



Slovak University of Agriculture
in Nitra



Università degli Studi del Molise



UTP, University of Science and
Technology in Bydgoszcz

International PhD Workshop on
Agriculture, Livestock and Food Technology and Biotechnology
Campobasso, November 26, 2015

Organized in the framework of
International PhD Programmes in
Agriculture Technology and Biotechnology
&
Welfare, Biotechnology and Quality of Animal Production

Book of Abstracts

Guest Editor:

Prof. Giuseppe Maiorano

International PhD Workshop

Campobasso, November 26, 2015

**Aula F. Silvestri, Department of Agricultural, Environmental and Food Sciences
University of Molise**

Organized in the framework of

**International PhD Programmes in
Agriculture Technology and Biotechnology
&
Welfare, Biotechnology and Quality of Animal Production**

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**The Workshop was supported by the
Department of Agricultural, Environmental and Food Sciences,
University of Molise**

Workshop programme

Wednesday 25th of November

Arrival of participants

Thursday 26th of November

08.00 – 8:30 Registration

08.45 – 09.15 Welcome and greetings

Prof. Gianmaria Palmieri – *Rector of University of Molise, IT*
Prof. Raffaele Coppola – *Head of Department of Agricultural, Environmental and Food Sciences, University of Molise, IT*
Prof. Giuseppe Maiorano – *PhD Coordinator, Department of Agricultural, Environmental and Food Sciences, University of Molise, IT*

09.15 – 10.30 *I Scientific session: Welfare, Biotechnology and Quality of Animal Production*

Chairman: Prof. dr. hab. Marek Bednarczyk, Prof. dr. Marcela Capcarová

10.30 – 11.45 *II Scientific session: Welfare, Biotechnology and Quality of Animal Production*

Chairman: Prof. dr. Peter Chrenek, Prof. dr. Branislav Gálik

12.00 – 13.00 *Lunch buffet*

13.00 – 13.45 *III Scientific session: Food Science, Technology and Biotechnology*

Chairman: Prof. Gianfranco Panfili, Prof. Fabio Pilla

13.45 – 14.30 *IV Scientific session: Welfare, Biotechnology and Quality of Animal Production*

Chairman: Prof. Fabio Pilla, Prof. Gianfranco Panfili

14.30 – 16.00 *V Scientific session: Sustainable Plant Production and Protection*

Chairman: Prof. Antonio De Cristoforo, Prof. Giuseppe Lima

16.00 – 16.30 *Closing ceremony*

Prof. Vincenzo Di Nuoscio - *Delegate for Doctoral and Postgraduate programmes, University of Molise, IT*
Prof. Giuseppe Maiorano – *PhD Coordinator, Department of Agricultural, Environmental and Food Sciences, University of Molise, IT*

Agenda for oral presentations

Thursday November 26

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Chairman: Prof. dr. hab. Marek Bednarczyk, Prof. Marcela Capcarová

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II Scientific session – Welfare, Biotechnology and Quality of Animal Production

Chairman: Prof. dr. Peter Chrenek, Prof. Branislav Gálik

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Chairman: Prof. Gianfranco Panfili, Prof. Fabio Pilla

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Chairman: Prof. Fabio Pilla, Prof. Gianfranco Panfili

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Chairman: Prof. Antonio De Cristofaro, Prof. Giuseppe Lima

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PREFACE

International PhD Programme in Agriculture Technology and Biotechnology

In the framework of the cooperation between Italy, Poland and Slovakia and on the basis of the internationalization process of the University education system, a three year International PhD Program is activated in “Agriculture Technology and Biotechnology (ATB). The Program will be organised between the following partners:

- Department of Agricultural, Environmental and Food Sciences, University of Molise;
- Faculty of Animal Breeding and Biology, UTP, University of Science and Technology in Bydgoszcz, Poland;
- Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra;
- Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra.

The PhD program is focused on the agrifood and environmental systems as encoded in Horizon 2020. Its objectives are sustainable agriculture, food safety and quality, animal welfare, green economy and environmental law.

The PhD program is organized into 3 *curricula*: Sustainable Plant Production and Protection; Food Science, Technology and Biotechnology; Welfare, Biotechnology and Quality of Animal Production. The program represents the continuation of first and second level degree courses (Agricultural Science and Technology; Food Science and Technology; Environmental Science and Forestry) activated by the Department of Agricultural, Environmental and Food Sciences.

The PhD program aims at providing specific skills and expertise for: the development of sustainable agriculture ensuring quality and safety of food from plants and livestock, food security, soil and environment protection, through innovative biotechnical and biotechnological means; the development, evaluation and monitoring of research and innovation projects of the agrifood sector. The educational goals of the program are designed to address the needs of fundamental and applied research, with particular emphasis on the latter (see notes in the *curricula*) The Doctoral program is based on courses, seminars and workshops also including soft skills (management of funds, financial and human resources; information management; planning capacity; fund raising; conceiving and preparing research and innovation projects; problem solving).

The *Curriculum* Sustainable Plant Production and Protection aims at providing skills and expertise for developing new technologies for the sustainable exploitation of agricultural resources, for the sustainable management of forest and agricultural ecosystems, and for the protection of biodiversity by reducing chemical inputs in agricultural ecosystems (Horizon 2020).

The *Curriculum* Food Science, Technology and Biotechnology aims at forming professionals that are able to develop, evaluate, monitor and manage research and innovation in the processes of food production.

The *Curriculum* Welfare, Biotechnology and Quality of Animal Production aims at providing skills and expertise ensuring the capability to compete in the area of animal production, and to develop biotechnological and technological innovation for optimizing animal production, health and welfare and to upgrade the products of animal origin. For these purposes, issues regarding economy, laws and regulations, sustainability, food safety and security are also taken into consideration.

PhD General Coordinator: Prof. Giuseppe Maiorano

Scientific Coordinator for the *Curriculum* Sustainable Plant Production and Protection: Prof. Raffaello Castoria

Scientific Coordinator for the *Curriculum* Food Science, Technology and Biotechnology: Prof. Gianfranco Panfili

Scientific Coordinator for the *Curriculum* Welfare, Biotechnology and Quality of Animal Production: Prof. Giuseppe Maiorano

International PhD Programme in Welfare, Biotechnology and Quality of Animal Production (WBQAP)

In the framework of the cooperation between Italy, Poland and Slovakia and on the basis of the internationalization process of the University education system, a four year International PhD Program is activated in “Welfare, Biotechnology and Quality of Animal Production”. The Program will be organised between the following partners:

- Department of Agricultural, Environmental and Food Science, University of Molise;
- Faculty of Animal Breeding and Biology, UTP, University of Science and Technology in Bydgoszcz, Poland;
- Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra;
- Faculty of Agrobiological and Food Resources, Slovak University of Agriculture in Nitra.

Aims:

To train, through studies and advanced research activities, young researchers on themes concerning animal productions, who are able to compete at an international level in the fields of research and its implementations, in order to promote and develop the technological innovation. Such innovation has to be considered the key to have success in the zootechnical systems, from a cellular, molecular, and managerial point of view, to optimize the performances, health and welfare of animals and to exploit the quality of products of animal origin, with a particular regard to the economic aspects, the safety and healthiness of food, such as the environmental defensibility.

Traineeship programmes:

First year: the acquisition of conceptual and operational tools with the aim to develop the scientific research and testing the activities; abilities to plan an individual research programme with a theme selection and enunciation of practical stages;

Second and third year: inquiry of the skills to realize the applied research and testing coherent with the general intended programme;

Fourth year: assessment of the skills in working out a research survey, organized and consistent, with the ability to communicate the meaning, the purposes and the most relevant results, both to the scientific community and to the young vocational students.

The traineeship programme also includes the participation of the graduate students to specific research programmes of reciprocal interest between the involved Universities.

Educational activities:

The educational programme of the International Research Doctorate envisages the following activities:

1. mobility of students and teachers;
2. outputs of advanced multidisciplinary and multifactorial researches, as understanding of the determinism in the global efficiency of breeding and the quality of products of animal origin;
3. training to the research activity through the participation of the graduate students to well defined research programmes of reciprocal interest within the proponent Institutions;
4. educational activities carried out in a coordinated way between the partner Universities;
5. testing of innovative methods in education, aimed to the Research Doctorate objectives.

The educational programme of the Research Doctorate is characterized by the combination of different ways of learning: course of lectures, conferences, seminars, workshops, summer school, master-classes, internships. The participation to the training activities of the WBQAP by the graduate students will be evaluated through the credit system (ECTS), as well as the university course degrees and post-graduate degrees.

General PhD Coordinator: Prof. Giuseppe Maiorano, University of Molise

Local Coordinator for the UTP, University of Science and Technology in Bydgoszcz, Poland: Prof. dr. hab. Marek Bednarczyk

Local Coordinator for the Slovak University of Agriculture in Nitra: Prof. dr. Peter Chrenek

Development and optimization of DNA microarray for assessment of the variability of genes affecting perception of taste

Tomáš Fekete

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The sense of taste plays an important role in food preferences and intake of nutrients. Perception of taste starts on the tongue and soft palate, where specific chemicals of food or beverages interact with taste receptors. The chemical compounds evoke five distinct taste sensations - sweet, bitter, sour, salty and „umami”, which are on the present accepted as five basic types of taste. However, people might differ in DNA sequences, which encode taste receptors or other downstream signalling molecules. These polymorphisms may result in variability in perception of food flavour. Differences in perception may lead to differences in food preference. As a result, eating habits might influence both nutritional and health statuses, as well as the risk of chronic disease (diabetes, obesity). Changes in taste perception were also reported in the ageing process and certain diseases (autism, Alzheimer). These conditions often require personalized diet, consumption of drugs or surgical intervention associated with the reduction of obesity, which can lead to temporary changes in nutritional status, immunity and quality of life. The subject of the study will be to develop the platform (DNA chip) for genotyping the single nucleotide polymorphisms (SNPs) of genes affecting the perception of taste. The purpose is to find the relationships among changes in perception of taste, ageing, food preference and health risks. The knowledge about the genetic conditionality of dietary preferences of seniors should be helpful to adjust the eating habits of each one and minimize the risk of chronic diseases (personalised food safety). The treatment of diseases would have been moved to the level of prophylaxis, which is a more economical solution. The study will include preparative operations, genotyping and association studies. The first one will consist of: a) searching the genes and associated SNPs (scientific papers, NCBI databases), b) self-designing of end-point (multiplex) PCR for target SNPs (*in silico* PCR validation and multiplexing, c) DNA chip manufacturing (APEX probes designing and spotting), d) data collection (sampling the DNA from buccal mucosa of the subjects, food frequency questionnaire). The genotyping will include: a) PCR of target SNPs, with 20 % fraction of dUTP, b) purification and fragmentation of PCR products (UNG enzyme), c) APEX - extension of probes arrayed onto the chip, which are complementary to target SNPs, d) genotyping - reading of chip (extraction of four colour signals) and subsequent base calling. The association studies will be performed based on obtained genotypes, frequency of food items intake data, age and other factors.

Acknowledgement

The research is supported by APVV-0629-12.

Monitoring ruminal pH of dairy cows from 4th to 18th week of lactation

Ondrej Hanušovský, Daniel Bíro, Milan Šimko, Branislav Gálik, Miroslav Juráček, Michal Rolinec

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The aim of the study was to continuously monitor reticuloruminal pH in 7 Holstein dairy cows during 15 weeks at University Experimental Farm in Oponice. Selected cows yielded in average 10175 kg milk per lactation with 3.94 % fat, 3.10 % protein and 4.7 % lactose. Animals were daily fed once with Total Mixed Ration (DMI – 24.45 kg; 153.86 MJ.kg⁻¹ NEL; 15.74 % CP; 24.35 % NDF; forage to concentrate ratio - 53:47) ad libitum (between 4:00 a.m. and 5:00 a.m.) and milked 3 times per day (6:00 a.m., 12:00 a.m. and 6:00 p.m.). Bolus for continual data measuring was implanted in each dairy cow 4 weeks after parturition through esophagus by a special balling gun. Used boluses (Devon, Ltd., UK) are characterized by limited dimensions (135 x 27 mm) and weight (207 g). Temperature and pH values were measured every 15 minutes (96 data points per day) with accuracy ± 0.1 for pH and temperature. Data were downloaded with the handset with antenna and dongle connected with USB dongle connector with the radio frequency 434 MHz in the milking parlor. Collected data were summarized with HathorHBClient v. 1.8.1 and statistically evaluated with IBM SPSS v. 20.0 (One-way ANOVA, Tukey Test). After the application of boluses, at 4th week of lactation an average pH of 6.31 ± 0.30 , which decreased ($P < 0.05$) to 6.24 ± 0.30 at the 7th week of lactation was found. Ruminal pH recovered up ($P < 0.05$) to 6.30 ± 0.25 in the 9th week compared to 7th week and dropped to 6.25 ± 0.29 in the 10th week of lactation ($P < 0.05$, compared to 9th week pH). In the 11th week of lactation ruminal pH increased to 6.31 ± 0.25 ($P < 0.05$) compared to 10th week, while in the 12th week of lactation pH was almost stable (6.33 ± 0.28) to compared to the previous week. At the 13th week of lactation it was found a weak decrease ($P < 0.05$) to 6.29 ± 0.40 , compared to the 12th week pH; then at the 14th week of lactation it was found an increased ($P < 0.05$) of the pH values (6.35 ± 0.43). The highest ($P < 0.05$) pH values were recorded from the 15th week of lactation (6.56 ± 0.46) till the 18th week (6.58 ± 0.55). Measured pH values ranged from 5.18 at 9:00 p.m. in 16th week of lactation to 7.57 at 10:00 p.m. in 18th week of lactation with total average for monitored period 6.36 ± 0.41 . Minimal and maximal pH values were affected by high outside temperature during the summer in July 2015. Overall, during the lactation pH values increased from the 4th week of lactation to 18th week of lactation by 4 %.

Acknowledgement

The project was supported by the Slovak National Scientific Grant Agency VEGA, Grant no 1/0723/15.

Influence of passaging rabbit mesenchymal stem cells on their viability

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Rabbit is a suitable biological model for stem cell experiments due to its cellular and tissue physiology that closely resemble human mesenchymal stem cells (MSCs). However, human MSCs are well defined by surface markers, the expression of specific markers is the matter of many studies of rabbit stem cells. Therefore, the aim of our preliminary study was to evaluate viability and apoptosis of MSCs from early passages (P1-P3) and to detect surface marker proteins that identify MSCs by flow cytometry. Briefly, we harvested rabbit mononuclear cells bone marrow of three New Zealand White line rabbits. Cells were separated by gradient density centrifugation. Cells from the suspension were counted, mixed with α -MEM supplemented with 20% FBS and seeded in tissue culture flasks. Medium was changed every 3-4 days. Upon reaching about 70% confluence MSCs were trypsinized. MSCs from passages P1, P2, P3 were stained by Annexin-V, Yo-Pro-1 and PI to test the viability and apoptosis by fluorescent microscopy and flow cytometry. Cells from P3 were used for detection of surface markers. Our MSCs were positive to CD44 and CD29 surface markers, commonly used to profile MSCs. We also used CD90 and CD45 markers as a negative control. Fluorescent microscopy revealed that early apoptotic cells had decreasing tendency from P1 to P3. The proportion of early apoptotic cells stained by Annexin-V was lower in P2 than in P1 ($P < 0.05$) and also decreased from P2 to P3 ($P < 0.01$). Using Yo-Pro-1, we revealed that apoptotic incidence was lower in P3 compared to P1 ($P < 0.05$). On the other hand, using flow cytometry we just observed statistically significant decrease in Annexin-V positive cells in P3 compared to P1 ($P < 0.01$). Fluorescent microscopy showed a decreasing trend in apoptosis rates related to passaging with more statistically significant results; but this trend was not observed using flow cytometry, which is considered more reliable and accurate, compared to fluorescent microscopy. That is why we cannot confirm that passaging of stem cells positively affects their viability.

Acknowledgement:

This work was supported by grant APVV-14-0043 coordinated by the Slovak Research and Development Agency (APVV) and VEGA 1/0611/15.

The effect of oils on fatty acids profile in table eggs

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The aim of study was to analyze the effect of pumpkin and flaxseed oils on fatty acids profile in table eggs of laying hens. At 38 weeks of age, Lohmann Brown Lite hens were housed in three-floor cages (943.2 cm² per hen), divided into three diet groups (C-control, E1-pumpkin oil (3%), E2-flaxseed oil (3%)). There were housed six hens in one cage. A total of 18 hens were monitored. In the control group, hens were fed with standard complete feed mixture for laying hens and in the experimental groups by feed mixtures with supplementation of pumpkin or flaxseed oils. Vitamin E (0.1g per 1 kg of feed) was added into feed mixture in the experimental groups. Used oils were obtained from business network in Slovakia. Analyzed oils are declared by the producer as pure and virgin oils which were pressed mechanically by cold technology. Laying hens in all groups received drinking water and feed mixture *ad libitum*. During experiment, the light regime was 16 hours. The experiment lasted 52 days. In the last week the eggs were collected and processed for chemical analysis. Twelve eggs from each dietary treatment were randomly selected and analyzed. The content of fatty acids was determine by Agilent 6890A GC (Agilent Technologies, USA) as a percentage in crude fat. Laboratory evaluation of fatty acids was carried out in the Laboratory of quality and nutritional value of feeds at the Department of Animal Nutrition at Slovak University of Agriculture in Nitra. Statistically significant ($P<0.05$) differences were found in contents of myristic, palmitic, stearic, heptadecanoic, oleic, linoleic, α -linolenic, arachidic, cis-11-eicosenoic, cis-11,14-eicosadienoic and nervonic fatty acid. Tendency of a higher concentration was observed in palmitoleic fatty acid after both oils supplementation, stearic fatty acid in the second experimental group, oleic fatty acid in both trial groups, α -linolenic and nervonic fatty acids after flaxseed oils supplementation. Appearance of γ -linolenic acid was detected only in the group without addition of oil in feed mixture. The best ratio (4.52) $\Sigma n6/\Sigma n3$ was found in eggs from the second experimental group. The supplementation of selected oils into diets led to decrease ($P<0.05$) of saturated fatty acids, and also of polyunsaturated fatty acids, which are very important for nutritional quality of table eggs. The concentration of monounsaturated fatty acids was higher ($P<0.05$) in the experimental egg samples especially due to high content of oleic acid. The addition of flaxseed oil had a positive effect on the content of important n3 fatty acid α -linolenic acid. Lower contents of linoleic and arachidonic fatty acids were detected in both the experimental groups.

Acknowledgement

This study was supported by Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak republic and Slovak Academy of Sciences (project No. 1/0723/15).

Effect of natural feed additives on performance of broiler chickens, quality and oxidative stability of chicken meat

Lenka Trembecká, Peter Haščík

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The ever-rising trend of poultry consumption shows the importance of meat quality evaluation for the poultry industry. Many natural substances used as alternatives to antibiotic in chicken nutrition have been shown to express positive effects on growth performance, meat quality and the other parameters. The aim of the study is to investigate the effect of probiotics applied *via* drinking water, propolis and bee pollen extract *via* feed mixture, in nutrition of broiler chickens with regard to the following: 1) meat performance of broiler chickens at the end of fattening and carcass processing; 2) technological, nutritional and sensory quality of chicken meat; 3) evaluation of *post mortem* changes during the maturation process in the most valuable parts of chicken carcass; 4) evaluation of oxidative stability of chicken meat during storage. The experiment will be conducted in the test poultry station of Slovak University of Agriculture in Nitra (Slovakia). One-day-old broiler chicks (Ross 308) will be divided into the control and experimental groups. The experiment will last for 42 days. The broiler chickens will be bred on breed litter (wood shavings), in a temperature-controlled room. The broilers will be provided with *ad libitum* access to feed and water and will be kept under constant light regime. The following will be examined: 1) Zootechnical indicators: mean body weight of chickens at the end of fattening; 2) Indicators of meat performance: carcass weight, giblets weight, breast and thigh muscle weight, abdominal fat weight, etc.; 3) Indicators of nutritional value of chicken meat: chemical composition (dry matter, protein, fat, amino acids, fatty acids, cholesterol, energy) of breast and thigh muscles; 4) Indicators of technological meat quality: colour, pH and oxidative stability during the storage; 5) Indicators of sensory quality of chicken meat: sensory evaluation of breast and thigh muscles (aroma, taste, juiciness, tenderness, total sensory evaluation). After application of above mentioned additives, we expect improvement in performance manifested by the higher body weight of chickens at the end of fattening, as a result of improving the feed utilization. We also expect percentage increase of most valuable parts of carcass (breast and thigh muscles) as well as the reduction of abdominal fat. In addition, we will consider it as being appropriate if the carcass yield is increased beyond the standardized value (72 %). Furthermore, the higher stability of meat during storage due to reduction in lipid oxidation is expected.

Acknowledgement

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Sperm competition and genetic distance between Tetra hens and four rooster breeds

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Sperm competition is an evolutionary mechanism through which natural selection of certain animal species was made for centuries. In nature, sperm competition takes place when spermatozoa derived from two or more males compete for fertilization of one ovum. Studies of varied animal species have shown that this mechanism is related to the selection of males which have features that ensure successful fertilization. The aim of this study was to determine the relation between genetic similarity of birds and test competition of sperm results. The research material consisted of Tetra SL laying hens and roosters derived from four breeds: Transylvanian Naked Neck Black (TNB), Transylvanian Naked Neck White (TNW), Hungarian Speckled (HS) and Green-legged Partridge-like (ZK). Eight microsatellite markers recommended by FAO/MoDAD Advisory group (<http://www.fao.org/dad-is>) for polymorphism testing in chickens were used for determining the genetic differences between populations. Among other things the expected and observed heterozygosity, inbreed coefficient, Polymorphism Information Content and Genetic distinction coefficient – F_{st} were calculated. Based on that the phylogenetic tree of the relations between the groups of birds was made. Further, six roosters from each breed of sperm donors and 12 Tetra SL recipient hens were selected so that previously identified polymorphism was a tool to identify offspring. For the sperm competition test - semen from each of the chosen roosters was standardized for the concentration, combined and used for insemination. Obtained chicks (100 individuals) were genetically analyzed using the same 8 panel of microsatellite markers to determine their paternity. Genetic variation analysis indicated that Tetra SL hens had the greatest distance to ZK roosters (0.5) and the lowest to TNB roosters (0.25). Genetic distance between Tetra SL hens and roosters from two other breeds was 0.33 for HS and 0.34 for TNW. Furthermore, paternity analysis indicated that majority of chicks (45%) inherited TNB genotype, followed by HS (32%) and TNW (14%), while ZK roosters had the lowest proportion of the progeny (8%). Positive correlation ($P < 0.05$) between high genetic similarity among males and females and sperm competition test has been demonstrated in this study. In the future, these results can be implemented in breeding programs to improve the reproductive traits of poultry.

Influence of *Lactobacillus* synbiotics administered *in ovo* on gene regulation in selected tissues of broiler chickens

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The avian digestive system is the habitat of a large, complex and dynamic microflora, which plays an important role in the stimulation of immune responses and maintenance of animal health, as well as in metabolic processes and nutrient digestion. The intestinal microflora may be modified as early as during embryonic development through *in ovo* application of synbiotics. Synbiotics can affect the chicken transcriptome by interacting with cellular receptors. The goal of study was to determine gene expression in broiler chickens treated with synbiotics *in ovo*. In this experiment, synbiotics were applied *in ovo* at the 12th day of embryo development. This involved randomly distributing embryos (Cobb500FF) into three groups which were injected with either SYN1 (*Lb. salivarius* IBB3154 + Bi²tos - Clasado, Sliema, Malta), SYN2 (*Lb. plantarum* IBB3036 + RFOs) or saline as a control. After hatching, birds were sexed and only roosters were reared. On the 7th, 14th, 21st and 42nd day post hatching, birds were sacrificed and cecal tonsils, spleen and liver were collected. Gene expression analysis was conducted using reverse transcription quantitative PCR (RT-qPCR) and two sets of genes. The first set was analyzed in cecal tonsils and spleen. It was composed of Th-1 (*INFγ*, *IL12*), Th-2 (*IL4*), antiviral (*IFNβ*), pro-inflammatory cytokines (*IL12*, *IL1B*) and chemokines (*IL8*). The second set was analyzed in liver and it consisted of the genes related to regulation of cholesterol level (*ABCG8*, *HNF4A*, *ACOX2*, *APBB1IP*, *BRSK2*, *APOA1*, *IRS2*) and other metabolic processes (*ITIH5*, *UPS18*, *GIMAP5*, *CCDC79*, *DIO2*). SYN1 stimulation caused down-regulation of gene expression in cecal tonsils and up-regulation in spleen at all-time points. SYN2 didn't affect any patterns of immune genes regulation in both lymphatic tissues. However, in the liver the two synbiotics had an opposite effect observed at the gene expression level. Genes down-regulated after SYN1 injection were up-regulated after administration of SYN2. Administration of synbiotics into the egg on 12 day of incubation can modulate the host transcriptome. This effect depends on the tissue investigated and the synbiotics used.

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***In ovo* validation model to assess the efficacy of commercial prebiotics in enhancing broiler performance and reducing lipid oxidation of meat**

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The objective of the study was to explore the potential of Bi²tos (BI)-Clasado Ltd., Malta; a non-digestive transgalacto-oligosaccharides, and DiNovo[®] (DI)-BioAtlantis Ltd., Ireland; carbohydrates derived from *Laminaria* spp.; injected *in ovo* with an automatized system, and determine their influence on performance, physicochemical properties (pH and water holding capacity) and lipid oxidation of meat in chickens reared in commercial conditions. A total of 350,560 Ross 308 crossbreed eggs were incubated under commercial-scale hatchery conditions, following an *in ovo* trial protocol. The eggs were randomly divided into 2 prebiotic groups, (BI) and (DI), and a control (approx. n= 27,000-29,000 eggs per group). On day 12 of incubation, the eggs were automatically injected *in ovo* into the air chamber, with 200 µl of physiological saline solution containing BI at dose of 3.5 mg/egg or DI at dose of 0.88 mg/egg. The control (C) group was injected with physiological saline. The research was performed in commercial farms: birds (approximately half males and half females) were reared at high density (22 chicks/m²). At slaughter (42 d of age), pectoral muscle (PM) was removed, from carcasses of 45 broilers, 15 birds (7 males and 8 females) per treatment, and weighted. Samples of breast muscle were taken for lipid oxidation analysis. Data on *in vivo* performance and mortality were analyzed by one-way analysis of variance. Slaughter performance data were evaluated by ANOVA, in a 3 × 2 factorial design. Lipid oxidation (TBARS values) was analyzed as a repeated-measures. Scheffé's test was applied to compare the mean values among the experimental groups. In general, the mortality percentage of the birds was low, both in the 1st week of life and at the end of the experiment (ranging from 3.56 to 4.55%), and it did not differ significantly among the groups. The final values of the performance were similar (P > 0.05) among the experimental groups. However, treatment with BI and DI were associated with slight increases in average body weight (2320.0 g and 2363.3g respectively) *versus* C (2310.0g). In addition BI and DI treatments were associated with slight reductions in the FCR compared to the C. In line with this, a trend towards increased European Broiler Index was observed for both BI and DI versus C. Slaughter traits results showed that chickens from the treatment groups were heavier (BI = +9.7%, P < 0.05; DI = +15.6%, P < 0.01) and showed a higher (P < 0.01) carcass weight and carcass yield compared with the C group. Moreover, the treated birds had higher PM weight (BI = +12.3%, P < 0.05; DI = +16.9%, P < 0.01) in comparison with those of the C group. There were no significant differences (P > 0.05) between the prebiotic groups. The TBARS values were lower in the meat of C birds in comparison with the prebiotic groups during storage (from 0 to 6 days). However, significant differences (P < 0.05) were only observed between the C and BI on day 4. In all experimental groups the TBARS values were similar between 0 and 2nd day of storage, followed by an increase of TBARS values after 4 days of storage with different magnitude in all groups. In conclusion, the values obtained for the meat at 6th day of storage (ranging from 0.18 to 0.032 mg MDA/kg) are below the critical value of 0.5 recognized for the production of rancid odour influencing negatively the taste of meat; *in ovo* administration of BI and DI was associated with significant improvements in a number of parameters of relevance to commercial poultry production, and thus, may offer a means for producers to increase productivity.

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Effect of dietary fermented and unfermented grape skin and vitamin E on digestibility of polyphenols, antioxidant and antimicrobial activity in chickens

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Grape skin is a source of polyphenols with antioxidant and antimicrobial properties. Little information is available regarding its application in animal feeds. An experiment was conducted to investigate the effect of inclusion of fermented (FS) and unfermented (UFS) grape skin at different doses (30 g/Kg, FS30 and UFS30, and 60 g/kg, FS60 and UFS60) and of α -tocopheryl acetate (200 mg/kg) in broilers fed a corn-soybean diet. Growth performance, ileal protein digestibility, ileal and excreta content, digestibility of total polyphenols and tannins, thigh meat lipid oxidation (at 1 and 7 days of storage) and intestinal microflora in one hundred and fifty 21-day-old broiler chickens were determined. Growth performances were negatively affected by the inclusion of FS60 and UFS60 in the diet with a worsening of weight gain and feed conversion ratio. Grape skin supplementation had no significant effect on protein digestibility, except in birds fed with UFS60, which showed a significant ($P<0.01$) decrease of protein digestibility. The inclusion of UFS grape skin in the diet increased significantly ileal and excreta total polyphenols content; whereas ileal and excreta tannins content increased in all experimental groups. The inclusion of FS and UFS grape skin in the diet did not affect ileal digestibility of total polyphenols, except in the case of birds fed UFS30 which showed a significant ($P<0.001$) higher value. Excreta digestibility of total polyphenols increased ($P<0.01$) in birds fed FS and UFS compared to the control group, with higher value ($P<0.001$) in birds fed UFS in comparison to those fed FS. Oxidative stability of thigh meat after 1 and 7 days of refrigerated storage, significantly increased with the dietary addition α -tocopherol, while grape skin did not exhibit any protective effect on meat lipid oxidation. Intestinal microflora was not affected by dietary treatment. In conclusion, grape skin (at 60 g/kg) impaired chicken growth performance and protein digestibility. Total polyphenols content was more bioavailable in the large intestine than in the small intestine. Antioxidant activity in chicken thigh muscle and intestinal microbiological activity were not influenced by grape skin diets. The antioxidant potential of FS and UFS grape skin was not effective as vitamin E.

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Polymorphism of prolactin gene and its association with growth and some biometrical traits in ducks

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The main aim of the study was to estimate the polymorphism of prolactin (*PRL*) gene and its relation with some morphological traits (body weight - BW, length of trunk with neck - LTN, length of trunk - LT, chest girth - CG, length of breast bone - LBB, length of shank - LS) of ducks. A secondary objective of this study was to evaluate, the effect of age, origin and sex on ducks growth performances. The experimental samples were composed of 53 Muscovy ducks (27 males, 26 females), 48 Pekin ducks AF51 (30 males, 18 females) and 45 Mulards (23 males, 22 females). The males and females of the Pekin ducks AF51 were evaluated for growth traits at 3, 5 and 7 weeks of age, the Muscovy females – at 3, 5, 7 and 10 weeks of age, the Muscovy males and Mulards (males and females) – at 3, 5, 7, 10 and 12 weeks of age. Prolactin genotypes were determined using the PCR-RFLP method. The multi-comparison Scheffe's test was used to separate the differences among the mean for statistical significance ($P < 0.05$). The digestion of the 417 bp PCR product that corresponds to intron 1 of the *PRL* gene with *Xba*I enzyme, differentiated two alleles (PRL^T and PRL^G), while the digestion of 400 bp PCR product corresponding to exon 5 of this gene with *Pst*I enzyme differentiated one allele (PRL^C). The PRL^G allele was detected in all examined groups of ducks, whereas the PRL^T allele did not occur at all in the Muscovy ducks. Three genotypes at locus *PRL/Xba*I and one genotype at locus *PRL/Pst*I were found. The analysis of χ^2 test showed that in the *PRL/Xba*I and *PRL/Pst*I locus the distribution of genotypes in those populations wasn't in Hardy-Weinberg equilibrium ($P < 0.01$). Pekin duck with the *PRL/TT* genotype in selected terms of evaluation were characterized by higher ($P < 0.05$) LS and LBB values than those with the *PRL/TG* genotype. In Mulard ducks *PRL/Xba*I polymorphism had an effect ($P < 0.05$) on BW and LS in birds aged 10 and 12 weeks. All growth traits examined significantly increased with age. There was a significant ($P < 0.01$) effect of the ducks' origin: until the seventh week of age, the Muscovies were lighter and had lower ($P < 0.01$) values of LTN, LT, CG, LBB, LS than Pekin and Mulard ducks. In next periods of evaluation an opposite trend was reported. At 10 and 12 weeks of age Muscovies were significantly heavier and had higher values for most of body parameters assessed in comparison with Mulards. The sexual dimorphism was clearly shown. The results confirm that there were significant associations between interaction of considered factors and estimated traits.

Development of an analytical method for the determination of Polycyclic Aromatic Hydrocarbons in large volume of surface water

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Polycyclic Aromatic Hydrocarbons (PAHs) are among the most diffused species worldwide: they are ubiquitous, come from incomplete combustion processes and cause serious effects on the human health. At authors' knowledge, studies on PAHs levels in surface waters are scarce. The main problem resides in the extraction procedures due to very low concentration of such compounds. This communication presents a new extraction protocol coupled with gas chromatography-mass spectrometry (GC-IT/MS) for simultaneous determination of nine PAHs in surface water. In particular, this technology has been successfully applied to PAHs determination in different surface water samples such as river water, tap water, spring water and water vending machine. The DLLME is a liquid-liquid extraction technique employing three solvents: the first is the aqueous phase, the second is the extraction solvent, immiscible in aqueous phase, which will extract the micro-pollutants, and the third is a dispersing solvent that favors the dispersion of the extraction solvent in aqueous phase. The combined action of these three solvents favors the formation of a stable and homogeneous emulsion. Iso-octane, showing a density lower than water, is used as extraction solvent. The sample volume of distilled water, choice of the extraction solvent, extraction solvent volume, extraction time, time required for the emulsion breaking and effect salting-out have been investigated. The important issue regards the procedure to form the emulsion without dispersing solvent but by means of ultrasounds. The optimum experimental conditions for the proposed protocol comprise 1000 mL of the distilled water sample, 300 μ L of iso-octane as extraction solvent, 2 min-ultrasound for the emulsion and 30 minutes on rotating plate to break the emulsion: the final solution appears quickly clear and separated into two layers. The upper layer (iso-octane) is collected and analyzed by GC-IT/MS. Under these conditions, this method provides a detection limit ranging between 0.001-0.009 ppb, Limit of Quantification between 0.003-0.022 ppb and recoveries ranging 101.2-110.3 % for all PAHs. The relative standard deviations (RSDs) are in the range 5.1-8.2 % (interday) for concentrations of 5-15 ppb. The important novelty of this method of extraction regards the enrichment factors, that was demonstrated to be significantly high (averagely 3000).

Southern Italian sourdoughs: technological characterization and exploitation of microbial biodiversity

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Southern Italy boasts of numerous typical breads obtained using traditional recipes and sourdough fermentation, one of the oldest biotechnological processes in cereal food production. Complex activities and metabolic interactions occur during fermentation and a complex and unique microbial ecosystem composition establishes, playing a key role in the definition of the final bakery products. Aim of the study was to characterize sourdoughs used for the production of southern Italian typical breads and to exploit the microbial biodiversity of sourdoughs using culture-dependent and independent methods. Twenty-eight sourdoughs from Campania region were analyzed for pH, titratable acidity (TTA), microbial composition. Total mesophilic count, lactic acid bacteria (LAB), yeasts, moulds, enterococci, *Enterobacteriaceae*, total and faecal coliforms were assessed by standard pour-plate technique. Furthermore, LAB biodiversity was investigated by culture-dependent and independent methods. Representative LAB were randomly selected and isolated from the samples and were identified by DGGE and sequencing of V1 region of 16S rRNA as described by Reale et al. (2011). At the same time, the total bacterial DNA was directly extracted from sourdoughs and DGGE was performed using universal primers for LAB. DNA was submitted to amplification by nested PCR: after the amplification of the gene encoding for 16S rDNA (Weinsburg et al., 1990), the amplicons were amplified for the region V3 (Muyzer et al., 1993), separated by DGGE and major bands were sequenced. Sourdoughs were characterized by a very low pH and high values of TTA. For most sourdoughs pH values ranging from between 3.51 to 3.72, while just seven samples from 4 to 4.5. TTA values varied from 4 to 15 ml of 0.1N NaOH/10 g sourdough, whereas most sourdoughs had TTA values ranging between 10 to 16 mL. LAB showed the highest microbial counts almost over time. LAB markedly varied between sourdoughs (10^5 to 10^9 CFU/g). In three samples, LAB were less than 3 log CFU/g. Seven sourdoughs showed higher yeast concentration in respect to the LAB count. Enterococci were found in only 4 samples of sourdoughs. No moulds and faecal coliforms were detectable in sourdough. *Enterobacteriaceae* were detected in six samples. Culture dependent methods allowed to identify numerous species of LAB. Out of 154 isolates, 118 were lactobacilli and 36 were cocci. Lactobacilli were identified mainly as *L. plantarum* (22%), *L. paralimentarius* (13%), *L. brevis* (5,8%), *L. pentosus* (5,8%), *L. rossiae* (5,2%), *L. zymae* (5,2%) and *L. sanfranciscensis* (5,2%). A minor presence was registered for *L. paracasei* (3,9%), *L. paraplantarum* (3,9%) and *L. sakei* (3,2%). Lactococci were identified as *Pc. pentosaceus* (8,5%), and *Lc. lactis* (4,5%), *Leuc. mesenteroides* (3,9%), *Weissella cibaria* (3,9%), *Kocuria kristinae* (0,7%), *Leuc. citreum* (0,7%) and *Leuc. pseudomesenteroides* (0,7%). Low isolates (about 4%) were not identified. The identification obtained by use of culture independent methods, DGGE-profiles of sourdoughs, allowed to ascertain the presence of sole four species isolated as such as *L. sanfranciscensis*, *Lc. lactis*, *Pc. pentosaceus* and *L. alimentarius*. Sourdoughs resulted populated by a mix of different and harmonious species of LAB, highlighting high biodiversity. The occurrence of LAB cocci, along with lactobacilli, was evident. The combined use of culture dependent and independent methods is essential to obtaining a complete profile of bacterial population occurring in fermented food.

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Influence of environmental pressures on the antimicrobial activity expressed by *Lactobacillus plantarum*

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Lactobacillus plantarum is a versatile and widespread microorganism found in different food-matrices and environments ranging from vegetables, dairy, and meat products, to the human gastrointestinal (GI) tract. Some strains of *Lb. plantarum* are known for their ability to produce several natural antimicrobial substances, such as bacteriocins, BLIS, phenyllactic acid, organic acids and hydrogen peroxide. The versatility of *Lb. plantarum* is at the root of its success in the industrial applications not only as starter culture but also as bio-protective agent. In this last field, the *in vitro* screening of bacterial protective properties is labour intensive and a large number of strains isolated from different food matrices is required. Therefore, it would be very interesting to ascertain the effect of different environments on the selection of strains able to exert antimicrobial activities. Nevertheless, the correlation between strain resistance to stress conditions and the ability to produce antimicrobial effects was poorly investigated. We sought more in depth knowledge of the relationship between antimicrobial properties of *Lb. plantarum* strains and their source of isolation. For this purpose, a total of 106 *Lb. plantarum* strains (producers) were tested against 33 undesirable microorganisms (indicators), including both moulds and bacteria. The antimicrobial activity exerted by growing cells was evaluated by the spot-on-the-lawn, while the activity of cell free supernatants (CFS), neutralized CFS (nCFS) and CFS treated with proteases (pCFS) was assessed by the agar well diffusion assay. The results evidenced a stronger antagonistic activity exerted by *Lb. plantarum* growing cells isolated from wines than that of cells isolated from the other fermented matrices. Moreover, 5 CFS from wine strains, as well as the corresponding nCFS and pCFS were able to inhibit different bacteria and moulds. Noticeable was the datum evidencing the relationship between the environment of isolation and antimicrobial properties of *Lb. plantarum* strains. Therefore, data reported in this study indicate that specific food conditions are able to influence the occurrence of certain strains able not only to respond to specific adverse conditions, but also to compete with other bacterial populations. So the most important scientific enrichment produced by this study is ascribable to results highlighting that the choice of the source of isolation could be an important preliminary tool for the individuation of antagonistic strains.

Effects of intramuscular Vitamin E multiple injections on growth performance, oxidative stability and sensory characteristics of Laticauda lamb meat

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Over the recent years, there has been growing pressure from the consumers demanding improvement in the quality of meat and meat products, especially of dietary fats. This has led to attempts to modify meat and meat products by dietary strategies guided towards increasing their PUFA contents. However, high unsaturated fatty acid content of meat can lead to alterations in the quality traits such as tenderness, flavour and muscle colour of meat due to their susceptibility to oxidation in the absence of antioxidants. The effects of multiple vitamin E intramuscular injections on growth performance, lipid oxidation, and sensory characteristics were studied in Laticauda lambs. Twenty four 15 day old Laticauda suckling male lambs were randomly divided into two equal groups and weekly intramuscular injections of DL- α -tocopheryl acetate (Control, 0 IU; Vitamin E treatment group, a total dose of 1500 IU) were given until the lambs were 57 days old. Lambs were slaughtered at 64 days of age. *Longissimus dorsi* and *Vastus lateralis* muscles were collected and analyzed for oxidative stability. Moreover, left pelvic limb was excised for sensory analysis. Vitamin E treatment did not influence growth, live weight, hot and cold carcass weights but lowered the hot (- 2.3 %; $P < 0.05$) and cold (- 2.2 %, $P = 0.07$) dressing percentages. There was no difference ($P > 0.05$) between the control and vitamin E groups on the pelvic, shoulder and loin weights as well as *longissimus* muscle area. Vitamin E improved lipid oxidative stability. Meat from vitamin E treated lambs received higher hedonic scores for tenderness and juiciness than the respective control. In conclusion, this study has shown that intramuscular vitamin E multiple injections reduce lipid oxidation, and increase consumer acceptability of lamb meat.

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Cryopreserving turkey semen in straws and nitrogen vapor: effect of two different cryoprotectants (DMSO or DMA) and their concentrations on the post-thaw semen quality

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This study was designed to identify the best cryoprotectant (DMSO or DMA) and the most suitable concentration (4 and 10% for DMSO; 8 and 18% for DMA) in order to obtain an effective freezing turkey semen protocol in straws exposed to nitrogen vapor. Semen samples were collected by abdominal massage and pooled (4-6 ejaculates/pool;) to avoid the effect of individual differences among males. Totally seven semen pools were used. The fresh semen quality was assessed in an aliquot taken from each pool, and the remaining pooled semen was cooled at 4°C for 25 min before freezing. After cooling the semen pools were diluted 1:1 (v:v) with a freezing extender composed of Tselutin diluent containing DMA or DMSO to give final concentrations of 8% or 18% DMA and 4% or 10% DMSO. Totally four semen aliquots were obtained. The semen was packaged in 0.25 mL plastic straws and equilibrated at 4°C for 20 min. Semen samples were frozen by exposure to liquid nitrogen vapor at the height of 10 cm for 10 min and then directly plunged into liquid nitrogen at -196°C. After thawing (50°C for 10 seconds), sperm motility, viability and membrane functional integrity were evaluated. Sperm motility was subjectively evaluated by use of phase-contrast microscope. Sperm viability was determined using two fluorescent stains SYBR-14 and propidium iodide (PI). Functional membrane integrity was determined by hypoosmotic swelling test (HOS). Although frozen semen quality decreased significantly in respect to that of the fresh semen, the cryosurvival of turkey sperm was significantly affected by the cryoprotectant (CPA) and its concentration. The 10% DMSO registered significantly higher values of sperm quality than the DMA (8 and 18%) and the 4% DMSO except for sperm membrane integrity (table 1). No significant differences between the two concentrations of DMA were observed.

Table 1. Effect of two cryoprotectants (DMSO or DMA) and their concentrations on the post-thaw semen quality.

Semen treatment			Sperm quality (mean ± SE)		
	CPA	CPA concentration (%)	Motility	Viability	Membrane integrity
Fresh			77.21 ± 2.04 ^a	78.84 ± 1.29 ^a	58.97 ± 2.20 ^a
Frozen (CPA)	}	DMA 8	16.92 ± 1.59 ^d	20.97 ± 0.20 ^d	13.24 ± 1.35 ^c
		DMA 18	21.28 ± 2.05 ^{cd}	26.73 ± 2.82 ^{cd}	15.40 ± 1.71 ^c
		DMSO 4	23.57 ± 3.05 ^c	31.09 ± 4.94 ^c	19.36 ± 2.61 ^{bc}
		DMSO 10	36.57 ± 1.79 ^b	42.09 ± 1.50 ^b	25.15 ± 2.65 ^b

a-d Different superscript letters within the same column indicate significant differences (P < 0.05).

CPA: cryoprotectant.

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Effects of intramuscular Vitamin E multiple injection on physicochemical and nutritional properties of Laticauda lamb meat

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The effects of intramuscular injection of vitamin E on physicochemical and nutritional properties of meat were studied in Laticauda lambs. Twenty four 15 days old Laticauda suckling male lambs were randomly divided into two equal groups and weekly intramuscular injections of DL- α -tocopheryl acetate (C, Control group, 0 IU; live weight: 11.29 ± 0.66 kg. V, vitamin E-treatment, a total dose of 1500 IU; live weight: 11.33 ± 0.70 kg) were given until the lambs were 57 days old. Lambs were slaughtered at 64 days of age. *Longissimus dorsi* (LD) muscle pH, water holding capacity (WHC) and colour were measured at 24h post-mortem. LD and *Vastus lateralis* (VL) muscles were removed from carcass for DL- α -tocopherol content. Moreover, fatty acid (FA) composition and cholesterol content were evaluated in LD muscle. The treatment did not affect ($P > 0.05$) pH₂₄, and WHC of LD muscle. The recorded pH values (5.68 and 5.66 in C and V groups, respectively) are within the acceptable range for commercial meats. Vitamin E treatment affected only the red index (a*), that was higher in the lambs of V group compared to those of the control (16.74 versus 15.51, respectively; $P < 0.05$). In both the LD and VL muscles, α -tocopherol concentrations were higher ($P < 0.05$) in DL- α -tocopheryl acetate treated lambs than in those of C group. Moreover, significant ($P < 0.05$) differences were observed in the α -tocopherol concentrations between the muscles of treated lambs (6.41 and 7.31 $\mu\text{g/g}$ in LD and VL respectively). Vitamin E treatment affected only the total PUFA content, which was higher in V group compared with the control one (15.58 versus 13.48, respectively; $P < 0.01$). In terms of individual SFA, heptadecanoic acid (C17:0) was higher ($P < 0.01$) in the C group, while lauric acid (C12:0) was slightly higher ($P = 0.077$) in V group. Among the single MUFA, meat from treated lambs had higher ($P < 0.05$) C22:1, and lower C17:1 ($P < 0.01$) and C18:1 n-9 *trans* ($P = 0.054$) compared with C group. Among the PUFA, in both experimental groups, the most abundant was C18:2 n-6 (7.77% and 7.92% for the C and V group, respectively; $P > 0.05$). The content of linolenic acid was increased (+ 0.37%; $P < 0.01$) by the vitamin E treatment. Vitamin E treated group had higher ($P < 0.01$) content of C 22:2n-6 and n-3 long chain PUFA: eicosapentaenoic fatty acid (EPA, C20:5n-3, $P < 0.05$), docosapentaenoic fatty acid (DPA, C22:5n-3, $P < 0.01$) and docosahexaenoic fatty acid (DHA, C22:6n-3, $P = 0.057$). Furthermore, the vitamin E treatment increased ($P < 0.01$) the PUFA to SFA ratio and lowered ($P < 0.01$) the n-6 to n-3 ratio and thrombogenic index. Cholesterol content (ranging from 78.79 to 79.0 mg/100g) was not affected by vitamin E treatment ($P > 0.05$). In conclusion, the results of the present study indicate that multiple intramuscular injections of DL- α -tocopheryl acetate maintained the redness of meat and produced meat with better nutritional characteristics with respect to the fatty acid profile, thrombogenic index and vitamin E content.

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Biological activity of extracts from wild hop *Humulus lupulus* (L.) against *Sitophilus granarius* (L.)

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Hop, *Humulus lupulus* (L.) is a high-climbing, perennial vine, utilized in the brewing industry to add flavor and bitterness to beer. Hop is counted in the category of aromatic plants containing substances rich in essential oil (EO) that has different biological properties. An interesting perspective in insect control is the use of natural products of plants as alternative ecologically compatible in substitution to the synthetic insecticides. Insect pests are responsible for the 20% loss of the world's annual crop production and 40% of food grains loss in granaries and storehouses. The granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae), is a serious pest of stored grains that causes major losses by its feeding activity and excretory products. The aim of this work is to deepen the knowledge on the biological activities of extracts from wild hops, by bioassays towards *S. granarius*. Aerial parts of hops were collected during the flowering stage in Bojano (Molise region, Italy). Plant extracts were obtained by solvents with different polarity (methanol, acetone and hexane) and by hydro-distillation in a Clevenger-type apparatus for 3 h. The major components were identified by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis. Bioassays tested were the topical application of extracts and EO on pronotum of *S. granarius* adults and flour discs treated with extracts for effects on nutritional indices and antifeedant activity. Contact toxicity bioassay showed that extracts and EO were toxic for *S. granarius* adults. Mortality rose with the increase of dose but did not show differences between 24 and 48 h after treatment. Mortality reached 100% for solvent extracts at the dose of 75 µg/adult, showing significant differences compared to control up to 9.37 µg/adult for alcoholic and acetone extracts and 18.75 µg/adult for hexane extract. EO reached 100% of mortality at the dose of 109.37 µg/adult and showed significant differences with the control up to 13.67 µg/adult. The highest activity of toxicity was observed for EO at LD₅₀ and LD₉₀ values of 13.3 and 40.2 µg/adult after 24 h of application, respectively, decreasing to 11.8 and 36.8 µg/adult after 48 h. Acetone extract was the most active among those obtained with solvents. LD₅₀ and LD₉₀ values were 16.2 and 32.2 µg/adult after 24 h of application, respectively, decreasing to 14.9 and 32.1 µg/adult after 48 h. Nutritional studies showed that the different extracts had significantly effects on growth rate, food consumption and food utilization. The greatest deterrence, 57% and 74% after 3 and 5 days respectively, was observed for methanol extract. The highest ingestion mortality, 52% at the concentration of 750 µg/disc after 5 days from treatment, was registered by acetone extract. This preliminary study indicated that wild hop produce substances acting on insects by contact and ingestion toxicity, which can be useful for pest control in storehouses when properly extracted.

Studies on insecticidal activities of *Lavandula angustifolia* Miller (Lamiaceae) essential oil against the stored grain pest *Sitophilus granarius* (L.) (Coleoptera, Curculionidae)

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Sitophilus granarius (L.), known as granary weevil, is an insect of stored grains that causes main quantitative and qualitative losses for its larval development within kernels, for feeding activity and excretory products. Recently there has been a growing interest in research concerning the possible use of plant extracts as alternatives to synthetic insecticides easily available at low cost and many essential oils provided potential alternatives to the currently used insect control agents. *Lavandula angustifolia* Miller, is an important aromatic species of the Lamiaceae family, widely distributed in the Mediterranean area, with essential oil (EO) of different biological properties, including insecticidal activity. In the present study, EO of lavender flower heads were extracted by hydrodistillation and its major components were identified by gas chromatography (GC) and gas chromatography- mass spectrometry (GC-MS) analysis. Lavender EO, at several concentration, was tested for contact and fumigant toxicity, repellent activity, effects on nutritional indices and antifeedant activity against *S. granarius* adults. Bioassays tested were: EO topical application on the pronotum adult for contact toxicity, EO impregnated filter paper for fumigant toxicity and repellent activity, EO treated flour discs for effects on nutritional indices and antifeedant activity. Contact toxicity bioassay showed that EO is toxic for *S. granarius* adults. Mortality rose with the increase of EO concentration after 24 and 48 h from application and reached mortality rates higher than 80% after the dose 224.5 µg/adult. LD₅₀ and LD₉₀ values were 83.8 and 379.7 µg/adult after 24 h of application respectively, decreasing to 58.3 and 208.3 µg/adult after 48 h. In fumigation chambers, EO showed toxicity on *S. granarius* adults with LC₅₀ values of 18.3, 2.6 mg/L air and LC₉₀ 80.0, 6.9 mg/L air in presence and in absence of wheat grains, respectively. Filter paper and arena bioassays both revealed EO repellent activity against granary weevil adults. In filter paper bioassay, the percentage of repellency was more than 90% (V repellency class) starting from the 0.44 mg/cm² dose at different observation times (5, 30, 60, 90, 120 min). In arena bioassay, in the presence of wheat grains, a significant EO repellent activity was found starting from the 1.1 mg dose. Nutritional studies showed that lavender EO did not significantly affect growth rate, food consumption and food utilization, whereas antifeedant activity was about 21% at 1.1 mg/disc. The results suggested that lavender EO, appropriately formulated, may be useful to protect stored grains against granary weevils as a possible alternative to chemical insecticides by contact, fumigant and repellent activities.

Identification of genes involved in resistance to *Fusarium oxysporum* in chickpea (*Cicer arietinum*) ecotypes from Central Italy

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Chickpea (*Cicer arietinum* L.) is a self-pollinating diploid ($2n=16$) cultivated species genetically distinguished in two subpopulations: Desi, which have purple flowers and small, dark, angular seeds with a higher fibre content, and Kabuli, which have white flowers and large, cream-coloured seeds. Desi is a kind of chickpea grown in India, Pakistan and East Africa while Kabuli is preferred in the Mediterranean and Central Asia and actually is spread around the world. Chickpea is the third most important grain legume in the world after soybean and beans, covering an area of 11.5 million ha. It is mainly used for food, and has unique physical characteristics, chemical composition and low content of anti-nutritional components compared to other legumes. In Italy, the annual mean yield of chickpea is about 1.9 t ha^{-1} and cultivated surface is actually increasing. The crop is mainly carried out by little farms so that the use of local ecotypes, which usually are identified with the name of the location where they are traditionally cultivated, is widely diffused. Due to the different ecological selective pressure these ecotypes could be differently affected by pathogens such as *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) and *Ascochyta rabiei* with significant reduction of yield too. The aim of this work was to characterize 18 chickpea ecotypes from Central Italy for genetic resistance to *Foc*, date of flowering and grow habit. The following studies were carried out: i) pathogenicity tests in growth chamber; ii) field pathogenicity tests and iii) genetic analysis by using molecular markers known to be associated to genome regions conferring resistance to diseases. The pathogenicity tests evidenced that only ecotype Longano displays full resistance to *Foc*, ecotype S.Elia a Pianisi shows a partial resistance, while all the other ecotypes are susceptible. Genetic cluster analysis showed the presence of two main groups of ecotypes. Moreover, in some ecotypes it has allowed the identification of the allele associated with resistance to *Foc* and the allele associated with susceptibility in the microsatellites TA27 and TA59, which are known to be associated with the QTL involved in the resistance to this pathogen. The results are discussed also in relation to the potential use of local genetic resources to improve chickpea cultivation and pathogen resistance. By molecular analysis we identified a genetic marker allele associated with resistance to *Foc* in ecotype Longano; in addition by comparison with several Spanish chickpea cultivars, some Italian ecotypes results more similar to Spanish lines than to the Italians populations analyzed.

Investigations on the transmission of apple proliferation phytoplasma by psyllids

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Phytoplasmas are microorganisms associated with severe plant diseases affecting many agricultural crops worldwide. Only phloem-feeding insects can potentially acquire and transmit these obligate parasites. Some species of the psyllid genus *Cacopsylla* (Hemiptera: Psyllidae) have been demonstrated to be involved in the transmission of important fruit tree phytoplasmas in Europe: *Candidatus* Phytoplasma mali associated with apple proliferation (AP), *Ca. P. prunorum*, the agent of European stone fruit yellows (ESFY), and *Ca. P. pyri*, the agent of pear decline (PD). The three phytoplasmas differ in serological comparisons and show clear differences in vector transmission and host-range specificity. Two psyllids, *C. picta* (Foerster) and *C. melanoneura* (Foerster), are confirmed vectors of '*Ca. P. mali*', but the studies conducted in different geographical regions show different transmission efficiency for the two species. Despite years of systematic control and the consequent strong reduction of psyllids population density, AP is still a major threat for apple production in Trentino-Alto Adige region and researches are ongoing in order to better understand the epidemiology of this disease. Here, we report the preliminary results of the investigations conducted in Trentino and Alto Adige on the capacity of acquisition and transmission of *Ca. P. mali* by *C. picta* and *C. melanoneura*. Transmission trials with both species (*C. melanoneura* and *C. picta*) collected in Valsugana (Eastern Trentino) were conducted under semi-field conditions in spring of 2015. The experiments involved overwintered adults, nymphs and new generation adults. Individuals of each stage involved were first confined on infected apple plants for phytoplasma acquisition and then moved to healthy test plantlets. At the end of the trials, the insects were recollected and analyzed by PCR and the plantlets were recovered in screen-house until November and then analyzed for the presence of *Ca. P. mali*. Recently, the phytoplasma transovarial transmission was investigated in *C. pruni*, which is the vector of *Ca. P. prunorum*, and in *C. melanoneura*. Therefore, preliminary studies were here started also on *C. picta*. Nymphs and adults derived from infected overwintering adults of *C. picta* were reared on non-infected apple saplings and tested by PCR for the presence of the phytoplasma. The results of this study indicate for the first time the possibility of a vertical transmission of *Ca. P. mali* in *C. picta* and thus provide the basis for a more detailed understanding of *Ca. P. mali* transmission by *C. picta*.

Innovative mechanical ventilation for a greenhouse microclimate control

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The increase of annual crop yield in greenhouse is possible only through a very close control of the internal conditions. Currently available conditioning plant solutions require high investment costs. In addition, high operational costs are required for an efficient solution without reducing crop yield or quality. Therefore, the conditioning of the internal air of a greenhouse occurs through the use of fossil fuels, especially during winter. Ventilation systems could allow proper control of temperature, relative humidity and CO₂ rate. For this purpose a research is being carried out. A prototype of a mechanical ventilation unit and two climate chambers for the reproduction of external and internal air was built in the laboratory. The recovery unit is equipped with a heat pump and is able to increase the thermal energy recovered, by the flow of exhaust air, through a high efficiency heat exchanger. The first study was carried out to evaluate the energy performances of the system during the control of temperature in winter season. Tests were performed at different temperature values of simulated external air T_E (-5°C, 0°C, 5°C and 10°C) and a fixed (reference) internal simulated greenhouse temperature (20°C). Each trial was performed with a ventilation flow rate of 535 m³/h. The resulting coefficient of performance of the overall system (COP_s) was 9.50 at 0°C, 8.86 at 5°C and 6.62 at 10°C respectively. It should be highlighted that during the trials conducted at -5°C the compressor behaved as an on-off type. This is due to a safety mechanism for the defrost of the evaporator. Also the ventilation flow rate was reduced to avoid a too low value of the supply in air temperature. For the other trials ($T_E = 0^\circ\text{C}$ or 5°C), the overall COP_s decreases when the external temperature increases, due to a lower difference between external and indoor air enthalpy. Ultimately it can be concluded that there exists an upper and a lower limit value of external temperature to which it is appropriate to change the operating setting. For values below the lower limit it is possible to operate with only the passive recovery and a heating system at service of greenhouse; while, for temperature values above the upper limit it is possible to operate with only the active recovery, until the external conditions will allow freecooling/freeheating.

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Mediterranean fruit fly in Trentino: studies on the varietal susceptibility in apple

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The Mediterranean fruit fly (Medfly), *Ceratitidis capitata* (Wiedemann), is one of the main insect pests worldwide, infesting the fruits of more than 300 species. Also in Italy, it develops on many ripening fruits, attacking also apples. The presence of *C. capitata* in our Country was registered for the first time in 1863, while in Trentino it was reported in 1990. After a period with no damages observed, *C. capitata* reappeared in 2010 and was found in the following years in the areas of Riva del Garda and Trento. The northern limit of the Medfly distribution was indicated around the 41° parallel and its presence above it, mainly due to the trade of infested fruits, is just occasional, as this species cannot stably survive in most of these areas. However, in some limited areas of Trentino, *C. capitata* was constantly reported in the last two years (2013 and 2014) The first step for studying the bioethology of the Medfly in Trentino is to know the susceptibility of the main apple varieties cultivated in the region and the duration of the developmental cycle of the larvae inside the fruits. Therefore, in this work we will show the preliminary results of the evaluation of preference for three different apple varieties (Royal Gala, Red Delicious and Golden Delicious) in field. Apples on the branch were selected at full ripening, or when they had reached the maximum sensitivity for the attack by this dipteran. Twenty-five cages were installed on different apples trees for each variety. Each apple selected was put inside a cage with 5 mature females (i.e. females that had started laying eggs since two days) and one male of *C. capitata* to ensure an adequate oviposition on apple. The J.R.C. ISPRA insect strain reared in the entomological laboratory at Fondazione E. Mach (San Michele all'Adige, Trento, Italy) was used for the experiments. For each variety, the number of eggs laid and the duration of larval stage (in days) were evaluated in relation to climate conditions (min. and max temperature, humidity, rain) and the physical-chemical characteristics of the ripen fruits (peel hardness, pulp hardness, % sugar, acidity, starch, color). The average number of bites/apple was calculate on 25 apples inside the cages; 10 were then inspected to count the eggs (evaluated with a direct count under stereomicroscope) and 10 were left in the field until the end of the larval cycle (from sleeves installation to the exit the mature larvae from apples). Considering the number of egg-laying bites, the variety Royal Gala seems to be the most sensitive to the Medfly, with an average of 2.4 bites/apple. The mean number of bites/apple recorded on Red Delicious and Golden Delicious were 1,3 and 1,6, respectively. So far, the evaluation of the duration of larval development in the orchard has been completed only for Royal Gala and Red Delicious because they are early varieties. The development of larvae lasts 28 days in Royal Gala and 44 days in Red Delicious.

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