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Nitra (SK)



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UTP, University of Science and Technology
in Bydgoszcz (PL)



International
PhD Programme
in Agriculture Technology
and Biotechnology

II International PhD Workshop on

Technology and Biotechnology in Agriculture, Livestock and Foods

14th December 2018

Book of Abstracts
Guest Editor:
Prof. Giuseppe Maiorano

II International PhD Workshop

Campobasso, December 14, 2018

**Aula G. Mendel, Department of Agricultural, Environmental and Food Sciences
University of Molise**

**Organized in the framework of the
International PhD Programme in
Agriculture Technology and Biotechnology**

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

Workshop programme

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- 09.30 – 10.00 Welcome and greetings
- Prof. Gianmaria Palmieri – *Rector of University of Molise, Italy*
Prof. Raffaele Coppola – *Head of Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy*
Prof. Giuseppe Maiorano – *PhD Coordinator, Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy*
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Chairman: Prof. Antonio De Cristofaro, Prof. Raffaello Castoria
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Chairman: Prof. Antonio De Cristofaro, Prof. Raffaello Castoria
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Chairman: Prof. Elena Sorrentino, Prof. Patrizio Tremonte
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- 17.15 – 17.30 *Closing ceremony*
Prof. Giuseppe Maiorano – *PhD Coordinator, Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy*

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Friday December 14

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Chairman: Prof. Antonio De Cristofaro, Prof. Raffaello Castoria

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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 Programme is activated in “Agriculture Technology and Biotechnology”. The Programme is organised between the following partners:

- Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy;
- 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 programme is focused on sustainable agriculture, food safety and quality, animal welfare, green economy and environmental law, aligned to Horizon 2020 priorities.

The PhD programme is organized into 3 *curricula*: Sustainable Plant Production and Protection; Food Science, Technology and Biotechnology; Welfare, Biotechnology and Quality of Animal Production. It represents the continuation of first and second level degree courses (Agricultural and Forestry Science and Technologies; Agricultural Science and Technology; Food Science and Technology; Forest and Environmental Science) activated by the Department of Agricultural, Environmental and Food Sciences. The PhD programme 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 agri-food sector. The educational goals of the programme are designed to address the needs of fundamental and applied research, with particular emphasis on the latter (see notes in the *curricula*) The Doctoral programme 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, the sustainable management of forest and agricultural ecosystems, and for the protection of biodiversity by reducing chemical inputs in agricultural ecosystems.

The *Curriculum* Food Science, Technology and Biotechnology aims to train professionals able to develop and apply new scientific knowledge in the field of food processing and to promote research, innovation and the technology transfer in food production processes.

The *Curriculum* Welfare, Biotechnology and Quality of Animal Production aims at providing skills and expertise in the area of animal production, and to develop biotechnological and technological innovation to optimize the production, health and welfare of the animals and enhance the quality of animal products. 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

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. Ing. Adriana Kolesarova

Preliminary evaluation of *Drosophila suzukii* females' behavioral responses to fruit odors of non-crop hosts

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Drosophila suzukii Matsumura (Diptera: Drosophilidae), is a highly polyphagous pest of fresh fruits. It has recently invaded both Europe and Americas, and it is causing serious losses in the production of soft fruits. Laboratory experiments were carried out to evaluate the behavioral responses of *D. suzukii* females to odors released by fresh and ripened fruits of non-crop hosts. Naive mated females were used in the study and fruits of wild European gooseberry (*Ribes rubrum*), wild European blackberry (*Rubus fruticosus*), wild cherry (*Prunus avium*), and wild white mulberry (*Morus alba*) were collected from different fields of the Molise region, Italy. The response of *D. suzukii* females to host fruit odors was investigated in two-choice assays using Y-tube olfactometer. The fruits were tested as non-sterilized and sterilized fruits. Sodium hypochlorite solution (1%) was used in the chemical sterilization of fruits. Ten flies were introduced at once to the stem of Y-tube and were observed for one hour. Each fruit species was tested separately in the olfactometer. The data obtained showed that all fruit types tested were significantly more attractive than the control. It was found that *D. suzukii* female flies were more attracted to non-sterilized fruits than sterilized fruits. Results depicted that each fruit type showed significant differences in response of the flies to sterilized and non-sterilized treatments. The highest attractiveness of fruits was recorded in wild European blackberry, and *D. suzukii* responses to four different species of non-crop host fruits can be ranked as follows: wild European blackberry \geq wild white mulberry \geq wild cherry \geq wild European gooseberry. The present study reports for the first time the behavioral response of *D. suzukii* to odors emitted by sterilized and non-sterilized fruits of non-crop hosts. Our bioassays data indicate that olfaction has a major role in host selection for feeding and oviposition in host fruits. The attractiveness of female flies towards non-sterilized fruits can be related to microorganisms associated with fruits surface and confirms that *D. suzukii* is able to infest a wide range of non-crop hosts, which may provide alternative potential fruits for *D. suzukii*. Our results contribute to the development of a new concept of trap, in which the attractiveness of commercial baits would be strongly increased by the combination of microorganisms releasing biologically active volatile compounds to *D. suzukii*. Traps baited with this new lure can contribute in setting up new environment-friendly control methods such as mass-trapping and attract and kill techniques.

Acknowledgements

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A database of the alimurgical plants in Italy

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The rediscovery of popular culture has become a scientific investigation, but also a preservation and an enhancement of the local traditions and of the "popular knowledge". This cultural context, arisen in the last decade, has exponentially increased the interest in food consumption of Wild Edible Plants (WEP).

The high consumption of fruit and vegetables, as a characteristic of the Mediterranean diet, has traditionally included wild fruits but especially wild vegetables, as reported by many ethnobotanical studies.

WEP, also known as alimurgical plants, played in the past an important historical role in integrating and enriching the diet based on basic agricultural food, considered an integral part of the Mediterranean basin diet. Although many species considered alimurgic are widespread throughout the Mediterranean basin, only few species (about 30) are currently used in the human food consumption.

The creation of a database of the Italian alimurgical flora (AlimurgITA) has therefore the aim to systematize the baggage of the extensive (and often disordered) knowledge on the WEP acquired in Italy in the last 100 years, creating an easy-to-read tool not only for purely speculative purposes but also for the practical ones (e.g. cultivation, presence of active compounds, etc.).

The design and population of the database was performed by means of the discovery and the analysis of bibliographic resources from 121 ethnobotanical and phytoalimurgical works published in Italy since 1918 to these days. For each of the 465 alimurgic species considered and reported in the database, 94 fields related to taxonomic, morphological, geographical and food use characteristics were considered.

The organization of this database and its internal structure will allow the retrieval of information through the online query, an evidence-based information resource, which will be available in the near future and useful for those scientific communities that will want to take advantage by having of a shared data set.

This study, aims to provide an useful tool to enhance the value of the alimurgical species, not commonly or rarely used but considered of a great nutritional and organoleptic value. The study presumes, also the creation of a WEP production chain (from population research to cultivation and marketing).

The website will be published at the beginning of 2019; further enrichments of data will allow to connect it to other scientific plant databases and bibliographic datasets. The data integration with global relevant data sources will allow AlimurgITA to enlarge its reference community, and to assist botanical investigations in retrieving locations of relevant bibliographic resources.

Development of attractant devices and an Automatic Trap for the Monitoring of the Olive Fruit Fly *Bactrocera oleae* (Gmelin, 1790) (Diptera: Tephritidae)

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The olive fruit fly *Bactrocera oleae* (Gmelin, 1790) (Diptera: Tephritidae) is considered the most serious pest of olive in many areas throughout the world, affecting the quality and quantity of oil and table olives during the infestations in late summer and autumn. The present research project focuses on the comparison of some commercial or modified traps, baited with different attractants, to be selected for the development of a semiautomatic e-trap for the monitoring of *B. oleae*. Various traps and attractants have been evaluated for selectivity toward predators and parasitoids. The experimental sites were located in an inner hilly area of the Campania region. Here the field trials carried out in 2018 are presented. Two tests were performed to compare the efficacy of Jackson traps (Jack) baited with pheromone (P) alone or in combination with ammonium carbonate (AC), using the green Jackson trap with yellow panel inside (JackY) or the yellow Jackson trap with yellow panel inside (JackYY) in a 4x4 latin square experimental design (not baited Jackson traps have been used as control). In both cases, the best performance was observed for Jackson+AC+P, followed by Jackson+AC and Jackson+P, not statistically different for Jackson trap not baited. Results were different in the two sexes: in males catches, JackY+P had the highest catches, not statistically different from JackY+AC+P, followed by JackY+AC and JackY not baited, statistically different one from the other. For the female catches, JackY+AC+P and JackY+AC had higher catches. JackY not baited and JackY+P had lowest levels, not statistically different one from the other.

A different test was carried out to evaluate a possible decrease of captures in the Jackson trap, due to the saturation of the sticky surface over time. Two yellow Jackson traps (JackYY) and two standard Jackson traps (JackY) were used in a 4x4 latin square design, where one of each model was cleaned after each control (every 3 days), and the other was left uncleaned. Among traps, cleaned Jackson had highest *B. oleae* adult catches and not cleaned Jackson had lowest catches. The results also showed a decrease in total catches as the checking days progressed, with the relative proportion of catches in cleaned traps increasing over time.

Two automatic traps (e-traps) were installed in the field from the end of August to the mid of November. The trapping component incorporated into the e-traps was obtained by an all yellow Jackson trap (JackYY), baited with ammonium carbonate, modified in the upper part to host an additional plastic part containing the digital camera. The image quality was evaluated by comparing the remote counting of *B. oleae* adults in the digital images and the number of flies counted by a scout in the field in the same trap and day. Results of the statistical regressions indicated a very high correlation between the two types of counts. The catches of olive fruit flies in the e-traps were contrasted with numbers of flies captured in conventional Jackson traps, with same color and size, run in parallel. Results showed higher catches in e-traps than in conventional Jackson, but differences were statistically not significant.

An approach based on Vis-NIR reflectance spectroscopy to characterize soil organic C dynamic in organic farming

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The capture and storage of soil organic carbon (OC) should improve the soil's quality and function and help to offset the emissions of greenhouse gases. However, to measure, model or monitor changes in OC caused by changes in land use, land management or climate, cheaper and more practical methods are needed to measure it and its composition. Conventional methods are complex and prohibitively expensive. Spectroscopy in the visible and near infrared (Vis-NIR) is a practical and affordable alternative.

The aims of this research, conducted in an organic farm specialized in production of bio-apples and located in north-west of Molise region, are to monitor "carbon dynamic" in the soil and its biological fertility, applying Diffuse Reflectance Spectroscopy, and to link the management of soil and crops, in order to optimize production and minimize environmental impacts.

After soil sampling (December 2017), measurements of soil parameters were made, such as temperature and relative moisture of top-soil, pH and electron conductivity (EC), clay content (pipette method), total CaCO₃ content, total organic carbon (TOC) content (Walkey-black and Loss On Ignition procedures) and determination of soil respiration rates (CO₂ ppm/g*h).

Firstly, soil sampled has been classified as Eutric Cambisols, in according to WRB-FAO classification.

From the principal component analysis (PCA) of the acquired spectra for the samples, it results that PC1 and PC2 explain 98% of the total variance; in particular, CaCO₃ content value is very well explained by PC2, such as soil respiration rate by PC1.

The obtained information was subsequently used for plotting of soil thematic maps through GIS software. The spatial distribution of the OC is due not only to the quantity and quality of the soil organic matter, but also to the variations of the temperature and humidity soil parameters: with decreasing of soil moisture both temperature and OC content increase. Further, a great amount of clay content corresponds to a great amount of OC, which is well correlated to soil respiration rates.

Moreover, in order to have a measure of the vegetative vigor of the plants, the circumference of the plants trunk was measured at the points where the soil samples were taken. In this case, maps do not show correlation between plants vigor and other parameters, such as OC or soil respiration rates.

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Phenolic fraction quantification in hop ecotypes (*Humulus lupulus* L.) in Central Italy

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Hop (*Humulus lupulus* L.), is an aromatic plant rich in many biologically active substances used in different fields. The female flowers localized in cone-shaped inflorescences (cones) that contain a yellow-orange coloured resin, referred to as lupulin, are mainly used in the production of beer. However, the high content of antioxidant substances is also of interest. In Italy, hop is not cultivated, but spontaneous plants are abundant and for this reason the interest in the identification and characterisation of new ecotypes is increasing. Recently, in the areas of Bojano (Campobasso province, at 480 m asl), 99 Cannelle (L'Aquila province, at 700 m asl), Bussi 1 and Bussi 2 (Pescara province, at 344 m asl), Onna (L'Aquila province, at 571 m asl), and Roio (L'Aquila province at, 714 m asl) were collected (in September, 2017) 3 samples each of wild hop cones and subsequently dried (room temperature, dark, for 7 days) and ground. The phenolic fraction was extracted (1.5 ml of ethanol 80%) from aliquots (0.1 g) of each sample, separated by centrifugation (20', 9168 x g, 5°C) and stored at -20° C until used. For each fraction the content in polyphenols ($\lambda = 765$ nm) and flavonoids ($\lambda = 510$ nm), both free and bound and the relative antiradical power (DPPH method, $\lambda = 517$ nm) were determined spectrophotometrically. The fractions obtained were also evaluated for their anticholinesterase activity ($\lambda = 412$ nm). The total polyphenol content ranged from 33.91 ± 0.78 (in Onna) to 78.28 ± 1.36 (in 99 Cannelle) mg/g dry weight (DW) (\pm ES), with statistically significant differences ($p < 0.05$) (ANOVA- Tukey HSD test) among some ecotypes, differentiable in 4 groups. With the exception of the sample collected in Molise (Bojano), the bound polyphenols, ranging between 10.99 ± 0.18 (in Bussi 2) and 29.26 ± 0.79 (in Bojano) mg/g DW, were on average lower than the free ones, ranged between 22.92 ± 0.91 (in Onna) and 50.32 ± 0.31 (in 99 Cannelle) mg/g DW. The total flavonoids, between 9.50 ± 0.30 (in Bussi 2) and 17.87 ± 0.34 (in 99 Cannelle) mg/g DW, represented 20-30% of the phenolic fraction, with significant differences ($p < 0.05$) among the ecotypes. The highest content of free flavonoids was recorded for the Roio samples (11.71 ± 0.79 mg/g DW) while the lowest for the Bojano samples (2.12 ± 0.13 mg/g DW). The bound flavonoids were found between 4.31 ± 0.15 (in Onna) and 9.40 ± 0.93 (in Bojano) mg/g DW. Maximum antioxidant capacity, measured as antiradical power (ARP), was found for the Bussi 1 samples (301.63 ± 1.52 mg /g) while the minimum for the samples of Roio (240.96 ± 3.92 mg/g). The contribution to ARP of the free fractions were on average higher than that deriving from the bound fraction, with significant differences ($p < 0.01$) for the samples of Bojano, Onna and Roio. The samples showed anticholinesterase activity, identified mainly in the free polyphenol fraction. The results confirm that the various ecotypes show significant differences in the polyphenol content, whose complete characterization is fundamental for their possible uses in the agri-food and/or pharmaceutical sectors.

Essential oils: their biological activity on *Philaenus spumarius* vector di *Xylella fastidiosa*

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Philaenus spumarius (L.) is a highly polyphagous species recently found to be the main vector of *Xylella fastidiosa* Wells *et al.* involved in the “Olive Quick Decline Syndrome” in the Salento Peninsula (Southern Italy).

The acquisition of knowledge about the perception of volatile compounds and the identification of behaviorally-active compounds to *P. spumarius* is of great practical interest. In fact, repellent compounds may be used to interfere with *P. spumarius* host selection process and to prevent sap sucking from olive plants, whereas attractant compounds could be useful to develop a suitable monitoring tool for this pest (Germinara *et al.*, 2017). Essential oils (EOs) of aromatic plants were traditionally used against economically important pests due to their capability to interfere with many physiological and behavioural functions in insects. Recently, antennae of *P. spumarius* adults were shown to possess a low number of antennal structures including few basiconic sensilla with a typical olfactory organization. In the present study, the capability of the peripheral olfactory system of males and females of *P. spumarius* adults to perceive volatile organic compounds (VOCs) of EOs was evaluated by electroantennographic analysis (EAG). The EOs were extracted from *Lavandula officinalis*, *Citrus aurantium* var. *bergamia*, *Cymbopogon nardus*, *Pelargonium graveolens*, *Salvia officinalis*, *Helichrysum italicum*, *Rosmarinus officinalis*, *Thymus vulgaris*, *Origanum vulgare* and *Santolina chamaecyparissus*, reported as repellents for many species of insect. In addition, four terpenes, myrcene, methyl salicylate, R-(+)-limonene and S-(-)-limonene were tested. Behavioral responses of *P. spumarius* to *L. officinalis*, *P. graveolens*, *C. nardus* and *C. a.* var. *bergamia* EOs were also evaluated using Y-tube bioassays. EAG demonstrated the capability of the peripheral olfactory system of female and male adults to perceive the EOs, and elicited dose-dependent EAG responses, in spite of the low number of antennal sensory structures described in *P. spumarius*. At certain concentrations, olfactory responses showed that males were attracted by *L. officinalis* and repelled by *C. a.* var. *bergamia* EOs. Females were attracted by *C. a.* var. *bergamia* at lower concentration and repelled at higher ones. Among alternative control strategies, the use of EOs extracted from plants seems to be promising. They are currently considered a new class of ecological products for controlling insect pests. *P. spumarius* responses to the tested EOs provide interesting insights from the perspective of practical EOs applications such as push and pull control strategies. More in-depth studies will be helpful to define the more suitable application procedure and doses.

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Assessing Lepidopteran Biodiversity along various land use types in Mounts Choke, Ethiopia

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Ethiopia is endowed with a widely diverse genetic resources and recognized as one of the twenty Mega diverse country in the world; however, its biodiversity is regarded as poorly investigated. In recent decades, the country has experienced a huge impact of anthropogenic disturbances leading to a strong loss of biodiversity. Insects provide a wide range of ecosystem processes and services to sustain human life and are also affected more severely and quickly than other taxa by changes in ecosystem. Lepidoptera is the second largest and most diverse order of the class Insecta, with approximately 160,000 living species described so far. Lepidopteran assemblages were sampled across five different land use types with different anthropic impact (natural forest, remain of natural forest, shrub and grazing land, crop fields, human settlement mosaic) in Choke Mountain, with the aim to assess the impact of human activities on the structure and richness of Lepidoptera community. Fieldwork was carried out from August 2017 to July 2018 using light traps operating during night-time simultaneously at four sites for each land use type. Similarly, in order to have a synthetic description of Lepidoptera communities in three natural forests of corresponding altitudes, collection was manually made using an illuminated white sheet with similar lamp and battery for three successive days. Since light trapping is highly dependent on weather conditions (temperature, wind, humidity and moonlight are the main drivers of moth activity at night), catches were restricted to periods without strong moonlight, windy days and heavy rains. Overall, approximately about 6,000 specimens were collected. Species diversity Shannon (H') and Margalef indices were calculated. Here, results from the first month of field activity were presented. A total of 465 specimens of Lepidoptera, belonging to 130 morphospecies, were collected. Biodiversity indexes showed that natural forest and remain of natural forest maintained higher and comparable diversity level, whereas in the other three land use types their values were lower, with human settlement mosaic the lowest. Our preliminary results suggested that the level of habitat transformation had a direct impact on biodiversity levels. Thus, a strong understanding of insect responses to anthropogenic activity is necessary.

Spectral data for the rapid characterization of compost-on-farm quality

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Compost is a variable mixture of organic residues and represents one of the most common ways to recycle green wastes in agriculture for fertilization, amendment, and erosion control. Farmers have started to produce compost on their farm (compost-on-farm) recycling their own agricultural wastes, often without a careful control of the process. In most cases, they use such compost-on-farm to improve their soils on the basis of their experience, without analysis of its chemical characteristics. An estimation of the compost quality during and at the end of its production process could result in a better quality compost thus fostering its use. Laboratory analysis are time-consuming and costly, therefore an alternative cheap and rapid method is desirable. Such a method is the Visible and near-IR diffuse reflectance spectroscopy (VisNIR). Studies on compost characterization by VisNIR are usually carried out on preprocessed samples after reducing them at a homogenous dimension. However, preprocessing represents a time and cost limit for farmers. In this study, we investigated the possibility to use VisNIR on samples without any preprocess. We compared the ability of Vis-NIR to discriminate samples of compost-on-farm with different chemical qualities, by acquiring spectra on sieved (2 mm) and raw samples. Twenty-one samples were split into 3 classes, which were clustered on the basis of total C, total organic C, total N, C/N, residual humidity and ashes content. In order to reduce the number of predictive variables, a Principal Components Analysis was run on spectra of raw and sieved samples. The principal components that mostly correlated with compost properties were selected and used to discriminate the clusters (table1). The discriminant analysis carried out on sieved samples resulted in a correct classification for 90% of the samples, whereas 80% of correct classification was achieved when raw samples were used. These preliminary results showed a limited loss of efficiency using row data. Improvement could be obtained with further studies over a larger dataset.

Table 1: percentage of correct classification of composts in quality clusters on the base of the spectral properties [Cluster 1: high quality compost consisting of mixed material (plants and manure), rich in total nitrogen and with a C/N ratio around 10; Cluster 2: low quality compost, very rich in ashes and scarce in total organic C and total nitrogen; Cluster 3: high quality compost, mainly consisting of plant products (prunings, grape residue), high C/N, Organic C and N, low ashes)].

	<i>Datasets</i>	
	<i>Raw</i>	<i>Sieved</i>
<i>Cluster 1</i>	91.7%	100%
<i>Cluster 2</i>	33.3%	66.7%
<i>Cluster 3</i>	80.0%	80.0%
<i>Total</i>	80.0%	90.0%

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Exploring *Trichoderma*-assisted phytoremediation potential of *Populus alba* clone (Querce) in Cadmium-contaminated soil

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The present study is focused on the phytoremediation of highly cadmium-contaminated soils by means of the tree species *Populus alba* L., clone “Querce”, in association with two Cd-tolerant strains of *Trichoderma* spp., rhizosphere fungi which had been previously reported to increase plant uptake of soil minerals. The tolerance of *T. polysporum* T60 (T1) and *T. harzianum* 908 (T2) to Cd was preliminarily assessed. Then, the effect on plant growth and Cd uptake by poplar plantlets inoculated and non-inoculated with the selected strains T1 and T2 was tested in pot assays in the presence of high Cd concentration (250 µM). The Cd uptake was determined by sampling plant tissues at a root, leaf and stem levels, which were then analysed by inductively coupled plasma optical emission spectrometry (ICP-OES). In order to gain insights into the poplar physiological response in the interaction with the beneficial microorganisms, the changes in the spectrum of plant-emitted volatile compounds (VOCs) inoculated with *Trichoderma* strains were also evaluated by GC-MS. Our results indicate that T2 treatment enhanced fresh root biomass by 44.3 % as compared to the control plants amended only with Cd and by 54.6% as compared to plants inoculated with T1. Although the “Querce” clone showed higher Cd content at the roots level when inoculated with T1 or in the control plants, compared to plants treated with the strain T2, these latter exhibited the highest Cd uptake in leaves and stems, indicating that this strain was effective in enhancing Cd phytoremediation. The tolerance index (Ti) indicated good tolerance of this clone under exposure to excess metal concentrations in presence of *Trichoderma* T2 inoculation, while T1 inoculation didn't show significant tolerance enhancement in the “Querce” clone compared with the control. Moreover, the plants treated with T2 exhibited a higher translocation factor (Tf) indicating a remarkable potential for a phytoextraction strategy.

Preliminary list of VOCs emitted by the “Querce” clone was obtained, resulting in the detection of 81 VOCs as a starting VOC pool necessary to further investigations.

The *Trichoderma harzianum* 908 properties highlighted in these experiments suggest that this fungal strain is a promising agent for enhancement of Cd-uptake by poplar plantlets, to be used in phytoremediation of highly Cd-contaminated soils.

Biological control against *Penicillium expansum*: influence of *Papiliotrema terrestris* on patulin biosynthesis and development of molecular genetics tools for validating biocontrol mechanisms

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Synthetic fungicides are the primary means to control postharvest diseases, but microbe-based biocontrol emerged as a promising alternative to chemicals. The study of the mechanisms of action of microbial biocontrol agents is pivotal to improve their performances. We focused on the mechanisms of the biocontrol agent (BCA) *Papiliotrema terrestris* LS28 against *Penicillium expansum*, the causative agent of blue mold disease on stored pome fruits, and producer of the mycotoxin patulin. We examined the influence of the BCA on *P. expansum*-driven acidification and on patulin (PAT) production. When *P. expansum* was grown in buffered media at pH values from 3 to 7, PAT production ($\mu\text{g/g}$ dried mycelium) was the highest at pH 5, while only traces were detected at pH 3 and pH 7. In unbuffered media, *P. expansum* led to a quick extracellular acidification to pH values below 3 after only 24 h. Conversely, the acidification was delayed to 120 h when the pathogen was co-cultured with the BCA. An analogous pattern was observed in dual culture in an apple extract. In vivo, a suboptimal BCA concentration lowered the disease but led to an increase of the specific rate of PAT biosynthesis in the apple tissue. The addition of an alkalinizing compound increased the biocontrol activity resulting in lower disease incidence, whereas the amendment with an acidifying compound had opposite effects, i.e. higher disease incidence but lower PAT biosynthesis. Our results suggest that pH modulation by LS28 is a new biocontrol mechanism that counteracts the acidification and the virulence of *P. expansum*. On the other hand, the BCA-based delay of acidification causes an increase of the specific rate of patulin biosynthesis ($\mu\text{g/cm}$ lesion diameter) by prolonging the time interval at which pH value is within the optimal range for PAT biosynthesis. In order to validate mechanisms of biocontrol and to identify the genes putatively involved in such mechanisms, we developed molecular genetics tools for *P. terrestris* LS28. Constructs for random insertional mutagenesis and for targeted mutagenesis were generated by cloning the marker genes NEO and HYG (conferring resistance to Neomycin and Hygromycin B) under the histone gene promoter and terminator of *P. terrestris* LS28. The BCA was transformed through biolistic, electroporation and *Agrobacterium*. Resistant colonies were selected from all the transformation systems. 672 random insertional mutants were in vitro tested with several stressing compounds related to biocontrol activity. Seventeen phenotypes of interest were identified and are currently under characterization. For the development of a targeted mutagenesis system, deletion cassettes harboring the HYG marker were designed for the genes YAP1 and RIM101, two transcription factors that are involved in the response to oxidative stress and to alkaline pH. Deletion mutants were obtained. Complementation cassettes were also designed, and complemented strains were obtained. Both the deletion mutants and complemented strains are currently being tested in biocontrol assays.

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Remote sensing technology in Agriculture for improving Total Factor Productivity: A review

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Remote sensing (RS) applications in agriculture are widely been modernized globally from the last decade. RS plays significant roles in securing the future of our food production and continuously rising world population. It is capable to provide results irrespective of the presence of clouds and detect standing water through emergent aquatic plants and forest canopies. It provides fine tools for reducing yield gaps at all scale levels (world, country, regional and farm), while reducing the inputs (water, nutrients, energy). RS has an irreplaceable role in sustainable agriculture, increasing the Total factor productivity (more output per input), contributing to mitigation of climate change. Besides the huge achievements produced by the adoption of techniques related to RS, authors outline positive or negative aspects significant to stockholders (in particular small farmers) lacking the digital revolution.

Adhesion properties of *Akkermansia muciniphila* DSM 22959 to human cell lines

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Akkermansia muciniphila is an intestinal, gram-negative, strictly anaerobic, non-motile bacterium; it has been found to inhabit the gastrointestinal tracts of more than 90% of adult subjects and constitutes 3 to 5% of the gut microbiota in both human and other mammalian species.

There is growing evidence that *A. muciniphila* is associated with gut health and its abundance has been inversely correlated to several disease states such as obesity, type 2 diabetes, inflammatory bowel diseases and liver disorders. Considering that *A. muciniphila* exerts a range of biological activities related to the host health, it was suggested as a biomarker for the state of health, as it could indicate the progress of diseases and recently, it has been proposed as a new beneficial microorganism for human health.

Notwithstanding the numerous evidences for the involvement of *A. muciniphila* in intestinal and metabolic health, the basic mechanisms underlying how *A. muciniphila* physiologically influences the human body have to be elucidated.

On the basis of these considerations, this study aimed at the understanding of the adhesive properties of an *A. muciniphila* strain on human cell lines and at evaluating some of its probiotic features.

The strain *A. muciniphila* DSM 22959, of human origin, was used in this study and *Lactobacillus rhamnosus* GG, a strain extensively used as probiotic, was used for comparative purposes.

In detail, survival at the gastrointestinal stresses, auto-aggregation ability, cell surface hydrophobicity and adhesive properties were assessed. Adhesion ability was assessed by *in vitro* methods, using three cell lines, Caco-2, HT-29 (human colon adenocarcinoma) and MIA-PaCa2 (human pancreas adenocarcinoma) and different bacterial loads.

Strains showed different responses to the stresses typical of the gastrointestinal tract. In detail, *A. muciniphila*, showed an excellent performance with high survival compared to *Lb. rhamnosus* GG that was more sensitive to exposure to low pH and bile salts.

The auto-aggregation ability showed positive trend over time for both strains, with better performances expressed by *A. muciniphila*.

With reference to the hydrophobicity tests, it was possible to observe a high cell surface hydrophobicity for *Lb. rhamnosus* GG, whereas *A. muciniphila* showed a medium cell surface hydrophobicity.

Results obtained by *in vitro* test of adhesive properties, expressed as percentage of adhesion, highlighted important differences among tested strains and cell lines. The adhesive properties of *L. rhamnosus* GG appeared influenced by the type of cell line used and strongly conditioned by the bacterial load, since significant adhesion levels were registered when low bacterial concentrations were used. When concentrations of *L. rhamnosus* higher than 5 log cfu/mL were used, a decrease in the adhesion to cell lines were detected. Contrarily, *A. muciniphila* showed a very high adhesion ability to all tested cell lines also when an initial bacterial charge of 8 log cfu/mL was adopted. This result is probably linked to metabolic activities of *L. rhamnosus* (lactic acid production) when in contact with cell lines. This activity probably provokes an acidification of the culture medium with consequent impairment of the adhesion ability to all human cells lines.

In conclusion, no link was found between adhesion to cell lines and other assays, such as cell surface hydrophobicity, often reported in literature as "predictive" for the adhesion potential of microorganism. Furthermore, the *A. muciniphila* has not been sensible to exposure to low pH and bile salts and it showed a high adhesion ability to all tested cell lines.

Use of microorganisms as cell factories to produce goods and services

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PhD research aims to optimize the production of goods and services by microorganisms useful in industrial biotechnology.

In this research project, selected strains belonging to *Candida rugosa* and *Bacillus amyloliquefaciens*, due to their large use in agricultural and biotechnology process, were assumed as target microorganisms. Preliminarily, the main limits to the scale up and industrialization of microbial cell production (to use as starter or protective culture in agriculture food process) have been identified. Data available in literature highlighted that the optimization of maximum biomass values and the improvement of stress tolerance in microbial strain of biotechnology interest represent the main issues.

On these bases, during the first year, the better conditions for growth and biomass accumulation by the strain *Candida rugosa* L_12 were investigated through experimental design of various critical medium components to obtain optimal medium combination of two independent variables such as carbon and nitrogen source. As for the variable carbon source, the effect of glucose, glycerol, tween80, palm and olive oil on growth kinetic parameters (lag and log phase, maximum growth rate, amount of final cells and maximum biomass) were estimated. To optimize the variable nitrogen source, the better combination of nitrogen and carbon sources was identified. For this purpose, kinetic growth parameters exhibited by *Candida rugosa* L_12 in conventional cultural condition or in presence of the optimized carbon source were compared with those obtained when the strain was cultivated in presence of ammonium sulphate or soy peptone.

Finally, the effect of two different temperatures (i.e. 28° C and 37° C) on biomass accumulation was assayed.

The results evidenced that all the variables (i.e. different carbon and nitrogen sources) were highly significant for biomass production according to their p values. Data obtained from the carbon source investigation highlighted that the maximum biomass values (CDW) were found when the fermentation in batch was carried out adding glucose in concentration of 10%. Moreover, the final maximum biomass accumulation was very close to the kinetic parameters. As for nitrogen source optimization, the combination of glucose (10%) and ammonium sulphate (5 g/L) showed the best results evidencing highest values both in CDW and in maximum growth rate values. In fact, this last combination optimizes the use of substrate producing a remarkable increase in fermentation yield.

Significant effects on growth kinetic parameters were also produced by the assayed temperature. Specifically, the fermentation at 28° C allowed to obtain values in CDW significant higher than those detected in the fermentation at 37° C. Therefore, results evidenced that the kinetic parameters and fermentation yield, are compatible with industrial processes.

Based on these results the scale-up and downstream process will be optimized evaluating the response and the adaptability of the strain to process stressors.

The influence of lipids on the oxidative stability of biscuits

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The term 'biscuit' is used for a variety of baked, mainly flour-based food products, so it includes biscuits, cookies, and crackers. Fats are the third major component used in biscuit production, after flour and sweeteners, however they are considerably more expensive than the other ingredients.

Palm oil is an affordable and versatile vegetable oil used as a raw material for food and non-food products. Before the "No palm oil" campaign promoted by the GIFT in 2014, this vegetable oil was the main fat in bakery products because of its high technological performance and high oxidation resistance.

Obviously, the GIFT campaign caused a reduction equal to 30-40% in its use. The goal of our research is to try and find an answer to the following question: are there vegetable oils or a blending of different lipids better than palm oil in terms of quality and oxidation stability?

Seven different biscuit brands and five different cracker brands were purchased from local supermarket, and their formulations - for biscuits: 1B: palm oil; 2B: high oleic sunflower oil; 3B: blending of coconut oil, sunflower oil and EVO oil; 4B: EVO oil; 5B: butter; 6B: sunflower oil; 7B: blending of palm oil, butter and cream; for crackers: 1C: EVO oil; 2C: palm oil; 3C: soybean oil; 4C: sunflower oil; 5C: blending of palm oil and coconut oil - have been studied. All samples are commercial products with similar ingredients and shelf-life.

The peroxide value with spectrophotometric analysis and the volatile compounds with SPME-GC-MS were analysed in order to evaluate the oxidative status of the samples.

The biscuit with EVO oil (4B) and butter (5B) and the crackers with soybean oil (3C) and EVO oil (1C) had the highest peroxide value (16.8, 7.5, 7.74 and 6.27 meq O₂/kg fat, respectively). These results could be probably due to the nature of fat used in samples. Indeed, these kinds of lipids did not undergo any previous refining, so their peroxide value can be higher at the beginning of biscuits and crackers' shelf-life.

The volatile profile showed a wide range of compounds in all samples, i.e. approximately 60 compounds in biscuits and 50 compounds in crackers. The major group of compounds identified are aldehydes, ketones and alcohols, followed by carboxylic acids, esters, furan compounds, pyrazines, sulfur compounds, aromatic hydrocarbons, terpenic compounds and others.

The next step of research will be to monitor the oxidative stability and the volatile profile during shelf life. In particular, the behaviour of several lipid oxidation-derived saturated and unsaturated alcohols and aldehydes - hexanal, 1-hexanol, 2-hexen-1-ol and nonanal- and several compounds responsible of off-flavours - heptanal, 2-heptenal and octanal - during the shelf life of the samples will be investigated.

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Differences in intestinal transcriptomic profile in two modern fast-growing chicken hybrids

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All fast-growing chicken lines are subjected to similar selective breeding processes mainly aimed at improving growth performance. However, considerable dissimilarities in performance between different lines are reported, even if the biological basis behind this are still not clear and poorly investigated. Given the key role of the small intestine in influencing animal growth and health, the physiological dynamics occurring at this level may be useful to better elucidate the different phenotypical responses observed among commercial chicken hybrids. Therefore, the aim of this study was to investigate intestinal transcriptomic profile of two fast-growing chicken hybrids (HA and HB) available for poultry industry and differing for growth performance.

A total of 1,170 one-day-old female chicks ($n = 585$ per genotype) was weighed and randomly divided into 18 pens (9 replications/group). At the processing (43 days), ileum mucosa was collected from 1 bird/replication. Total mRNA was extracted to perform microarray analysis (Chicken Gene 1.1ST Array Strip), and an exploratory gene set analysis was conducted (Gene Set Enrichment Analysis software). The normalized enrichment score (NES) was calculated for each gene set and the respective p -values obtained applying a gene set-based permutation procedure. Gene sets were considered significantly enriched when both NES p -value and False Discovery Rate q -value were lower than 0.05. Significantly enriched gene sets were then classified according to their main biological function.

In the HA line, a high percentage of biological gene sets involved in cellular energy metabolism and mitochondria structure and functionality were observed (43% and 23% of the total, respectively). Other significantly enriched gene sets were related to ribosome structure and protein synthesis (11%), cell structure and integrity (8%), as well as antioxidant and detox mechanisms (6%). On the other hand, a significant enrichment in gene sets related to immune system activation (28% of the total) was observed in the ileum mucosa of HB birds. Moreover, an increased expression of gene sets involved in signal transduction and cell signaling (20%), DNA remodelling and replication–chromatin/histone modification (15%), cell activation, migration and adhesion (12%), inflammation (7%) and bone remodelling (4%) was detected in the HB group.

Results seem to reflect an overall healthy condition of the ileum mucosa in HA birds mainly supported by a high energy production at the epithelial level, which is necessary to maintain a proper gut structure, integrity, and functionality. HB birds have shown upregulation of gene sets related to inflammation and immune response: considering the good health status of these animals along with their higher feed intake compared to HA, it is possible that these birds may have suffered gut overload resulting in a chronic low-grade inflammation triggered by excess in nutrient intake known as “metabolic inflammation”. In conclusion, these results indicated that the two chicken genotypes differing for growth patterns are characterized also by different gene expression profiles in the intestinal mucosa.

Effect of diet based on yellow lupine on the in vivo performance, carcass traits and meat quality of fattened geese

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Poland is one of the leading producers of goose meat in Europe. The breeding of geese is inscribed in Polish tradition and over the years goose meat was recognized as a specialty of domestic agriculture. The aim of the research was to compare the production results and quality of meat of oat fattening geese fed with a complete feed based on native sources of plant protein (legume seeds) as an alternative to the commonly used soybean meal. Two-hundred 1-day-old “oat geese” (half males and half females) were divided into two equal feeding groups based on: soybean meal (Control group, C); yellow lupine (YL). From 13 weeks of age both groups were fed only with oat (fattening period) until slaughter (16 weeks of age). Feed and water were offered *ad libitum*. At slaughter, live weight was recorded and carcass traits (10 from each group) were evaluated. From each carcass, breast muscle samples were collected for meat analysis, proximate composition (water, protein, lipid and salt), collagen content, water holding capacity (WHC) and drip loss. Data were analyzed by one way analysis of variance. Final body weight, carcass weight and carcass yield were not significantly affected by diet. Compared with group YL, geese from group C showed higher weight of drumstick muscle (518.54 g vs 470.63 g; $P < 0.05$) and its percentage based on hot carcass weight (12.00% vs 10.78%; $P < 0.05$); breast, neck with skin, and wings weight and their percentages, as well as total fat were similar ($P > 0.05$) between groups. The proximate composition of the breast muscle was affected by diet. In particular, water and protein content was higher ($P < 0.05$) in C group, while fat in YL group; differently, the amount of salt was not affected by diet ($P > 0.05$). Diet did not influence ($P > 0.05$) the WHC of meat and intramuscular collagen amount; while, drip loss decreased ($P < 0.05$) in meat of geese fed with yellow lupine. While the diet with yellow lupine reduced ($P < 0.05$) the drip loss of the meat. In conclusion replacing the soybean meal by yellow lupine in the diet of fattening geese for 13 weeks and the next 3 weeks with oat we had observed: i) no negative effect on the in vivo performance, ii) a reduction of the weight and percentage of an important cut, as the drumstick, iii) an effect on the proximate composition of meat; iv) a reduction of the drip loss. Therefore, it is possible that the legume seeds could be alternative to commonly used soybean meal in fattening geese rearing.

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The relationship between the environment and genes methylation profile in chicken –epigenetic approach

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Understanding the environmental impact on genes makes it possible to determine its contribution to the phenotypic variability. Hence, this will facilitate the selection of desirable properties, which might have an impact on animal production. Epigenetics can be defined as a change in the phenotype of an individual caused by mechanisms that do not lie under the DNA sequence. Epigenetic regulation of gene expression is a form of interaction of the environment during transcription and translation of information encoded in nucleic acids. It includes the inheritance of changes in gene expression that do not occur as a result of modification of the DNA sequence. Under the influence of various external factors (nutrition, sanitary conditions, stress, climate), a multi-stage process of prescribing genetic material into a protein undergoes changes, which may result in modulation of the phenotypic traits. The main aim of this research is estimation of methylation level of CpG islands in candidate genes in chicken research model. Analysis will be done at three different levels: (1) *in vitro* cultures, (2) embryo development and (3) adults chickens stimulated *in ovo* with bioactive substances. In this abstract we present the concept of epigenetic research using the chicken model and the methodology of DNA methylation, which is a specific chemical modification of a nucleic acid based on methylene group attached to cytosine or adenine. Materials for analysis are: primordial germ cells (PGCs) subjected to short- and long-term *in vitro* culture, PGCs isolated from gonads of embryos, immune and metabolic tissues (spleen, cecal tonsils, liver) isolated from adult chickens stimulated *in ovo* on day 21 of egg incubation with bioactive substances (prebiotic - GOS, probiotics - lactic acid strains of bacteria, synbiotics – combination of GOS with lactic acid strains of bacteria). Isolated DNA from cells and tissues will be subjected to methylation analysis using the BC-MSP method (methyl-specific PCR preceded by bisulfite DNA conversion). Due to this modification, unmethylated cytosine is deaminated, resulting in formation of uracil. Therefore, specific PCR primers for methylated and unmethylated DNA make it possible to visualize this DNA modification by the qPCR. Pairs of primers - specific for methylated and unmethylated DNA - are designed for each gene using the MethPrimer tool. The conversion is carried out using the EpiJet Bisulfite Conversion Kit (Thermo Fisher Scientific, Waltham, MA, USA). qPCR reactions are subsequently performed for developed panel of genes based on previous expression microarray studies for which gene expression was silenced. Bioinformatics analysis allowed to select a panel of genes silenced at the DNA *in silico* analysis caused selection of promoter sequences of genes. The research proposed in this project will provide new insights into gene silencing mechanisms. The results of the proposed experiments will greatly expand the knowledge of epigenetic approach embryo development influence of bioactive substances in poultry and it will verify its epigenetic character. Such studies have not been carried out so far.

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Studies on sustainable feedstuff management in the livestock production systems of Centre-South Italy

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Analysing the changes of the world agricultural sector, it is necessary investigate the presence and the possible use of new crops that, due to their agronomic, pedological, climatic and employment characteristics, can guarantee less depletion of available resources while maintaining an excellent productive attitude. The goals of this work are: re-evaluating the role of the meadow and the pasture in marginalised areas of Molise region, and the study and the application in the animal nutrition of a totally innovative forage obtained from the use of a "non-food" plant. In the first case, based on data available in literature from 1990 up to 2016, the evolution of pasture areas in Molise has been drawn and a series of floristic and pedological samplings are planned to evaluate any changes occurred in the same areas over a period of about thirty years. This would provide a possible correct management for pastures and marginal areas. The second topic to be developed aims to investigate the possible use of the patented crop *Nicotiana tabacum* L., cv Solaris as a sustainable feedstuff for herbivores. This cultivar, nicotine free and no GMO, has many inflorescences and presents an excellent adaptability to the various cultivation areas. Between 2016 and 2018 the chemical composition of Solaris biomass was studied, according to the official methods (AOAC 1995, 2000). In 2017, through ensiling practice, has been carried out the first trial of the conservation of Solaris biomass (named SiloSolaris). At the end of ensiling period, the SiloSolaris, was administered to growing Friesian heifers in a field trial. Sixteen heifers were divided into two group that received diets differentiated for including (SS) or not (Control) ensiled Solaris whole plant. Specific scores related body condition, fecal consistency, and deambulatory capacity were assigned to each animal, according to the literature. In addition, the investigated consumption of feedstuffs (offered-refusals), body weight gain, average daily gain are presented. Data resulting from the first trial period (d 0-49), analyzed by ANOVA, show that is possible to preserve the Solaris biomass by ensiling and that the SiloSolaris is appreciated by Frisian heifers. Moreover, due to the high energy content, the treatment with the SiloSolaris tendential improved feed conversion rate, without affecting the animal welfare. This study represents the first attempt of the use in ruminant nutrition of a product obtained from an innovative crop able to recover the "know-how" of the tobacco cultivation of Centre-South Italy.

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Pathways Interaction Analysis (PIA): an R package for post GWAS analysis

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Data mining of massive outcomes obtained with high-throughput genomic analysis (as GWAS, ROH, selection signature) represents a mounting challenge to researchers. In fact, among the list of significant genes obtained it is difficult to recognize those with a biological significance connected to the trait of interest. In this regard, the traditional bibliographic research remains the last ‘validation’ step, often arduous and time-consuming. At the same time, grouping long lists of individual genes into a smaller set of related ones, sharing the same functional context (i.e. pathway), is a common approach to reduce the complexity of this challenge. This method, known as ‘pathway analysis’, has become popular during the last years and many tools have been developed for this purpose. Nevertheless, the majority of them perform the analysis within a single item (i.e. pathway), not providing information about possible interactions among multiple pathways of interest and related group of genes. This aspect represents a disadvantage particularly when a complex physiological phenomenon is under investigation.

Here we introduce Pathways Interaction Analysis (PIA), an R package designed for the biological classification of functionally related genes into dependent network of pathways in interaction. PIA result help to infer possible candidates among a list of genes and more broadly give insight into biology of condition under study. In fact, the interactions among genes and pathways at different levels provide good clues for the data interpretation from ‘big picture’ perspective and may help to uncover ‘flow’ of information between biological processes. The tool has no species constraint and is completely based on information available on Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. The method was validated using a publicly available dataset from a genome-wide association (GWA) study on bovine milk fatty acids in Italian Simmental and Italian Holstein. Among the list of 220 possible positional candidates, PIA highlighted 22 functional candidate genes. Some of those are well-known genes involved in fatty acid metabolism, as *DGATI*, *SCD* and *FASN*. The association of the remaining genes was confirmed by bibliographic research.

Effects of prebiotic on meat quality in slow-growing broiler chickens exposed to heat stress

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The balance of poultry intestinal microbiota can be compromised by a lot of environmental factors. Among them, heat stress induced by elevated ambient temperatures has a strong and immediate effect on performance and welfare of the birds. The aim of this study was to evaluate the physico-chemical (pH, color, proximate composition, cholesterol) and collagen properties of pectoral muscle (PM) in slow-growing chickens stimulated *in ovo* with GOS prebiotic and exposed to heat stress. On day 12 of incubation 3000 eggs (Hubbard JA57) were randomly divided into three experimental groups: C, control group (uninjected); S, injected with 0.2 ml of physiological saline solution; GOS, injected with 0.2 ml of a commercial prebiotic (Bi²tos, Clasado Ltd., Malta) containing 3.5 mg/egg of trans-galactooligosaccharides. After the injection, each hole was sealed with organic glue and the incubation was continued until hatching. Each experimental treated group (C, S and GOS) was divided into 2 groups raised in thermoneutral (25°C, TN) and heat stress conditions (HS), respectively. Heat stress was applied for 14 days from the 35 days, with a constant exposure at a temperature of 30°C for 8 hours per day. Animals were fed *ad libitum* with commercial diets and had free access to water. At slaughtered (50 days of age), right PM was removed from 45 carcasses randomly chosen from each group (n =15), for meat analysis. Data were analyzed by ANOVA in a 3 × 2 factorial design. Carcass weight was not affected (ranging from 1603.9 vs 1659.1 P > 0.05) by prebiotic treatment. Ultimate muscle pH was affected by prebiotic treatment, it was higher (P < 0.05) in GOS group in comparison with C, while S group presented intermediate values (P > 0.05). Meat color was significantly affected by treatment. In particular, lightness (L*) value was lower in GOS group (52.32) in comparison with C (54.11; P < 0.05) and S (54.93; P < 0.01), while, meat redness (a*) was higher in GOS group in comparison with S (2.09 vs 1.31, respectively; P < 0.01), intermediate value (P > 0.05) was observed in C group. Compared with thermoneutral conditions, heat stress reduced carcass weight (1706.7 vs 1555.9, TN and HS respectively; P < 0.001) and the redness of meat (P < 0.01), while it did not influence both lightness and yellowness of meat. Heat stress increased the moisture (P < 0.001) and reduced the protein (P < 0.01) content, while no effects (P > 0.05) were found on lipid, ash and cholesterol content, and intramuscular collagen properties. Proximate composition, cholesterol content (ranging from 37.07 to 40.05 mg/100g) and intramuscular collagen properties of PM muscle were similar (P > 0.05) among the treatment groups. Strong interactions (P < 0.01 and P < 0.001) between the GOS and temperature were observed in case of carcass weight, and protein and lipid content. In conclusion, the results obtained showed that *in ovo* injection had no negative effect on physico-chemical and nutritional properties of meat produced by slow-growing broilers chickens reared on intensive system. Differently, heat stress affected negatively carcass weight and meat quality.

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Analysis of abnormalities in chicken breast muscle in commercial conditions

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An investigation was conducted to estimate the incidence of the three emerging abnormalities of poultry meat (White Striping - WS, Wooden Breast -WB and Spaghetti Meat - SM) in commercial conditions, and to evaluate the effect of some *ante mortem* factors on the incidence of the above-mentioned muscle abnormalities. A total of 18000 boneless and skinless *Pectoralis major* muscles were obtained from 90 flocks of broiler chickens reared and harvested under standard commercial conditions. Fillets were selected at 3 h *post mortem* in the deboning area of the commercial processing plant and classified by one experienced person through manual palpation and visual appearance, according to the presence of muscular abnormalities WS, WB and SM. The fillets were categorized as normal, moderate and severe based on: 1) the size and distribution of the gross white striations on the surface; 2) on the diffuse hardened areas and pale ridge-like bulges at the caudal end; 3) on the presence of an overall impaired integrity and tendency toward separation of the muscle fiber especially within the cranial part of the fillet. The *ante-mortem* variables that were recorded were: genetic type (Ross 308); sex; feeding (exclusively vegetable or with animal fats); broiler average live weight; age at slaughter; transport time; pre-slaughter time. The data collected during the investigation for the assessment of the incidence of muscle abnormalities were included in a database subsequently used for statistical-descriptive analysis. Data were analysed using the one-way ANOVA, considering the *ante-mortem* factors. The results showed that 43% of the analysed breasts showed at least one of the moderate-stage defects and 23% of breasts had at least one severe defect. The WB condition presented the highest incidence with a total percentage of 60% (42% moderate and 18% severe). The incidence of breasts with WS defect was equal to 31% (22% moderate and 9% severe). 21% of breasts presented SM defect (17% moderate and 4% severe) and it was more pronounced in females. An exponential increase in the incidence of WS condition was observed when the weight of breasts was higher. An even more drastic increase was observed the WB condition related with the increased breast weight, while no relationship with the SM condition emerged. In conclusion, *ante mortem* factors, such as transport time and arrest at the slaughterhouse, do not play an important role in the appearance of breast abnormalities. On the contrary, the growth rate of animals seems to be a possible cause especially for the WB and SM, which are the most common defects in heavy chickens. Besides, it is hypothesized that another critical factor is the genetic selection of birds as a consequence of the demand of the poultry industry to produce heavier birds, this has led to an increased pressure on muscle development rate and on the appearance of breast abnormalities.

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Safeguard of the Mediterranean Brown Trout (*Salmo cettii*) biodiversity: effects of basic extender on post-thaw quality and fertilizing ability of the cryopreserved semen

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The native Mediterranean brown trout (*Salmo cettii*) of Biferno river is declining as a result of the river pollution, poorly regulated fishing activities and the introduction of allochthonous strains for recreational purposes, with a consequent genetic introgression.

The creation of a cryopreserved sperm bank is an effective strategy to protect the biodiversity of the local brown trout population and provides the opportunity to preserve the sperm samples of the most valuable males, which can be used in restocking programs.

In this regard, the development of an effective freezing protocol for *Salmo cettii* semen represents an important purpose. Therefore, this study was carried out to optimize the freezing procedures of *Salmo cettii* semen, evaluating the effects of two basic extenders on the post-thaw quality and fertilizing ability of the cryopreserved semen.

Semen was collected from thirty native males from Molise rivers. Six pools were used in total. Each pool was split into two aliquots and diluted 1:3 in the extenders composed by 300 mM glucose (extender A) or 75 mM NaCl, 70 mM KCl, 2 mM CaCl₂, 1 mM MgSO₄ and 20 mM Tris (extender B) combined with 10% egg yolk and 10% dimethylsulfoxide (DMSO). The diluted semen was packaged in 0.25 mL plastic straws and equilibrated at 4°C for 10 min, then the straws were frozen through the exposure at 5 cm above the liquid nitrogen level for 10 min. Lastly, the straws were dipped and stored in the liquid nitrogen. Before the analysis, the semen samples were thawed at 30°C for 10 sec. The post-thaw sperm quality was evaluated by the measurement of the sperm motility parameters (total motility, VAP, VCL, VSL) by computer-assisted sperm analysis (CASA), the spermatozoa movement duration (SMD), the sperm viability (SYBR-PI) and the DNA integrity (Acridine Orange).

The data obtained *in vitro* showed a significant effect of extender used on all sperm motility parameters evaluated, indeed significantly higher values of total motility, VAP, VCL, VSL and SMD were recorded in semen frozen with extender A than extender B, while no significant differences were registered for sperm viability and DNA integrity.

Fertilization trials were performed using three groups of eggs (around 100), one group was inseminated with fresh semen and other two groups with frozen semen using extender A or B. Fertilization and hatching rates were significantly higher in the fresh semen compared to the frozen one. No significant differences emerged when we compared the frozen semen using extender A or B, although, the higher percentage of fertilization and hatching rates were recorded in eggs fertilized with extender A ($38.5 \pm 9.3\%$ and $34.8 \pm 8.3\%$) compared to extender B ($27.5 \pm 4.1\%$ and $22.7 \pm 2.7\%$). In conclusion, according to the *in vitro* and *in vivo* results, the glucose-based extender (A) was more effective for the cryopreservation of *Salmo cetti* semen. Our results can contribute to the development of an effective freezing protocol that will allow creating the first sperm cryobank in Europe for the native Mediterranean brown trout inhabiting Molise river.

Migratory abilities of chick PGCs isolated from blood or gonads and cultured *in vitro*

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Long-term cultivation and proliferation of chick primordial germ cells (PGCs) *in vitro* might be useful for their biotechnological usages and for the conservation of endangered other avian species. Though there were several reports regarding the culture of chicken PGCs *in vitro*, the condition of the culture did not be certainly established until now. Additionally, the procedures of PGCs culture were complicate and it took long time. The purpose of the present study was to develop a simple method of chick PGCs culture *in vitro*. Additionally, our study aims at investigating the effect of this culture on migratory ability of cultured cells.

According to the previous reports, we had fixed the culture conditions and resources of the PGCs were originated from embryonic gonads before the 4th day of incubation (gonadal PGCs; gPGCs) and circulating blood of 2.5th day of incubation (circulating PGCs; cPGCs). Because the number of gonadal PGCs are much higher than those of previous embryonic stages though there is the possibility that gPGCs are already committed as the next developmental phase. Regarding the cPGCs, there seems no chance to be committed for the further development though their number is not so much.

The future gonadal regions were collected from stages 17 to 18, dispersed with Trypsin-EDTA solution and incubated modified KAv-1 medium at pH 8.0 at 38°C. In case of the cPGCs, embryonic blood was sucked out from stages 14 to 15 and stained with PKH26 fluorescent dye just before the culture. Both gonadal and cPGCs was seeded on the embryonic feeder cells with modified KAv-1 medium containing 10 µl/ml FGF-basic, 2% chick embryo extract from 4 days' embryo, and 5% KnockOut Serum Replacement), and one of two volumes of the culture medium was changed with fresh medium. Cultured gPGCs were collected after the detach from the feeder layer and labelled with fluorescent dye PKH26. Five to eight hundred of labeled gPGCs were injected into the bloodstream of stages 14-16 HH recipient embryos. After the injection, the window was sealed with adhesion tape and glue. Cultured cPGCs were also injected into the recipient embryonic bloodstream with the same manner.

Throughout the cultivations, the growth ratio of cPGCs was much higher than that of gPGCs in the present study. Both gPGCs and cPGCs cultured in this system retain their pluripotency and the unique ability of migration, and were detected in the recipient gonads as PKH26 positive cells.

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