Giornata di studio:

Innovazione negli allevamenti per la prevenzione

Firenze, 15 settembre 2015

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Introduzione

Prima di dare inizio al workshop, non posso tralasciare di ringraziare il collega accademico Alessandro Nardone che ha presieduto, con la consueta efficacia, il Comitato Consultivo "Allevamenti e Prodotti animali" dell'Accademia dei Georgofili, dai cui lavori sono già scaturiti, oltre a questo odierno, molti altri interessanti workshop su tematiche di grande interesse e attualità, il collega accademico Giuseppe Pulina, che ha con me condiviso l'onere di organizzare l'odierno workshop, i tre colleghi relatori, e cioè, il prof. Fabrizio Ceciliani, dell'Università di Milano, e i proff.ri Gianfranco Greppi e Antonio Pazzona, dell'Università di Sassari, insieme ai rispettivi collaboratori che hanno contributo alla stesura delle relazioni che a breve ascolteremo. Un ringraziamento sincero, infine, al'Accademia dei Georgofili che ancora una volta ci ospita in questo luogo suggestivo.

Il mondo dell'agricoltura in generale, e la zootecnia nella specifico, dovranno nei prossimi decenni cercare, più che in passato, nell'innovazione tecnologia gli strumenti per raggiungere due e apparentemente contrastanti obiettivi: aumentare significativamente la disponibilità di alimenti e di proteine nobili, soprattutto per soddisfare le crescenti richieste dei paesi emergenti, e contestualmente ridurre l'impiego delle risorse non rinnovabili e l'impatto complessivo su ambiente e clima.

Nondimeno, soprattutto nei Paesi sviluppati, dovrà essere in grado di aumentare i già elevati livelli di sicurezza degli alimenti, di tracciabilità e rintracciabilità delle derrate, di miglioramento della qualità dietetico-nutrizionale degli alimenti. Non solo, dovrà anche saper rispondere in maniera convincen-

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te alle crescenti richieste di rispetto dei principi etici nella manipolazione e nell'allevamento degli animali di interesse zootecnico, tutelandone il benessere e preservandone lo stato di salute.

Nel far questo gli imprenditori, i tecnici e i ricercatori che operano all'interno del comparto zootecnico non dovranno dimenticare di operare in un contesto accidentato in cui una comunicazione impropria e faziosa fa spesso avvertire l'innovazione tecnologica come nemico del buono, del sano, del genuino.

Compito del mondo scientifico, quindi, per le sue competenze e per i suoi doveri deontologici, è quello di sperimentare le innovazioni tecnologiche, di favorirne l'applicazione quando mature e di illustrarne, in maniera obiettiva, i benefici e gli eventuali limiti.

La finalità di questo workshop appare pertanto evidente: approfondire la conoscenza di alcune tecnologie innovative in grado di intervenire virtuosamente nel processo produttivo del comparto zootecnico e farsi strumento di comunicazione obiettiva presso una comunità che travalichi i confini del mondo accademico.

In tale contesto si inquadra il titolo che si è voluto dare al workshop di questa mattina, giacché è innegabile che le tante crisi che hanno, negli ultimi decenni, colpito la zootecnia nazionale e mondiale sono spesso derivate dall'incapacità di prevedere e di prevenire i rischi per la salute degli animali e dell'uomo, l'insorgenza e la diffusione di patologie e di zoonosi, l'effetto di mutamenti strutturali, produttivi, demografici e ambientali sulle attività zootecniche.

In uno scenario caratterizzato da rapidi e spesso profondi mutamenti delle dinamiche di mercato, del mondo del lavoro e delle produzioni e delle condizioni climatiche, obiettivo è quello di fornire alcuni utili spunti di riflessione in merito all'adozione, anche in campo zootecnico, degli strumenti resi disponibili da settori innovativi della ricerca scientifica e tecnologica. Uno sguardo in avanti, insomma, per prevenire (o almeno affrontare meglio) i problemi e le sfide che la zootecnia dovrà fronteggiare in un immediato futuro. Verranno, nel dettaglio, discussi il potenziale applicativo delle tecniche proteomiche nel campo delle scienze zootecniche con particolare riferimento alla tutela del benessere animale; esposti i risultati della più recente ricerca nanotecnologica nel campo del risanamento ambientale e delle produzioni animali; discusse le principali applicazioni della "sensoristica" per lo sviluppo della zootecnia di precisione e quindi di strumenti di gestione volti al monitoraggio automatico del benessere, della salute degli animali, dell'impatto ambientale, della sicurezza del consumatore e della produzione in tempo reale.

Come avrete modo di ascoltare, le innovazioni tecnologiche delle quali oggi si discuterà verranno presentate non solo nei loro aspetti direttamente coinvolti con l'efficientamento delle attività zootecniche, ma anche, in una visione più ampia, nei loro risvolti sul contenimento della spesa energetica e idrica, sulla riduzione della produzione di reflui e di scarti di produzione, sulla riduzione dell'uso di presidi sanitari e chimici antiparassitari, sulla riduzione di matrici non biodegradabili.

Diagnosticare precocemente, monitorare, correggere tempestivamente sono quindi le parole chiave di questo workshop. Riscoprire il ruolo essenziale della ricerca di innovazione è l'imperativo che vorremmo trasmettere a quanti, appartenenti al mondo delle istituzione e della politica, dovessero trovarsi tra le mani gli atti di questo workshop. Investire in ricerca e in ricerca di base, soprattutto, non può essere infatti considerato un lussuoso accessorio per un Paese progredito e sviluppato e che voglia continuare a progredire e a sviluppare le sue potenzialità. Con estrema onestà dobbiamo però anche riconoscere che, anche nel nostro campo, si produce forse troppo sviluppo, perfezionamento, adeguamento delle tecnologie esistenti e forse troppo poca innovazione. L'auspicio è quindi che soprattutto i nostri giovani ricercatori, i nostri allievi, che hanno il compito di continuare sulla strada della ricerca da noi intrapresa, scoprano o riscoprano l'importanza e il fascino di pensare, scoprire, generare innovazione.

RIASSUNTO

La finalità di questo workshop è approfondire la conoscenza di alcune tecnologie innovative in grado di intervenire virtuosamente nel processo produttivo del comparto zootecnico e farsi strumento di comunicazione obiettiva presso una comunità che travalichi i confini del mondo accademico.

Verranno discussi il potenziale applicativo delle tecniche proteomiche nel campo delle scienze zootecniche con particolare riferimento alla tutela del benessere animale; esposti i risultati della più recente ricerca nanotecnologica nel campo del risanamento ambientale e delle produzioni animali; discusse le principali applicazioni della "sensoristica" per lo sviluppo della zootecnia di precisione e quindi di strumenti di gestione volti al monitoraggio automatico del benessere, della salute degli animali, dell'impatto ambientale, della sicurezza del consumatore e della produzione in tempo reale. Come avrete modo di ascoltare, le innovazioni tecnologiche delle quali oggi si discuterà verranno presentate non solo nei loro aspetti direttamente coinvolti con l'efficientamento delle attività zootecniche, ma anche, in una visione più ampia, nei loro risvolti sul contenimento della spesa energetica e idrica, sulla riduzione della produzione di reflui e di scarti di produzione, sulla riduzione dell'uso di presidi sanitari e chimici antiparassitari, sulla riduzione di matrici non biodegradabili.

ABSTRACT

The purpose of this workshop is to deepen the knowledge of some innovative technologies that virtuously affect the production process of the livestock industry and be objective communication tool in a community that goes beyond the confines of academia.

The potential application of proteomic techniques in the field of livestock sciences with particular respect to the protection of animal welfare will be discussed; the results of the most recent nanotechnology research in the field of environmental remediation and animal products will be presented. Finally the main applications of the "sensors" for the development of precision livestock will be discussed and the management tools designed to automatically monitor the welfare, animal health, environmental impact, safety of the consumer and of the production in real time.

As you will hear, the technological innovations of which we will discuss today will be presented not only in their aspects directly involved with the efficiency of livestock activities, but also, in a broader view, in their implications on containment of energy and water spending, on reducing waste production, on reducing use of drugs and chemical pesticides, on the reduction of non-biodegradable matrices.

Fabrizio Ceciliani*, Cristina Lecchi*

Proteomic and animal health

proteomics: the magic world of proteins

The term proteomics refers to the large-scale study of proteins, including their structures and functions. The proteome defines the set of proteins expressed by the genetic material of an organism under given environmental conditions (Schlieben et al., 2012). Proteomic science emerged as a distinct field of research during the last twenty years (Thanomsridetchai et al., 2011) and, following a slow start, it has developed rapidly, driven by improvements in electrophoresis techniques and mass spectrometry analyses. Being a proteome more complex than its encoding genome (Corthals et al., 2000) the complete characterization of the proteins that compose even simple biological systems is hardly achievable, as opposed to the determination of full genomes (Burgess and Burchmore, 2012), on the background that the proteins are also present across a broad dynamic range. These issues are compounded by regulation of protein expression, in response to developmental and environmental stimuli, which results in a dynamic proteome. Nevertheless, the importance of proteins as the primary effector molecules of biology, which are also the major drug targets and antigens, has triggered strong interests and investments in proteomics, and the field continues to develop rapidly.

Proteomic techniques

Proteomics involves the resolution of a complex mixture of proteins into com-

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ponents that can then be identified, matching protein to encoding gene, and possibly quantified. As opposite to genomics or transcriptomics techniques, proteomics is unique in providing detection of post-translational protein modifications, such as phosphorylation or glycosylation. Protein characterization is carried out by mass spectrometry, which is generally performed after initial fractionation, on the background that even simple prokaryote proteomes comprise thousands of proteins and multicellular species may comprise greater than 100.000 proteins. The type of fractionation depends on the complexity of the proteome and the specific research question but must be compatible with the downstream mass spectrometry (MS). Major MS platforms employed for proteomics differs by the mechanism through which ions are generated and include matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). MALDI instruments receive analytes in the solid state, while the sample is delivered to ESI instruments in a volatile solvent.

The protein fractionation systems can be either electrophoretic (usually applied to intact proteins) or chromatographic (usually applied to peptides generated by protein cleavage). Orthogonal separation approaches are often utilized to enhance resolution, and the archetypal orthogonal separation in proteomics is 2-dimensional electrophoresis (2DE) (Gorg et al., 2004). Conventional 2DE involves separation by isoelectric focusing in the first dimension, followed by sodium dodecyl sulphate electrophoresis in the second, both dimensions being performed in a polyacrylamide gel matrix, and the proteins migrate on 2-dimensional gels as spots according to isoelectric point and apparent molecular weight. The resulting spot map are visualized by protein staining and are able to resolve several thousand protein species. Spots can then be excised directly from the gel and identified by means of mass spectrometry.

2DE remains the highest-resolution protein separation approach and is inherently quantitative. The separation of intact proteins by charge and mass provide information about post-translational modifications that would not be evident in the lower resolution 1-dimensional electrophoresis or in peptide-based separations (Rogowska-Wrzesinska et al., 2013). Yet, 2DE tends to under-represent those proteins that are of relatively low abundance, very large, or highly charged. Prefractionation to enrich proteins of interest or by focusing 2DE on specific charge and/or mass ranges can circumvent some of these issues. Hydrophobic proteins may be refractory to solubilization in the nonionic conditions that are required for isoelectric focusing and alternative detergents or 2-dimensional separations, such as the BAC/SDS-PAGE system (Bridges et al., 2008; Hinz et al., 2012) come useful in this context.

The heterogeneity of intact proteins provides a serious challenge the resolution of chromatography for proteomic workflows. Proteins can also be fragmented to peptides and then separated by chromatography. Reversed-phase chromatographic separation of peptides is ideally suited to proteomics because peptides can be trapped and desalted before elution and because the mobile phase comprises volatile solvents that can be evaporated in the ESI source. Chromatography can thus be directly coupled to ESI-MS, Multidimensional chromatographic separation is increasingly employed (Yates et al., 2009), being automated and because even highly charged or hydrophobic proteins will likely generate some peptides that can be identified on MS analysis. Ion exchange is mostly used as a first dimension. Chromatographic approaches can be more sensitive than electrophoresis because there is no requirement to recover proteins or peptides from a gel matrix. Combinations of electrophoresis and chromatography are among the most efficient fractionation systems (Xie et al., 2011). Although targeted approaches such as subcellular fractionation or affinity purification of protein complexes can result in greatly enhanced coverage of a subproteome.

Proteins obtained from a biological source are fractionated by electrophoresis, peptides are generated after trypsinization and further fractionated by high-performance liquid chromatography (LC) before analysis by an ESI-MS which allows the identification of the peptides (Burgess and Burchmore 2012). The resulting data are analyzed with the support of dedicated softwares or search engines, such as for example Mascot (Matrix Science Ltd), which generates in silico MS data for the specified genome sequence database and looks for statistically significant matches with the experimentally generated MS data. The data output provides a list of potential matches, ranked by confidence, to proteins that may be components of the sample.

Proteomics can also provide protein relative quantitation, which can be achieved by a diversity of comparative approaches. Relative quantitation of intact proteins can be carried by gel-based methods, such as 2DE using semi-quantitative protein stains, or protein-labeling strategies, such as difference gel electrophoresis (DiGE) (Alban et al., 2003). DiGE techniques have increased the utility of 2DE for quantitative proteomic analysis, allowing the direct comparison on a single gel of samples that are differentially labeled by fluorophores that are mass and charged matched but spectrally discrete.

Quantitation at the peptide level can be achieved by stable isotope-labeling approaches or by label-free comparison. Isolated proteins or tryptic peptides are chemically labeled before separation [iTRAQ], dimethyl labeling (Hsu et al., 2006) or proteins can be metabolically labeled with heavy and light amino acids (stable isotope labeling with amino acids in culture [SILAC]) (Ong et al., 2002). An appropriate software deconvolutes from the resulting MS data the relative abundance of specific proteins in each sample. Of the protein-labeling approaches, SILAC is incorporated during growth, avoiding the possibility of introducing artifactual changes in protein abundance during the sample preparation step, although chemical labeling of proteins can be performed with proteins from any source.

Label-free approaches involve serial LC-MS analysis of multiple unlabeled samples and are becoming commonplace as the robustness of chromatographic separation improves and facilitates the alignment of data sets that is essential for comparison. Label-free approaches are more costly in instrument time, as unlabeled samples cannot be multiplexed – an important consideration, as LC-MS instrumentation is costly to maintain. Regardless of the quantitation approach, comparative proteomics experiments have the potential to highlight key proteins in phenotypes of interest and thus have tremendous potential to highlight drug targets and biomarkers and elucidate biological mechanisms (Burchmore, 2006). A brief summary of the two proteomics workflows is provided in figure 1.

PROTEOMICS IN VETERINARY AND ANIMAL SCIENCES

Several proteomic techniques have been applied to the understanding of protein pathways involved in host and pathogen interactions during diseases. Pathogens and immune defenses adapt to each other, due to the regulation of the expression of several genes of both sides, to cope with changing stimuli. The fine-tune of gene expression has been studied by Next Generation Sequencing techniques, in particular for what concerns host-pathogen relationship (Ojha and Kostrzynska, 2008). However, since the correlation between DNA levels and actual protein expression is poor (Griffin et al., 2002) and integration between the two techniques, genomics and proteomics, is required.

Given the background of economic needs, most of proteomics investigation explored the pathogenesis of mastitis, in bovine milk in particular, because of the relative ease of sample collection (Bohemer, 2011).

Proteomics was very useful to describe the modification of milk proteins during mastitis in cows with naturally occurring infection (Smolenski et al., 2007) as well as in experimentally induced coliform mastitis (Danielsen et al., 2010), providing a list of milk proteins that can be found in milk and that

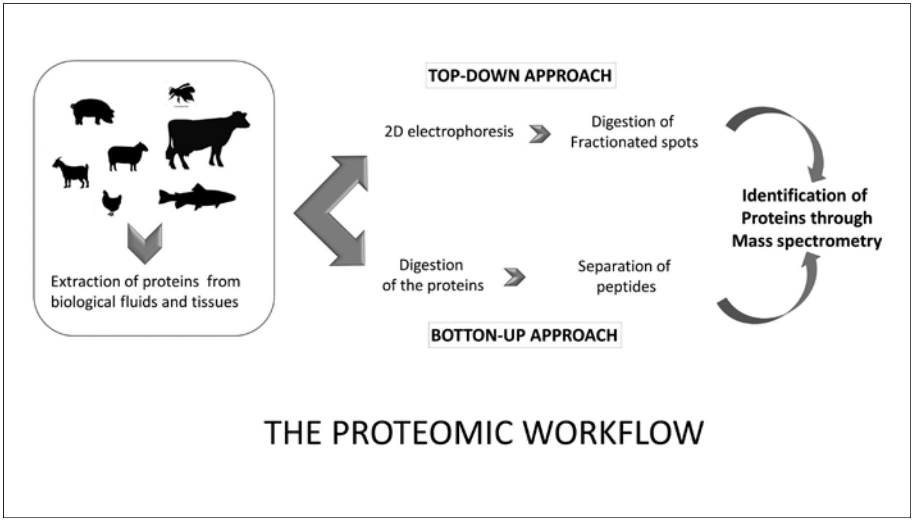

Fig. 1

can be possibly used as biomarkers for early diagnosis of mastitis.

Proteomic techniques have been widely applied to the pathogenic mechanisms of bacterial infection in farm animal diseases, focusing, again, on pathogen responses during clinical intramammary infections (Tedeschi et al., 2009), providing the identification of immunogenic proteins in bovine mastitis *S. aureus* isolates involved in virulence. *M. avium subsp. paratuberculosis* was also investigated, identifying among the others a set of 10 proteins whose expressions are upregulated during natural infection (Hughes et al., 2007), as well as the proteome of pathogenic leptospires, the causative agent of leptospirosis, expressed during urinary excretion from reservoir hosts of infection (Nally et al., 2007).

Beside bovine species, a significant amount of proteomic studies has been performed on other farm animals, such as porcine and caprine species (de Almeida and Bendixen, 2012; Ceciliani et al., 2014). In addition to its role in meat production, the porcine species is an important animal model for the study of disease in humans.

An interesting field of application of proteomics is the study of the pathogenesis of infectious disease of poultry, which has an impact on both the production and the need to study avian diseases as zoonosis, such as for example avian flu, where human host adaptation signatures have been identified (Miotto et al., 2010).

Fish diseases are responsible for the main economic losses in aquaculture.

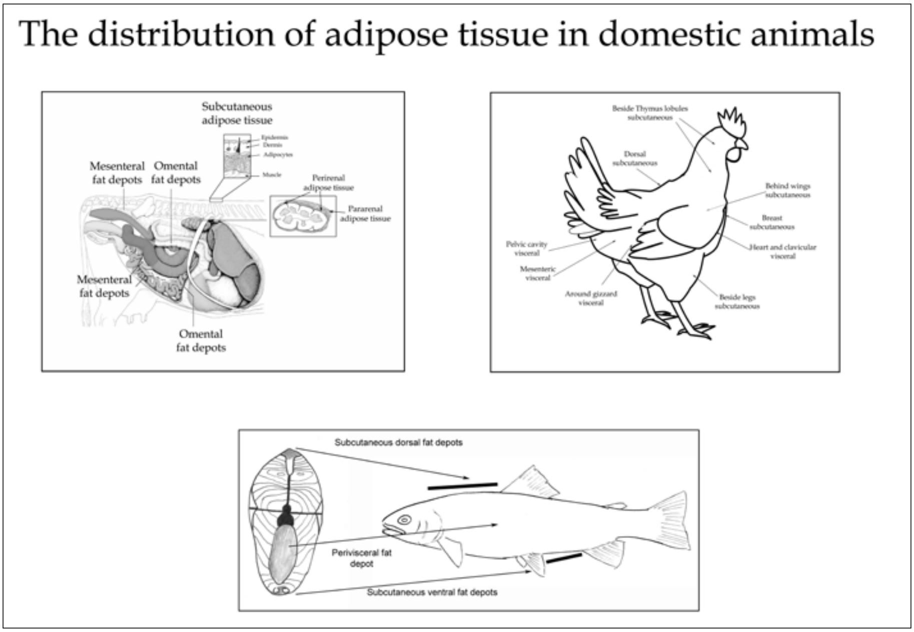

Fig. 2

Proteomics techniques have been used to address this issue, in particular for the development of new vaccines and disease diagnostics. Finally, the complete proteomic atlas of bee has been recently published (Chan et al., 2013), providing a deep insight into organ-level resolution of protein expression.

Due to its involvement in complex processes like reproduction, inflammation and immune response, also adipose tissue has been analyzed by applying proteomic techniques (Sauerwein et al., 2014), presenting the evidence that different adipose tissue depots express a different protein pattern.

THE ADIPOSE TISSUE: NEW CONCEPTS FOR AN OLD TISSUE

The importance of adipose tissue as energy store was already well established many centuries ago. In farm animal physiology, most of the studies on adipose tissue were aiming to understand the mechanisms underlying the mobilization of lipids in lactating animals to fulfill the energy requirements of milk production or the deposition of fat in different depots, in particular those associated with muscle. Since the discovery of leptin in 1994 (Zhang et al., 1994), adipose tissue was no longer considered as a mere source of energy but was increasingly recognized to be actively involved in complex regulatory processes, e.g. regulating appetite, energy expenditure, body weight, inflammation and reproduction via its synthesis and secretion of messenger molecules which are now collectively referred to as "adipokines". There are several types of adipose tissue depots (fig. 2).

Priorities in adipose tissue research in farm animals are different from the focus of human biomedical research. Most of the information available in mammalian adipose tissue biology has been linked with obesity and metabolic diseases in humans, where the specific term "obesidomics" was coined to define proteome and secretome in pathological obesity (Pardo et al., 2012). Although several animal models of obesity were developed (Lutz et al., 2012), obesity is hardly an issue in farm animals due to their controlled feeding according to well defined needs.

Transcriptomics has provided important advancement in understanding the functions of adipose tissue. Yet, the major limit of transcriptomics is that it does not provide any hint about the effective expression of the proteins, the correlation between mRNA level and actual protein expression being poor (Griffin et al., 2002). Therefore, integration between genomics and techniques focused on protein expression is required. Within the past decade proteomics has emerged as an accessory technique to transcriptomics. Hereby are provided examples of how adipose tissue features can be explored by means of applying proteomic techniques, focusing on goat adipose tissue.

ADIPOSE TISSUE PROTEOMICS

Adipose tissue is distributed as fat depots throughout the whole body, and is classified mainly as subcutaneous (SAT) and visceral (VAT) adipose tissue (fig. 2). In ruminants, SAT includes depots located beneath the skin, e.g. the armpit cavity, the subcutaneous areas over the sternum and the withers and the base of the tail, while VAT is located in the intra-abdominal cavity, surrounding specific organs, such as kidney and heart, or distributed among peritoneum layers, such as mesenteric and omental fat. Therefore, adipose tissue should not be considered a single endocrine organ located in different region of the body, but a group of endocrine organs with specialised and location specific endocrine function (Kershaw and Flier, 2004). In fact, VAT AND SAT are distinguished according to their different metabolic characteristics and by their ability to release inflammatory cytokines. In farm animals, and particularly in ruminants, the regulation of lipid metabolism is of key importance not only for animal health, but also for production of meat and milk. Nevertheless, a systematic investigation of the molecular mechanisms of adipose tissue has not yet been undertaken in these species. Transcriptome studies of adipose tissues from mice, rat, sheep and cow have shown that visceral and subcutaneous adipose tissue depots differ in mRNA abundance, highlighting the importance of sampling site in studies of e.g. metabolic pathways in AT (Mukesh et al., 2010).

Proteomic studies have been limited to bovine species, and include a comparative study of adipogenic differentiation of preadipocytes in the omental, subcutaneous and intramuscular tissues (Rajesh et al., 2010), a time resolved investigation of embryonic fat deposition (Taga et al., 2012) and of intramuscular fat depots of Korean steers (Zhang et al., 2010). LC-MS/MS was applied to characterize and compare the proteome composition of SAT (base tail and sternum) and VAT (perirenal and omentum) of young goats (Restelli et al., 2014) providing the first adipose tissue proteome of goat. The proteomic analysis of different SAT AND VAT deposits showed protein expression's differences, confirming also in goat-kids the importance of sampling site when studying adipose tissue's metabolic roles. The protein expression characteristics of adipose tissues was determined by quantitative RT-PCR and confirmed that adipose tissues seems to play a central role in control of inflammation, detoxification and coagulation pathways, as well as for regulation of body fat mobilization in dairy animals. These findings were of particular interest in farm animals where health and production traits are important for animal welfare and for economic gain. A number of 761 proteins were found to be uniquely produced by adipose tissue. Of them, most were involved in metabolic processes, dominating both VAT and SAT tissues, although significantly more in VAT (73.5%) than in SAT (54.5%). Structural proteins represented 20. 4 % of the observed adipose tissue proteome, being more abundant in visceral AT than subcutaneous AT (30.1% and 19.6%, respectively), and particularly so for sternum deposits (48.5%). In our studies, 71 out of 761 adipose tissue proteins relate to this group (9.3%) (Restelli et al., 2014). Other proteins were involved in proteins involved in toxic response and folding, including families such as HSP, chaperons and peroxiredoxins. The last group of proteins were involved in immune and inflammatory response, and represented 6.5% of the total.

In a second experiment, the impact of different diet was studied (Restelli et al., manuscript in preparation). The comparative investigation of visceral adipose tissue proteomes of goat-kids with different high-fat fed mothers was perfomed. Periparturient goats ere fed with fish oil and stearic acid enriched diets, and a quantitative 2D-LC-MS/MS analysis was carried out, using iTRAQ labelling, in order to evaluate the possible influence of different diets on kids' omentum protein expression. The involvement of adipose tissue (AT) in several physiological and pathological processes, such as appetite regulation, reproduction, and inflammatory and immune response, is well recognized. In humans, adipose tissue has a key role in obesity, while in farm animals, where obesity is not an issue due to the controlled environment in which they live, particular focus has been given to adipose tissue's influence on animal health and meat quality. Indeed, it has been demonstrated that adipose tissue within the muscle (i.e. marbling fat) strongly influence meat quality and composition, by affecting parameters such as tenderness, juiciness and taste (Wood et al., 2008). On the other hand, in dairy animals AT metabolism gained particular interest for its essential role in the transition period when a hormonally-controlled lipid mobilization is established in order to support milk synthesis (Shirley et al., 1973; Contreras and Sordillo, 2011). The active role of adipose tissue in regulating the wide range of body functions is explicated by its ability to produce and secrete adipokines. Adipokines are signalling molecules with endocrine, autocrine or paracrine functions, secreted in response to stimulus coming from the hormone system and the central nervous system (Harwood, 2012). Taking into account the profound relationship between body fat reserves and food, it is not surprising that adipose tissue's transcriptomic profile can be modified by diets or feed deprivation. Indeed, as demonstrated in goats, 48 h of feed deprivation alter the expression profile of several genes in omental and perirenal AT deposits, with omentum more sensitive to feed deprivation than perirenal areas (Faulconnier et al., 2011). In addition, Ebrahimi et al. (2013) demonstrated that linseed oil supplementation to Boer goats' diet, leads to changes in fatty acid profile of subcutaneous adipose tissue and expression of genes related to fat metabolism such as PPARα, PPARγ and stearoyl-CoA desaturase. Few proteomic studies are regrettably available.

Another aspect to consider when studying the relationship between diets and adipose tissue, is the different impact that distinct fat sources have on adipose tissue itself, as demonstrated by Thering et al. (2009), which investigated the effect of fish and soybean oils or saturated lipids enriched diets on lipogenic and adipogenic gene expression in cow's tailhead adipose tissue. Fish oil is particularly rich in eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) that can positively influence animal health due to their involvement in innate immune pathways (Pisani et al., 2009; Lecchi et al., 2011). On the other hand, Bueno et al. (2010) demonstrated that diets enriched with coconut oil or lard, both rich in saturated fatty acids, can modify the pro-inflammatory environment of white adipose tissue in rats, by upregulating haptoglobin expression.

Adipose tissues originate during early life from mesenchymal stem cells, which can differentiate into adipocytes, osteoblasts, chondrocytes, and myoblasts (Lee et al., 2013). It has been shown that a diet based on milk or milk replacer can influence meat quality and fat composition of suckling kids (Bañon et al., 2006), therefore the next step to be investigated was the influence that the maternal diet has on kid's adipose tissue characteristics. UCP1 expression and thermogenesis can be modulated by high fat diets, in perirenal adipose tissue of newborn lambs (Chen et al., 2007), while overfeeding sheep during late gestation, enhances adipogenesis in lamb's fetal muscles (Tong et al., 2008). In addition, fish oil enriched diets increases the amount of n-3 PUFAs in colostrum and mature milk in pregnant dairy goats (Cattaneo et al., 2006), and a specific involvement in fetal and neonatal development has been recognized for DHA (Innis, 2000). Although proteomics could be of great help in understanding the relationship between maternal diet and kids' adipose tissues' characteristics, mainly transcriptomic studies have been carried out. The few existing proteomic experiments have been performed in rodents, aimed to evaluate the effect of high fat diets on the protein expression of insulin target tissues in mice (Schmid et al., 2004) or on the expression of adipose tissue proteins between obesity-susceptible and obese-resistant rats (Joo et al., 2011). In farm animals, few information are available, among which, the demonstration of the influence of maternal diets on adipose tissue proteome in newborn pigs (Sarr et al., 2010).

The results demonstrated that at least 30 proteins were differentially expressed as a consequence of different diets administered to the mothers, belonging to different families, including immune related proteins, fatty acid related metabolism and oxidative stress.

HEADING TOWARD NEW HORIZONS: THE FUTURE OF PROTEOMICS

Better biomarkers are urgently needed in both veterinary medicine and animal sciences for diagnosis and prognosis of diseases, and for phenotyping of QTL needed to provide breeds more resilient and resistant to diseases. The research world is entering a postgenomic era, which provides great opportunities in the pursuit of new biomarkers. Despite its importance in animal health, the application of proteomics in animal and veterinary science is still lagging, if compared with proteomics in humans and mice. There is an evident need for proteomics to be included in future investigation of animal health, welfare and production. It is clear that valuable information on the molecular mechanism of diseases of animals of veterinary interest is being and will be generated in the future as the technology becomes more applicable in studies designed to explore and explain the pathology of veterinary diseases and animal productions. Initial proteomic studies, when applied to novel areas, have tended to focus at first on describing the proteome of a particular tissue or fluid. Then the power of the techniques is recognized, and experiments to compare and quantify protein changes in experimental procedure or in comparison of disease to healthy samples, or to different farming conditions, become more common.

One of the reasons why proteomics has played a limited role in veterinary medicine and diagnostics, and animal sciences, beside the economic one, is the scarce genomic and proteomic data available as compared with rodents and humans. The recent publication of genomes from pig and cow as well as the growing availability of proteomic reference maps of companion animal tissues and biological fluids will probably overcome these technical barriers. The cost of proteomics experiments is decreasing as well. Given these premises, the still-limited number of proteomic maps is expected to increase, providing new opportunities to utilize proteomic information for diagnosis of animal diseases and better identification of animal productive traits. Technological advances in proteomics have expanded the dynamic range of detection for low-abundance proteins, allowing the detection of disease-specific proteins to be used as potential biomarkers in veterinary medicine as well. The ultimate goal should be to develop diagnostic protocols on clinical samples at multiple levels, including transcriptome (RNA) and proteome (proteins).

RIASSUNTO

La proteomica è quell'insieme di tecniche che permettono di studiare il proteoma, ovvero l'intero insieme di proteine che costituiscono un dato organismo o un sistema biologico (cellula/tessuto/organo). Il potenziale applicativo delle tecniche proteomiche nel campo delle scienze zootecniche è enorme. Le tecniche proteomiche sono complementari alle tecniche genomiche, con il valore aggiunto che sono le uniche che permettano la

caratterizzazione delle modificazioni post traduzionali delle proteine, come per esempio quelle che intercorrono nella maturazione del formaggio, oppure nella trasformazione da muscolo a carne.

Nella presente relazione viene presentata in una prima parte una ampia descrizione dello stato dell'arte sulla applicazione delle tecniche proteomiche che vengono comunemente utilizzate nel campo delle scienze zootecniche. La seconda parte è invece focalizzata su alcuni esempi di come le tecniche proteomiche possano essere applicate alle scienze animali. In modo particolare vengono evidenziati i risultati di un esperimento sugli effetti di differenti diete, arricchite di acidi grassi a catena lunga saturi e polinsaturi, sulla modificazione della espressione di proteine presenti nel tessuto adiposo della capra da latte.

Vengono inoltre presentati alcuni i risultati ottenuti applicando tecniche proteomiche alla caratterizzazione delle modificazioni post-traduzionali (glicosilazioni e fosforilazioni) di proteine coinvolte nella immunità innata del bovino e nella capra.

ABSTRACT

Proteomics allows the study of proteins present in a given tissue or fluid (the proteome). Proteomics is of significant importance to several scientific areas, including veterinary and animal sciences. Application of proteomics to animal sciences has been limited due to the cost and lack of genomic data from livestock. The present report provide examples of successful applications of proteomics in animal production and health with insights into adipose tissue.

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Nanotechnology in agriculture and food sciences

Historically, agriculture preceded the industrial revolution of about 90 centuries; the history of the technological innovation in agriculture and agri-food have produced profound changes in production, in the landscape and environment, and in significant socio economic relationships, revolutionizing farming operations with less dependence on farm workers to specific tasks. The latest series of technological innovations include the new genetics, biotechnology, intensive farming of animals and new techniques of cell reproduction. The products of these technological innovations have affected differently large segments of agriculture, new varieties of seeds and animals, new varieties of chemical products, more pesticides, fertilizers and veterinary drugs. Nanotechnology, as an emerging technology, presents an important opportunity for the scientific and business community. Industrial development-intensive chemical agriculture in recent decades has produced high environmental costs associated with the loss of biodiversity, toxic pollution of land and waterways, increased salinity, erosion and decreased soil fertility. Nanotechnology is now imposing, albeit with light and shade and not in the all areas, as an element of development in modern agriculture and in the food sector where it can be a driving economic force in the near future. Nanoscience and nanotechnology are new frontiers of this century (Raliya et al., 2013). Nanotechnology enables plants to use water, pesticides and fertilizers more efficiently; industrial development aims important role in the development of novel methods for the production of new products, to replace existing production plants and to

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reformulate new materials and chemicals with improved performance resulting in lower consumption of energy and materials, reduced damage to the environment, environmental remediation, sustainability and enhancement of nutritional food, including crops intended for human consumption and animal feed. Ultimately, nanotechnology could be described as the science of designing and building machines in which every atom and chemical bond is precisely specified (Ditta, 2012). According to another definition, "nanomaterial" means a natural, incidental, or manufactured material containing particles in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution one or more external dimension is in the range 1-100 nm. Nanoscale materials exhibit novel properties such as increased strength, enhanced optical features, antimicrobial properties, and superconductivity. Nanotechnology is unlike some other sectors of the chemical industry, where significant capital is already invested in the form of large plants and established supply chains in which production techniques are technologically and culturally embedded. It is not a set of special techniques, devices or products, but the set of capabilities that we have when technology is approaching the limits of the atomic physics. However, while research in nanotechnology began to grow for industrial applications almost half a century ago, the momentum for the use of nanotechnology in agriculture came only recently (Agrawal and Rathore, 2014) respect to their use in drug delivery and pharmaceutical products. Engineered nanoparticles (NPs) are now present in matrices that can interfere with food production (Sonkaria et al., 2012). In fact their industrial use for a wide range of potential applications led to the contamination of environmental media (water, air, soil) with nanomaterials so it may raise concerns related to environmental risk. Between 2006 and 2011, reports have shown that the number of nanotechnology-related products across the world grew by 521 percent. By 2015, the market for nano products was expected to hit $ 2.4 trillion. Nanotechnology use may bring potential benefits to farmers through new agrochemical agents and new delivery mechanisms to improve crop productivity. Furthermore it promises to reduce pesticide use, increase food production and to improve the food industry through the development of innovative products for preservation and packaging uses. Applications include nanoparticle-mediated gene or DNA transfer in plants for the development of insect-resistant varieties, food processing and storage, nanofeed additives, increased product shelf life, nanosensors/ nanobiosensors for detecting pathogens, for soil quality and for plant health monitoring, nanoporous zeolites for slow-release and efficient dosage of water and fertilizers for plants and release of nutrients and drugs for livestock, nanocapsules for agrochemical delivery in form of green slow-release fertilizer (Kottegoda et al., 2011), biofuels, nanocomposites for plastic film coatings used in food packaging, antimicrobial nanoemulsions for applications in decontamination of food, nanobiosensors for identification of pathogen contamination, and improving plant and animal breeding (Espitia et al., 2013). Nanomaterials can enter the water cycle in various ways: for example, domestic sewage may be affected by textiles, detergents, cosmetics, pharmaceuticals or building materials, while bathing waters by sun protection products, and groundwater by industrially manufactured or processed nanomaterials which are discharged into various water cycles, or by fertilizers and landfill leachates. Nanotechnology promises to accelerate the development of biomass-to-fuels production technologies. Experts feel that the potential benefits of nanotechnology for agriculture, food, fisheries, and aquaculture need to be balanced against concerns for the soil, water, and environment and the occupational health of workers. Raising awareness of nanotechnology in the agri-food sector is one of the keys to influencing consumer acceptance. While the successful implementation is important for the growth of the global economy, nanotechnology offers much promise, in fact these novel properties and behaviors may also pose new risks so there is also a need to consider the possible environmental health and safety impact. On the basis of only a handful of toxicological studies, concerns have arisen regarding the safety of nanomaterials, and researchers and companies will need to prove that these nanotechnologies do not have a negative impact on the environment. There is increasing concern of the toxicity of engineered nanomaterials and their effects on biological systems and environment, which remain largely unknown (Podila and Brown, 2013). Nanomaterials possess physical and chemical properties that can have an unpredictable impact on safety and human health; biological naturally occurring nanoparticles nanoclay, tomato carotenoid lycopene, many chemicals derived from soil organic matter, lipoproteins, exosomes, magnetosomes, viruses, ferritin, have diverse structures with wide-ranging biological roles; biological nanoparticles are often biocompatible and have reproducible structure (Giordani et al., 2012). The interaction of these nanomaterials with human organs and tissues initially aroused scientific interest for possible applications in biomedicine, later began a major concern in both scientific organizations and health-conscious environment. Population exposure to nanoparticles may occur directly or indirectly. Indirect exposure occurs both by nano particles produced by natural processes such as fires, earthquakes and volcanic eruptions, both from nano particles from air pollution caused by technological advances that led to the accumulation of large amounts in the environment and can cause changes in the final products or changes of metabolites that may also lead to high risks (Cushen et al., 2012). A number of recent reports and reviews have identified the current and short-term projected applications of nanotechnologies in the food sector (Groves, 2008; Kuzma and VerHage, 2008; Kuzma, 2010; Mura et al., 2013; Bouwmeester et al., 2007). There are already identified potential uses of nanotechnology in virtually every segment of the food industry with four key focus areas:

(i) agriculture-pesticide, fertilizer or vaccine delivery; animal and plant pathogen detection; targeted genetic engineering; nanoagrochemicals and water pollution,

(ii) food processing-encapsulation of flavor or odor enhancers; food textural or quality improvement; new gelation or viscosifying agents in nanofood,

(iii) food packaging-pathogen, gas or abuse sensors; anticounterfeiting devices, UV-protection, and stronger, more impermeable polymer films, in agri-environment

(iv) nutrient supplements-nutraceuticals, cosmetic with higher stability and bioavailability.

Finally, the use of nanomaterials in the environment can cause changes in the final products or changes of metabolites that may also lead to high risks. On basis of these stresses a new discipline called "nanotoxicology" is born who is trying to study the interactions of these nanostructures with biological structures with the laying of a certain question about the gap: science and ethics in nanotechnology. Significant evidence indicates that manufactured nanomaterials and combustion-derived nanomaterials elicit toxicity in humans exposed to these nanomaterials. The toxicology studies worked on in vitro cytotoxicity studies of cells, lately also in vivo studies have increased. The number of studies that have been published on the topic of nanosafety speaks for itself. We have seen an almost exponential rise over the past 15 years or so in the number of articles on nanotoxicology. Although only a couple of hundred papers had appeared on the topic of "Nanomaterials: environmental and health effects" before 2000, this number has exploded to over 10 000 since 2001. Most of these studies, however, do not offer any kind of clear statement on the safety of nanomaterials. On the contrary, most of them are either self-contradictory or arrive at completely erroneous conclusions (Krug, 2014). The epidemiological studies are very complicated, for the interaction of multiple components and biological events that occur in vivo. The environmental effects require further research to determine whether the assessment methods currently used (organisms, cell cultures, exposure regimens, analytical methods) are applicable to the testing of nanomaterials in standardized toxicity tests to determine the effects of nanomaterials in ecosystems (Stanley, 2014). Their absorption, distribution, metabolism and excretion (ADME) evaluates these parameters for different nanomaterials in order to examine the interaction of nanomaterials with model ecosystems (Fedeel et al., 2015).

NANOAGROCHEMICALS

A doubling in global food demand projected for the next 50 years poses huge challenges for the sustainability both of food production and of terrestrial and aquatic ecosystems and the services they provide to society. Recent agricultural practices associated with the Green Revolution have greatly increased the global food supply (Brennan, 2012). They have also had an inadvertent, detrimental impact on the environment and on ecosystem services, highlighting the need for more sustainable agricultural methods (Gogos et al., 2012). It is well documented that excessive and inappropriate use of fertilizers and pesticides has increased nutrients and toxins in groundwater and surface waters, incurring health and water purification costs, recreational opportunities, and decreasing fishery in Developing Countries (Chaudhry and Castle, 2011). Agricultural practices that degrade soil quality contribute to eutrophication of aquatic habitats and may necessitate the expense of increased fertilization, irrigation, and energy to maintain productivity on degraded soils (Marchiol, 2012). Agriculturalists are the principal managers of global usable lands and will shape, perhaps irreversibly, the surface of the Earth in the coming decades. Degraded ecosystems have become a serious threat to human health and civilization. The benchmark for ecosystem degradation is linked to its failure to retain carbon and prevent escape of various forms of nitrogen from the soil to water bodies and the atmosphere. It leads to increased pests, reduced availability of clean water and biodiversity loss. Land degradation is often the result of land mismanagement, including: deforestation, overgrazing, monoculture, salinization, pollution of land and water sources by agriculture or industries, misuse of fertilizers and/or chemicals, poor farming practices, and soil erosion. Farmland is a fundamental resource for human survival and development, however, farmland fragmentation has become a serious problem, causing ecological damage and low crop production efficiency in many parts of the world (Cheng et al., 2015). Despite many adjustments to agricultural policy, intensification of production in some regions and concurrent abandonment in others remain the major threat to the ecology of agro-ecosystems impairing the state of soil, water and air and reducing biological diversity in agricultural landscapes. The impacts also extend to surrounding terrestrial and aquatic systems through water and aerial contamination and development of agricultural infrastructures (e.g. dams and irrigation channels). Improvements are also documented regionally, such as successful support of farmland species, and improved condition of watercourses and landscapes. All of this increases food insecurity and makes the affected areas, their populations and business operations more vulnerable to climate change. Manufactured nanoparticles can be produced from nearly any chemical; however, most NPs that are currently in use have been made from transition metals, silicon, carbon (carbon black, carbon nanotubes; fullerenes), and metal oxides; few of these nanoparticles have been produced for several decades on an industrial scale, but various new materials such as carbon nanotubes, fullerenes or quantum dots have only been discovered within the last two decades. Agrochemical companies are reducing the existing chemical emulsions to the nanoscale and substituting active ingredients with their encapsulated nanosized equivalents in attempt to bring a number of benefits into potential applications of nanotechnology to pesticides, and other agrochemicals such as fertilizers and plant growth regulators (DeRosa et al., 2010). New incentives and policies for ensuring the sustainability of agriculture and ecosystem services will be crucial if we meet the demands of improving yields without compromising environmental integrity or public health (Garcia et al., 2010). Nanotechnology can improve crops yield, germination, nutritional values (Khodakovskaya et al., 2009), and can offer added value to crops or environmental remediation (El-Ramady, 2014). Particle farming is one such fields, which yields nanoparticles for industrial use by growing plants in gold rich soil. The gold nanoparticles can be mechanically separated from the plant tissue following harvest (Owolade et al., 2008). New applied research also aims to make plants use water, pesticides and fertilizers more efficiently, to reduce pollution and to make agriculture more environmentally friendly. Smaller companies are forming alliances with major players such as LG, BASF, Honeywell, Bayer, Mitsubishi, and DuPont to make complete plant health monitoring systems in the next 10 years using nanotechnologies. Opportunities for applying nanotechnology in agriculture lie in the areas of genetic improvement of plants, delivery of genes and drug molecules to specific sites at the cellular level in plants (Giraldo et al., 2014), plant nanobionics approach to augment photosynthesis and biochemical sensing, nanoarray-based technologies for gene expression in plants to overcome stress and development of sensors and protocols for its application in precision farming, management of natural resources, early detection of pathogens and contaminants in food products, smart delivery systems for agrochemicals like fertilizers and pesticides (Vidyalakshmi et al., 2009), and integration of smart systems for food processing, packaging, and monitoring of agricultural and food system security. Precision farming has been a long-desired goal to maximise output (i.e. crop yields) while minimising input (i.e. fertilisers, pesticides, herbicides, etc) through monitoring environmental variables and applying targeted action. Precision farming makes use of computers, global satellite positioning systems, and remote sensing devices to measure highly localised environmental conditions thus determining whether crops are growing at maximum efficiency or precisely identifying the nature and location of problems. By using centralised data to determine soil conditions and plant development, seeding, fertilizer, chemical and water use can be fine-tuned to lower production costs and potentially increase production all benefiting the farmer. With nanofertilizers emerging as alternatives to conventional fertilizers, buildup of nutrients in soils by eutrophication and contamination of drinking water may be eliminated (Manimegalai et al., 2011). Pesticides are commonly used in agriculture to improve crop yield and efficiency, smart delivery system has a huge potential for improving efficiency of fungicides in agriculture systems. Development of these technologies in plant protection would allow their use in crop protection. The application of smart delivery systems for improving treatment of plant diseases with chemicals (fungicides, insecticides, herbicides) could be immediate (Rai et al., 2012). However, the more complex part is the translocation of the substances within the plant to reach the action point. Nanopesticides are one of a new strategy being used to address the problems of non-nanopesticides and enables companies to manipulate the properties of the outer shell of a capsule in order to control the release of the substance to be delivered. 'Controlled release' strategies are highly prized in medicine since they can allow drugs to be absorbed more slowly, at a specific location in the body or at the say-so of an external trigger. Nanopesticides cover a wide variety of products, some of which are already on the market, U.S. EPA statement, several manufacturers have been interested in releasing nanoscale pesticides. Nevertheless, almost no major agrochemical companies, except Syngenta, have announced that they are manufacturing products, which contain nanomaterials having a diameter less than 100nm. Syngenta has been selling its Primo MAXXR for several years. Primo MAXXR is by far the most widely used Plant Growth Regulator (PGR) by golf course superintendents and other professional turf managers since its introduction in 1993. Syngenta claims that the particle size of this formulation is about 250 times smaller than typical pesticide particles. According to Syngenta, it is absorbed into the plant's system and cannot be washed off by rain or irrigation. In 1998 Monsanto entered an agreement with Flamel Nanotechnologies to develop "Agsome" nanocapsules of Roundup, which might have been more chemically efficient than the conventional formula. They cannot be considered as a single entity; rather such nanoformulations combine several surfactants, polymers (organic), and metal nanoparticles (inorganic) in the nanometer size range (Ray, 2013), it is marketed as a "micro-emulsion" concentrate. The lack of water solubility is one of the limiting factors in the development of crop-protecting agents. Microencapsulation has been used as a versatile tool for hydrophobic pesticides, enhancing their dispersion in aqueous media and allowing a controlled release of the active compound. Polymers often used in the nanoparticle production have been reported (Perlatti et al., 2013), potential applications across the food chain (in pesticides, vaccines, veterinary medicine and nutritionally-enhanced food), these nano and micro-formulations are being developed and patented by agribusiness and food corporations such as Monsanto, Syngenta and Kraft. Researchers have reported various aspects of nanoparticle formulation, characterization, effect of their characteristics, and their applications in management of plant diseases. First of all, polycaprolactone and poly(lactic) acid nanospheres were used for encapsulation of the insecticide ethiprole, silica nanocapsules were prepared by a recently reported emulsion and biomimetic dual-templating approach under benign conditions and without using any toxic chemicals (Wibowo et al., 2014). Nanonization is an attractive solution to improve the bioavailability of the poorly soluble drugs, to improve therapies, in vivo imaging, in vitro diagnostics and for the production of biomaterials and active implants (Sheth et al., 2012). Nanoparticles in the pharmaceutical industry and the use of supercritical fluid technologies for nanoparticle production in drug delivery, application of nanotechnology is commonly referred to as Nano Drug Delivery Systems (NDDS). In this case, results indicated that nanospheres do not provide a controlled release of agrochemical active ingredients but, due to their small size, they enhanced the penetration in the plant compared to the classical suspension (Boehm et al., 2003). In vivo experiments carried out with Egyptian cotton leaf worm Spodoptera littoralis larvae indicated that the toxicity of nanoparticles of novaluron resembled that of the commercial formulation (Elek et al., 2010). Nanomaterials serve equally as additives (mostly for controlled release) and active constituents (Adak, 2012), controlled-release (CR) formulations of imidacloprid (1-(6 chloro-3-pyridinyl methyl)-N-nitro imidazolidin-2-ylideneamine), synthesized from polyethylene glycol and various aliphatic diacids using encapsulation techniques, have been used for efficient pest management in different crops. The bioefficacy of the prepared CR formulations and a commercial formulation were evaluated against major pests of soybean, namely stem fly, Melanagromyza sojae Zehntmer and white fly, Bemisia tabaci Gennadius. Most of the CR formulations of imidacloprid exhibited better control of the pests compared with its commercial formulations; however, of the CR formulations, poly(poly(oxyethylene-1000)-oxy suberoyl) amphiphilic polymer-based formulation performed better than others for controlling of both stem fly incidence and Yellow Mosaic Virus infestation transmitted by white fly. In addition, some of the developed CR formulations recorded higher yield over commercial formulation and control (Adak et al., 2012a; Adak et al., 2012b). CR formulations of carbofuran and imidacloprid provided better or equal control against the aphid, Aphis gossypii and leafhopper, Amrasca biguttula Ishida on potato crop, than commercial formulations (Kumar et al., 2011). Nanoparticles in insects and their potential for use in insect pest management have been reported (Elek et al., 2010; Al-Samarrai, 2012). The residue of carbofuran and imidacloprid in potato tuber and soils was not detectable at the time of harvesting in any one of the formulation (Jdyalakshmi et al., 2009). Nanomaterials including polymeric nanoparticles, iron oxide nanoparticles, gold nanoparticles, and silver ions have been exploited as pesticides. Nanoencapsulation helps slow release of a chemical to the particular host for insect pest control through release mechanisms that include dissolution, biodegradation, diffusion, and osmotic pressure with specific pH (Barik et al., 2008). Nanoparticles loaded with garlic essential oil proved effective against Tribolium castaneum Herbst. The use of amorphous nanosilica as biopesticide has been reported (Jayaseelan et al., 2011). Nanocopper particles suspended in water have been used since at least 1931, in a product known as Bouisol as fungicide in the growing of grapes and fruit trees (Hatschek, 1931). In the research and development stage, nanosized agrochemicals or nanoagrochemicals are mostly nano-reformulations of existing pesticides and fungicide. (Green et al., 2007; Kah et al., 2013). Nanoformulations are generally expected to increase the apparent solubility of poorly soluble active ingredients, to release the active ingredient in a slow/targeted manner, and/or to protect against premature degradation (Kumar et al., 2010). Nanopesticides offer a way to both control delivery of pesticide and achieve greater effects with lower chemical dose. Agrochemical companies are reducing the particle size of existing chemical emulsions to the nanoscale, or are encapsulating active ingredients in nanocapsules designed to split open, for example, in response to sunlight, heat, or the alkaline conditions in an insect's stomach. The smaller size of nanoparticles and emulsions used in agrochemicals is intended to make them more potent. Many companies make formulations that contain nanoparticles within the 100-250 nm size range that are able to dissolve in water more effectively than existing ones, thus increasing their activity (Perez-de-Luque et al., 2009). Other companies employ suspensions of nanoscale particles (nanoemulsions), which can be either water-based or oil-based and contain uniform suspensions of pesticidal or herbicidal nanoparticles in the range of 200-400 nm. Potential advantages described by the research community are the solubilisation of hydrophobic pesticides (hence no need for toxic organic solvents). However, it should be noted that manufacturing opportunities are not developed, as the precise mechanisms by which nanoemulsions form and how their properties controlled are still the subject of intense basic research. The benefit of nano-emulsions over coarser systems is not so clear. Information from our interviews with industrial representatives suggests that the use of tailor made adjuvants together with micron particles is likely to override the nano-emulsions which are much more complicated with regard to preparation as well as stabilisation (Anton and Vandamme, 2011). Nanocapsules can enable effective penetration of herbicides through cuticles and tissues, allowing slow and constant release of the active substances. Viral capsids can be altered by mutagenesis to achieve different configurations and deliver specific nucleic acids, enzymes, or antimicrobial peptides acting against the parasites (Perez-de-Luque et Rubiales, 2009). The ultimate expression of this technology would be development of a vector that encapsulates, protects, penetrates, and releases DNA-based BW [biological warfare] agents into target cells but is not recognised by the immune system. Such a 'stealth' agent would significantly challenge current medical counter measure strategies (Defense Intelligence Agency analysts, US government, Washington, DC). Silver nanoparticles at 100 mg/kg inhibited mycelia growth and conidial germination on cucurbits and pumpkins against powdery mildew (Afrasiabi et al., 2012). Silver nanoparticles have received significant attention as a pesticide for agricultural applications. The potential of nanomaterials in insect pest management as modern approaches of nanotechnology, has been reported (Rai et Ingle, 2012). Nanoencapsulation is currently the most promising technology for protection of host plants against insect pests. With nanoencapsulation techniques it is possible to step down the chemical release under controlled situations, reducing the current application dosage and improving efficiency. Nanoparticles can be used in the preparation of new formulations like pesticides, insecticides, and insect repellants (Peisker and Gorb, 2013). Treatment of Bombyx mori leaves with grasserie disease with ethanolic suspension of hydrophobic alumina–silicate nanoparticles significantly reduced the viral load (Goswami et al., 2010). DNA-tagged gold nanoparticles are effective against Spodoptera litura and would therefore be a useful component of an integrated pest-management strategy (Chakravarthy et al., 2012). Development of nanobased viral diagnostics including kits can help to detect the exact strain of virus and identify differential proteins in healthy and diseased states during the infectious cycle and the stage of application of therapeutics to stop disease, thus increasing speed as well as power of disease detection (Scrinis and Lyons, 2007). Nanosilica has been successfully employed to control a range of agricultural insect/pest and ectoparasites in animals. Such nanoparticles get absorbed into cuticular lipids (used by insects to prevent death from desiccation) by physisorption and cause insect death by physical means when applied on leaves and stem surfaces. Antifungal activities of polymer-based copper nanocomposites against pathogenic fungi, and silica–silver nanoparticles against Botrytis cinerea, Rhizoctonia solani, Calllectotrichum gloeosporioides (Cioffi et al., 2004; Jo et al., 2009). Bipolaris sorokiniana, and Magnaporthe grisea have been reported. Copper nanoparticles in soda lime glass powder showed efficient antimicrobial activity against gram-positive and gram-negative bacteria and fungi (Esteban-Tejeda et al., 2009). A novel photodegradable insecticide involving nanoparticles has been reported (Guan et al., 2008). Specific nanoencapsulated pesticides will have the ability to kill targeted insects only, thereby reducing the effective dose when compared to traditional pesticides (Park et al., 2006). Further, these are absorbed on the surface of the plant, facilitating a prolonged release that lasts for a longer time compared to conventional pesticides that wash away in the rain. Significant mortality of two insect pests, *Sarocladium oryzae and Rhyzopertha dominica*, after 3 days' exposure to nanostructured alumina-treated wheat was reported (Dimkpa et al., 2013). Halloysite nanotube has potential to be applied as a nanocontainer for encapsulation of chemically and biologically active agents such as agromedicines and pesticides (Naderi and Danesh-Shahraki, 2013). It is essential to remove weeds for increasing the yield of any crop and weeding using nanoherbicides is seen as an economically viable alternative. Conventional herbicides have proved highly effective in controlling weeds without damage to crops or environment. However, chemical weed management under rain-fed areas depends on the moisture availability during the application of herbicides. Lack of moisture limits the use and efficiency of the application. The nano-silicon carrier comprising diatom frustules (pore size 1-100 nm) has been used for delivery of pesticides and herbicides in plants as well as in hormonal wastewater treatment. CR formulation is superior to its counterpart and results in a higher yield and better crop quality. Such a formulation also finds use in active-agent herbicides, pesticides, and plant growth regulators. The potential application of a layered single-metal hydroxide, particularly zinc-layered hydroxide, as the host for the preparation of a nanohybrid compound with a tunable CR property containing two herbicides simultaneously has been demonstrated. In this context, a nanohybrid containing both herbicides (4-(2,4-dichlorophenoxy) butyrate [DPBA] and 2-(3-chlorophenoxy) propionate [CPPA]) labeled as ZCDX was found a suitable host for the CR formulation of two herbicides, namely DPBA and CPPA, simultaneously. The monophasic, well-ordered zinc-layered hydroxide nanohybrid containing two herbicides, CPPA and DPBA, was found to be composed of a higher loading of DPBA compared to CPPA between the zinc-layered hydroxide inorganic interlayers, with percentage contributions of 83.78% and 16.22%, respectively. The release rate of both CPPA and DPBA was found to be different, suggesting that the anionic guest molecules' sizes and the interactions between the host and guest could control the release kinetics. Researchers reported a functional hybrid nanocomposite based on the intercalation of two herbicides' anions (2,4-dichlorophenoxy acetate and 4-chlorophenoxy acetate) with zinc–aluminum-layered double hydroxide. CR formulations of nanocomposites such as 4-chlorophenoxy acetate–zinc–aluminium-layered double hydroxide and 4-dichlorophenoxy acetate–zinc–aluminum-layered double hydroxide were reported. Researchers reported manganese carbonate core-shell nanoparticles loaded with pre-emergence herbicide pendimethalin programmed to release smartly based upon the requirements. Researchers have reported nanosilver and titanium dioxide nanoparticle applications in management of plant diseases (Rao and Paria, 2013). Fungicidal efficiency of sulfur nanoparticles against two phytopathogens has been reported: *Fusarium solani* (isolated from an infected tomato leaf, responsible for early blight and Fusarium wilt diseases) and *Venturia inaequalis* (responsible for the apple scab disease) (Soni and Prakash, 2012). Pheromones are naturally occurring volatile semiochemicals and are considered ecofriendly biological control agents. Pheromones immobilized in a nanogel exhibited high residual activity and excellent efficacy in an open orchard (Bhagat et al., 2013). Environment-friendly management of fruit flies involving pheromones for the reduction of undesirable pest populations, responsible for decreasing yield and crop quality, has been reported. The development of nanocomposites is a new strategy to improve physical properties of polymers, including mechanical strength, thermal stability, and gas barrier properties (Kumar and Krishnamoorti, 2010). The most promising nanoscale size fillers are montmorillonite and kaolinite clays. Graphite nanoplates are currently under study. In food packaging, a major emphasis is on the development of high barrier properties against the migration of oxygen, carbon dioxide, flavor compounds, and water vapor. Decreasing water vapor permeability is a critical issue in the development of biopolymers as sustainable packaging materials. The polymer composites incorporating clay nanoparticles are among the first nanocomposites to emerge on the market as improved materials for food packaging. Nano-layer structure of clays increases the path of diffusion of gases or other substances that penetrating significantly improve the polymer's barrier properties. The nanoscale plate morphology of clays and other fillers promotes the development of gas barrier properties. Several examples are cited. Challenges remain in increasing the compatibility between clays and polymers and reaching complete dispersion of nanoplates (Pandey et al., 2013). Challenges remain in processing of these nanodispersions and in maintaining stability over longer durations. Commercial products (e.g. ImpermR, AegisR or DurethanR) are included into two general categories: regular and high load. Regular products have nanoclay loading in the 2-4% range and high load 5-8%. Regular load products bring 2 times barrier improvement for oxygen and water vapour. The food contact materials based on metal/metal oxide nanoparticles use especially Nano-Silver, Nano-Titanium, Nano-Aluminium and Nano Zinc Oxide. Nano-Silver particles can significantly reduce bacteria and insure safer, fresher and tastier food (Boholm and Arvidsson, 2014). Nano-Titanium is used in filtration systems in fridges and vacuum cleaners. Nano-Aluminium enables to improve properties of the foil surface, for instance to develop anti-adhesive coating or black coating of baking foil which does not reflect heat in an oven. Nano ZnO is used as a non-organic antibacterial agent, which does not discolour nor does not need ultra-violet light to be activated. Products based on metal or metal oxide nanoparticles used for food contact materials are already in the market, e.g. food containers, cutting boards, refrigerators, kitchenware and tableware, aluminum foil or plastic wrap. Recently a method has been reported combining a processing technique of modified emulsion templating and freeze drying; the resulting powder composites are stable, highly porous and form nanodispersions when added to water. The technique has been demonstrated with the antimicrobial agent Triclosan (Liu et al., 2009). There has been considerable research into the use of nanosized quantum dots (QDs) to detect foodborne pathogens. These semiconductor nanocrystals have been used as fluorophores for cellular imaging, as they possess superior properties to conventional fluorophores. QDs have been coupled with specific antibodies to facilitate detection of organisms, including the parasites *Cryptosporidium parvum* and *Giardia lamblia* and the bacteria *Mycobacterium bovis*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, and *Shigella*. Indeed, a modified cellphone has been used as a detection system for *E. coli*. Toxins, including shiga-like toxin, cholera toxin, and ricin, have been detected using a QD protocol (Billington et al., 2014).

NANOTECHNOLOGY AND AGRI-ENVIRONMENT

The use of pesticides and fertilizers to improve food production leads to an uncontrolled release of undesired substances into the environment. Recent decades have revealed the high environmental costs associated with industrial scale chemical-intensive agriculture, including biodiversity loss, toxic pollution of soils and waterways, salinity, erosion and declining soil fertility. Effect of carbon nano materials on pesticide residue in zucchini, corn, tomato and soybean has been investigated by Torre-Roche et al. It was found that pesticide residue uptake by the above plants was reduced in presence of carbon nanotubes. Today, nanotechnology represents a promising approach to improve agricultural production and remediate contaminated soil and groundwater. Researchers reported the recent applications of nanotechnologies in agro environmental studies, with particular attention to the fate of nanomaterials once introduced in water and soil (Gruere et al., 2014). They showed that the use of nanomaterials improved the quality of the environment and helped detect and remediate polluted sites; however, only a small number of nanomaterials demonstrated potential toxic effects (Parda Saradhi, 2014). Carbon/ fullerene nanotechnology is a rapidly growing area of research which finds use in plant, medicine and engineering. Carbon nanotubes (single-wall carbon nanotubes and multi-wall carbon nanotubes) in many cases can penetrate the seed coat and plant cell wall which depends on their size, concentration and solubility. The size of carbon nanotubes alone is of great significance in agriculture and biotechnology, the penetration of carbon nanotubes into the plant system can bring changes in metabolic functions leading to an increase in biomass and fruit/ grain yield (Serag et al., 2013). The impact of iron nanoparticles on terrestrial plants revealed that orange–brown complexes/ plaques, formed by root systems of all plant species from distinct families tested, were constituted of nanoparticles containing iron. Further, the formation of iron nanoparticles/ nanocomplexes was reported as an ideal homeostasis mechanism evolved by plants to modulate uptake of desired levels of ionic iron (Husen and Siddiqi, 2014). Copper is an essential element in the cellular electron-transport chain, but as a free ion it can catalyze production of damaging radicals. Researchers showed using synchrotron microanalyses that common wetlands plants, as Phragmites australis and Iris pseudoacorus, transformed copper into metallic nanoparticles in and near roots with evidence of assistance by endomycorrhizal fungi when they are grown in contaminated soil in the natural environment (Manceau et al., 2008). Converting carbon dioxide to useful chemicals in a selective and efficient manner remains a major challenge in renewable and sustainable energy research. Silver electrocatalyst converts carbon dioxide to carbon monoxide at room temperature; however, the traditional polycrystalline silver electrocatalyst requires a large overpotential. A nanoporous silver electrocatalyst enables electrochemical reduction of carbon dioxide to carbon monoxide with approximately 92% selectivity at a rate (that is, current) over 3,000 times higher than its polycrystalline counterpart under moderate overpotentials of <0.50 V. The improved higher activity is a result of a large electrochemical surface area and intrinsically higher activity compared with polycrystalline silver (Rou et al., 2014). Growing and harvesting organic nanoparticles from plants represents an important step in the development of plant-based nanomanufacturing (Xia et al., 2010). It is a significant improvement on the exploitation of plant systems for the formation of metallic nanoparticles. An enhanced system for the production of English ivy adventitious roots and their nanoparticles by modifying GA7 Magenta boxes and identifying the optimal concentration of indole-3-butyric acid for adventitious root growth was developed, it represents a pathway for the generation of bulk ivy nanoparticles for translation into biomedical applications (Burris et al., 2011). Recent research has demonstrated that the adventitious roots of English ivy are responsible for the production of an adhesive compound composed of polysaccharide and spherical nanoparticles 60-85 nm in diameter (Xia et al., 2011). The recent advances brought into methodology for biological and eco-friendly synthesis and characterization of herbal and medicinal plant-mediated nanoparticles were reported (Thul et al., 2013; Chauhan et al., 2012).

NANOBIOTECHNOLOGY IN AGRI-FOOD PRODUCTION

Nature is a great teacher, and nanotechnology applications in agriculture can be successful if natural processes are simulated in greater scientific sophistication/articulation for successful implementation. For example, the goal might be to

Antibodies attached to fluorescent nanoparticles to detect chemicals or foodborne pathogens
Antimicrobial and antifungal surface coatings with nanoparticles
Biodegradable nanosensors for temperature, moisture and time monitoring
Cellulose nanocrystal composites as drug carrier
Delivery of growth hormones in a controlled fashion
Electrochemical nanosensors to detect ethylene
Lighter, stronger and more heat-resistant films with silicate nanoparticles
Modified permeation behaviour of foils
Nanocapsulated flavour enhancers
Nanocapsule infusion of plant based steroids to replace a meat's cholesterol
Nanocapsules for delivery of pesticides, fertilizers and other agrichemicals more efficiently
Nanocapsules to deliver vaccines
Nanocapsules to improve bioavailability of nutraceuticals in standard ingredients.
Nanochips for identity preservation and tracking
Nanoclays and nanofilms as barrier materials to prevent spoilage and oxygen absorption
Nanocochleates (coiled nanoparticles) to deliver nutrients more efficiently without affecting colour or taste
Nanoemulsions and nanoparticles for better availability and dispersion of nutrients
Nanoencapsulation of nutraceuticals for better absorption, better stability or targeted delivery
Nanoparticles to deliver DNA to plants (targeted genetic engineering).
Nanoparticles to selectively bind and remove chemicals or pathogens from food
Nanosensors for detection of animal and plant pathogens
Nanosensors for monitoring soil conditions and crop growth
Nanosize powders to increase absorption of nutrients
Nanotubes and nanoparticles as gelation and viscosifying agents
Single molecule detection to determine enzyme/substrate interactions
Vitamin sprays dispersing active molecules into nanodroplets for better absorption

Tab. 1 *Examples of potential applications of nanotechnologies in Agrifood sector*

make soils more capable in order to improve efficient nutrient use for greater productivity and better environmental security (Haghighi and Pourkhaloee, 2013). In a recent article in the journal Nature Materials, a researcher at the Cavendish Laboratory of Cambridge University urged her material scientist colleagues to consider agriculture not as a "feedstock with an essentially uncontrollable composition," but as "a rich and diverse category of materials", many of them "nanostructure composites, in which self-assembly may play a key role (Athene, 2004). Nanobiotechnology opportunities include food, agriculture and energy applications. Kraft, Nestle, Unilever and others are employing nanotech to change the structure of food – creating "interactive" drinks containing nanocapsules that can change colour and flavour (Kraft) and ice creams with nanoparticle emulsions (Unilever, Nestle) to improve their texture. Others are inventing small nanocapsules that will smuggle nutrients and flavours into the body (what one company calls "nanoceuticals"). As noted earlier, nanotechnology has the potential to revolutionize agricultural and food (agrifood) production as illustrated in Tab. 1. Potential applications of the technology include controlled nutraceutical delivery systems for food; on farm applications to deliver drugs or pesticides to livestock or crops; and smart-sensing devices for agriculture environment interactions (Huang et al., 2009). Nutrient management with nanotechnology must rely on two important parameters, ie, ions must be present in plant-available forms in the soil system, and since nutrient transport in soil-plant systems relies on ion exchange (eg, NH_4^+, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, Zn^{2+}), adsorption-desorption (eg, phosphorus nutrients) and solubility-precipitation (eg, iron) reactions, nanomaterials must facilitate processes that would ensure availability of nutrients to plants in the rate and manner that plants demand (Cao et al., 2011). Nanobiotechnology provided industry with new tools to modify genes and even produce new organisms (Knauer and Bucheli, 2009). This is due to the fact that it enables nanoparticles, nanofibers, and nanocapsules to carry foreign DNA and chemicals that modify genes (Torney et al., 2007). In addition, novel plant varieties may be developed using synthetic biology (a new branch that draws on the techniques of genetic engineering, nanotechnology, and informatics). In a recent breakthrough in this area, researchers completely replaced the genetic material of one bacterium with that from another transforming it from one species to another (Galbraith, 2007). Using a medicinally rich vegetable crop, bitter melon, researchers demonstrated the accumulation of carbon-based nanoparticle Fullerol ($C_{60}(OH)_{20}$) in tissues and cells of root, stem, petiole, leaf, flower and fruit at particular concentrations, as the causal factor of increase in biomass yield, fruit yield, and phytomedicine content in fruits. Fullerenes are a relatively new group of compounds and represent a class of sphere-shaped molecules made exclusively of carbon atoms. Since their discovery in 1985, many aspects of both fullerene and its analogues have been intensively studied to reveal their physical and chemical reactivity, as well as potential use in biological systems (Injac et al., 2013). Fullerol treatment resulted in increases of up to 54% in biomass yield and 24% in water content. Increases of up to 20% in fruit length, 59% in fruit number, and 70% in fruit weight led to an improvement of up to 128% in fruit yield (Kole et al., 2013). Further, contents of two anticancer phytomedicines, cucurbitacin-B and lycopene, were enhanced up to 74% and 82%, respectively, and contents of two antidiabetic phytomedicines, charantin and insulin, were augmented up to 20% and 91%, respectively (Kresma, 2007). Chemists have successfully made DNA crystals by producing synthetic DNA sequences that can self-assemble into a series of three-dimensional triangle-like patterns. When multiple helices are attached through single-stranded sticky ends, a three-dimensional crystal is formed. This technique helps in improving important crops by organizing and linking carbohydrates, lipids, proteins, and nucleic acids to this crystal (Zeng et al., 2009). Chemically coated mesoporous silica nanoparticles help in delivering DNA and chemicals into isolated plant cells, these are various ways in which nanoparticles enhance drug delivery, and these include encapsulation against immune response, tissue penetration, target selectivity and specificity, delivery monitoring, promoting apoptosis, and blocking pathways (Chandolu and Dass, 2013). The coating triggers the plant to take the particles through the cell walls, where the genes are inserted and activated in a precise and controlled manner, without any toxic side or after effects. This technique has been applied to introduce DNA successfully to plants, including tobacco and corn plants (Park et al., 2008). An International Federation on Organic Agriculture Movements Position Paper on the Use of Nanotechnologies and Nanomaterials in Organic Agriculture rejected the use of nanotechnology in organic agriculture (IFOAM 2011). However, Nano Green Sciences Inc. sells a nanopesticide that they claim is organic (GMO Report 2009). Canada has banned nanotechnology in organic food production. An amendment was added to Canada's national organic rules banning nanotechnology as a "Prohibited Substance or Method".

NANOREMEDIATION AND WATER PURIFICATION

Nanotechnology has played a very important role in developing a number of low-energy alternatives in remediation, among which three are most promising: 1) protein–polymer biomimetic membranes; 2) aligned-carbon nanotube membranes; and 3) thin-film nanocomposite membranes. Nanoremediation methods entail the application of reactive nanomaterials for transformation and detoxification of pollutants (Tratnyek and Johnson, 2006). These nanomaterials have properties that enable both chemical reduction and catalysis to mitigate the pollutants of concern (Zhang and Elliott, 2006). For nanoremediation *in situ*, no groundwater is pumped out for above-ground treatment, and no soil is transported to other places for treatment and disposal (Otto et al., 2008). Many different nanoscale materials have been explored for remediation, such as carbon nanotubes and fibers, enzymes, various noble metals [mainly as bimetallic nanoparticles (BNPs)] and nanoscale zeolites (Manikandan and Subramanian, 2014), nanostructures like titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles and nanowires offer large surface to volume ratio to attract higher probability of the organic molecules to come in contact with the metal oxide molecules residing on the surface of the nanoparticles metal oxides. Nanotechnology can be applied simultaneously to remove the harmful effects of highly toxic organic pesticides and increasing the fertility of the soil through photocatalysis. An attractive part of photocatalysis is that the end products are carbon dioxide that escapes into the atmosphere, water and mineral salts that are added for the fertility of the soil. Photocatalysis degradation process has also gained popularity in the area of wastewater treatment. Of these, nanoscale zero-valent iron (nZVI) is currently the most widely used (Theron et al. 2008 and Zhang 2003). Macro-scale zero-valent iron (ZVI) has been recognized as a good electron donor with a property to release electrons in aquatic environments. ZVI has been used as a reactive material in subsurface permeable reactive barriers to degrade groundwater pollutants since the early 1990s. ZVI is very active in transforming halogenated compounds, polychlorinated hydrocarbon pesticides and dyes (Mueller et al., 2012). Nanotechnology can also be used to clean ground water e.g. the use of aluminum oxide nanofibres (NanoCeram) can remove viruses, bacteria and protozoan cysts from water (Thornton, 2010). Nanocheck, a commerical lanthanum nano-particle product that absorbs phosphates from aqueous environments, is utilized for cleaning fish ponds and swimming pools effectively (Senturk et al., 2013). Water purification using nanotechnology exploits nanoscopic materials such as carbon nanotubes and alumina fibers for nanofiltration (Cohen-Tanugi and Grossman, 2012). Nanofiltration is a relatively recent membrane filtration process used mostly to remove solids, including bacteria and parasites, in surface and fresh groundwater. The solar-powered system uses nanofiltration membranes to treat the local brackish (saline) water, resulting in high-quality desalinated irrigation water. The first field application was reported in 2000 (Zhang, 2005). Nanoparticles have been shown to remain reactive in soil and water for up to 8 weeks and can flow with the groundwater for > 20 m. In one study, Zhang (2003) produced a 99% reduction of TCE within a few days of injection. Trichloroethylene (TCE) is a halogenated aliphatic organic compound which, due to its unique properties and solvent effects, has been widely used as an ingredient in industrial cleaning solutions and as a "universal" degreasing agent. TCE, perchloroethylene (PCE), and trichloroethane (TCA) are the most frequently detected Volatile Organic Chemicals (VOCs) in ground water. Nanomaterials have shown great potential in a wide range of environmental applications due to the extremely small particle size, large surface area, and high reactivity. Nanoscale iron–manganese binary oxide was an effective sorbent for removal of arsenic (III) and arsenic (V) from both synthetic and actual field groundwater (Kong et al., 2013). Calcium–alginate polymer is an excellent choice as an entrapment medium as it is nontoxic and has little solubility in water. The use of nanoscale zero-valent iron (diameter 10-90 nm with an average value of 35 nm) entrapped in calcium–alginate beads showed great promise for aqueous arsenic treatment (Bezbaruah et al., 2014). A water-cleaning product for swimming pools and fishponds called "Nano-Check" (Altair Nanotechnologies, Reno, NV, USA) uses 40 nm particles of a lanthanum-based compound which absorbs phosphates from the water and prevents algae growth. Lanthanum oxide nanoparticles were utilized to scavenge phosphate from microbial growth media for the use of targeted nutrient starvation as an antimicrobial strategy (Gerber et al., 2012). The effect was shown on *Escherichia coli*, *Staphylococcus carnosus*, *Penicillium roqueforti*, and *Chlorella vulgaris* (Li et al., 2014). Nanotechnology can be used to clean ground water. The US company Argonide (Sanford, FL, USA) is using 2 nm diameter aluminum oxide nanofibers (NanoCeram) as a water purifier. Filters made from 2 nm diameter aluminum oxide nanofibers (NanoCeram) can remove viruses, bacteria, and protozoan cysts from water. Nanoscale iron oxide particles are extremely effective at binding and removing arsenic from groundwater. GeohumusR, a product of Geohumus International is a soil enhancer with water storage capacity based on nanotechnology, which can be also used as a mineral repository in agriculture. It has a larger water storage capacity than previous wetting agents and a product lifetime of 3–5 years. GeohumusR is a high-efficiency polymer that consists of a water storing hybrid material, volcanic rock flour and plant available colloidal silicate. Nanoscale Fe(oxy)hydroxide phases are among the most common natural mineral nanoparticles formed by precipitation from solution after oxidation of aqueous ferrous Fe (Van der Zee et al., 2003), although Fe is an essential element for growth in nearly all species, an abundance of free chelating Fe has been linked to DNA damage, lipid peroxidation, and oxidative protein damage in vivo (Valko et al., 2005). Particle coating, surface treatments, surface excitation by ultraviolet radiation, and particle aggregation can modify the effects of particle size, suggesting that some nanoparticles could exert their toxic effects as aggregates or through the release of toxic chemicals (Nel et al., 2006). The inevitable release of engineered silver nanoparticles (AgNPs) into aquatic environments has drawn great controversy over antibacterial silver: implications for environmental and sustainability assessments toxicity and safety (Boholm and Arvidsson, 2014). Although aggregation and transformation play crucial roles in the transport and toxicity of AgNPs, how the water chemistry of environmental waters influences the aggregation and transformation of engineered AgNPs is still not well understood (Yu et al., 2013). The iron nanoparticles as catalysts are reported (Stein et al., 2011) in reaction catalysis such as asymmetric transfer hydrogenation of ketones, alkene, alkyne hydrogenation, carbonyl reductions, and hydrogenation of several functional groups such as aldehydes, ketones, imines, and amides, and breakdown of organic contaminants such as trichloroethene, carbon tetrachloride, dioxins, and PCBs (Polychlorinated biphenyl) to simpler carbon compounds which are much less toxic (Rangheard et al., 2010). Nanoscale iron oxide particles can effectively bind and remove arsenic from groundwater and can help to develop potable water problems in the developing world (Otto et al., 2008). The European Commission requested EFSA (Question number: EFSAQ-2007-124a) to conduct an initial scientific opinion of the risks arising from nanoscience and nanotechnology in food and feed with respect to human health, safety and environmental quality. EFSA which started the process in November 2007 requested from industry the following information:

– Data on the safety of nanomaterials used in food and feed.
– Environmental studies performed on nanotechnologies and nanomaterials used in food and feed.
– Food and feed applications and products containing or consisting of nanomaterials.
– Methods, procedures and performance criteria used to analyse nanomaterials in food and feed.
– Other data of relevance for risk assessment of nanotechnology and nanomaterials in food and feed.
– Risk assessments performed on nanomaterials used in food and feed.
– Toxicological data on nanomaterials used in food and feed.
– Use patterns and exposure to humans and environment.

NANOTECHNOLOGY FOR AQUACULTURE AND FISHERIES

Aquaculture plays an important role in global food production through genetic improvement of plants and animals along with cellular level delivery of genes and drug molecules to specific sites in plants and animals (FAO 2011). Aquaculture is the fastest growing food-producing sector in the world, the world's fastest growing area of animal production is the farming of fish (Defra, 2009), crustaceans and mollusks and the highly integrated fish farming industry may be among the first to incorporate and commercialize nanotech products (Lead and Wilkinson, 2006). According to the FAO there were 45.7 million tonnes of aquaculture production in 2000 and it is growing at a rate of more than 9% per year. With a strong history of adopting new technologies, the highly integrated fish farming industry may be among the first to incorporate and commercialize nanotech products expanding and intensifying as novel tool for aquaculture and fisheries development in almost all regions of the world (Rather et al., 2011). The global population is increasing, thus, the demand for aquatic food products is also increasing. Production from capture fisheries has leveled off and most of the main fishing areas have reached their maximum potential (Subasinghe et al., 2014). Nanotechnology has a wide usage potential in aquaculture and seafood industries (FAO, 2011). The shelf life of fish and shellfish may be improved with the use of antibacterial nano-coatings, and transparent polymer films that can help exclude oxygen from around the food product. Nanosensors on the food packaging can also be used to report the deterioration of the fish or shellfish. A public engagement programme is needed to ensure public confidence in the food uses of nano-technology by the industry. Little is known about the effect of nanoparticles on aquatic organisms (Handy, 2012). There is an immense opportunity to use the nanoparticles to deliver nutraceuticals in fish feed and neutrogenomics studies (Can et al., 2011). Moreover, various nanoformulations of feed help to maintain better consistency and taste of feed (Rather et al., 2011). For fish health in aquaculture, nanotechnological applications include antibacterial surfaces in the aquaculture system, nanodelivery of veterinary products in fish food using porous nanostructures, production from heterotrophic microalgae through transesterification, nanosensors for detecting pathogens in the water, nanopurified water could be used for irrigation and fish culture (Zhang et al., 2013). Scientists from the Russian Academy of Sciences have reported that young carp and sturgeon exhibited a faster rate of growth upon iron nanoparticle feeding furthermore a nanoselenium-supplemented diet could improve the final weight, relative gain rate, antioxidant status as the glu-tathione peroxidase activities and muscle selenium concentrations of crucian carp (*Carassius auratus gibelio*) (Zhou et al., 2009), moreover, nanoselenium was found more effective than organic selenomethionine in increasing muscle selenium content (Zoho, 2009). Further, the growth and performance of the fish which were experimented, were found higher at nanolevel delivery of these nutraceuticals (Rather et al., 2013). Direct use of silver nano-particles in water to treat a fungal disease has been found toxic to young trout, but a water filter coated with silver nanoparticles prevented fungal infections in rainbow trout farmed indoors (Johari et al., 2013). Not surprisingly, a great deal of government funded research in nanosensors aims to detect minute quantities of biowarfare agents such as anthrax or chemical toxins to counter terrorist attacks on US soil as well to warn soldiers on a battle field of pos-sible risks. For example, the US government's "SensorNet" project attempts to cast a net of sensor across the entire United States that will act as an early warning system for chemical, biological, radiological, nuclear and explosive threats (Handi et al., 2011; Rather et al., 2011). Pretreatment of rare earth oxide nanoparticles with phosphate in a neutral pH environment prevented their biological transformation into urchin shaped structures and profibrogenic effects. Nanocochleates are unique lipid-based supramolecular assemblies composed of a negatively charged phospholipid and a divalent cation. Nanocochleates, 50 nm cylindrical (cigarlike) nanomaterials, can be used to deliver nutrients such as vitamins, lycopene, and omega fatty acids more efficiently to cells, without affecting the color or taste of food. Researchers have met with moderate success at developing nanoencapsulated vaccines against the bacterium Listonella anguillarum in Asian carp (Rajesh et al., 2008), and white spot syndrome virus in shrimp (Rajesh et al., 2009). Nanoparticles have promise for improving protection of farmed fish against diseases caused by pathogens. Chitosan nanoparticles are promising carriers for an oral plasmid DNA vaccine. The major advantages of encapsulating agrochemicals and genetic material in a chitosan matrix include its ability to function as a protective reservoir for the active ingredients, protecting the ingredients from the surrounding environment while they are in the chitosan domain, and then controlling their release, allowing them to serve as efficient gene delivery systems (Kashyap et al., 2015). For example, oral administration with chitosan/ pDNA induced an antibody immune response in fish against *Vibrio parahaemolyticus (OS4)* (Li et al., 2013; Myhr et al., 2011).

NANOTECHNOLOGY IN ANIMAL PRODUCTION/REPRODUCTION AND ANIMAL NANOFEED APPLICATIONS

Many diverse opportunities for nanotechnology exist to play an important role in food production as well as in livestock production (Mura et al., 2014). The potential uses and benefits of nanotechnology are enormous (Verma et al., 2012). Several types of nanostructures and NPs have been developed and have revolutionized the approach to animal sciences. In particular, nanotechnologies were applied to the development of novel drug delivery systems and nanosensors for the diagnosis and treatment of diseases. Emerging evidences indicate that nanotechnology may represent a promising approach to develop new and specific products for animal nutrition (Ross et al., 2004). Although there are not many studies on this topic, many advantages can be obtained by applying this technology to animal production (Scott, 2005), and to improvement of reproductive performance in beef and dairy cattle (Sutovsky et al., 2013). Reproduction management is an important part of the sustainable production of livestock. It has become evident that advances in farm animal reproduction have become increasingly dependent on advance scientific research in addition to an understanding of the physiological processes involved in reproduction. The use of assisted reproductive techniques (ART) has helped owners to produce offspring from valuable farm animals that were considered infertile using standard breeding techniques. Recently in some of these fields remarkable progress has been made. Implanting tracking devices in animals is nothing new - either in pets, valuable farm animals or for wildlife conservation. Injectable microchips are already used in a variety of ways with the aim of improving animal welfare and safety - to study animal behaviour in the wild, to track meat products back to their source or to reunite strays with their human guardians. In the nanotech era, however, retrofitting farm animals with sensors, drug chips and nanocapsules will further extend the vision of animals as industrial production units. None the less, imperfections are remaining and sustained efforts will be required to optimize existing and invent new technologies (Verma et al., 2012). Microfluidic biochips are being used to segregate male sperm from female eggs for sex selection for animal breeding. Microfluidic devices can not only sort sperm and eggs, but also bring them together in a way that mimics the movement of natural reproduction. This technique would make mass production of embryos cheap, quick and reliable (Studnicka et al., 2009); this study evaluated a nanoparticle-based magnetic purification method that removes defective spermatozoa (~30% of sample) from bull semen and improves sperm sample viability and fertilizing ability in vitro and in vivo. Nanotubes linked to nutrients can be administered to animals and released in specific sites, thus allowing the maintenance of high levels for a long time; this approach should avoid the degradation of nutrients and increase their availability (Ross et al., 2004). Sodium selenite NPs coated with metacrylate copolymers, sensitive to variations of pH, were orally administrated to ruminants and the improvement of selenium absorption was evaluated (Romeo-Perez et al., 2010). Silver NPs and Cu-montmorillonite NPs were used as feed additives to increase the average daily weight gain of pigs (Fondevila et al., 2009; Tong et al., 2007). With funding from the US Department of Agriculture (USDA), Clemson University researchers are feeding bioactive polystyrene nanoparticles that bind with bacteria to chickens as an alternative to chemical antibiotics in industrial chicken production. The FAO has estimated a contamination of 25% of worldwide cereals stockpile by mycotoxins each year with an enormous economic effect (Jelinek et al., 1989; Lindemann et al., 1993; Kim et al., 2012). Regarding this topic, nanoabsorbents composed of magnesium oxide and embedded by silica nanoparticles has been used as effective adsorbent agents as a way to remove aflatoxins from wheat flour (Luo et al., 2004; Masoero et al., 2007; Moghaddam et al., 2010). Shi et al. (2009) reported the use of a modified montmorillonite to decrease the toxicity in feeds of chicks.

NANOFOOD

The term 'nanofood' describes food which has been cultivated, produced, processed or packaged using nanotechnology techniques or tools, or to which manufactured nanomaterials have been added (Joseph and Morrison, 2006). In the food processing industries, a few of the most common usages of nanobiotechnology in quality monitoring of food products may be enumerated as nanosensors/ nanobiosensors and bacteria identification; furthermore this technology provides barriers to oxygen and carbon dioxide, thus protecting food quality. The nanosensors can be utilized to detect the presence of insects or fungus accurately inside the stored grain bulk in storage rooms. Researchers suggested models for use of nanobiotechnology, either on a standalone basis or through complementarity with the existing technologies (Sastry and Rao, 2013). Cellular "injection" with carbon nanofibers containing foreign DNA has been used to genetically modify golden rice. Many natural foods contain nanoscale components and their properties are determined by their structure (Dingman et al., 2008). Research into naturally occurring nanostructures in foods is mainly designed to improve the functional behavior of the food (Momin et al., 2013). These have been eaten safely for generations; future generations of humanity will be able to eat any food, no matter how rich; sugar, salt, fat, cholesterol — all the things we love but have to consume in moderation now will have no restrictions on them in future. All food will be nutritious; the sole criterion for choosing meals will be taste. Nanotechnology also holds out the promise of 'interactive' foods able to change their nutritional profile in response to an individual's allergies, dietary needs or food preferences. The purpose of nanofood is to improve food safety, enhance nutrition and flavor, and cut costs. Although nanofood is still in its infancy, nanoparticles are now finding application as a carrier of antimicrobial polypeptides required against microbial deterioration of food quality in the food industry (Cao et al., 2008). Nanofood has, in fact, been part of food processing for centuries, since many food structures naturally exist at the nanoscale.

Currently, the number of food products using nanotechnology of any kind is relatively small. Most of the nanotechnology is still only a promise for enabling new food products: some or many years in the future (Chen et al., 2014). Nanotechnology may revolutionize the food system and has the potential to influence the science of food in a positive way, as it could generate innovation in food texture, taste, processability, and stability during shelf life (Rao, 2009). The benefits of nanofood, for instance, include health-promoting additives, longer shelf lives or new flavor varieties. Researchers examined the encapsulation and controlled release of active food ingredients using nanotechnological approaches (Huang et al., 2009). The dairy industry utilizes three basic microsized and nanosized structures (casein micelles, fat globules, whey proteins) to build all sorts of emulsions (butter), foams (ice cream and whipped cream), complex liquids (milk), plastic solids (cheese), and gel networks (yogurt) (Semo et al., 2007). In fact, dairy technologies not just a microtechnology but also a nanotechnology has existed for a long time. The present research has been focused on modifying food substances to produce nanoparticles that have a different function from the original substance. Early examples from the patent literature and marketing brochures are a number of oxides, such as titanium dioxide and silicon dioxide. TiO_2 is in the top five of NPs used in consumer products, accounting for 70% of the total production volume of pigments and consumed annually at about 4 million tons worldwide, the former has conventionally been used as a color and the latter as a flow agent in foods. Nano-emulsions can encapsulate functional ingredients within their droplets, which can facilitate a reduction in chemical degradation. Nanolamination is a technique for protecting the food from moisture, lipids and gases (Chen et al., 2006). Examples of nano-ingredients and manufactured nanomaterial additives include nanoparticles of iron or zinc, and nancapsules containing ingredients like co-enzyme and lipds (Magnuson et al., 2011). Food industries argue the addition of micro and nanocapsules to processed foods that will improve both the availability and delivery of nutrients, thereby enhancing a food's nutritional status (Kuzma and VerHage, 2006). For example, a recent study claimed that the encapsulation in nanoemulsions of curcumin, the phytochemical found in tumeric and claimed to have antitumor and anticarcinogenic properties, increased the bioavailability of this compound (Wang, 2007) and hydrophobically modified starch formed micelles encapsulated curcumin (Huang et al., 2010). Dairy products, cereals, breads and beverages are now fortified with vitamins, minerals, probiotics, bioactive peptides, antioxidants and plant sterols (Kumar and Rai, 2009). Some of these active ingredients are now being added to foods as nanoparticles or at particles of a few hundred nm in size (Shefer and Shefer, 2003). Active ingredients including vitamins, preservatives and enzymes have recently been added to foods in microscale capsules. For instance, many of the commonly used Omega-3 food additives are micrometres in size, such as the 140-180 micron microencapsulated tuna fish oils, which are used by Nu-Mega Driphorm to fortify Australian bread (Mozafari et al., 2006). A coating of starch colloids filled with antimicrobial substance, such that if microorganisms grow on the packaged food they will penetrate the starch releasing the antimicrobial agent. Reports on nanofoods are covered by the popular media. Octenyl succinic anhydride-ε-polylysine has the potential to become a bifunctional molecule that can be used as either surfactants or emulsifiers in the encapsulation of nutraceuticals or drugs or as antimicrobial agents. Lipid-based nanoencapsulation systems enhance the performance of antioxidants by improving their solubility and bioavailability, in vitro and in vivo stability, and preventing their unwanted interactions with other food components (Mozafari et al., 2008). The main lipid-based nanoencapsulation systems that can be used for the protection and delivery of foods and nutraceuticals are nanoliposomes, nanocochleates, and archaeosomes. Nanoliposome technology presents exciting opportunities for food technologists in areas such as encapsulation and controlled release of food materials, as well as the enhanced bioavailability, stability, and shelf-life of sensitive ingredients. The application of nanoliposomes as carrier vehicles of nutrients, nutraceuticals, enzymes, food additives, and food antimicrobials was reported (Mozafari et al., 2008). Nanotechnology can provide manipulation of food polymers and polymeric assemblages to provide tailor-made improvements to functional food quality and food safety (Momin et al., 2013). Further, foods among the nanotechnology-created consumer products coming onto the market include a brand of canola cooking oil called Canola Active Oil (Shemen Industries, Tel Aviv, Israel), a tea called Nanotea (Qinhuangdao Taiji Ring Nano-Products Co., Ltd., Hebei, People's Republic of China), and a chocolate diet shake called Nanoceuticals Slim Shake Chocolate (RBC Life Sciences Inc., Irving, TX, USA). The canola oil contains an additive called "nanodrops" designed to carry vitamins, minerals, and phytochemicals through the digestive system and urea. Experts envision numerous nanoparticulate agroformulations with higher bioavailability and efficacy and better selectivity in the near future. Multidisciplinary approaches could potentially improve food production, incorporating new emerging technologies and disciplines such as biochemical biology integrated with nanotechnologies to tackle existing biological bottlenecks that currently limit further developments. European Food Safety Authority (EFSA) in its opinion on the potential risks arising from nanotechnologies on food and feed safety uses term engineered nano materials (ENM). An engineered nanomaterial is any material that is deliberately created such that it is composed of discrete functional and structural parts, either internally or at the surface, many of which will have one or more dimensions of the order of 100 nm or less. The safety of a given compound engineered in a food should not automatically apply to a nanoversion of the compound, due to possible novel properties and characteristics (Rico et al., 2011), interaction of nanoparticles with edible plants and their possible implications in the food chain. The term "engineered" as used in this opinion is equivalent to the term "manufactured" as used in other reports. Insufficient scientific data prevents FDA from extending GRAS (generally recognized as safe) status of an ingredient to its nanosized version. A significant segment of the public does not want its food "engineered"– bio, nano, GM or otherwise (Kahan et al.,2008).

Reason 1: Toxicity risks of nanofoods and nano agrochemicals remain very poorly understood. The current scientific evidence of the risks associated with nanomaterials is sufficient to warrant a precautionary approach to their management. However significant knowledge gaps remain, presenting a barrier to the development of effective regulation to manage nanofoods and nano agrochemicals.

Reason 2: Nanomaterials are not assessed as new chemicals. Existing regulations do not treat nanomaterials as new chemicals. If a chemical has been approved in larger particle form, the new use of the substance in nanoparticle form does not trigger any requirement for new or additional safety testing (Cushen et al., 2012). This has been recognized by the United Kingdom's Royal Society and Royal Academy of Engineering as a critical regulatory gap (Coles and Frewer, 2013). They recommended that all nanomaterials be assessed as new chemicals (U.K. RS/RAE 2004).

Reason 3: Current methods for measuring exposure are not suitable for nano. Existing regulations are based on the mass of the material as a predictor for expected exposure rates. This approach is completely inappropriate for nanomaterials as the toxicity can be far greater per unit of mass (Reijnders, 2006). Scientists have suggested that nanoparticle surface area or the number of nanoparticles is a more valid metric for measurement of nano exposure (Nel et al., 2006; SCENIHR 2006).

Reason 4: Current safety testing is not suitable for nano. Even if a nanomaterial triggered new safety testing, current test guidelines are inadequate for nanomaterials as they do not assess key properties that influence nanotoxicity. These include: shape, surface, catalytic properties, structure, surface charge, aggregation, solubility and the presence or absence of 'functional groups' of other chemicals (Magrez et al., 2006; Nel et al., 2006). Nanomaterials must also face full life-cycle assessment, which existing regulation does not require.

Reason 5: Many safety assessments use confidential industry studies. Past assessments of nanomaterials safety by the European Scientific Committee on Cosmetics and Non-food Products and the United States Food and Drug Administration have relied on proprietary company studies (Innovest, 2006). There is often no requirement for the safety of nanomaterials to be assessed by independent nanotoxicologists or for the results and methodology of this safety testing to be made public.

CONCLUSION AND PERSPECTIVES

Coming nanotechnologies in the agricultural field seem quiet promising. However, the potential risks in using nanoparticles in agriculture are not different than those in any other industry. Through the rapid distribution of nanoparticles to food products – whether it be in the food itself or part of the packaging – nanoparticles will come in direct contact with virtually everyone. The editors of Nature estimated that any technology takes some 20 years to emerge from the laboratory and be commercialized. Technological innovation has played an important role in shaping the development and characteristics of the agri-food system over the past century and more (Goodman et al.,1987). The emergence of the new biotechnologies of food production since the 1980s — such as genetic engineering, tissue culture and other cellular and genetic level techniques — have been identified as the basis of a new technological paradigm, and as framing the restructuring of contemporary agri-food systems. In the agricultural sector in particular, this has variously been referred to as a new 'bioindustrial paradigm' (Goodman and Wilkinson, 1990; Wilkinson, 2002b), a 'genetic-corporate paradigm' (Scrinis, 1995; 2007), or more generally in terms of a shift from a Green Revolution to a Gene Revolution form of agricultural production (Chauhan et al., 2012). Since successive waves of technology, from tractors and combine harvesters to herbicides and GM crops, agriculture have moved ever closer towards an industrial ideal in which agricultural production more closely mirrors the factory system and agricultural labourers are left under-paid, under-employed and unemployed. Nanotechnology in agriculture might take a few decades to move from laboratory to land, especially since it has to avoid the pitfalls experienced with biotechnology. As we are still in the relatively early stages of research and commercialization of nanotechnology, there is considerable potential for civil society groups, workers' unions, farmer and producer organizations, environmental and consumer groups, to challenge and shape the development and implementation of this technology, and to thereby support alternative applications, regulatory regimes, and techno-economic paradigms of development. With the production of engineered nanoparticles we are confronted with a new class of materials that have novel properties compared to bulk material. Information describing the health risk of engineered nanoparticles is only evolving and many questions are still open. For this to happen, sustained funding and understanding on the part of policy planners and science administrators, along with reasonable expectations, would be crucial for this nascent field to blossom. The opportunity for application of nanotechnology in agriculture is prodigious. Research on the applications of nanotechnology in agriculture is less than a decade old. Nevertheless, as conventional farming practices become increasingly inadequate, and needs have exceeded the carrying capacity of the terrestrial ecosystem. We have little option to explore nanotechnology in all sectors of agriculture. It is well recognized that adoption of new technology is crucial in accumulation of national wealth (Knauer and Bucheli, 2009). As the excitement of nanotechnology began to grow, the initial approach to address the potential toxicity of engineered nanomaterials was to assume that these novel materials will behave like their bulk counterparts. A strong dismissive tone regarding potential hazard reigned supreme. It was apparent that material scientists were guiding safety assessment in the early stages of this field. Inevitably, biologist and toxicologist became involved and took a new leadership role in the safety evaluations of nanomaterials. Unfortunately, out of the gate there were missteps. There is an urgent need to develop human resources with an understanding of the complexities of the agricultural production system to serve nanotechnology applications in agriculture successfully. By and large, agricultural education has not been able to attract sufficient numbers of brilliant minds the world over, while personnel from kindred disciplines might lack an understanding of agricultural production systems (Brock et al., 2011). Instruction programs in agricultural nanotechnology, if initiated, might fill this void by fulfilling the twin goals of attracting brilliant learners and developing a body of skilled farm-focused personnel.

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RIASSUNTO

La nanotecnologia è una tecnologia emergente e può rappresentare un'opportunità importante per la comunità scientifica e le imprese. Essa si basa sullo sviluppo di "nanomateriali" che includono materiali naturali, accidentali, o ingegnerizzati, contenenti particelle (non legate, aggregate o agglomerate) in cui il 50% o più in numero e distribuzione, hanno una dimensione esterna nel range 1-100 nm. I nanomateriali posseggono nuove proprietà, come maggiore forza, caratteristiche ottiche avanzate, proprietà antimicrobiche e superconduttività. Attualmente esistono applicazioni in campi diversi, ma ci aspettiamo che la nanotecnologia diventerà una forza economica trainante per lo sviluppo della moderna agricoltura e nel settore alimentare. Le nanotecnologie, infatti, sono in grado di utilizzare in modo più efficiente acqua, pesticidi e fertilizzanti; inoltre possono essere sviluppati nuovi metodi di produzione per sostituire gli impianti di produzione esistenti e riformulare nuovi materiali e sostanze chimiche con prestazioni migliorate con conseguente minore consumo di energia, di materiali, ridotto danno per l'ambiente, e per una bonifica ambientale. In questo lavoro verranno analizzati i recenti progressi nello sviluppo di prodotti nanoagrochimici e le applicazioni delle nanotecnologie in campo agroambientale, per la produzione agroalimentare, per le nanobonifiche e depurazione delle acque, per l'acquacoltura e la pesca, per la produzione/ riproduzione animale, per lo sviluppo di nano-cibo e per l' alimentazione animale.

ABSTRACT

Nanotechnology is an emerging technology and can represent an important opportunity for the scientific and business community. It is based on the development of "nanomaterials" that include natural, incidental, or manufactured materials containing particles (unbound, aggregated or agglomerated) where 50% or more of them, in number and size distribution, have an external dimension in the range 1-100 nm. Nanomaterials exhibit novel properties such as increased strength, enhanced optical features, antimicrobial properties, and superconductivity. Actually exist different applications in different fields but we expect that nanotechnology will become a driving economic force for the development of modern agriculture and in the food sector. Nanotechnology in fact can enable plants to use water, pesticides and fertilizers more efficiently; furthermore novel methods of production can be developed to replace existing production plants and to

reformulate new materials and chemicals with improved performances resulting in lower consumption of energy and materials, reduced damage to the environment, and for environmental remediation. In this work recent advances on the development of nanoagrochemicals, and applications of nanotechnology in agri-environment, agri-food production, nanoremediation and water purification, aquaculture and fisheries, animal production/ reproduction, nanofood and animal nanofeed will be analyzed.

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Il ruolo dei sensori nella zootecnia di precisione per il benessere animale e la sostenibilità ambientale

introduzione

Le sfide che attendono le scienze agro-zootecniche sono riassunte in un rapporto della FAO recante un titolo volutamente esplicativo: *Come possiamo sfamare il mondo nel 2050?* (FAOSTAT, 2009). Le stime di questo rapporto indicano per il 2050 una crescita della popolazione mondiale di oltre 9 miliardi, oggi siamo poco più di 7 miliardi, di cui oltre il 70% si collocherà nella fascia urbanizzata, nel 2009 era il 49%, e il maggior contributo alla crescita demografica mondiale sarà dato dai paesi in via di sviluppo. Un rapporto successivo, *La Zootecnia nel Mondo* (McLeod, 2011), stima un incremento del consumo di carne del 73% circa entro il 2050, mentre quello di prodotti caseari salirà del 58% circa. Per soddisfare una tale richiesta di proteine animali, allo stato attuale di efficienza del settore zootecnico, si dovrebbe incrementare del 100% l'allevamento di pollame, dell'80% quello dei piccoli ruminanti, del 50% quello dei bovini e del 40% quello dei suini, senza mutare l'attuale livello di sfruttamento delle risorse naturali. Diversi autori (Avery, 2001; Reilly e Willenbockel, 2010; Pulina et al., 2011) hanno evidenziato la costante riduzione della superficie disponibile ed edibile pro capite, dovuta all'incremento demografico e, in maggior misura, alla diversa destinazione d'uso di queste superfici (naturalistica, urbana, energetica). In un mondo globalizzato la sicurezza alimentare deve essere affrontata anche sotto l'aspetto qualitativo e la Direzione Generale per la salute dei consumatori dell'Unione Europea, ben consapevole di questo, nel rapporto EU 2011 manifesta viva preoccupazione per le malattie che possono trasmettersi dagli animali all'uomo e per l'eccessivo impiego di antibiotici negli allevamenti.

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AREE DI APPLICAZIONE DELLA PLF	FREQUENZA DI CAMPIONAMENTO
Parametri biologici	
Incremento ponderale giornaliero (IPG)	Giornaliero
Indice di conversione alimentare (ICA)	Giornaliero
Consumo mangimi (CM)	Orario
Indice di massa corporeo	Giornaliero
Comportamento normale/nervoso	Giornaliero
Rilevamento calori	Orario
Condizioni ambientali	
Condizioni climatiche ambientali esterne e interne (locali, sale)	Orario
Temperatura e umidità del pavimento	Orario
Velocità e ricambio aria	Orario
Livello Gas, ad esempio CO_2 e NH_3	Orario
Livello polveri	Orario
Tracciabilità e trasporto	
Codice elettronico univoco	-
Condizioni ambientali di trasporto	Orario
Codice univoco per alimentazione e medicinali.	-

Tab. 1 *Sintesi delle possibili variabili ambientali e gestionali che potrebbero essere misurate, registrate e analizzate nella Plf (Durack, 2002; Berckmans, 2014)*

Il consumatore medio risulta sempre più attento alla qualità e alla provenienza delle derrate alimentari e se in passato i prodotti provenienti da sistemi che garantivano elevati livelli di benessere animale non superavano il 10% della fetta di mercato (Webster, 1999), oggi questa percentuale tende ad aumentare. Significativo, in tal senso, il risultato di uno studio (Furnols et al., 2011) che indica come il consumatore non acquisti le carni di agnello di dubbia provenienza. Termini quali: aflatossina, diossina ed Escherichia coli iniziano a entrare nel linguaggio del consumatore medio, portandolo a una spesa più attenta e ricercata.

Allo stato attuale delle conoscenze scientifiche, e data l'estrema eterogeneità di progresso tecnologico e gestionale degli allevamenti, esiste ancora un ampio margine per aumentare l'efficienza dell'intera filiera agrozootecnica. La ricerca scientifica ha applicato diverse tipologie di sensori e tecnologie di trasmissione dei dati al fine di aumentare l'efficienza degli allevamenti. La miniaturizzazione e la riduzione dei costi di produzione di queste tecnologie consentono il monitoraggio in *real time* dei parametri ambientali e biologici del comparto zootecnico. In particolare i sensori in grado di misurare sia parametri ambientali (polveri, CO, CO_2, NO_2, O_3, CH_4, H_2S, NH_3, temperatura e radiazione), che biologici (frequenza di respirazione, temperatura corporea, posizione dell'animale, attività muscolare e cardiaca) sono oggi gestibili da un unico microcontrollore e disponibili a prezzi accessibili.

La zootecnia di precisione Plf (Precision livestock farming) è la disciplina che racchiude quest'ottica di gestione aziendale (Berckmans, 2014) e consente di monitorare in tempo reale i numerosi parametri biologici e ambientali inerenti ciascun singolo componente della mandria. Un sistema Plf è formato sempre da tre parti: una parte fisica, detta *hardware*; una parte per l'elaborazione e la presentazione dei dati, detta *software;* una parte per la trasmissione dei dati, detta *rete*. L'hardware è rappresentato dai sensori, dai calcolatori e/o microcontrollori, dai sistemi di trasmissione e ricezione dati e dagli attuatori. I modelli matematici per l'elaborazione dei dati e l'interfaccia di presentazione dei dati sono inseriti nel software caricato all'interno del microcontrollore. In tabella 1 sono riportate delle numerose applicazioni della Plf e la loro possibile frequenza di monitoraggio (Durack, 2002; Berckmans, 2014).

Diversi autori hanno sviluppato le tematiche di interesse della Plf, quali: sistemi avanzati per il controllo dei parametri ambientali (Banhazi et al., 2009), sensori per la valutazione del benessere e del comportamento animale (Shao e Xin, 2008), sistemi per la valutazione in tempo reale del peso corporeo degli animali (Kollis et al., 2007), monitoraggio dei consumi in tempo reale dell'acqua e del mangime (Madsen e Kristensen, 2005; Madsen et al., 2005), utilizzo dei sensori per la diagnosi e il monitoraggio delle patologie (Maatje et al., 1997; Eradus e Jansen, 1999), identificazione elettronica (Naas, 2002; Samad et al., 2010), registrazione e interpretazione dei versi animali (Holst, 1999). L'inarrestabile sviluppo tecnologico offre, costantemente, possibili miglioramenti e opportunità ai sistemi implementati, anche di ultima generazione.

Un esempio eccezionale di nuove tecnologie indossabili sono gli occhiali a realtà aumentata. Questi dispositivi hanno integrato al loro interno un microcontrollore, un display, una telecamera, un microfono, una memoria e la possibilità di una connessione wireless e 3G (fig. 1). L'allevatore può visualizzare direttamente nel display integrato negli occhiali le informazioni trasmesse dal pc aziendale sullo stato dei singoli capi, senza interrompere la normale routine di lavoro. Esistono varie case produttrici di occhiali a realtà aumentata, come ad esempio i Google Glass della Google Inc. (Mountain View, California, Stati Uniti), i Moverio BT-200 della Seiko Epson Corporation (Suwa, Nagano, Giappone), gli Smart Eye Glass della Sony Corporation (Tokyo, Giappone). Le varie aziende produttrici promettono la commercializzazione, per il grande pubblico, di questi dispositivi entro il 2016. Google inc., nel febbraio 2013, ha pro-

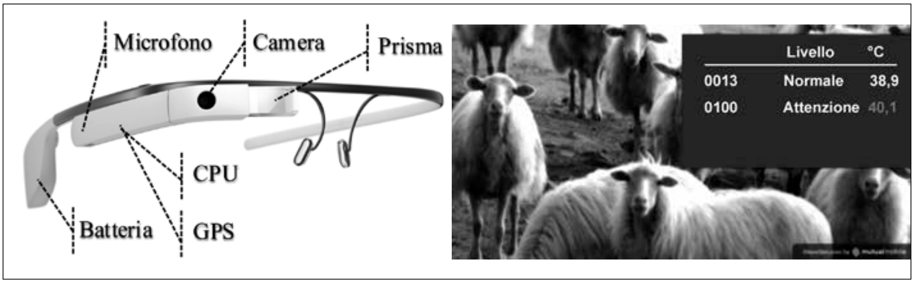

Fig. 1 *A sinistra, una veduta dei Google Glass, versione Explore 2.0. Gli occhiali a realtà aumentata sono composti da una batteria, una CPU con integrato un GPS, un microfono, una videocamera da 5 Mpx e un prisma che funge da display. Gli occhiali risultano leggeri ed ergonomici. A destra, una simulazione (http://glasssim.com/ http://www.sardiniapost.it/) dell'esperienza di visione da parte dell'utente che indossa i Google Glass*

mosso una versione prototipale degli occhiali, i "Google Glass Explorer Edition" (8.000 esemplari), e nell'aprile 2014, per un breve periodo, ha ampliato la vendita nei soli Stati Uniti. Nel gennaio del 2015, ha concluso il progetto Explorer per avviare una nuova fase di sviluppo degli occhiali con l'obiettivo della commercializzazione di massa. Tra gli 8000 esploratori Google Inc. ha selezionato Teunis van de Zandschulp proprietario di un'azienda zootecnica (circa 130 vacche da latte) nel Lunteren, Paesi Bassi. L'allevatore si è dichiarato entusiasta[1] dei Google Glass, in quanto gli permetteva di ricevere aggiornamenti reali circa lo stato dei suoi capi mentre continuava la normale routine di lavoro. I Google Glass sono stati recentemente testati da alcuni autori in diversi campi, principalmente in quello medico (Muensterer et al., 2014; Glauser, 2013; Feng et al., 2014, McNaney et al., 2014; Ishimaru et al., 2014).

Un obiettivo dello studio è stato quello di realizzare un'applicazione per i Google Glass in grado di mostrare, sul display integrato, la temperatura corporea di un animale. Lo studio descrive, inoltre, lo stato dell'arte dei principali componenti della Plf: il software, l'hardware e la trasmissione dei dati, focalizzando l'attenzione sulle problematiche relative alla modularità hardware e alle differenze tra i software con licenza e non.

Nei sistemi Plf il trattamento delle immagini rappresenta una delle tecniche maggiormente utilizzate poiché consente la rilevazione di parametri comportamentali, biologici e patologici, senza interferire con le attività routinarie dell'animale. Un altro obiettivo è stato quello di calcolare l'area occupata da

[1] Dairy Global, De Boerderij krant, De Boerderij magazine, Rabobank magazine.

una carcassa d'agnello utilizzando un software *open source* per l'analisi immagine, CellProfiler[2] (Jones et al., 2008).

STATO DELL'ARTE DELLA PLF

Sensori

Nei sistemi Plf i sensori si possono suddividere in due grandi categorie: quelli atti a rilevare grandezze ambientali e quelli atti a rilevare grandezze biologiche. Tipici parametri ambientali sono: la temperatura, l'umidità, la velocità dell'aria, la concentrazione di polveri, di CO_2, di NH_3, il livello di odore e la qualità dell'acqua. I principali parametri biologici sono la temperatura corporea, l'estro, i battiti cardiaci, il respiro, la ruminazione, l'attività cerebrale, l'attività motoria, le infezioni, le zoppie e l'alimentazione. La Plf rappresenta un tipico sistema di controllo automatico nel quale sono presenti un ingresso e un'uscita. In un sistema atto a rilevare l'estro, ad esempio, si può utilizzare un podometro che, monitorando la crescente attività motoria della bovina, segnala l'evento all'allevatore. Questo è un sistema semplice da implementare sia a livello hardware che software, ma in un sistema reale, come è noto, possiamo avere una bovina affetta da zoppia che si trova nella condizione ottimale di inseminazione. In questo caso il podometro registra una ridotta o assente attività motoria della bovina e potrebbe non individuare l'estro. Pertanto, un sistema in grado di monitorare tutte queste variabili interpretando gli scostamenti dai valori attesi, risulta complesso come hardware e come software. Per realizzare un controllo preciso risulta fondamentale la creazione di un modello matematico che includa il maggior numero possibile di variabili rappresentative dell'animale monitorato. Le principali caratteristiche che un sensore deve possedere per l'impiego nei sistemi Plf sono: l'accuratezza, l'affidabilità, la robustezza e la possibilità di registrare e trasmettere i dati. L'accuratezza di un dispositivo, che misura lo scostamento dal valore atteso, deve essere di norma inferiore al 5%. In commercio per un prezzo maggiore vi sono dispositivi più costosi in grado di trasdurre la stessa grandezza ma con accuratezza inferiore all'1%, pertanto la scelta della percentuale di accuratezza deve essere dettata dalla tipologia di grandezza da monitorare. In merito all'affidabilità e alla robustezza, vi è da rilevare che alcuni sensori in commercio non risultano idonei all'utilizzo in azienda zootecnica e necessitano, quindi, di una ripro-

[2] www.cellprofiler.org

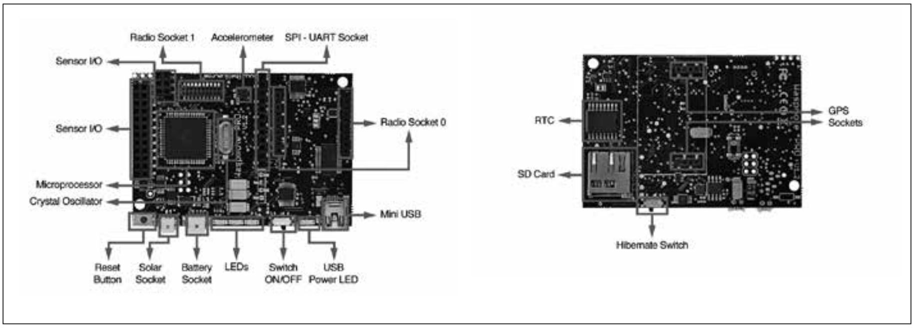

Fig. 2 *A sinistra una vista dall'alto di un microcontrollore, il Waspmote, indicati dalle frecce abbiamo, partendo dal basso e in senso orario: il pulsante per il reset della scheda, il microprocessore, i pin per l'inserimento dei sensori, l'accelerometro, i pin per la connessione dei moduli per trasmissione dei dati, una porta mini-USB per la connessione al pc e all'alimentazione dalla rete e infine la presa per il collegamento all'alimentazione da batteria o da pannello fotovoltaico; a destra una vista dal basso, dove è possibile individuare: l'ingresso per la scheda di memorizzazione SD e i pin per l'inserimento del GPS*

gettazione hardware per essere, ad esempio, impermeabili all'acqua, al fango e alla polvere. Inoltre, assai spesso si rende necessaria la riprogettazione software dei sensori per rendere possibile la registrazione dei dati.

Microcontrollori

I microcontrollori costituiscono un sistema che integra in uno stesso chip il processore, la memoria permanente, la memoria volatile e i canali d'ingresso-uscita (I/O), oltre a eventuali altri blocchi specializzati (fig. 2). Questi dispositivi sono in grado di ricevere le informazioni in arrivo dai sensori e trattarle preliminarmente con gli opportuni blocchi di filtraggio e con l'eventuale conversione analogico-digitale. In seguito i microcontrollori, dopo l'elaborazione dei dati con lo specifico algoritmo, sono in grado di azionare eventuali attuatori.

Un tipico sistema di controllo automatico è composto da: un trasduttore, che acquisisce la grandezza del sistema da controllare; un convertitore analogico-digitale (A/D); un microcontrollore, che elabora la grandezza trasdotta e invia l'opportuno segnale di controllo all'attuatore (fig. 3).

Un esempio semplice di controllo automatico è dato dalla regolazione della temperatura e dell'umidità all'interno della stalla/cuccetta in funzione dalla temperatura corporea dell'animale (fig. 4).

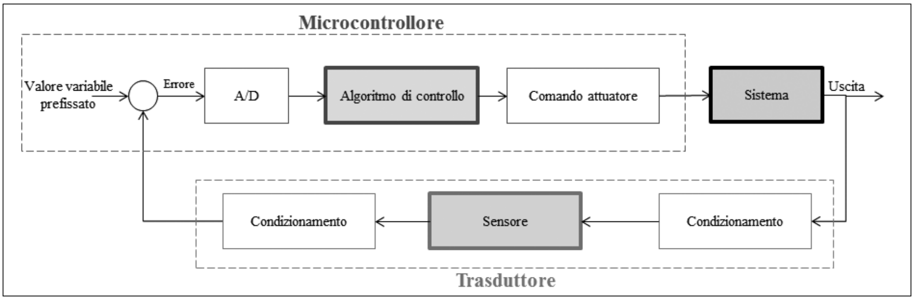

Fig. 3 *Schema a blocchi di un tipico controllo automatico. Il microcontrollore ha la funzione di acquisire dai traduttori le informazioni, per poi elaborale tramite l'algoritmo di controllo e infine di trasmettere gli opportuni comandi all'attuatore per minimizzare l'errore tra il valore desiderato e quello misurato della variabile controllata*

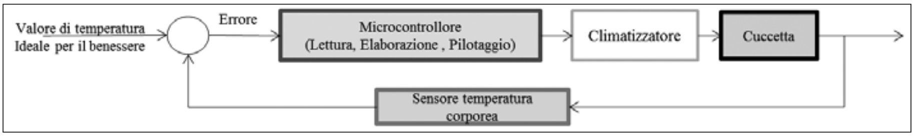

Fig. 4 *Schema a blocchi di un controllo automatico per il benessere termo-igrometrico dell'animale in una cuccetta. Questo è un tipico esempio di sistema a singolo ingresso e a singola uscita, detto SISO (Single Input Single Output)*

Al modificarsi della temperatura corporea dell'animale, il microcontrollore gestisce il sistema di raffrescamento o riscaldamento della cuccetta. Questo è un tipico esempio di sistema a singolo ingresso e a singola uscita, detto SISO (*Single Input Single Output*). Esistono altri tre sistemi: SIMO (*Single Input Multiple Output*), singolo ingresso e uscite multiple; MISO (*Multiple Input Single Output*), ingressi multipli e singola uscita; MIMO (*Multiple Input Multiple Output*), ingressi multipli e uscite multiple. Le reali potenzialità della Plf possono essere sfruttate solamente se si utilizza un sistema di tipo MIMO (fig. 5).

L'innalzamento della temperatura corporea degli animali stabulati, ad esempio, non sempre può essere controllato col raffrescamento della stalla perché possono intervenire diversi fattori, quali: la risposta del sistema immunitario (infezioni o ferite), il calore o altri eventi esterni. In questo caso il sistema deve essere in grado di gestire più parametri contemporaneamente al fine di individuare la reale causa dell'innalzamento della temperatura corporea e compiere la corretta azione di controllo.

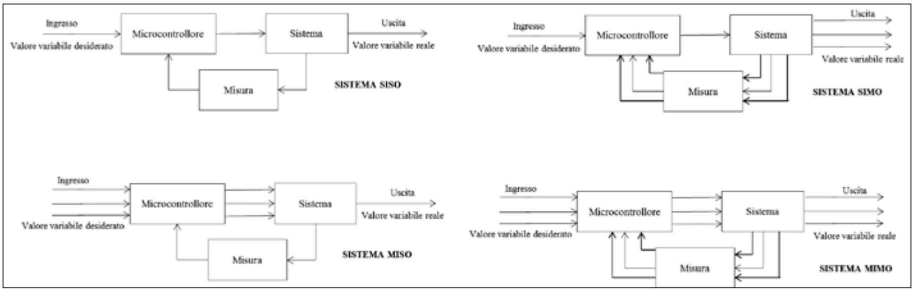

Fig. 5 *Rappresentazione schematica dei sistemi SISO (ingresso singolo e uscita singola), SIMO (ingresso singolo e uscite multiple), MISO (ingressi multipli e uscita singola) e MIMO (ingressi multipli e uscite multiple)*

SOFTWARE REVIEW

La Plf è una tecnologia embrionale che possiede grandi potenzialità, ma che richiede ancora un'intensa attività di ricerca e sviluppo prima di poter maturare ed essere utilizzata appieno (Wathes et al., 2008). La mancata modularità hardware e i relativi software *closed source* (software commerciale o con licenza) dei sistemi automatici proposti dalle case produttrici costituiscono i principali fattori che condizionano lo sviluppo della Plf. Molti dei sistemi oggi in commercio, infatti, non consentono di aggiungere ulteriori sensori al sistema stesso, se non quelli con il medesimo marchio commerciale e spesso le case produttrici non offrono una gamma completa di tutte le grandezze trasducibili in azienda (tab. 2). L'obiettivo della ricerca deve pertanto essere quello di creare una piattaforma libera e modulare, aperta all'evoluzione delle nuove tecnologie. La ricerca scientifica ha già proposto in altri settori sistemi modulari e liberi (*open-source)* che hanno riscosso un grande successo, se ne ricordano alcuni: VEGA (Pedretti et al., 2004), piattaforma aperta per lo sviluppo di applicazioni bio-informatiche, con architettura *plug-in* e programmazione di *script*; NA-MIC (Pieper et al., 2006), piattaforma aperta per la comunità informatica di immagini mediche; il robot umanoide iCub (Metta et al., 2008), piattaforma aperta per la ricerca sui processi cognitivi.

MODELLI MATEMATICI

Un punto chiave della Plf è la scelta dell'algoritmo che deve rappresentare, il più fedelmente possibile, la realtà del fenomeno osservato/rilevato dai sensori. La modellizzazione matematica risulta cruciale nella valutazione o clas-

	Temperatura stalla	Umidità stalla	Velocità del vento stalla	Direzione del vento stalla	Temperatura esterna	Umidità esterna	Velocità del vento esterna	Direzione del vento esterna	Livello polveri stalla	Livello CO_2 stalla	Livello NH_3 stalla	Livello odore stalla	Livello odore esterno	Mangime consumata	Acqua consumata	Peso maiale	Livello sudorazione	Registrazione audio	Registrazione video	Livello ingrassamento (back fat)	Calore	**********	Feedback del mercato	Supporto	Controllo automatico	Accesso privato	Rete	Ingresso dati manualmente	**********	Caricamento nel Pc	Software di analisi	Importa	Esporta	Conserva	Protocollo
Farmex	√	√	√	√	√	√	√	√	•	•	•			√	√	•	•	•	•	•	•	*	•	√	√	√	√		*	√	√	√	√	web	
Environdata	√	√	√	√	√	√	√	√														*		√		√			*	√	√			pdb	rs232, modem
Ruddweigh																√						*		√		√			*	√				csv	rs 232
Skov	√	√	•	•	√	√	•	•	•	•	•			√	√	√				•	•	*	•	√	√	√	√		*	√	√	√	√	pbd	
Piggery Systems & design	√				√																	*		√	√	√			*	√					
Hotraco (B&M Slots)	√	√		√	√	√		√		√	√			√	√							*		√	√	√	√		*	√	√		√	asc	rs232/modem
Big Dutchman	√	√		√	√									√	√	√						*		√	√	√	√		*	√					
Veng System	√	√	√	√	√	√	√	√	√	√	√			√	√							*			√	√	√		*	√			√		rs232
Multifan	√	√			√	√																*			√		√		*	√					
Microfan	√	√			√																	*			√	√			*	√					
Rotem	√	√		√	√									√	√							*			√	√			*	√					cable, modem
Fancom	√	√			√	√				√	√											*			√	√			*	√	√				cable, modem
Watchport	√	√			√	√																*				√	√		*	√					usb/ ethernet
Phason	√				√									√	√							*			√	√	√		*	√	√				
bsmagri	√																					*			√				*						
chretronics	√	√																				*			√	√			*	√	√				cable, modem
Ozonaire												√	√									*			√				*						
Biocurtain												√	√									*							*						
VIA - (osborne)																√			√			*				√			*	√	√				cable
Ethovision																			√			*				√			*	√	√				cable
Farmweld																√						*			√				*	√	√				wireless
Osborne	√	√												√		√					√	*			√	√			*	√	√	√	√	csv	rs232
tru-test scales																√						*							*	√				csv	rs232 + rs485
skiold/ sorti-pen																√						*			√				*	√					
IVOG														√		√					√	*							*						
Mannebeck														√						√	√	*		√		√		√	*	√					
Sono-grader																				√		*							*	√	√	√	√	csv	handheld upload
Lean meater																				√		*							*						
Pigtog 105																				√		*							*	√					
Super tester																				√		*							*						

Tab. 2 *Comparazione dei sensori di diversi sistemi Plf per allevamenti suinicoli (Banhazi et al., 2003), Farmex e Skov rappresentano i sistemi più forniti rispetto alle grandezze trasducibili, ma nessun sistema in tabella è in grado di misurare il livello di odore all'interno e all'esterno della porcilaia*

sificazione di una variabile o di un evento. Nella tabella 3 sono comparati i risultati ottenuti da due sistemi per la predizione dell'estro nella bovina: l'HR Tag (SCR Engineers, Ltd., Israel) che monitora il movimento del capo e la ruminazione e l'IceQube sensor (IceRobotics, Ltd., Scotland) che monitora l'attività motoria. La specificità indica la proporzione/probabilità che una bovina priva di calori risulti negativa al test (no estro), mentre la sensibilità indica la probabilità che una bovina con l'estro risulti positiva al test (estro). I migliori risultati sono forniti dalle reti neurali, tecniche di apprendimento automatico che arrivano al 100% della sensibilità con la combinazione di entrambe le tecnologie. Recentemente si stanno sviluppando algoritmi di autoapprendimento o di apprendimento supervisionato per la regressione e la classificazione, quali le macchine a vettori di supporto e le reti neurali. I modelli di autoapprendimento, attraverso i quali si crea una sorta di memoria virtuale e un esperienza nella catalogazione molto simile a quella dell'allevatore, diventano più precisi ed efficienti all'aumentare dei dati a disposizione. Questi modelli, che richiedono un'enorme mole di dati, sono implementati su calcolatori sempre più miniaturizzati e potenti.

MODELLO MATEMATICO	TECNOLOGIA	SENSIBILITÀ[1]	SPECIFICITÀ[2]
Foresta casuale	HR Tag	44,4%	95,3%
	IceCube	88,9%	98,2%
	HR Tag + IceCube	88,9%	98,2%
Analisi discriminante lineare	HR Tag	77,8%	88,8%
	IceCube	77,8%	98,2%
	HR Tag + IceCube	77,8%	97,6%
Rete neurale	HR Tag	55,6%	91,8%
	IceCube	88,9%	93,5%
	HR Tag + IceCube	100,0%	96,5%

[1] Sensibilità = Veri positivi / (Veri positivi + Falsi negativi) x 100
[2]Specificità = Veri negativi / (Veri negativi + Falsi positivi) x 100

Tab. 3 *Confronto della precisione di corretta classificazione dell'estro della bovina per diversi modelli matematici a parità di sensore (Borchers, 2015). Il modello a rete neurale permette, utilizzando contemporaneamente i dati del sensore IceCube e del HR Tag, la perfetta individuazione di una bovina con estro*

STATO DELL'ARTE DEI SENSORI NELLA PLF

L'allevatore monitora la sua mandria tramite l'analisi sensoriale ed è guidato nelle scelte decisionali dall'esperienza maturata negli anni (Wathes et al., 2008), ma l'incremento del numero di capi per allevamento rende sempre più difficile il monitoraggio diretto (Guarino, 2005). La Plf permette l'implementazione di sistemi automatici in grado di monitorare, fra l'altro, l'attività motoria degli animali (Cangar et al., 2008; Aydin et al., 2010), la tracciabilità (Barcos, 2001; Kashiha et al., 2013), il comportamento (Leroy et al., 2006), il benessere (Song et al., 2008; Poursaberiet al., 2010; Viazzi et al., 2011) e il tasso di crescita (De Wet et al., 2003; Demmers et al., 2012). Uno degli scopi principali della Plf è quello di fornire un supporto all'allevatore nell'osservazione della mandria e del singolo animale, utilizzando sensori e tecniche di monitoraggio quali, ad esempio, l'analisi dell'immagine e del suono. Queste tecniche consentono di ottenere le informazioni necessarie senza sottoporre l'animale a metodiche invasive di rilevamento che, sottoponendo l'animale a stress, rischiano di falsare le misure (Cangar et al., 2008).

L'ANALISI IMMAGINE IN ZOOTECNIA

L'analisi immagine è una tecnica non invasiva per acquisire informazioni comportamentali, produttive e patologiche nelle diverse specie. Alcuni studi hanno utilizzato le tecniche di analisi immagine per valutare, ad esempio, il confort termico che influisce in misura significativa sulla produzione di latte e sul comportamento nei bovini (Kadzere et al., 2002; Bohmanova et al., 2007), nei suini (Shao e Xin et al., 1998; Xin e Shao et al., 2008) e nei pulcini (Cassuce et al., 2013), mentre non sono presenti studi negli allevamenti semi-stabulati e allo stato brado. Attraverso la valutazione di specifiche regioni anatomiche dell'animale è possibile calcolare il peso in vivo o lo stato d'ingrassamento – Body Condition Score (BCS) – nei suini (Brandl e Jorgensen, 1996), nei bovini da latte e da carne (Ferguson et al., 2006; Bewley et al., 2008; Roche et al., 2009; Azzaro et al., 2011), nelle bufale (Negretti et al., 2008) e nei polli (De Wet et al., 2003), mentre non sono presenti studi riguardanti gli allevamenti equini e ovi-caprini, per questi ultimi si utilizza il metodo a mano (Santucci e Maestrini, 1985; Thompson e Meyer, 1994).

Il monitoraggio del BCS e del peso dell'animale rappresenta un importante indicatore per la gestione aziendale: una bovina troppo grassa, ad esempio, può incorrere in parti difficili, mentre un'eccessiva magrezza può causare una diminuzione delle produzioni e delle qualità lattiere. Recentemente, utilizzando algoritmi e software sempre più efficienti, sono stati proposti sistemi di misura del peso estremamente accurati nei suini e nei bovini (Wang et al., 2008; Kashiha et al., 2014; Ozkaya et al., 2015). Tecniche di analisi immagine sono state utilizzate anche per il monitoraggio del comportamento e per valutare stati di panico o malessere nei suini (Costa et al., 2014), nei bovini (DeVries et al., 2004; Stubsjøen et al., 2009) e nei polli (Vranken et al., 2005). Con l'analisi immagine è possibile, inoltre, monitorare alcuni stati patologici, come ad esempio le zoppie nei cavalli (White et al., 2008) e nei bovini (Van Hertem et al., 2015). Di pari importanza risulta la possibilità di monitorare il tempo passato dagli animali in mangiatoia e la quantità di foraggio ingerita negli allevamenti avicoli (Mehdizadeh et al., 2015) e nei bovini da latte (Porto et al., 2014; Shelley e Anthony, 2013).

Esistono numerosi software con licenza per l'analisi immagine che garantiscono funzionalità diverse legate al prezzo del prodotto, mentre quelli *open source* sono: ImageJ[3], Fiji[4], OpenCV[5] (il più completo ma sprovvisto di interfaccia grafica), Simplecv[6] e CellProfiler[7]. Quest'ultimo è nato per i biologi privi di una formazione nella programmazione informatica per analizzare le immagini con particolare riferimento alle cellule. Il software è dotato di

[3] http://rsb.info.nih.gov/ij/
[4] http://fiji.sc/Fiji
[5] http://opencv.org/
[6] http://www.simplecv.org/
[7] http://www.cellprofiler.org/

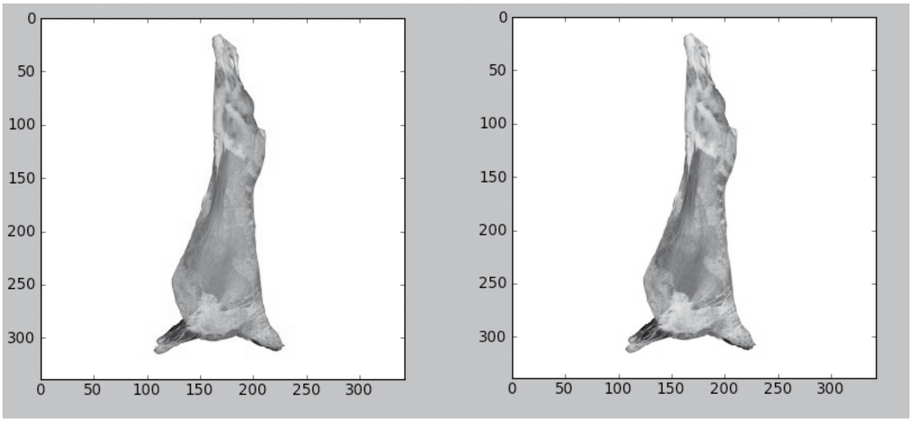

Fig. 6 *Trattamento dell'immagine prima della sua elaborazione; a sinistra l'immagine originale, a destra la carcassa dell'agnello dopo la conversione da RGB a scala di grigi*

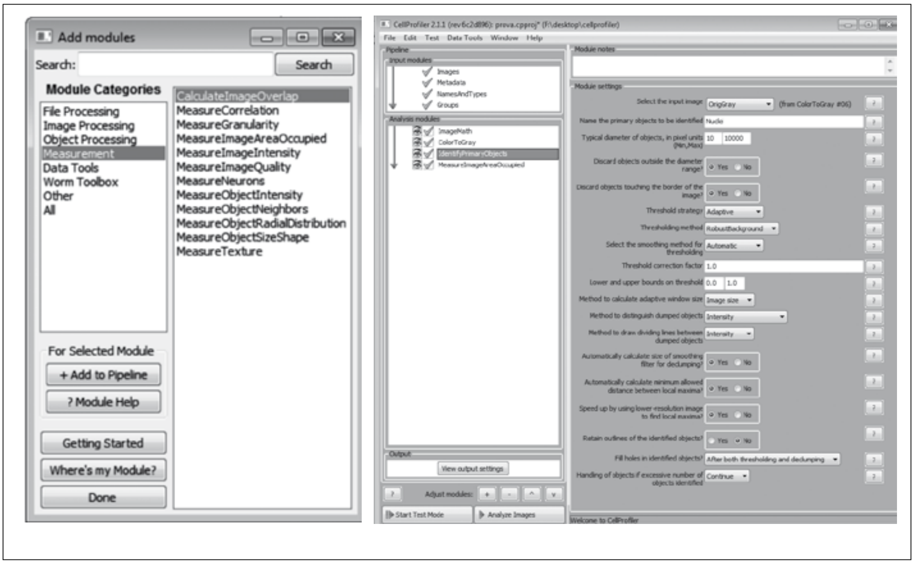

Fig. 7 *A destra la schermata del software per l'inserimento dei moduli per l'analisi immagine, a sinistra la schermata con la selezione dell'algoritmo e di altri parametri per l'analisi dell'area occupata dalla carcassa dell'agnello*

una serie di moduli che consentono di rilevare diverse grandezze dall'analisi dell'immagine, ma una delle potenzialità più interessanti del software è la possibilità di modificare moduli o di crearne di nuovi per soddisfare particolari esigenze (Carpenter et al., 2006; Lamprecht et al., 2007; Jones et al., 2008;

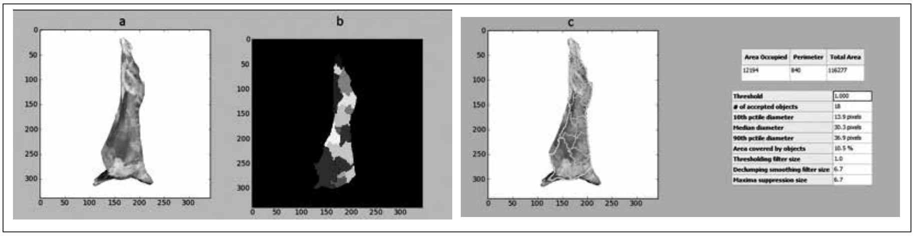

Fig. 8 *Esempio di calcolo dell'area occupata nell'immagine dalla carcassa d'agnello tramite il software CellProfiler*

Stöter et al., 2013; Bray e Carpenter, 2015). Si è scelto a titolo d'esempio l'analisi immagine di una carcassa d'agnello[8] che è stata selezionata utilizzando il motore di ricerca specifico di Google. Prima di procedere al calcolo dell'area occupata dalla carcassa è necessario trasformare l'immagine da RGB a scala di grigi (fig. 6), opzione effettuabile tramite i moduli di trasformazione delle immagini disponibili all'interno del software CellProfiler. Nel software si possono selezionare dei moduli che permettono l'estrazione di diverse misure quali, ad esempio (figg. 7 e 8): l'area, l'intensità, il perimetro, la granularità.

ANALISI SUONO

L'analisi del verso degli animali è un metodo non invasivo che consente la valutazione di diversi parametri come il benessere e il comportamento (Watts e Stookey, 2000; Chung et al., 2013). Questa tecnica consente di monitorare parametri comportamentali, patologici e produttivi quali, ad esempio, la quantità di sostanza secca ingerita dall'animale (Schirmann et al., 2009), il comportamento (Deshmukh et al., 2012), l'identificazione e la localizzazione del singolo capo (Tamaki et al 1993; Jahns et al., 1999; Ikeda et al., 2003), il tempo passato nella masticazione (Laca e Wallis De Vries, 2000), l'estro (Kim et al., 2010), le zoppie (Whay et al., 1998; Rajkondawar et al., 2002) e i livelli di emissione di gas metabolici responsabili dell'inquinamento dell'aria (Van Hirtum e Berckmans, 2002). Gli studi finora condotti si sono concentrati soprattutto negli allevamenti stabulati dei suini (Weary et al., 1998), degli avicoli, dei bovini (Lee et al., 2015) e solo in parte nel settore equino (Moehlman, 1998), ovino (Walser et al., 1980; Kendrick et al., 1995) e caprino (Shelton, 1980).

[8] http://hashimidrisfood.com/product/lamb-carcass-1-kg/

MICROCONTROLLORE	ATMEGA1281
frequenza	14MHz
SRAM	8KB
EEPROM	4KB
FLASH	128KB
Scheda SD	2GB
Peso	20gr
Dimensioni	73.5 x 51 x 13 mm
Clock	RTC (32KHz)
consumo acceso	15mA
consumo elettrico a riposo	55uA
consumo in ibernazione	0.7uA

Tab. 4 *Caratteristiche tecniche del microcontrollore Waspmote: leggero e dai bassi consumi si presta agevolmente per l'utilizzo in sistemi Plf*

IL PROBLEMA DELLA MODULARITÀ HARDWARE E DEL SOFTWARE PROPRIETARIO

La mancanza di un software libero seguito da una minima modularità hardware rappresenta un freno per la diffusione della Plf. In commercio sono disponibili diverse piattaforme per la prototipazione rapida, tra le quali: Arduino (Interaction Design Institute, Ivrea, Italia), Waspmote (Libellium CTO, Calle Escatrón, 16, 50014 Zaragoza, Spagna), Raspberry Pi (PiRaspberry Pi Foundation, Caldecote, South Cambridgeshire, Regno Unito). Queste piattaforme sono modulari, a basso costo e con una grande comunità attiva sul web, che spazia dall'ingegneria all'agricoltura. Il microcontrollore Waspmote, ad esempio, caratterizzato da elevata velocità e bassi consumi**,** ha integrato un sensore di temperatura che consente una lettura da -40°C a +85°C con un'accuratezza di 0,25°C (tab. 4). All'interno della scheda è compreso un accelerometro ±2g/±4g/±8g e ha la possibilità di avere integrato il Gps modello JN3 –Telit, che consente di ottenere informazioni quali: latitudine, longitudine, altitudine, velocità, direzione, data/ora.

La caratteristica più interessante del microcontrollore Waspmote è costituita dalle schede aggiuntive disponibili per il monitoraggio di diversi parametri ambientale e biologici (tab. 5). La connessione intuitiva delle schede al microcontrollore richiede minime conoscenze tecniche, mentre la programmazione delle schede richiede più tempo. Grazie ai numerosi componenti aggiuntivi è possibile misurare diversi parametri ambientali, chimici (fig. 9), biologici, fisici e meccanici quali: l'inquinamento dell'aria, la qualità dell'aria, le emissioni in azienda, i valori ambientali in serra, le grandezze di controllo

Fig. 9 *La scheda per il monitoraggio dei Gas è già cablata e deve solo essere inserita negli appositi pin dello Waspmote, inoltre i sensori sono calibrati e forniti di certificazione, parametri fondamentali che distinguono un sensore per misure hobbistiche da uno per quelle scientifiche*

industriali e per gli incendi boschivi, la potabilità dell'acqua o il livello di inquinamento nei fiumi o nei mari, la localizzazione delle merci o la tracciabilità degli animali, la vibrazione e la pressione, il livello di liquidi e o solidi, grandezze metereologiche, il diametro dei frutti, il livello di bagnabilità fogliare e l'umidità del terreno.

Fondamentale poi la scheda per la prototipazione rapida che consente la realizzazione e l'integrazione all'interno del microcontrollore Waspmote di qualunque sensore, questa scheda rappresenta la soluzione al problema della modularità hardware, uno dei punti critici dei sistemi Plf ossia di inserire al loro interno nuovi sensori disponibili grazie alle innovazioni future senza stravolgere il sistema già integrato in azienda.

PARAMETRI CHIMICI / FISICI				MECCANICI	AMBIENTALI
Monossido di carbonio	CO	Calcio	$Ca2^{+}$	Pressione	Aria di temperatura / umidità
Diossido di carbonio	CO2	Floruro	F^{-}	Inclinazione	Terreno di temperatura / umidità
Ossigeno molecolare	O2	Fluoborato	$BF4^{-}$	Vibrazione	Bagnatura fogliare
Ozono	O3	Nitrato	$NO3^{-}$	Impatto	Anemometro
Monossido di azoto	NO	Bromuro	Br^{-}	Effetto Hall	Radiazione solare
Biossido di azoto	NO2	Cloruro	Cl^{-}	Luminosità	Radiazione ultravioletta - UV
Diossido di zolfo	SO2	Cuprico	$Cu2^{+}$	Temperatura	Diametro tronco
Ammoniaca	NH3	Ioduro	I^{-}	Presenza liquidi	Diametro stelo
Metano	CH4			Perdite liquidi	Diametro frutto
Idrogeno molecolare	H2	pH		Livello liquidi	Segnavento
Idrogeno solforato	H2S	Ossigeno dissolto		Peso	Luminosità
Cloruro di idrogeno	HCl	Conducibilità		Suoni	Ultrasound (distance measurement)
Acido cianidrico	HCN	Temperatura			Potenziale di Ossidazione-Riduzione
Fosfina	PH3	Torbidità			Pressione atmosferica
Ossido di etilene	ETO				
Cloro	Cl2				
Polveri	PM 1/2,5/10				

Tab. 5 *Parametri chimici, fisici, meccanici e tecnologici che possono essere misurati con le diverse schede già assemblate del microcontrollore Waspmote*

La parte concernente la trasmissione dei dati rappresenta uno dei punti più delicati dei sistemi Plf. Il microcontrollore Waspmote offre estrema flessibilità nella trasmissione e ricezione dei dati tramite svariate protocolli di comunicazione, quali: 802.15.4 / ZigBee, LoRa 868 / 915MHz, Bluetooth Low Energy (BLE) 4.0 e Wifi (tab. 6). Sono disponibili sistemi di comunicazione in grado di trasmettere a grandi distanze come ad esempio, il modulo XBee-900 che è in grado di ricevere e comunicare dati oltre i 10 km. Inoltre, vi è la possibilità di criptare la comunicazione, qualora vi fosse la necessità, con chiave AES 128b.

MODELLO	PROTOCOLLO	FREQUENZA	TX POTENZA
XBee-802.15.4	802.15.4	2.4GHz	100mW
XBee-ZB-Pro	Zigbee	2.4GHz	50mW
XBee-868	RF	868MHz	315mW
XBee-900	RF	900MHz	50mW

Tab. 6 *Panoramica dei diversi protocolli di comunicazione, di tipo Xbee, disponibili per il microcontrollore Waspmote. All'aumentare delle distanze di trasmissione dei dati corrispondono moduli di comunicazione necessariamente più energivori. Il consumo elettrico è un parametro da tenere sotto controllo durante la progettazione dei sistemi Plf*

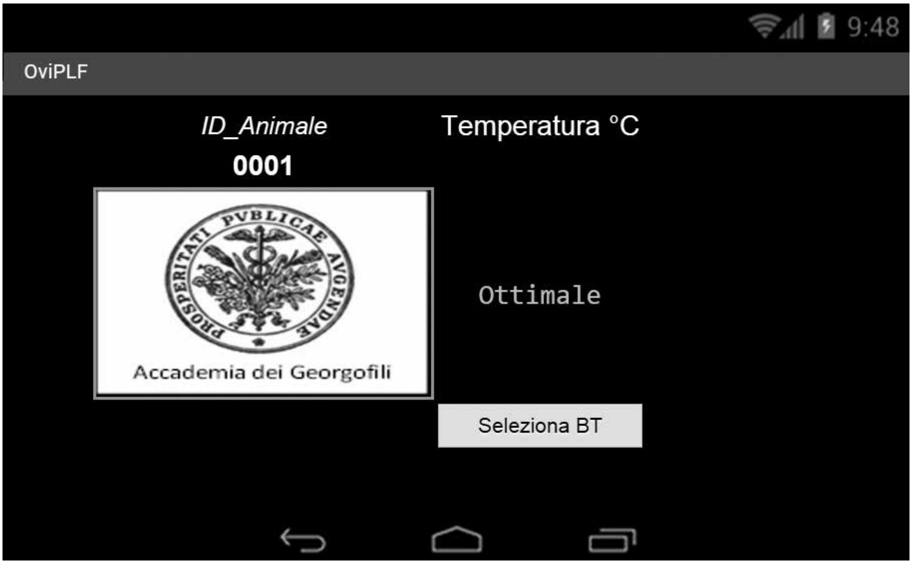

Fig. 10 *La schermata dell'app Oviplf. In alto a sinistra il codice identificativo dell'animale in alto a destra la temperatura, monitorata in tempo reale, e in basso a destra il pulsante per selezionare il Bluetooth, che permette di comunicare con il sistema per la rilevazione della temperatura corporea dell'animale*

I GOOGLE GLASS PER IL MONITORAGGIO DELLA TEMPERATURA CORPOREA DELL'ANIMALE

Il monitoraggio della temperatura corporea dell'animale rappresenta un importante parametro per la gestione aziendale poiché è quasi sempre associato a problematiche patologiche, produttive e fisiologiche (Tucker et al., 2008, Suthar et al., 2011, Badakhshan e Abshenas 2015). Il monitoraggio in tempo reale dello stato degli animali è oggi possibile, durante la normale routine di lavoro, grazie all'utilizzo degli occhiali a realtà aumentata. La sezione di In-

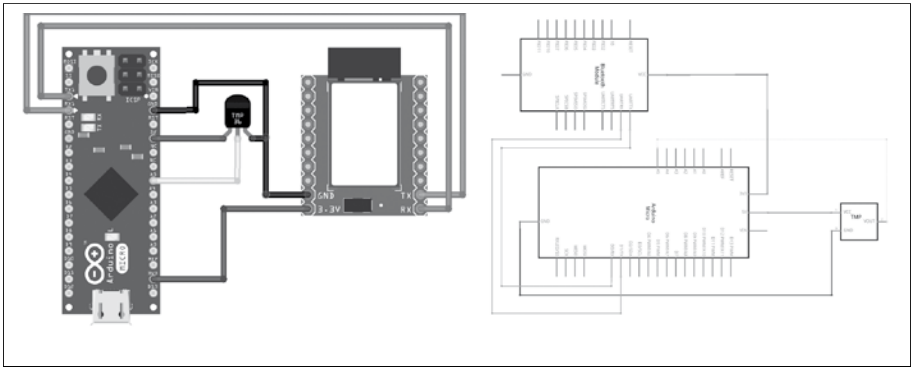

Fig. 11 *A sinistra una rappresentazione del sistema per la rilevazione della temperatura costituito da: un microcontrollore Arduino, un sensore di temperatura analogico LM35 e un modulo di trasmissione dati Bluetooth; a destra lo schema del circuito elettronico*

gegneria del Territorio del Dipartimento di Agraria dell'Università di Sassari ha realizzato un'applicazione, *Oviplf*, che mostra, nel display integrato dei Google Glass, la temperatura corporea dell'animale (fig. 10).

L'applicazione è stata realizzata utilizzando il software gratuito *MIT app inventor*[9], ambiente di sviluppo per applicazioni *Android* gestito dal Massachusetts Institute of Technology. La rilevazione della temperatura è stata effettuata tramite un sensore analogico LM35, in grado di rilevare la temperatura in un intervallo compreso tra i 2°C e i +150°C con un'accuratezza pari a ±0,5°C. Il valore trasdotto viene inviato ai Google Glass tramite il modulo Bluetooth HC-06 del microcontrollore Arduino. Il circuito e lo schema elettronico (fig. 11) sono stati realizzati utilizzando il software per la progettazione elettronica Fritizing (Knörig et al., 2009) versione 0.9.2b. *Oviplf* è un'applicazione estremante modulare e risponde a una delle principali necessità della Plf, ossia fornire un supporto all'allevatore nella gestione del singolo animale in mandrie che, col passare degli anni, diventano sempre più numerose.

ABSTRACT

FAO estimates predict for 2050, as a result of a global growth in population, an increase in the demand for animal protein, especially in developing countries. It appears, therefore, the need to increase efficiency and reduce waste in the agro-livestock and to this end it is essential a real-time monitoring of many biological and environmental parameters.

9 http://appinventor.mit.edu/explore/

One of the disciplines that can operate in this direction is the Plf (Precision livestock farming). This study describes the state of the main components of Plf: software, hardware and data transmission, focusing on issues related to the modular hardware and the differences between the licensed software and not. It has been developed, finally, an application, Oviplf, highly modular, able to detect the animal's body temperature and display it in real time in the integrated display of Google Glass, augmented reality glasses.

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Conclusioni

A conclusione della giornata di studio su *Innovazione negli allevamenti per la prevenzione*, organizzata su proposta del "Comitato consultivo per gli allevamenti e le produzioni animali" dei Georgofili, si possono trarre le seguenti brevi conclusioni.

Il presidente onorario, prof. Franco Scaramuzzi, ha sottolineato l'importanza che l'innovazione ha sempre rivestito per l'Accademia e la sensibilità plurisecolare che l'Istituzione ha mostrato verso l'avanzamento delle conoscenze in tutti i campi delle Scienze Agrarie. Egli ha ribadito, inoltre, la particolare esigenza di nuove forme di produzione e di prevenzione nel campo della zootecnia, anche alla luce della crescente domanda di prodotti di origine animale nel mondo.

Il prof. Agostino Sevi, dell'Università di Foggia, ha introdotto i lavori riprendendo quanto accennato dal presidente onorario e ha ricordato la necessità di rapide innovazioni nel settore zootecnico che consentano di rispondere alla domanda sempre crescente di prodotti di origine animale (+70% di carne e + 35% di latte nel 2050), con una riduzione degli impatti ambientali e un minor impiego di risorse.

In particolare, il prof. Sevi ha sottolineato che in un contesto di rapidi e spesso profondi mutamenti delle dinamiche di mercato, del mondo del lavoro e delle produzioni e delle condizioni climatiche, obiettivo della giornata di studi è stato quello di fornire alcuni utili spunti di riflessione in merito all'adozione, anche in campo zootecnico, degli strumenti resi disponibili da settori innovativi della ricerca scientifica e tecnologica. Uno sguardo in avanti, insomma, per prevenire (o almeno affrontare meglio) i

* *Università di Sassari*

problemi e le sfide che la zootecnia dovrà fronteggiare in un immediato futuro.

La prima relazione, *Proteomica e salute animale* svolta dal prof. Fabrizio Ceciliani dell'Università di Milano, si è incentrata sull'applicazione della proteomica, la scienza che studia le proteine, al settore veterinario. Dopo aver ricordato il ruolo di questi indispensabili composti nel metabolismo animale, con particolare enfasi ai processi infiammatori, il relatore si è concentrato sui risultati di studi recentissimi che hanno evidenziato nel tessuto adiposo uno degli organi più rilevanti nella sintesi di composti proteici coinvolti nell'infiammazione.

La seconda relazione, *Nanoparticelle nelle produzioni agricole: applicazioni e prospettive* svolta dal prof. Gianfranco Greppi, in collaborazione con le dott.sse Stefania Mura e Iva Chianella dell'Università di Sassari, ha esplorato l'affascinante mondo del molto piccolo. Il focus della comunicazione ha riguardato l'uso delle nanotecnologie nella depurazione delle acque, aspetto di particolare rilevanza nei Paesi in via di sviluppo, e i nano-feed, cioè l'applicazione delle nanoparticelle quale veicolo di *nutrients* o di aromi per la mangimistica animale.

La terza relazione, *Il ruolo dei sensori nella zootecnia di precisione per il benessere animale e la sostenibilità ambientale* svolta dal prof. Antonio Pazzona, in collaborazione con l'ing. Giovanni Chessa dell'Università di Sassari, ha preso in esame la *Precision Farm System*, o Zootecnia di Precisione, quale strumento per migliorare le *performances* aziendali e ridurre gli impatti ambientali della zootecnia. In particolare nel corso dell'Adunanza è stata mostrata per la prima volta in Italia una applicazione dei *Google Glass*, la periferica a forma di occhiali che consente di proiettare le immagini elaborate in prossimità degli occhi, al settore agricolo e presentata una piattaforma *open access* (PFN-open) per gestire la complessa sensoristica necessaria per la conduzione di una moderna azienda zootecnica.

In conclusione, le relazioni presentate nel corso dell'adunanza hanno consentito di comprendere meglio il potenziale applicativo delle tecniche proteomiche nel campo delle scienze zootecniche con particolare riferimento alla tutela del benessere animale, i risultati della più recente ricerca nanotecnologica nel campo del risanamento ambientale e delle produzioni animali nonché di valutare le principali applicazioni della "sensoristica" per lo sviluppo della zootecnia di precisione e quindi di strumenti di gestione volti al monitoraggio automatico del benessere, della salute degli animali, dell'impatto ambientale, della sicurezza del consumatore e della produzione in tempo reale.

L'applicazione di questi nuovissimi campi della conoscenza alla zootecnia costituirà certamente una delle sfide tecnologiche più rilevanti per l'ottenimento di maggiori produzioni da animali più sani salvaguardando nel contempo il loro benessere e la sostenibilità ambientale dei relativi sistemi di allevamento.