (H4). Salinity decreased the emergence and leaf length by 17 and 35%, respectively; while increased the total chlorophyll content by about 18%. The H1 strain increased the plant emergence by 40% in presence of NaCl, but delayed leaf development compared with the control. In presence and absence of salt, H4 increased the leaf number and photosynthetic pigments contents. The H10 strain decreased the seedling emergence by 35%. Obtained results showed that barley plants exhibit different responses to endophytic fungi and salinity. Results could also suggest that H1, H10 correspond to different fungi species.

A65

ORGANIC SOLUTES CONTRIBUTION TO THE OSMOTIC ADJUSTMENT IN *Prosopis alba* G. UNDER SALINE STRESS

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The algarrobo blanco (*Prosopis alba* G.) possesses high quality wood, edible fruits, and forage-apt leaves; it is highly saline-tolerant due to its ability to perform osmotic adjustment though osmocompatible solutes have not been reported as taking part in the process. This work aimed at determining the contribution of organic solutes to such osmotic adjustment in seedlings of *P. alba* under saline stress. Two week old seedlings were sown in the presence of 0, 100, 200, 300, 400 or 500 mM NaCl solutions. Their osmotic potential (Ψ_s); osmotic adjustment and soluble sugars, proline, and glycinebetaine concentrations were quantified 14 days later. An entirely randomized experimental design with four repetitions was used and the results were analyzed using the test of Kruskal-Wallis. The saline treatments brought about Ψ_s decrease and osmotic adjustment increase. The organic solutes contribution to osmolarity decreased as the saline concentration in the solution increased. While these solutes represented 29% of the osmolarity in the control, their contribution in seedlings incubated in 500 mM NaCl solutions was of just 18%. The contribution of proline was lower than 1% in all these treatments and though the contribution of soluble sugars diminished with saline stress, it was this solute the one that accumulated in higher concentrations (26% of the osmolarity in the control, 11% in 500 mM NaCl solution). The glycinebetaine increased its contribution responding to stress ranging from 2% for the control to 7% for the maximum saline concentration. It is concluded that soluble sugars and glycinebetaine are organic solutes involved in the osmotic adjustment in seedlings of algarrobo blanco under saline stress.

A66

CORRELATIONS BETWEEN PHOTOSYNTHESIS AND LEAF ANATOMY IN “QUINOA”

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Quinoa (*Chenopodium quinoa* Willd, Chenopodiaceae) is a C3 species with a photosynthetic assimilation (PA) ranging between 14.8 and 34.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (González *et al.*, 2010). PA depends on biochemical and morpho-anatomical factors of the leaf. The relationship between maximum PA (Amax), stomatal conductance (gs), perspiration (Tr), internal CO₂ concentration (Ci), carboxylation capacity and leaf anatomy, stomatal density (ED), stomatal size (ES), tissue thickness (TT) and specific leaf area (SLA) were studied. Seven quinoa varieties, cultivated in Amaicha del Valle, 22° 31'S, 65° 59'W, 1985 m asl, Tucumán, Argentina, were analysed. Kancolla, Sayaña (Bolivian Altiplano, 3850 m), Quinoa blanca (Cochabamba, Bolivia, 2,570 m), Quinoa roja (Cangrejillos, Argentina, 3,700 m), CICA (Peru, grown in Argentina at 2000 m) and CO-407 (Chile, 140 m). All varieties show amphistomatic leaves. The ED was negatively correlated with the size of the stomata. A significant correlation was found between total ED, Amax ($r= 0.854$) and carboxylation capacity ($r= 0.855$). The SLA was negatively correlated with the thickness of the leaf blade ($r= -0.620$), particularly the palisade mesophyll ($r= -0.767$). Stomatal conductance (gs), Tr and Ci showed no correlation with ED. Amax was greater in isolateral leaf varieties. The presence of amphistomatic leaves, isolateral or bilateral mesophyll with well developed palisade tissue, high Amax and ED, indicates adaptations to environments with high irradiation and low humidity, pointing Quinoa as a species of high tolerance with phenotypic plasticity to adapt to a wide range of environments.

A67

MORPHOLOGICAL STUDY OF THE CORATINA VARIETY OF OLIVE TREE (*Olea europaea* Surges L.) IN THE POMAN DPT. PROVINCE OF CATAMARCA

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In the province of Catamarca the cultivation of the olive tree has grown notably, the varieties present a great capacity for adaptation to the region. The botanical characteristics of the variety coratina cultivated in the Pomán area have been evaluated. This study has been considered pertinent because the morphological characteristics are related with the variety, the ecological and cultural conditions.

We carried out a quali-quantitative study. The population study is all the plants of coratina olive tree variety cultivated. A random sample was obtained. The analysis units were each one of the plants, chosen at random; being the observation units their vegetative structures (tree and leaves). For the morphological characterizations the outline has been used proposed by Barranco & Rallo (1984) modified by Tous Martí and Romero Aroca (1993), corresponding nine vegetative characters: four from tree and five from mature leaf.

All plants present a more vigorous growth in their arboreal aspect, in connection with their similar ones analyzed for example in other countries of the world like Italy, where behavior of half vigor was registered (world catalog of olive tree). With regard to the leaves, it is evidenced that coratina shares the same foliaceous characteristic that the opposing ones in the Central Valley of Catamarca (Romero BN *et al* 2012).

According to the results we can conclude that the plants of the variety coratina, offer a remarkably vigorous growth, which could have direct relationship with their adaptation to the prevailing favorable environmental conditions in the region.

A68

PRELIMINARY STUDY ON Cr(VI) REDUCTION BY EXTRACTS FROM *Salvinia minima* FRONDS AND LACINIAS

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The resistance to Cr(VI) of terrestrial and aquatic living organisms (animals, plants, bacteria, fungi, etc.) is wide and varied with different mechanisms to limit and/or reduce its toxicity. Among these mechanisms, widely distributed, the reduction of Cr (VI) to Cr (III) by both enzymatic (redox type enzymes) and non-enzymatic (chemical) pathways seems to be very important. In several bacteria and fungi the presence of a NADH dependant chromate reductase activity (CR) specific and highly efficient to reduce the Cr (VI) has been demonstrated. Although this activity has not yet been demonstrated in plants, recently, from *Arabidopsis thaliana* leaves a NADPH dependant quinone reductase activity (NQR) has been isolated which is able to reduce Cr (VI). In this way the existence of a CR activity acting parallel to non-enzymatic reduction of Cr (VI), can also be possible in plants accumulating high chromium concentrations. The aim of this study was to analyze the presence of a CR activity in floating (fronds) and submerged (lacinias) leaves of *S. minima*, a floating Cr(VI) hyperaccumulator fern. CR activity was assayed in frond and lacinia extracts obtained with phosphate buffer 0,2 M, pH 7,0 using NADH as electron donor. Incubations were performed at 37 °C for 30, 60 and 120 min. CR activity was estimated by the determination of remaining Cr(VI) in reaction mixture with 1,5-diphenylcarbazide. Results showed an increased Cr activity in lacinias, where the Cr (VI) is mostly accumulated. Based on obtained results it can be concluded that there would be a CR activity in *S. minima* leaves, but more studies are needed to fully confirm its presence in plants.

A69

APPLICATION OF COMPLETE FACTORIAL DESIGN FOR OPTIMIZATION OF CULTURE MEDIUM FOR Cr(VI) REMOVAL BY INDIGENOUS YEASTS

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Chromium pollution is increasing incessantly due to continuing industrialization. Of various oxidation states, Cr(VI) is very toxic due to its carcinogenic and mutagenic nature. Microbial reduction of toxic chromium offers a potential cost-effective bioremediation approach. In order to reduce operating costs in a large scale process, composition and concentration of the components of a culture medium were studied. Complete factorial design was employed to optimize Cr(VI) removal (1 mM initial concentration) at 24 h by indigenous yeasts *Cyberlindnera jadinii* M9 and *Wickerhamomyces anomalus* M10. The interaction between four variables (SO₄(NH₄)₂; CaCl₂; NaCl, and inoculum) for M9, and three variables (sucrose, KHPO₄, and inoculum) for M10 were studied and modelled. In both strains, total removal of Cr(VI) was achieved at 24 h. Also, removal time at 12 h was optimized. The optimum combination predicted was confirmed through experiment, whereby maximum removal of Cr(VI) reached at 12h was 73.91% and 87.32% for M9 and M10, respectively. In the case of M9, NaCl could be excluded from the medium formula because it did not present any effect on Cr(VI) removal. The final composition of optimized medium for M9 is (g/L): sucrose, 60; KHPO₄, 1; yeast extract, 1; Mg(SO₄)₂, 0.5; SO₄(NH₄)₂, 1.05; CaCl₂ 0.12; inoculum, 20% and for M10 (g/L): sucrose, 90; KHPO₄, 1.2; SO₄(NH₄)₂, 1.2; yeast extract, 1; Mg(SO₄)₂, 0.5; CaCl₂, 0.1; NaCl, 0.1; inoculum, 20%.

These results strongly suggest that Cr(VI) removal could be reached in a short time indicating that this economic medium formulated could be used in developing a bioremediation process at large scale.

A70

ISOLATION AND IDENTIFICATION OF *Caesalpinia gilliesii* BIOACTIVE METABOLITE

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In this work, antimicrobial activity of *Caesalpinia gilliesii* leaf infusion (CGI) was studied. Previous reports about this activity were not found. The **aims** of this work were to: evaluate CGI and methanolic fraction (MF) antibacterial activity (ABA) over pathogenic ATCC bacteria isolated from skin wounds; determine CGI and MF toxicity; purify and identify bioactive compounds through bioguided procedures. CGI showed ABA over the tested bacteria. CGI lethal concentration 50 (LC₅₀) was less than 1000 µg/mL of extracted material (EM)/mL, showing lack of toxicity. During bioguided purification, MF presented ABA in puntual bioautography assays. Seven fractions (FI – FVII) were obtained through column chromatography-Sephadex-LH20. FIII and FIV were chemically analyzed and two quercetin glycosides were identified: quercetin-3-O-rutinoside and quercetin 3-glucoside. FI showed ABA over *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 by bioautography. The purification and identification of the compounds responsible of the ABA are under study. *C. gilliesii* is presented as a potential source of antibacterial compounds against pathogen bacteria.

A71

METABOLISM OF ISOCHROMAN BY BIOTRANSFORMATION WITH FILAMENTOUS FUNGI

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Isochroman and its derivatives are substances of natural origin, such as compounds obtained by the cultivation of microorganism and of plant origin. The increasing studies during the last 20 years indicated the undoubted value of the

isochroman ring as the structural basis of a large number of biologically active compounds. In the present work we present the methods used to catalyze the biotransformation of isochroman using two experimental conditions: growing and resting cells with strains of *Aspergillus flavus*, *A. terreus*, *A. niger* and *Cunninghamella* spp. Three bio-transformation products from the selective oxidation of isochroman at C-1 were purified from the bio-reaction media and their structures were elucidated by spectroscopic methods and mass spectrometry. The technique coupled with gas chromatography was used to analyze the time course of the bio-transformation. The use of cytochrome P450 enzyme inhibitors, as well as working under poor oxygen conditions allowed us to inquire about the type of enzymes involved in the process. The results also establish that *A. niger* was the best biocatalyst for the diversity of compounds obtained. The bio-transformation with *A. flavus* showed the presence of two compounds from substrate, whereas other filamentous fungi gave a high yield of a particular metabolite. Finally, these results provide tools to handle bio-reaction methods and particularly to choose the proper incubation times to obtain better yields of a particular metabolite. Bio-catalytic development described here can serve as a starting point to optimize conditions so as to obtain isochroman derivatives through such environmentally friendly procedures.

A72

ANTIFUNGAL ACTIVITY TESTING OF PLANT EXTRACTS IN *Aspergillus* SAMPLES

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This study was aimed at testing antifungal activity of plant extracts of *Flourensia blakeana* Dillon compared to *Aspergillus ochraceus* and *Aspergillus parasiticus*. *Aspergillus* samples were isolated in the Laboratory of Food Microbiology at the UBA, from paprika samples taken in the Calchaquí Valley (NW Argentina). Extracts were prepared with dry aerial parts sprayed in different solvents (chloroform, ethanol, acetone and hexane). Antifungal activity was determined through inhibition of radial growth method in trays, using as a purpose medium potato dextrose agar (PDA), with various extract concentration (10, 50,100 and 200µg/mL) and seeded with the *Aspergillus* species. In order to test antifungal activity, 7-day-old discs of the *Aspergillus* samples (5mm diameter) grown in PDA trays were worked on. Results obtained show that, in the case of chloroform extract, this presented a moderate activity as compared to *A. parasiticus* and a significant inhibitory activity over *A. ochraceus*. Then, as to ethanol, acetone and hexane extracts, they also showed an important inhibitory effect over *A. ochraceus* and a low activity over *A. parasiticus*. As a conclusion, *A. ochraceus* turned out to be the most sensitive family to the studied plant extracts and this allows for the prediction that this species of *Flourensia* can be of interest in the search for natural antifungal substances applicable to food preservation. Work is now on the isolation and identification of antifungal compounds.

A73

ANTIFUNGAL ACTIVITY OF EXTRACTS FROM *Amphilophium cynanchoides* ON *Aspergillus niger* AND *Aspergillus carbonarius*

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The *Aspergillus* black rot causes grape contamination with mycotoxins such as ochratoxin A. There is a need for new molecules to control this disease. In this work, the antifungal activity of leaves and stems of *Amphilophium cynanchoides* was evaluated on growth of *A. niger* y *A. carbonarius*. Aerial parts of *Amphilophium cynanchoides* collected in Trancas (Tucumán) were separated in stems and leaves, grounded and extracted with a sequence of solvents (dichloromethane, ethyl acetate and methanol). The organic extracts were evaporated to dryness, and a known amount of each dry residue was dissolved in ethanol, obtaining the fractions fChCl₂, fAcet and fMeOH, respectively. The last ones were assayed by the micro-dilution method in YES semi-liquid medium (2500-2.4ppm) on 10⁴ conidia/mL. Fungal biomass was determined by reading absorbance at 620nm. The IC₅₀ values of fChCl₂, fAcet and fMeOH from leaves and fMeOH from stem were higher than 2500ppm for both fungal species. The fChCl₂ and fAcet from stems showed CI_{50s} of 2178ppm and 1631ppm on *A. niger* and no activity on *A. carbonarius*. Our results indicated that the stem extracts of low and middle polarity contained allelochemicals with selective antifungal activity on *A. niger*.

A74

**ANTIFUNGAL ACTIVITY OF 4-HYDROXY-3-(3-METHYL-2-BUTENYL)ACETOPHENONE
AGAINST *Candida albicans***

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C. albicans is opportunistic fungal pathogen, and a common cause of invasive fungal infections in humans, producing infections that can involve any organ. The most used drug for candidiasis is fluconazole (FLU), an azole drug with fungistatic action, an aspect that favors the development of drug resistance. To overcome drug resistance a combination of drugs is suggested to be used. The main secondary metabolite of *Senecio nutans* Sch. Bip (Asteraceae) aerial parts is 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (4HMBA), preliminary reported as antifungal, which could be an alternative for fungal infections treatments, alone or in combination with FLU. 4HMBA antifungal activity against *C. albicans* (ATCC10231) was preliminary evaluated by disk diffusion assay with and without FLU (6 mg/L) to statistically analyze synergistic, additive or antagonist effects. Minimal inhibitory concentrations (MICs) and fractional inhibitory concentration (FIC) of 4HMBA and FLU (alone and combined) were obtained by microdilution assay. The interpretation of FIC was: ≤ 0.5 , synergistic effect; >0.5 to < 4.0 , additive effect; ≥ 4.0 , antagonistic effect. There were significant differences ($p < 0.05$) between the growth inhibition zones diameters for 4HMBA against *C. albicans* with and without FLU (13.0 ± 0.2 mm and 8.0 ± 0.1 mm respectively). The MIC for 4HMBA (without FLU) was 40 mg/L and the FIC=0.133, which indicated synergistic effect between 4HMBA and FLU. As 4HMBA turns FLU action from fungistatic to fungicidal, this combination could overcome the occurrence of fungal resistance derived from FLU fungistatic action.

A75

**ELUCIDATION OF THE PHYSIOLOGICAL ACTIVITY OF LEAF METABOLITES FROM *Schinopsis*
ON *Fusarium graminearum*, ETHIOLOGICAL AGENT OF CEREAL EAR ROTS**

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Schinopsis species from northwest Argentina contain alkylcatechols and terpenoids with inhibitory activity on mycelia growth and spore germination of *Fusarium*. The action mechanism of these substances could be different or similar to that of commercial fungicides. The aim of this work was to characterize the impact of these metabolites on physiological processes of *Fusarium graminearum*. Fractions ALK (mix of alkylcatechols), LUPf (lupeol) and M1 (a mix of alkylcatechols and lupeol) were obtained from leaves of *Schinopsis lorentzii* and *S. marginata*. The effect of these fractions (250, 125, 62 y 31 $\mu\text{g/ml}$) was evaluated on suspensions of 10^6 macroconidia/ml on *F. graminearum* after 4, 12 and 24 hs. Spore germination and production of H_2O_2 were evaluated at each time. Production of deoxynivalenol (DON) was determined at the same concentrations after 7 days of fungal growth in trichothecene inducing liquid medium. Prothioconazol was included as positive control. At sublethal dosis, ALK, LUPf and M1 inhibited DON production. With respect to the negative control, ALK and M1 produce a decrease in the release of H_2O_2 while LUPf had no a significant effect. Prothioconazol, at the same concentrations and conditions, increased DON accumulation and H_2O_2 production. ALK, M1 and prothioconazol did not change the spatial pattern of emergence of the macroconidial germ tubes. The antifungal activity of alkylcatechols and prothioconazol was related to a break in the growth dominance exerted by the end cells in the macroconidia. However, the mentioned phenolic lipids had a different mechanism of antifungal activity because they did not trigger an oxidative burst as prothioconazole did during or before DON production.

A76

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST SPOILAGE AND PATHOGENIC MICROORGANISMS ASSOCIATED TO TOMATO PURÉE

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The new trend in food industry is the use of natural preservatives to replace traditional methods. Application of essential oils (EOs) is a very attractive method for controlling postharvest diseases and spoilage in fruit and vegetables. The aim of this study was to evaluate the antimicrobial activities of different concentrations (50-300 ppm) of EOs against several microorganisms isolated from tomatoes and belonging to collection cultures. Antimicrobial activity of lemon, oregano, onion or garlic EOs was tested against *Escherichia coli* ATCC 25922, *Klebsiella ornithinolytica*, *Listeria monocytogenes* and *Candida tropicalis* by the paper disc agar diffusion method. First, the sensorial acceptability of the EOs was evaluated in TP by an untrained panel using a hedonic scale of four points. Lemon EO was deemed the most acceptable for all tested concentrations except 300ppm, while onion EO was not acceptable under any condition. Garlic EO did not show, in general, antibacterial activity being only active against *C. tropicalis* while all tested microorganisms were sensible to lemon and oregano EOs at 300ppm, which was considered organoleptically unacceptable. Among them lemon EO was the only capable of inhibiting the growth of *E. coli* ATCC 25922, *K. ornithinolytica* or *L. monocytogenes* at 150-200ppm. At these concentrations the organoleptic contribution of lemon EO to the flavor was satisfactory. In conclusion, lemon EO may be useful as natural and safe additives for promoting the safety and quality of minimally processed fruit products.

A77

STUDIES OF RESISTANCE OF *Aspergillus niger* TO THE TOXICITY OF Cr(VI)

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The resistance of microorganisms to Cr(VI) benefits environmental biotechnology because they are employed for remediation of waste, water and metal-contaminated soils. The objective is to study the resistance of *Aspergillus niger* to the Cr(VI) and toxicity on the growth of the fungus during the remediation of contaminated waste.

We studied: 1) resistance of the fungus to the Cr(VI) (5; 10; 20 and 50mg/L). 2) effect of Cr(VI) (50mg/L) on: a) protein profile PAGE-no denaturant and unfolded protein. b) production of polygalacturonase and naringinase. In the assay lemon pulp was used as a substrate and waste contaminated with metal, then added, g/g: urea (0.12); (NH₄)₂SO₄ (0.25) and KH₂PO₄ (0.07); 2.10⁶ conidia/mL of *A. niger* and incubated at 30°C for 96h. Growth controls were carried out in pulp without Cr(VI). *A. niger* developed at all concentrations of Cr(VI) without showing alterations in the mycelium. Macroscopically early black coloration of the conidia occurred (48h) compared to control (72h). Fungal culture developed with Cr(VI) showed that: a) the concentration of protein (0.22mg/L) was 12% lower; b) processing the PAGE — no denaturant showed fewer protein bands and, 3) poligalacturonase and naringinase, production declined, 24 and 85%, compared to the control.

In conclusion, *A. niger* is resistant to the Cr(VI) at the concentrations tested and can be used in bioremediation processes. The presence of the metal and not the concentration induced conidia early maturation, whereas minor alterations at the level of protein content were observed with 50mg/L of Cr(VI), but was significant on the synthesis of naringinase.

A78

ANTIBACTERIAL ACTIVITY OF AERIAL PARTS OF *Aspidosperma quebracho-blanco*.

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Aspidosperma (Apocynaceae) comprises 24 species distributed from México to Argentina. Several species of *Aspidosperma* showed antimicrobial activity. The antibacterial activity of *A. quebracho-blanco* was not yet characterized. In this work, the effect of aerial parts of *A. quebracho-blanco* was evaluated on several phytopathogenic bacteria. Leaves and stems of *A. quebracho-blanco* were separated and ground. These materials were sequentially extracted with dichloromethane, ethyl

acetate and metanol. The organic extracts were evaporated to dryness and known amounts of the dry residues were dissolved in ethanol, obtaining fractions fCH₂Cl₂, fAcet y fMeOH, respectively. The last ones were assayed by the microdilution method with Mueller-Hinton semiliquid medium. (4000-2,9 ppm) on strains of CECT 225 (*Erwinia carotovora* var *carotovora*), CECT 124 (*Pseudomonas corrugata*), CECT 126 (*Pseudomonas syringae* pv *tomato*) and *Agrobacterium tumefaciens* using suspensions of 10⁷ UFC/mL. The IC₅₀ was calculated by probit analysis. Ciprofloxacin was used as positive control (250-62 ppm). The CI₅₀s of fCH₂Cl₂, fAcet and fMeOH from stem, and fCH₂Cl₂ and fAcet from leaves were higher than 4000 ppm for *Agrobacterium tumefaciens*, *Erwinia carotovora* and *Pseudomonas syringae*. The fMeOH from leaves showed IC₅₀ of 3261 ppm on *Pseudomonas syringae*. In the case of *Pseudomonas corrugata*, the IC₅₀ of fCH₂Cl₂ from stems and leaves, and fAcet and fMeOH from leaves were 2460, 3138, 2728 and 3258 ppm, respectively. The isolation and identification of the bioactive molecules is in progress.

A79

ANTIFUNGALS FROM *Zuccagnia punctata*: POTENTIAL OF CHEMOSENSITIZATION OF ANTIMICOTIC ACTIVITY OF FOOD GRADE PRESERVATIVES

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The use of food preservatives offers difficulties in the food industry including undesirable effects on microbial processes involved in food production and alterations in sensorial characteristics of food products. For this reason, its use and doses must be reduced. The aim of this work was to evaluate the joint action of leaf chalcones from *Z. punctata* on growth of *Fusarium* species. Ethanolic extracts from leaves of *Z. punctata* were partitioned with diethyl ether. The ethereal extract was fractionated by column chromatography in silica gel. The chalcones were detected in the fractions eluted and analyzed by TLC and visualization with NP/PEG+UV-365 nm. Purification was completed with HPLC, and identification was confirmed with GC-MS and UV-VIS spectra (200-600 nm). The bioactivity of the chalcones alone and in mixtures with salts of organic acids (sodium sorbate, calcium propionate and sodium benzoate) was determined by the chessboard assay on *F. graminearum sensu stricto* and *F. verticillioides*. The LC₅₀ of chalcones and their binary mixtures with the xenobiotics was determined in lethality test of *Artemia salina*. The 2',4'-dihydroxychalcone (DC) and 2',4'-dihydroxy-3'-methoxychalcone (DMC) were purified from the leaves. Both chalcones showed synergic interactions with the salts of organic acids. Nevertheless, the highest synergistic activity was obtained for DMC. The binary mixtures synergistic were not toxic. The chalcones potentiated the antifungal effect of the food preservatives on *Fusarium*, indicating that they exerted different antifungal mechanisms from those of the xenobiotics. The absence of toxicity of the mixtures suggests that the chalcones can be used as additives of the salts of organic acids in foods, a situation that allows to reduce the doses of these xenobiotics required to inhibit *Fusarium*.

A80

STEROID-INDUCED MEIOSIS IN *Rhinella arenarum* OOCYTES: MPF ACTIVATION

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There is controversy about the role of steroids in amphibian oocyte maturation and no direct evidence exists of progesterone (P₄) being the natural inducer-steroid. However, androgens may induce oocyte maturation in other taxa. The oocyte maturation is regulated by Maturation Promoting Factor (MPF). The pre-MPF activation depends on its dephosphorylation by cdc25 phosphatase that catalyzes its conversion to active MPF. This work analyzes the effect of various steroids on *in vitro* maturation of *R. arenarum* oocytes and the participation of MPF in this process.

Mature females of *R. arenarum* harvested throughout the year in Tucumán. Dose (10⁻⁹-10⁻⁵ M) and time response curves with oocytes or follicles in the presence of different steroids (P₄, T = testosterone, DHEA = dehydroepiandrostenodione, AD = androstenedione, and P₅ = pregnenolone) were performed. The MPF participation on steroid-induced oocyte maturation was analyzed by inhibition of the cdc25 phosphatase with NaVO₃ (0.1-1.0 mM) and through cytoplasmic microinjection experiments.

The results indicate that the tested steroids induce follicle and oocyte GVBD in a dose and time dependent manner. During the breeding season P₄ and P₅ were the most effective drugs (EC₅₀ = 7 and 25 nM respectively). During the non-reproductive

period oocyte response to all steroids was markedly lower. While the response to androgens was variable in no case did it exceed that induced by P_4 . Inactivation of *cdc25* with $NaVO_3$ inhibited GVBD induced by steroids in a dose-dependent manner. Microinjection of cytoplasm matured with DHEA, AD or T induced maturation of immature oocytes. In *R. arenarum* oocytes the androgens may induce resumption of meiosis and can activate the MPF. Nevertheless P_4 sensitivity is higher, especially during the breeding season.

A81

EFFECT OF STEROID HORMONES ON AMPHIBIAN OVIDUCTAL SECRETION

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In *Rhinella arenarum* we determined variations in the secretion profile of proteins and glycoproteins throughout the reproductive cycle. We demonstrated that the 74kDa (gp74) glycoprotein present in the secretion and in the jelly surrounding the oocytes is highly diffusible and participates in fertilization. At present the endocrine regulation of the secretion of proteins and glycoproteins that constitute the oocyte jelly is unknown. The aim of this study is to analyze by SDS-PAGE electrophoresis the influence of steroid hormones estradiol (E_2), progesterone (P), dihydrotestosterone (DHT) and testosterone (T) on the secretion profile of the PC in castrated and control adult females (with and without hormone treatment). The results revealed that with P at the ovisac level and with DHT in the PC, the secretion of all the proteins and glycoproteins that form the jelly surrounding the oocytes was stimulated. The basal level of secretion of gp74 increased with P and DHT treatment. With P, but not with DHT, secretion was abundant and represents a dense, opalescent, sticky material which is stored in the oviductal ovisac. In the PC the secretion induced by this hormone is scarce, watery and practically with no proteins. In castrated untreated animals, the profile revealed the presence of gp74 and of a non-diffusible 300 kDa glycoprotein (gp300). Similar data were obtained with E_2 . With this hormone we observed that, when the secreted material was abundant, it became organized, forming a clear thin stringy mucus, with hydration capacity, characteristics which are similar to those of human cervical mucus in the preovulatory stage. T is not involved in secretion. These unpublished results in amphibians demonstrate that the jelly secretion of proteins and glycoproteins is regulated by P and DHT. The arrangement of the jelly matrix would depend on factors possibly regulated by E_2 .

A82

ULTRACYTOCHEMICAL OF CALCIUM AND Ca^{2+} -ATPase IN AMPHIBIAN TESTIS

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The aim of this work was to evaluate the subcellular localization of calcium (Ca^{2+}) and Ca^{2+} -ATPase activity in adult *Leptodactylus chaquensis* testis from the breeding season. For subcellular localization of Ca^{2+} , testis samples were fixed in a solution of potassium pyroantimonate 2% and osmium tetroxide 1% (pH 7.8). They were then washed, dehydrated in alcohol series and acetone 100% and embedded in epoxy resin. For localization of Ca^{2+} -ATPase, the samples were fixed in glutaraldehyde 1% - paraformaldehyde 1% in cacodylate buffer 0.1M (pH 7.4). The testis sections were incubated in the presence of EGTA 10mM, washed in cacodylate buffer without EGTA and incubated in medium containing glycine-KOH 250 mM (pH 9.0), ATP 3mM, $CaCl_2$ 10mM as activator, lead citrate buffer 2 mM and L-tetramisole 2.5 mM as alkaline phosphatase inhibitor. Samples were postfixed with osmium tetroxide 1%, dehydrated in a series of alcohols and acetone 100% and embedded in epoxy resin. The observations, made with transmission electron microscope, showed in the sperm Ca^{2+} deposits in acrosome and subacrosomal space and in axoneme of the flagellum. Ca^{2+} -ATPase activity was revealed in plasma membrane, the inner and outer acrosomal membranes and the mitochondria. In the Sertoli cells, deposits of Ca^{2+} were observed in the nucleus, cytoplasm, mitochondria and lamellar bodies. In the same cells, the Ca^{2+} -ATPase activity was detected in the plasma membrane. The results suggest that the Ca^{2+} -ATPase, located in sperm head, would be involved in Ca^{2+} sequestration in order to prevent acrosome reaction, while the presence of Ca^{2+} in the axoneme would be related to the flagellar movement. In the Sertoli cells, mitochondria and lamellar bodies would represent Ca^{2+} deposits.

A83

EFFECT OF A SESQUITERPENE LACTONE (DHL) ON THE ACROSOME REACTION OF SPERMATOZOA OF *Chinchilla lanigera*

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The Dehydroleucodine (DhL) is a sesquiterpene lactone isolated from *Artemisia douglasiana* Besser. The DhL induces exocytosis of cortical granules (CG) in amphibian oocytes by a process dependent on the release of calcium from intracellular stores and has been used to activate protoplasts during cloning in bovine, where they are also involved in the GC. Likewise, the acrosome reaction (AR) is a calcium ion dependent phenomenon, for this reason, the aim of this work is to study the effect of DhL on the acrosome exocytosis, epididymal sperm of *Chinchilla lanigera*.

The samples obtained by puncture of the caudal epididymis region from 8 adult males, were washed and trained in THFm oven for 2.30 h with 0.5% CO₂ in air at 37°C, and then treated with DhL (0.1-2mM) at different times (10-40min). Viability, motility pattern and AR were analyzed in all treatments. The reactions were stopped with 4% formaldehyde and 2.5% glutaraldehyde and then processed using Coomassie Blue staining and SEM

Results: The viability of the samples showed no significant changes in treatments with 0.1mM DhL with different incubation times. At higher concentrations cell viability decreased in connection with incubation time. Simultaneously the assessment of the pattern of motility recorded significant decreases in the times of incubation concentrations of 0.5; 1 and 2mM DhL. With respect to the action of DhL on the AR, the results indicate an inducer of the same effect even with the lowest concentrations tested.

DhL has an inducing effect of AR, however, it has a negative effect directly related to cell motility, which does not affect sperm viability to the same extent. Being the first time that DhL was used as an inducer of the acrosome reaction, further studies would be necessary to assess the cellular mechanisms involved in their action.

A84

EFFECT OF NERVE STIMULATION IN THE AMPHIBIAN OVIDUCT SECRETION

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Previous work in our laboratory showed that the *Rhinella arenarum* oviduct is innervated by the autonomic nervous system. Based on the above, the aim of this study was to determine the effect of nerve stimulation on the secretory cells of the oviduct of adult females captured during the breeding period of the species. For this, same animal was used as control and experimental. Once oviducts were exposed one of them was stimulated with an electrical stimulus of an intensity of 7mA, a frequency of 10Hz for 1min on nerves that run through the mesentery, while the control duct was preserved of the stimulus. Subsequently pars convolute (PC) samples of the two oviducts were taken and then fixed and processed following routine techniques for transmission electron microscopy.

Observations have revealed that in the stimulated oviduct: a) secreting epithelial cells, loaded with secretory granules, are located near the surface and even protrude into the lumen of the duct. Secretion of these cells includes flocculent material and granules. b) The glandular secretory cells release only secretory granules many of which show an electron-dense core. c) The release of cell contents would be carried out mainly by merocrine mechanism, however apocrine or holocrine secretion was observed also in some cells. d) At the duct lumen level the secretion exhibits structured materials (granules of various shapes, sizes and electrodensity with and without dense core) as well as unstructured ones with flocculent appearance. Ultrastructural analysis allows us to suggest that stimulation of autonomic nerves innervating the oviduct promotes the release of contents from both epithelial secretory cells as well as glandular secretory cells in the oviductal mucosa.

A85

MYOCARDIAL AND KIDNEY ANATOMO-PATHOLOGICAL EVALUATION IN AN ARSENIC AND LEAD EXPOSED EXPERIMENTAL MODEL

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There are environment cumulative toxins, such as lead and arsenic, capable of generating risk, and cardiovascular disease. They begin this process of disease causing endothelial dysfunction and cardiovascular risk. **Objectives:** to evaluate changes in myocardial and kidney anatomo-pathology in a lead and arsenic intoxicated experimental model. **Material and methods:** we worked with adult white Wistar rats with n=6 each group: one treated with lead acetate in drinking water at a concentration of 1000ppm; group 2: treated with 0.1ppm of arsenic in drinking water; group 3: 1000ppm of lead acetate with 0.1ppm arsenic in drinking water, and a control group. Determination of lead in blood and arsenic in urine were done. Hematoxylin-eosin technique for optic microscopy was used to evaluate myocardial and renal histological alterations.

Results: Blood lead: 16.5 ± 3.8 µg/dl, qualitative determination of arsenic in urine: positive. Animals exposed to 1000ppm found in kidney: congestion with hyaline deposits in tubules, hemorrhage in myocardium. Group 2 in kidney: congestion with extravasation of red blood cells without necrosis and chronic focal hemorrhage without necrosis myocardium. Group 3: kidney congestion with minor bleeding. In myocardial coagulation necrosis focalised with extravasation of red blood cells. Control group: preserved myocardial and kidney histoarchitecture.

Conclusions: this study shows that lead associated with arsenic concentrations similar to those found in endemic areas, produces necrosis which produces great myocardial damage. On the other hand, both toxins are capable independently of causing myocardial and kidney damage.

A86

APPENDIX CECAL TUMORS (ACT): CLINICAL PATHOLOGICAL ANALYSIS OF A RARE INJURY

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Background: ACT is a rare condition and most of the time, an intraoperative finding. They correspond to 0.4 to 0.5% of all gastrointestinal tumors and prognosis depends on the type of tumor. The most common malignant ACT are carcinoid (CT) occurring in 0.1-5% and adenocarcinoma (ADca) in 0.2% of all appendectomies.

Objectives: To analyze the characteristics of ACT and its clinicopathological forms of presentation.

Material and Methods: Type of study: retrospective, observational, longitudinal and descriptive. Population Studied: clinical (Sex, age -over or under 40 years, clinical presentation) and pathological features (type of tumor) of patients with biopsies from archives of the Pathology Department Hospital CSZ Santillán, a 14 year period (2000- July 2014). **Results:** 13 (0.15%) patients had diagnosis of ACT in 8,371 appendectomies identified in 27,737 biopsies; 10 were primary tumors (PT): 7/10 (70%) CT; 2/10 (20%) mucinous ADca and 1/10 (10%) Mucocele and 3 secondary (ST): (2 regional engagement of colon cancer and 1 metastasis of gastric cancer). In PT patients were: 7 women and 3 men; in ST patients were: 2 women and 1 male. Patients older than 40 years: 5/10 PT patients and 3/3 ST. All PT patients consulted for appendicular acute condition, ST patients were oncologic resections findings. **Discussion:** The reported incidence of ACT is very low and the macroscopic suspicion during surgery is the only thing of value to treat it, clinical symptoms require imaging studies. The most common clinical presentation and diagnosis in ACT patients is an acute appendicitis. Sometimes incidental findings in imaging tests performed in study of other diseases. Diagnosis is important to allow a proper treatment.

A87

DAMAGE IN SPERM INDUCED BY CADMIUM IN POISONING SUB-CHRONIC RAT MODEL

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This study was to analyse the first signs of toxicity of cadmium in rats' sperm exposed to low doses. Wistar rat were treated with 10 mg/kg CdCl₂ for 3 months. Each month the sperm was obtained from the epididymis tail to determine a) sperm viability with eosin stain b) sperm motility, being classified as immobile, progressively motile or non-progressively motile and c) sperm morphology with methylene blue and May-Grünwald-Giemsa and transmission electron microscopy (MET). The data was analysed by analysis of variance and with the method Student-Newman-Keuls. All statistical comparisons were performed with a confidence level of 95%. Animals treated during two months showed, at the MET level, sperm with aberrant mitochondria and displacement of outer dense fibers in relation to the central axoneme. A significantly higher percentage of abnormal sperm was evident during the 3rd month of treatment compared with controls. The motility of sperm from animals treated during three months showed a significant decrease in the straight progressive motility of sperm while there were increases in immotile sperm and with movement in situ with regard to the controls. The sperm viability in controls and treated animals showed no significant differences throughout the experimental period. In conclusion, from the second month of treatment with 10 mg/kg CdCl₂ the metal produces structural alterations in the tail of the sperm disturbing its motility.

A88

HISTOLOGY OF OVARIES AND OVIDUCTS -PREOVULATORY PERIOD- IN *Oreobates discoidalis* (ANURA, TERRARANA)

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The *Oreobates discoidales* species lays its eggs out of water in forest environments with direct development, metamorphosing juvenile without a tadpole stage; they are endotrophic. Ovaries and oviducts of mature females were fixed in 10% formalin in phosphate buffer at pH 7, dehydrated in ethyl alcohol battery up to 96° and transferred to n-butyl-alcohol. It was included in Histowax; 5µm cuts were stained with Hematoxylin-Eosin (H-E), Mallory trichrome, PA-Schiff (PAS) in combination with Alcian Blue (AB) at different pH for differential identification of glycoconjugates, Toluidine Blue (TB) with different pH. Ovaries with 4-6 vitellogenic follicles are distinguished: germinal vesicle in the animal pole, no nucleoli observed but chromosomes compacted. Acidophilic cytoplasm with yolk platelets of different sizes arranged in layers in follicles in more advanced state platelets forming compacted homogeneous yolk oocytes. We distinguish: vitelline envelope; granulose cells and thecas. Oviducts: granules with protein cephalic region; the remaining areas with acid glycoconjugates with metachromasia resistant alcohol; serous abundant collagen fibers and smooth muscle, vascular plexus in the mucosa. The direct development of this species involves: decreased number of follicles, homogeneous yolk, granulose and theca well developed, conspicuous vascularity of ovarian and oviductal; protein presence; acid proteoglycans perhaps to capture the ions spawning medium that would facilitate the development process. Histological examination of this species shows significant differences with others studied. This is important because it is a vulnerable species by anthropic activity.

A89

MACRO AND MICROSCOPIC STUDY OF *Chinchilla lanigera* OVIDUCT

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The oviduct is involved in the transport of gametes, sperm capacitation, fertilization and early embryonic development. The aim of this work is to achieve a macroscopic and microscopic description of the oviduct of *Chinchilla lanigera*. The oviducts from multiparous adult females were isolated, fixed and processed using routine histological techniques or for ultrathin sections; stained with hematoxylin-eosin and toluidine blue. The oviduct is a winding and opaque tube, surrounded by abundant adipose tissue and mesentery. Its length is 44-43 mm. The infundibulum is an incomplete C-shaped tube

surrounding the ovary and emits long processes or fimbriae, with an epithelium ciliated pseudo stratified. One can observe ciliated columnar cells with prominent elongated nuclei in central position and non-ciliated cells with apocrine secretion and rounded basal nuclei. The apocrine secretions are observed free and bound to the epithelium. The smooth muscle layer is sparse or absent. The ampulla is a hollow tubular structure whose light is fully occupied by large primary folds with numerous secondary folds. The epithelium is pseudo stratified ciliated similar to infundibular epithelium. The muscle layer is continuous, thin and circular. The isthmus has a narrow light and scarce primary folds. The epithelium is simple columnar in which ciliated and secretory cells interspersed. The secretory cells have abundant clear cytoplasm and secretory vesicles. The ciliated cells have narrower and darker cytoplasm. The muscular layer is well developed with an inner layer with circular fibers and an outer layer with longitudinal fibers. Based on this study we suggest that the macro and microscopic structure of the oviduct of *Chinchilla lanigera* would be similar to that described for other histricomorfos rodent.

A90

ULTRASTRUCTURAL STUDY OF THE OVIDUCTAL MORPHOLOGY OF *Tupinambis merianae*

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Previous anatomic-histologic studies of the oviduct of *Tupinambis* allowed us to determine the oviductal areas: infundibular area, uterine tube, uterine-tubal union, uterus, and vagina. The presence of spermatozoa in the area of the uterine-tubal union made us consider this zone as the storage place for spermatozoa, given the fact that in this species, copulation takes place while follicles are still in growing stages.

This current study analyzed the internal surface of the oviduct of the oviparous reptile *Tupinambis merianae*, using scanning electron microscope (SEM), and characterized the ultrastructure of the previously defined regions. For that purpose, a female specimen in reproductive phase was killed, 5 days after copulation. The oviducts were dissected and fixed in Karnovsky; they were processed with conventional technique for scanning electron microscopy, and they were studied with a JEOL 35 CF microscope. The whole length of internal oviductal surface showed the presence of cells with cilia and cells with microvilli, in different proportions. The infundibular area presented an epithelium with a high proportion of cells with cilia, which diminished towards the area of the uterine tube. The uterine-tubal union area showed a predominance of cilia cells, and spermatozoa could be seen in the oviductal folds. Cilia cells decreased in proportion in the uterine area. The vaginal zone showed the presence of secretory material. This study allowed us to observe the variations in the oviductal epithelium of *Tupinambis* with SEM, for the first time. The finding of spermatozoa in the uterine-tubal region would reinforce the previous proposal considering this the storage area for spermatozoa in this species.

A91

NUTRITIONAL STATUS OF THE SOUTH AMERICAN MALE FRUIT FLY *Anastrepha fraterculus* (Diptera: Tephritidae) AT TIME OF EMERGENCE

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Anastrepha fraterculus (Wiedemann) is an important plague that is distributed in southern U.S. and Argentina. One way to reduce the use of insecticides is the implementation of the sterile insect technique (which is based on the release of sterile males in order to mate with wild females and transfer sterile sperm). This requires a massive rearing where the nutrients provided in the diet of larvae are optimized. The aim was to compare the nutritional status of *A. fraterculus* males from a rearing laboratory (L) and from guavas collected in the wild (wild, W). For the study, individuals at -20°C on the emergence day without any diet were retained. Adults were dried at 50°C for 5h to obtain the dry weight. Glycogen level, sugar and lipid were measured by Van Handel's method, and total protein by the Bradford method. From each source 10 individuals were analyzed and means were compared by t test for independent samples. L males were heavier than W males. Protein concentration, sugars and glycogen were the same for both sources, whereas lipids were significantly higher in L males. The data reveal that when larvae ingest artificial laboratory diet (rich in lipid) obtained heavier individuals and with greater lipid load than those fed guava during the larval stage. The lower accumulation of lipid in S males is attributable to the chance of

getting this nutrient in nature during the larval stage. Data found for this species are novel to investigate the effect of this lipid reserve laboratory individuals on the reproductive success of sterile male way of contributing to the management of this plague.

A92

IMPACT OF DIET ON NUTRITIONAL STATUS IN MALES OF *Anastrepha fraterculus* (Diptera: Tephritidae)

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The South American fruit fly *A. fraterculus* (Wiedemann) is an important pest of fruits. A control tool is the Sterile Insect Technique (SIT), which requires that laboratory males compete with males of nature and transfer sterile sperm to wild females. The mating success of males depends on their nutritional status, however it is unknown how the rearing conditions affect the way flies manage their nutritional reserves. The effect of adult diet on the nutritional status of males of *A. fraterculus* was evaluated. Two sources: a wild (W, collected from guava) and laboratory (L) population were analyzed. Adults were fed for 15 days with 2 types of diet: sugar (S) or a mixture of sugar and yeast hydrolysate (S+P). Sugar, glycogen, lipids (Van Handel method) and protein (Bradford reagent) were determined. Three batches of 10 individuals for each case were analyzed. Data analysis consisted of a mixed model for each source where diet was the fixed component and batch was random. Diet had a significant effect on body weight. S+P fed males had higher dry weight for both sources. Differences for amount of proteins, sugar, glycogen and lipid between flies W and L were found. Protein content was higher in S+P fed L males, while diet did not affect the amount of sugar, glycogen and lipids. Protein content in W males was similar for both diets, while a higher content of sugar, glycogen and lipid in S fed males was observed. The existence of a differential nutritional assimilation capacity dependent on both, diet and individual source is suggested. It remains to explore which nutritional requirements contribute to increase reproductive success, in order to implement improvements in SIT programs.

A93

ISOLATION AND PARTIAL CHARACTERIZATION OF IMMUNOGLOBULIN G FROM SOUTH AMERICAN TAPIR (*Tapirus terrestris*) COLOSTRUM

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Newborn of all mammals depend on colostrum and milk of their mother to obtain nutrients and protective factors, to ensure proper development. Perissodactyla and other mammalian groups present diffuse epitheliochorial type placental structure that prevents the passage of large molecules from mother to fetus. Secretory IgA is the predominant immunoglobulin in the milk and colostrum of many species, but in hoofed mammals colostrum contains mainly IgG. The aim of this work was to isolate IgG from tapir colostrum and partially characterize them, in order to provide information about one of the major whey protein of this mammal. A pool of colostrum samples from 3 tapirs were use. A gel filtration chromatography, SDS-PAGE in reducing and non reducing conditions, western blots and immunodots were performed. Results showed that IgG is the dominant immunoglobulin in South American tapir colostrum. Analysis by SDS PAGE showed a protein band of 160 kDa, which is recognized by the anti horse IgG antibody. Under reducing conditions a heavy chain of 57kDa and a light chain of 27 kDa were demonstrated. From cross-reactivity assays, a slight positive reactivity with anti-rabbit IgG and anti-goat IgG was observed. These results allow to identify the IgG as the dominant immunoglobulin in tapir colostrum, partially characterizing their structure and suggesting that common conformational antigenic determinants were recognized. This finding corresponds to what happens in the milk of different species that do not transfer passive immunity to the offspring through placenta.

A94

**THE CHIA (*Salvia hispanica*) OIL CONSUMPTION IMPROVES ALTERATIONS
HAEMATOLOGICAL GENERATED BY A RICH CHOLESTEROL DIET**

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The aim of this study was to evaluate the effect of consuming chia oil (rich in ω 3 fatty acids) in the morphological alterations and the fatty acid composition and membrane fluidity of an animal model of hypercholesterolemia caused by a diet high in cholesterol. The rabbits were divided into 4 groups: 1) Fed with control diet (DC), 2) DC supplemented with 1% cholesterol (DH), 3) DC added with chia oil 10% (DC-CH), 4) DH chia oil added with 10% (DH-CH) for 6 weeks. After this period blood was taken and analyzed for plasma triglycerides (TG) and cholesterol (TC, LDL, HDL). Erythrocyte membranes were removed and fatty acids were measured by gas chromatography and membrane fluidity by fluorescence polarization. The blood smear and leukocyte formula was performed. The chia oil decreased TG levels (mg/dl) (DH: 222.3 ± 32.6 vs DH-CH: 91.7 ± 14) and the presence of acanthocytes (2% vs 10.15%), echinocytes (23% vs 60.55%) and anisocytosis (55.6% vs 83.16%) in rabbits with DH. Also rouleaux not characteristic of DH were observed. Adding chia oil decreased the fluorescence polarization (DH: 0.385 ± 0.033 vs DH-CH: 0.305 ± 0.01 vs DCH: 0.301 ± 0.008) and % saturated fatty acids (SFA) in erythrocyte membranes (DH: 70 vs DH-CH: 57 vs DC: 56) and increased the % of unsaturated fatty acids (UFA) (DC: 38 and DH-CH: 40 vs DH: 27) decreasing the SFA / UFA in DC: 1.5 and DH-CH: 1.6 vs DH: 2.6 and atherogenic index (AI) in DC: 0.82 and DH-CH: 0.83 vs DH: 1.78. In conclusion, the addition of chia oil protects erythrocytes of deleterious changes induced by hypercholesterolemia increasing membrane fluidity by incorporating UFA to it. This improves the morphology of erythrocytes.

A95

ENZYME IN PLASMA AND LIVER OF RATS TREATED WITH LOW-DOSE CADMIUM

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In mammals, the exposure to cadmium (Cd^{2+}) is common in low doses with variations in the activity of enzymes. This enzymatic variation is indicative of liver damage in plasma. Until now, the relationship between intra and extracellular activities enzymes has not been investigated. The objective of this work was to evaluate the aminotransferase aspartate (AST), aminotransferase alanine (ALT), alkaline phosphatase (FAL) and pseudocolinesterase (pChE) activities enzyme in plasma and liver of rats exposed to Cd^{2+} . Male Wistar rats were treated with 10 mg CdCl_2/kg administered orally for 3 months, as the control water distiller was used. Each month controls and treated rats were sacrificed. Blood was collected by cardiac puncture and plasma was obtained. Liver was dissected to prepare a homogenate followed by differential centrifugation. The enzyme activity was determined by colorimetric methods for AST, ALT and FAL, and kinetic method for pChE. The result were evaluated by variance analyze and post-test Student-Newman-Keuls. The Pearson coefficient was used to evaluate the correlation between variables. The statistical study was performed with $P \leq 0.05$. The result in liver homogenate showed an increased of AST and pChE activities enzyme from the first month while the ALT activity increased from the second month. FAL showed no significant differences in any of the periods studied. A significant increase in plasmatic level of AST and pChE was observed from the second month and from the third month an increase in ALT and FAL was also observed. A positive correlation was observed between AST, ALT and pChE activities in plasma and liver tissue. This result indicated that in liver treated rat the capacity of enzyme synthesis was altered, reflecting this effect in the plasma with a time delay.

A96

ANTIOXIDANT TREATMENT REVERSES INCREASED BASAL TONE CALCIUM DEPENDENT ON A MODEL OF HYPERTENSION BY CHRONIC DEFICIENCY OF NITRIC OXIDE

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In previous works we showed that the model of hypertension (HT) by chronic deficiency of nitric oxide (NO) LNAME (N-nitro-L-arginine methylester) rats (LR) basal tone (BT) blood was increased. **Objective.** Analyze in HT rat aortas by this model the effect of Ca-free medium, the action of antioxidants on the BT and levels of NO. **Methods.** LR were treated with: tempol (T): LR-T or Vitamin C: LR-VC for 6 weeks. Mean arterial pressure (MAP) was measured. Aortic segments were subjected to treatment with sodium nitroprusside (SNP) or removal of Ca from the incubation medium (without Ca ringer+EGTA: 0 Ca) able to assess unstimulated BT. Endothelial function (EF) was also measured in response to acetylcholine (AC) in pre contracted rings with noradrenaline, and nitrite levels (NL) by Griess reaction. Compared to control rats (CR). **Results.** There were no changes in weight of the rats, the intake of food and water, and creatinine clearance. LR developed hypertension. T and VC did not change the values of PAM in any case. LR showed higher response to SNP ($p < 0.001$). Similar results were obtained with 0Ca. In LR-T this response decreased. VC had less effect on BT ($p < 0.01$ compared to RL). In vitro administration of T, but not VC also produced BT decrease. The LR had lower response to AC (RC: -893 ± 225 mg vs RL: 19 ± 8 mg, $p < 0.001$). LR-T showed a partial reversal of this response ($p < 0.01$ compared to CR). LR had lower levels compared to RC nitrite ($p < 0.001$). Only LR-T showed similar NL to CR. **Conclusions.** Finding similar relaxing effects of SNP and 0 Ca indicates that increased BT in LR is due to an increase in intracellular Ca. In this alteration of BT (HT-dependent) there would be decrease of the bioavailability of NO, which would involve alterations in compensatory mechanisms of superoxide dismutase.

A97

HISTOLOGICAL ALTERATIONS OF RENAL MORPHOMETRY IN TWO MODELS OF HYPERTENSION

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Introduction. N ω -nitro-L-arginine methylester (L-NAME) produces chronic inhibition of nitric oxide (NO) by inhibiting the NO synthase enzyme. This increases vascular basal tone which rises as well periferic resistance. Deoxycorticosterone acetate (DOCA) associated to NaCl consumption produces Na retention and an increase in circulating blood volume. Both of this substances are used to produce hypertension (HT) models. **Objective.** Study the histopathological lesions in two models of HT. **Materials and methods.** Wistar Kyoto rats were divided in groups: L-NAME Rats (LR): L-NAME (50mg/100ml) was administered in drinking water for 50 days. DOCA Rats (DR): DOCA (12,5 mg/Kg. for 2 days) and 1,5% of NaCl were administered in drinking water for 50 days. Control Rats (CR). Mean arterial pressure (MAP), plasma and urinary Na, creatinine clearance, Na and free water clearance were measured in each rat. A bilateral nephrectomy was also performed and the kidneys were fixed in 10% buffered formalin. Glomerulus, tubular (TA) (including lumen and epithelial cells) and vascular areas (VA), and Bowman's space (BS) were measured. **Results.** MAP was increased in LR and DR rats, being bigger in LR than in DR ($p < 0,01$). Plasmatic Na levels were also increased in LR and DR. Na clearance was only higher in DR. A decrease in glomerular size was found in LR and DR (Δ according to CR, RL $28 \pm 3\%$; $p < 0,001$ and 20 ± 5 ; $p < 0,001$ respectively). There was a reduction in BS area too. LR and DR also presented a decrease in TA. Only in LR VA was than CR ($14 \pm 4\%$; $p < 0,05\%$). **Conclusions.** Vascular damage was greater in LR. Some similarities were found in the histological analysis of glomerular and tubular damage, being independent of the type of HT. This indicates that HT per se produces glomerular damage, regardless of underlying physiopathology.

A98

METABOLIC, HAEMATOLOGICAL AND LIVER ALTERATIONS ASSOCIATED WITH FAT DIETS

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The aim of this work was to evaluate in young rabbits the effect of consumption of a DG with cholesterol added for a short period of time on cardiometabolic risk factors and their relation to hematologic and hepatic abnormalities. Male rabbits were fed: balanced food (DC), 2-enriched 1% cholesterol (DH), 3-enriched fat to 18% (DG), 4-enriched 18% fat and 1% cholesterol (DG-H) for 6 weeks. Tolerance test pre and post the glucose feed was performed, the animals were sacrificed, blood was drawn and the liver and the visceral fat were weighed. Total cholesterol (TC), HDL, LDL, TG, blood smears and leukocyte formula was performed. Increasing TC levels (mg / dl) (DH: 872±114, DG-H: 1016±240 vs DC: DH 59± 5.6) was observed; LDL (DH: 666±92, DG-H: 836±244 vs DC: 23.8±3.2) in DH and DG-H while TG increased all DG (DG-H: 641±371; DG: 182±57, DH: 222± 33 vs DC: 105.7±32.4). intolerance was generated to glucose (mg / dl) in DG y DG-H (DG: 0' pre 118±7.65 vs post 126.7±11; 60': pre 172±12 vs post 208±13; 120': pre 142±7 vs post 172±6 and DG-H: 0': pre 127±4 vs post 136±4; 60' pre 181±9 vs post 251±34; 120' pre 153±7 vs post 184±9). DG-H: neutrophil increase was observed in 50%; DH: 38%; DG: 35% vs DC: 23.4%; lymphocytes decreased DG-H: 47% vs DC: 79.67%. Blood smears revealed alterations in the morphology of erythrocytes with increased echinocytes (%) in DH: 60.5; DG: 47.2 and DG-H: 26 vs DC: 3 and anisocytosis. The liver weight / body weight ratio increased in rabbits fed DH: 4.62±0.36; DG-H: 4.59±0.31; DG: 5.12±1 vs DC: 3.19±0.14 and had a close correlation with plasma TG: r = 0.99 in DG-H. Visceral fat / body weight ratio increased DG: 0.65±0.48 and DG-H: 0.43±0.06 vs DH: 0.11±0.003 and DC: 0.39±0.22. We observed that both the DG and DG-H generated increased neutrophils, hepatomegaly and altered morphology of erythrocytes.

A99

ADAPTATION AND TUNING KJELDAHL METHOD FOR MEASURING TOTAL NITROGEN IN BALANCED BEEF FOOD

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In previous works a cattle diet with citrus peel was formulated, created as a sustainable environmental solution to the disposal of this waste of the citric industry. Finding out the amount of proteins this food had was of major importance since proteins are essential for knowing the nutritional value this food has. In this paper, we present the adaptation and tuning of an official technique for the determination of total nitrogen, the method Kjeldahl. The sample of the balanced food contains: orange peel, grains of corn, treacle, cream of yeast, urea. Due to the heterogeneity of the ingredients, it was necessary to use a mill for triturating, and thereby making the sample more homogeneous. As a catalyst, selenium and potassium sulfate were used, and for the digestion, concentrated sulfuric acid. Direct distillation was used instead of entrainment by steam. To verify if the modifications of this technique were correct, we realized experiments in food with a well-known concentration of nitrogen such as: milk powder and cereals with sugar, and a sample was sent to a certified laboratory, obtaining in all cases the same results. This showed that it is possible to adapt a standard technique for the determination of total nitrogen, considering that the crushing of the material allows us to take representative samples of such foods, the use of selenium decreased digestion time, and that potassium sulfate elevated the boiling point of the acid. The direct distillation allowed us to process a major quantity of sample for digestion. For this, we conclude that the technique Kjeldahl modified can be used for the determination of total nitrogen and proteins of balanced cattle food. **Keywords:** adaptation, method, determination, protein food.

A100

HEMATOLOGIC VARIABLES DETERMINED IN PORCINE PRODUCTION ANIMALS OF ESTABLISHMENTS IN LITORAL REGION OF ARGENTINA

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In this publication, hematologic variables of two breeds of porcine production animals were determined: first (CH) and multigiven birth sows (G). Blood samples were taken from eyes veins of 35 CH and 38 G females butcher's shops one Entre Ríos and two center region Santa Fe, in winter 2013.

The parameters decided were: Hto, GR, Hb, VCM, HCM, CHCM; GB; and the leucocitary formula: N, E, B, L y M; manual methods used: Microtechnology with the capillaries for Hto; Neubauer's chamber for count of GB - GR and method cianmetahemoglobin colorimetric for Hb. May Grünwald-Giemsa's tint was applied for the leucocitary formula. The hematimetric indexes were calculated. The method ANOVA was applied, in analysis of the variance. The mean values and standard deviations in CH and G were: GB(/mm³) 13.953,4 ± 4.877,5; 10.167,6 ± 3.728,17; N(%) 29,31 ± 6,36; 34,26 ± 7,29; E(%) 2,46 ± 1,04; 2,37 ± 1,26; B(%) 0,37 ± 0,49; 0,13 ± 0,33; L(%) 65,89 ± 7,47; 61,68 ± 7,55; M(%) 1,94 ± 0,83; 1,71 ± 0,73; GR(/mm³) 6.279.496 ± 620.780; 5.747.166 ± 669.416; Hb(g/dL) 11,94 ± 0,56; 11,30 ± 1,39; Hto(%) 32,43 ± 5,00; 30,68 ± 5,06; VCM(fL) 51,42 ± 3,64; 53,50 ± 4,75; HCM(pg) 19,17 ± 1,40; 19,70 ± 1,2; CHCM(g/dL) 37,53 ± 4,83; 37,18 ± 3,06, respectively. Statistical differences (p <0,05) could be observed in parameters: GB, N, GR y Hto. The mean values of hematologic profile were observed inside values of reference in bibliography. In later publications, hematologic variables of other farms will be analyzed in Entre Rios and Santa Fe on summer station, which is going to allow to know values for Argentina.

A101

STUDY OF MINERAL PROFILE DURING THE TRANSITION IN DAIRY CATTLE OF LAS COLONIAS DEPARTMENT - SANTA FE

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In different physiological states of dairy cows, there are important mineral modifications seeking fetal development, birth, breeding, and the initiation and maintenance of milk production. The highest incidence of disease production occurs in the transition period or peripartum. The aim of our study was to characterize the blood levels of macro and micro minerals in 58 Holstein Friesian dairy cows during the transition period in the Las Colonias Department during the fall and spring 2013-2014. The blood was drawn from jugular vein with 20 mL syringes, the serum was separated from these samples. Macrominerals: calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K); and trace elements: iron (Fe), zinc (Zn) and copper (Cu) and phosphorus (P) were determined by atomic absorption spectroscopy. The equipment used is a Perkin Elmer model 3110 ANOVA statistical method was used to treat the data. The mean values of serum mineral profile in dairy cattle were within the range reported by the national and international literature as a reference. There is significant difference between the mean values of Cu in autumn and spring because the availability of the mineral in the food varies by season. The low values of Fe and Zn in both seasons are due to lactogenesis. Normal value of Ca was noted in the transition due to the smooth operation of homeostatic mechanism. The values of Mg in autumn and spring are in lower limit of reference range in precalving, this is due more to a failure in absorption of mineral rumen, associated with diminished intake by high stocking or depression appetite for pregnancy. Both input values as related to the homeostatic mechanism regulating levels of blood macrominerals were adequate and allowed optimal nutritional metabolic balance, as such, except in isolated cases, no production diseases were observed.

A102

PRELIMINARY METABOLIC EVALUATION OF CALVES DIET MADE WITH CITRIC INDUSTRY WASTE

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We formulated a balanced food for cattle containing wet orange peel. Once the formulas were analyzed nutritionally and microbiologically, it was necessary to perform an actual test on animals. We show the metabolic evaluation of calves which tried such diet. The trials were performed in Trancas (Tucumán), in 2013 during the period of citric production and natural grass shortage. Four calves of dutch-argentinian breed, clinically healthy, with age and weight of sixty days and 85kg, respectively were used. Two homogeneous groups were formed (two calves per group) to test the following treatments: 1) Experimental: wet orange peel (52%), corn (7.4%), yeast (35.2%), molasses (7.4%), and urea (0.4%), and natural forage. 2) Witness: commercial balanced food of soy pellets (70%), corn (30%), and natural forage. Three blood samples were taken monthly out of each calf during the experiment. It was analyzed: hematocrit, hemoglobin, glycemia, urea, total proteins, and calcium. We used for glycemia, urea and calcium, an enzymatic method, and for total proteins the Biuret system.

In all the parameters studied, the numeric results obtained were within a normal range, in both witness and experimental animals. In the case of the urea, high plasmatic concentrations were observed in the experimental animals, 0.33% g/l while in witness animals was of 0.23% g/l. This indicated an excess of proteins in the experimental diet, and a deficit of energy in the degradation of the protein and energy in each portion. On the basis of these results, we considered important to determine the cause of the elevated values of urea in blood, and thereby reformulate another diet to carry out another live experiment.

Keywords: metabolic evaluation, waste, diets, citric.

A103

DEHYDROFUKINONE, MAIN METABOLITE FROM *Senecio punae* IMPROVES CIPROFLOXACIN ACTIVITY AND STIMULATES LACTOBACILLI BIOFILMS

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A pale yellow oil identified as dehydrofukinone (D) was isolated from the aerial parts of *S. punae* with a high yield (1.7%) and purity. We present here the results of the synergic effects of different ciprofloxacin and D mixtures against Gram (+) and (-) bacteria. The amount of biofilm formation and the antibacterial effects of the mixtures were evaluated with micro-dilution method. We employed four pathogenic strains: *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* isolated from skin injuries, *Staphylococcus aureus* ATCC 6538 P and an isolate methicillin resistant strain (MRSA) from clinical specimen. Probiotic bacteria were *Lactobacillus acidophilus* ATCC 521, *L. casei* ssp. *paracasei* C2 and *L. plantarum* CE 105. The tensioactive activity of non pathogenic strains was also evaluated employing the "Oil Spreading" technique.

Compound D has weak antibacterial and antibiofilm effects against both strains of *P. aeruginosa* but a mixture of D (23µg/ml) and C (0.25µg/ml) displays a strong synergic effect for both strains (FIC=0.38) and reduces significantly the biofilm formation compared to control. *S. aureus* ATCC and MRSA showed MIC values of 3.00 and 1.50mg/ml, respectively. Sub-lethal doses of D (50 and 25µg/ml) also diminish significantly *S. aureus* biofilms (87.46 and 68.12%, respectively). On the other hand, D at 23µg/ml improved the antibiotic action of C (FIC=0.25), and the inhibition of biofilm.

The results show that different associations of D and C have no antibacterial effects against lactobacilli and strongly stimulate their biofilm formation (218-142%) and the tensioactive activity (117-104%). Our results are promising for design of selective antimicrobial containing protective effects for beneficial lactobacilli known as health promoters.

A104

SAFETY EVALUATION OF WILD STRAIN *Enterococci* FROM GOAT MILK

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In artisan cheese made from raw milk, the enterococci play an important role in flavor and texture development, also in the natural preservation of the cheese. In spite of their beneficial activities *Enterococci* may present risks to human health, so wild strain safety must be considered if further technological application were desired. The objective is to evaluate safety conditions of 40 isolation of *Enterococcus* sp (16S ARNr) obtained from goat raw milk. Assessment of resistance to antibiotic, hemolytic activity and gelatinase production were selected as safety indicators. Antibiotic-containing disks (Britania) were those of ampicillin (AMP 10µg), chloramphenicol (CMP 30µg), clindamycin (CLI 2µg), penicillinG (PEN 10IU), tetracycline (TET 30µg), the minimum inhibitory concentration (MIC) of vancomycin was also determined as an antibiotic commonly used in hospital infections; the diffusion method in agar (Mueller-Hinton) was used according to the NCCLS. Hemolytic activity was performed by Columbia agar supplemented with 5% v/v sheep blood; the development of β-hemolysis was weighted by the presence of halo around the colonies. The gelatinase production was performed by agar Todd-Hewitt supplemented with gelatin (30 g/l). The hydrolysis was determined by a transparent halo around cells to develop the plates with solution development (HCl HgCl₂). None of the strains showed hemolytic activity and gelatin hydrolysis. Results showed that every isolated tested were sensitive to vancomycin, MIC < 2.5µg/ml was found to 85% of the strings; 89% of enterococci evaluated were sensitive to AMP, TET, PEN y CMP and 79% to CLI. The results show that there are strains with resistance to certain antibiotics, all were susceptible to vancomycin due to which the result was satisfactory.

A105

INFLUENCE OF AUTOCHTHONOUS CULTURES ON FATTY ACID PROFILE, ESTERASE AND ACCEPTABILITY OF ARGENTINEAN GOAT CHEESES

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Artisanal goat cheeses from Santiago del Estero are characterized by a typical flavor nice to consumer. Flavor development is due to glycolysis, proteolysis and lipolysis carried out by enzymes of lactic acid bacteria (LAB). Lipolysis plays an important role in: 1) flavor development by esterase enzymes which release short chain fatty acids (SCFA), 2) bioactive lipids generation as conjugated linoleic acid (CLA) produced by isomerases and desaturases. The aim of this work was to evaluate the influence of autochthonous starter and adjunct cultures, isolated dairy goats, and a commercial starter culture on goat cheese lipolysis. Three semi-hard cheeses were made: CC with commercial culture, CS with goat starter culture, CA with goat starter culture and adjunct. Chemical composition, esterase activity, fatty acid profile, sensory evaluation desaturase and atherogenic indexes at initial and 60 days were determined. The results showed statistically significant differences on fatty acid profile and esterase activity, without observing significant changes on the chemical composition. In CS and CA cheeses, SCFA and polyunsaturated fatty acids contents were higher than in CC. The incorporation of indigenous cultures improved the flavor and atherogenic index at 60 days. CLA concentration increased during ripening in the CS and CA cheeses from 0.6 to 1.0g/100g of fatty acids, containing between 417-427 mg of CLA/100 g of cheese. The use of native BL as starter and adjunct cultures in goat cheese manufactured could have an impact on the development of dairy products with functional and nice sensory properties.

A106

EVALUATION OF THE CONTENT OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF WINES. EFFECT OF MALOLACTIC FERMENTATION

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Red wine is a source of phenolic compounds which are recognized by their antioxidant properties and their probable role in the prevention of various diseases associated with oxidative stress. Malolactic fermentation (MLF) conducted by lactic acid bacteria can produce changes in its composition. In this study we investigated the changes in the content of phenolic compounds and antioxidant activity of red wine Malbec variety, obtained by microvinification after MLF induced by *Oenococcus oeni* MS46 isolated from fermented must of our region. At the same time, we compared the results obtained with commercial red wines made from grapes of the same variety. The phenolic compounds, expressed as mg/l of equivalent of Gallic acid (GAE) were determined by the Folin-Ciocalteu method and the antioxidant capacity by measuring the reducing activity of ferric iron (FRAP) and capture of free radicals 2, 2-diphenyl-1-picrilhidracilo (DPPH) and 2,2-azino-di-3-etilbenzotiazolin sulfonic acid (ABTS). At the end of MLF the MS46 strain grew 1.58 logarithmic units and L-malic acid (2 g/l) was completely consumed. At this time the total phenolic compounds content (2670 mgGAE/ml) increased 15% compared with the alcoholic fermentation end and it was similar to the concentrations found in the commercial wines. In all cases the flavonoid fraction was majority. FRAP, DPPH, ABTS methods showed that, in general, the wine obtained by microvinification had the highest antioxidant activity, which was superior than that obtained in commercial wines (among 15 and 25%). In conclusion, the FML induced by the MS46 strain increased the content of phenolic compounds which was related to its higher antioxidant activity, representing this property a benefit to consumers' health.

A107

DESIGN OF A VECTOR-HOST SYSTEM FOR SURFACE DISPLAY OF HETEROLOGOUS ANTIGENS ON *Bacillus subtilis* SPORES

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The safety, stability and easy handling of *Bacillus subtilis* spores makes this system of particular interest to express in the surface recombinant antigens capable of triggering a protective immune response. In order to obtain a novel surface display system based on the use of bacterial spores, we carried out a transcriptional fusion between the genes encoding a protein of the *Bacillus subtilis* spore coat, CotB and a immunogenic protein of the casual agent of American Tegumentary Leishmaniasis *Leishmania (V.) braziliensis*, named LbAg3. The structural gene of CotB was amplified from *B. subtilis* 168 and subsequently reamplified using specific primers that incorporated a small polylinker toward the 3' end. The amplicon was cloned in the pRSETa plasmid. The LbAg3 coding region was amplified by PCR from pIVEX-LbAg3 and subsequently reamplified adding toward the 3' end a *Xma*I site restriction, a coding sequence for six histidine residues and a *Bam*HI restriction site. This amplicon was cloned directionally downstream of *cotB*. The obtained fusion *cotB-polylinker-lbAg3-6His* was digested with enzymes *Hind*III and *Bam*HI and cloned into the integration vector pDG364 obtaining the vector called pSPOK. The efficient surface presentation of the heterologous protein, together with the stability are being evaluated. This vector allows the integration of the genetic construction in the genome of *Bacillus* and through the multiple cloning site will allow working with genes of other antigens.

A108

BACTERIOCIN PRODUCTION BY LACTIC BACTERIA ISOLATED THE PATAGONIAN MARINE ENVIRONMENT

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The Patagonian marine environment provides a habitat with physicochemical and biological characteristics allowing the isolation of lactic acid bacteria (LB) with particular physiological properties. LB produce a variety of compounds with antimicrobial activity, specifically bacteriocins are of great interest for its application in food biopreservation. Foods with high water content are easily damaged by the action of bacterial flora itself and contaminating microorganisms such as *Listeria monocytogenes* and *Pseudomonas* sp. The objective of this work was to study the bacteriocin production by LB in different culture media and incubation temperature. The 50 strains of *Enterococcus* sp were isolated from Patagonian marine environment and different tests were performed: resistance to vancomycin (6µL/mL), hemolysis, gelatinase, exopolysaccharide production and bacteriocins production. Ten bacteriocin producer strains (TW6, Tw56, Tw222, 278, 452, 465, 471, 492, 802, 807) active against *L. innocua*, which did not exhibit any of the virulence factors tested, were selected. Different incubation temperatures (25, 30 and 37°C) in MRS and LAPTG liquid medium were used and it was verified that the addition of 0.5 mg / mL of cysteine increases the amount of detectable bacteriocins in up to 32 times in the culture media. This behavior is related to the decrease of the redox potential of the culture medium and demonstrates that under low oxygen level in the medium the production of bacteriocins can be enhanced. The maximum bacteriocins production was achieved at 30°C, temperature that did not agree with optimal bacterial growth temperature (37°C). The results indicate the importance of temperature and addition of cysteine to the bacteriocins production by LB.

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IDENTIFICATION OF ENZYMES PRODUCED BY *Aspergillus niger* IN MEDIA AMYLOLYTIC WITH HYDROLYZED POTATO (*Solanum tuberosum*) DISCARD

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The enzyme α -amylase (EC 3.2.1.1) and glucoamylase (EC 3.2.1.3) are imported into Argentina at high cost. The objective is to identify activities amylolytic produced by *Aspergillus niger* using potato of scrap as a substrate for low cost. We studied: 1) kinetics of production amylolytic enzymes with 200 g hydrolyzed potato, and: KH_2PO_4 , 1; NaNO_3 , 3; CaCl_2 , 2 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/l (pH 4). 2) Effect: maltose; dextrans and soluble starch (1 g/L) on the production. All assays are conducted with $2 \cdot 10^6$ conidia of *A. niger*/mL; were incubated at 25°C, 250 rpm during 48 h. 3) identification of the activity of enzymes by chromatography on paper (CP); layer fine (CCF) and HPLC. One unit of amylase is the amount of enzyme which liberates 1 µmol of reducing sugars per minute.

Results: 1) the maximum production of amylases (128 U/L) by *Aspergillus niger* coincides with the phase of decelerating growth, which has lower specific growth rate ($\mu = 0,002 \text{ h}^{-1}$) and high specific speed of formation of product ($q_p = 1,82 \text{ U/gh}$). 2) In media with dextrin; soluble starch and maltose were obtained 75; 45 and 30 U/L. 3) by CP and CCF, spots dextrans and maltose were determined, end products of α -amylase and glucose by glucoamylase. HPLC confirmed release of glucose by the action of glucoamylase on maltose. In conclusion *A. niger* is a potential organism, which in low-cost media, produced α -amylase and glucoamylase of great industrial importance.

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MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT *Staphylococcus aureus* CLINICAL ISOLATES IN A PAEDIATRIC HOSPITAL

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Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most important agent in nosocomial (HO-MRSA) and community *S. aureus* infections (CO-MRSA). The CO-MRSA infection is an emergent entity, associated with skin infections, cellulite, abscesses and also with severe complications such as sepsis and pneumonia. Several characteristics differentiate HO-MRSA's from CO-MRSA: absence of risk factors associated with previous hospitalizations, antibiotics sensibility to non β -lactámics, different genotypes from those of the hospital strains, presence of smaller SCCmectype IV or V, genes that codify toxins as leucocidina of Panton Valentine. Many CO-MRSA like HO-MRSA can circulate in the community and in the hospitals. Considering the high frequency of these infections in paediatric patients our aim was to do the molecular characterization of clinical isolates of MRSA in a Paediatric Hospital. **Materials and methods:** 29 MRSA isolated from skin and soft parts were studied to determine the structural type of the genetic region that codifies resistance to meticilin (Staphylococcus Cromosomal Cassette mec) SCCmec. The type of SCCmec was identified by Multiplex PCR (Oliveira et al, 2002), we differentiated the I, IA, the II, IIIrd, IIIA and the IVth cassettes, *mecA* gene. PVL's detection was done using specific primers (Lina et al, 1999). **Results:** 28 clinical isolates were positive for the *mecA* gene, Panton-Valentine leucocidin and the IVth allele. **Conclusions:** we can conclude that there is a high prevalence of CA-MRSA in the paediatric patients studied. **Keywords:** molecular characterization, *S. aureus* meticilin resistant.

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CO-CIRCULATION OF INFLUENZA B/YAMAGATA AND B/VICTORIA LINEAGE VIRUSES DURING THE 2012 AND 2013 INFLUENZA SEASONS IN TUCUMAN

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Influenza A and B virus causes severe respiratory infections in humans. While IA viruses comprise a large group of different subtypes, IB viruses formed a homogenous group and diverge into two antigenically distinguishable lineages: Yamagata and Victoria. Since 1999 Tucuman Virology Laboratory is component of the National Network of Influenza and Respiratory Virus Surveillance System which provides information on regional strains circulation. The purpose of this study is to describe IB lineages cocirculation in 2012 -2013 and the relationship with vaccines strains. 8,788 respiratory samples were taken from hospitalized and outpatient and were tested with immunofluorescence for antigen detection. 40.4% were positive for some type respiratory virus (Influenza, Parainfluenza, Respiratory Syncytial Virus, Adenovirus, Metapneumovirus). IB was identified in 225 patients (6.3%). Negative samples were further processed by real time PCR and revealed 98 IB positive cases. Reference National Laboratory identified influenza B lineage: 90.7% Victoria and 9.3% Yamagata during 2012, in 2013 were 23% Victoria and 77% Yamagata. Global Influenza Surveillance and Response System demonstrated in northern and southern hemispheres the two influenza B lineages co-circulated during 2012 and 2013 influenza seasons. For this reason WHO recommended a tetravalent flu vaccine. Effective surveillance is essential for understanding influenza seasonality and for determining the best strategies for influenza control. National authorities approve the composition and formulation of vaccines used in each country. They are responsible for making recommendations regarding the use of the vaccine.

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DIFFERENTIAL *Salmonella* serovar *Gallinarum* IDENTIFICATION BY POLYMERASE CHAIN REACTION

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Avian salmonellosis is a highly contagious disease that causes significant economic losses. Fowl typhoid and pullorum disease are caused by *Salmonella gallinarum* and *Salmonella pullorum*, respectively and affect birds of any age with a variable mortality from 4 to 50%. Bacteriological diagnosis methods require time, are laborious and the information obtained is limited. It is necessary to standardize techniques for rapid and specific surveillance of these pathogens. **Objective:** To optimize the differential *S.pullorum* and *S.gallinarum* identification, by PCR in stool samples. **Methodology:** *Salmonella* detection was done by real-time PCR (rt-PCR) by designing specific primers based on the gene InvA. Reference *Salmonella* strains biovar *pullorum* and *gallinarum*, to determine optimal conditions for amplification of the gene, were used. *Escherichia coli* strains as negative control were used. Samples of chicken feces of 5 farms were taken. After a pre-enrichment in buffered peptone water and enrichment in selenite broth, DNA extraction was performed. Differential identification of *S. pullorum* and *S. gallinarum* was performed by duplex-PCR from positive samples for *Salmonella* spp. **Results:** *Salmonella* spp was identified in stool samples by rt-PCR. The duplex-PCR allowed to identify *Salmonella enterica* serovar *gallinarum* in 8 samples. **Conclusions:** rt-PCR method for detection of *Salmonella* spp was optimized from samples of chicken feces. Only biovar *gallinarum* through conventional duplex-PCR was identified in those samples positive for *Salmonella* by rt-PCR. The specific detection of *Salmonella* will allow to implement early preventive strategies to control infections caused by this pathogen, which would have a high positive impact on poultry production.

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RECURRING STOMATITIS IN *Tupinambis* LIZARDS BRED IN CAPTIVITY

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Infectious stomatitis is a common disease in captive reptiles that occurs as a result of contaminated food products or in situations of stress or malnutrition, where these microorganisms can act pathogenically, favoring disease development. The aims of this study were to evaluate the presence and identification of pathogens in lizards *Tupinambis merianae* with clinical signs of disease, evaluate and implement appropriate drug therapy according to pathogen identification, and implement prophylactic measures to prevent disease in the general population's measures.

We worked with 4 adult specimens of both sexes at the Experimental Breeding of Facultad de Agronomía y Zootecnia, UNT, which had been isolated for manifest signs of clinical disease. Buccal and pharyngeal mucosal swabs for microbiological culture were performed and blood sampled for hematology. After identification of the pathogen, the animals were treated pharmacologically and monitored until they came out of hibernation.

Microbiological culture showed *Proteus vulgaris* and *Pseudomonas aeruginosa* and sensitivity to amikacin, ciprofloxacin, gentamicin, ceftazidime and cephapine as well as resistance to other antibiotics such as ampicillin and cephalexin. Enrofloxacin was administered 5 mg/kg intramuscularly for 5 days, and the antiparasitic albendazole 50 mg/kg orally for 3 consecutive days. Then the animals were isolated and observed until they entered hibernation. Prophylactic measures such as vitamin supplementation and disinfection of the facilities with quaternary ammonium were taken. After hibernation the animals again had the same pathogens and showed different stages of infection. Two of these animals died and the remaining two responded to treatment. However, in subsequent cultures resistance to the antibiotics used was observed. For more satisfactory responses to treatment, we should reconsider the dose and duration of antibiotic therapy.

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COMPARATIVE STUDY ABOUT THE OPSONOPHAGOCYtic ACTIVITY BETWEEN HEALTHY AND IMMUNODEFICIENT PATIENTS

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Polymorphonuclear (PMN) neutrophils form a fundamental part of innate immunity and they are well-known for their ability to phagocytise and kill invasive microorganisms. They are the first line of defense of the host organism against infections produced by bacteria and fungi. Immunodeficient patients are characterized by the presence of variations in phagocytic, humoral or cellular immunity that condition a high risk of developing infections or opportunistic processes. The aim of this work was to evaluate *in vitro* the opsonophagocytic activity of PMN neutrophils in immunodeficient patients and compare them with a healthy patient control groups.

For this, several female patients were taken peripheral venous blood samples and some parameters were determined. 20 immunodeficient patients and 20 healthy patients were selected followed the same criteria.

PMN neutrophils were incubated in microscope slides in contact with *Candida albicans* suspension (2×10^6 yeast/ml) during 30 minutes in a 37°C humid atmosphere. They were immediately coloured with Giemsa and observed by immersion in the light microscope. The number of phagocytized yeasts per hundred neutrophils (opsonophagocytic index) and lysis percentage (as the percentage of *Candida* lysed in relation to which they were phagocytosed) were determined. With ANOVA test significant differences were found ($p < 0.05$) in absolute values of leukocytes and PMN neutrophils, as in phagocytic (IOF) and lytic (%L) activity in immunodeficient patients with respect to the control group. Distribution of IOF and %L were also verified and compared in both groups of patients studied. In conclusion, the opsonophagocytic activity of the PMN neutrophils in immunodeficient patients was less with respect to the healthy ones.

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