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State of art of nanotechnology applications in the meat chain: a qualitative synthesis

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Abstract

Background Nanotechnology is a promising area in industry with a broad range of applications including in the agri-food sector. Several studies have investigated the potential benefits deriving from use of nanomaterials in the context of the whole food chain drawing scenarios of benefits but also potential for concerns. Among the agri-food sector, animal production has potential for nanomaterial application but also for safety concerns due to the possibility of nanomaterial accumulation along the farm-to-fork path.

Scope and Approach The aim of this work was to define the state of the art of nanomaterial applications in the animal production sector by assessing data belonging to recently publishes

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studies. To do this, a qualitative synthesis approach was applied to build a fit-for-purpose framework and to summarise relevant themes in the context of effectiveness, feasibility and health concerns.

Key Findings and Conclusions Nanomaterials have potential for use in a wide range of applications from feed production and farming to food packaging, including several detection tools designed for the benefit of consumer protection. The current high degree of variability in nanomaterials tested and in study designs impairs external validation of research results. Further research is required to clearly define which safe nanomaterial applications have the potential to reach the market.

Keywords

Nanomaterial, framework synthesis, food safety, nanoparticle

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Introduction

Nanotechnology is a leading research area with great potential for application in different industrial sectors, including agricultural and food production (agri-food) systems. The term "nanotechnology" refers to the "application of scientific knowledge to manipulate and control matter at the nanoscale in order to make use of size and structure dependent properties and phenomena, as distinct from those associated with individual atoms or molecules or with bulk materials"(ISO, 2015). Thus, nanotechnology can be described as the fabrication, characterisation and manipulation of particles with sizes <100 nm (ASTM, 2012).

Currently, according to the EFSA Nano Inventory, 276 nanomaterials are available on the market, but this number rises to 633 if we also consider applications at the research and development stage. Limiting the research to agri/feed/food fields reduced this count to 55 (RIKILT and JRC, 2014). The extrapolation of nanotechnology applications from the food to the feed sector is also possible; however, few data have been obtained in this context to date.

The study of nanotechnology applications in the agri-food sector is of particular interest due to its economic importance and to its direct relationship with the environment and public health. Moreover, food safety issues are of great public opinion interest. Several studies have covered the application of nanotechnology in the agri-food sector beyond the detailed EFSA Nano Inventory, resulting in a number of applications intended for different stages of the agri-food chain. However, within the "farm to fork" path, the animal production system has potential for bio-accumulation of chemicals, including nanomaterials, deriving from both direct applications and environmental contamination.

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Knowledge about specific nanotechnology applications in the meat chain is of great interest for consumers and policymakers as it can allow evaluation of the potential short-term impact of nanotechnology in this sector in terms of benefits, risks and regulatory needs. However, to our knowledge, only two scientific works specifically deal with nanotechnology applications in the meat chain (Baltić *et al.*, 2013; Ramachandraiah *et al.*, 2015).

The present work aims to add to the current knowledge by means of an innovative approach relying on qualitative synthesis, inspired by the "best fit" framework synthesis (*Carroll et al.*,, 2011). Using this approach, the status of nanotechnology research in the meat chain was addressed through a systematic evaluation of scientific literature.

Methods

Study design

An approach inspired by the "best fit" framework synthesis (Carroll *et al.*, 2011) was applied to code primary research describing nanotechnology applications in any stage of the meat supply chain against an *a priori* framework built on recent reviews dealing with nanotechnology in the whole agri-food sector. To our knowledge, this is the first attempt to apply a similar method in the field of food science and technology, as framework synthesis falls into the group of methodologies intended for qualitative synthesis, was primarily conceived for social sciences, and is currently under development (Thomas and Harden, 2008). The choice of this method was based on the possibility that framework synthesis would offer a highly structured approach to organise and analyse data (Carroll *et al.*, 2011). Indeed, it allows the step-by-step construction of a coherent picture starting from an overwhelming number of studies as the reviewers become more familiar with the literature being reviewed.

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Moreover, the major themes presented in the final selection of studies were summarised through an iterative, full text-based coding process, to describe the status of scientific research on nanotechnology in the meat supply chain. A full picture of the current study design is presented in Figure 1.

Identification of a priori framework

To generate the *a priori* framework by means of a transparent approach, a literature-based search of published reviews was carried out. Due to the inability to use the two review papers which directly dealt with meat for such a purpose, all recent reviews of nanotechnology applications in the whole food supply chain were collected by searching three scientific databases (Web of Science Core Collection, PUBMED, CAB Abstracts).

Eligible reviews were written in English, published after 01/01/2010 and should report an overview of nanotechnology applications in the agri-food sector. Keywords used for the literature searches are reported in Table 1. Two reviewers (FG, SB) independently screened (single coding) the *Title/Abstract* and the *Full Text* to find eligible reviews.

Data were collected from the selected reviews to obtain a list of nanotechnology applications in the agri-food sector, grouping together similar items. That information was then used to build the *a priori* framework on the basis of the defined farm-to-fork flow. Two reviewers independently coded a subset of the studies.

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Definition of the Framework

A second literature search was carried out to fill in the *a priori* framework with applications referring to the meat supply chain. Three databases were interrogated on 1 November 2015: Web of Science Core Collection, PUBMED, CAB Abstracts. The terms included in the search string are detailed in Table 2 and were chosen according to the list of applications obtained from the *a priori* framework. Eligible studies described primary research, were written in English and were published after 1 January 2011.

In detail, selection criteria for the studies were that:

-the applied technology be consistent with the *nano* definition, as reported in the *Introduction* section, with at least one dimension of the studied material being below 100 nm;

-the nanotechnology described be applicable in the meat production chain (from farm, including also feed production, to fork);

-the nanotechnology be directly tested under a real life scenario or under simulated *in vitro* conditions reproducing such a scenario.

Both *Title/Abstract* and *Full Text* screening were performed by three reviewers (SB, FG, CL). Single coding was applied. Research studies identified in the frame of the second literature search were then coded against the *a priori* framework to describe the area of the meat supply chain covered by nanotechnology research. Data were extracted by three reviewers (SB, FG, CL). A quality appraisal of the included studies was conducted based on the presence of the benchmarking of nanomaterial characterisation as defined by EFSA (EFSA, 2011). However, studies not reporting a coded characterisation were retained as a conservative choice.

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Assessment of the research state of the art

To evaluate the likelihood of nanotechnology applications reaching the market, and thus affecting the environment and public health in the future, a thematic summary was conducted targeting the following themes, which had been defined iteratively during *Full Text* evaluation:

-rationale of the application: the ability of the application to go beyond existing limitations;

-effectiveness: ability of the application to obtain the desired effect;

-health concerns: the possible negative impact on human health and/or the environment due to exposure to nanomaterials or to ascertained side effects;

-feasibility: likelihood of the nanotechnology application to be implemented, with respect to cost, time and technical constraints.

The absence of these themes in the *Discussion* section of the selected studies were classified as data gaps and used to suggest further studies or implementation needs.

Results and discussion

Definition of the Framework

Forty-three reviews satisfied the inclusion criteria (Figure 2a) to generate and inform the *a priori* framework (The full list of references is available in the Supplementary Materials). The complete framework is shown in Figure 3, where nanotechnology applications in the meat supply chain in the context of agri-food background are illustrated.

The second literature search was limited to the meat chain and it did not identify any nanotechnology application in addition to those already determined by the *a priori* framework,

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but conversely, some agri-food applications described in the *a priori* framework were not covered by any recent primary study focused on the meat chain.

Quality appraisal

Due to the different nature of nanomaterials compared to bulk materials, it is necessary to fully characterise them in terms of physico-chemical properties. The fundamental required information is assessment of size and shape. To assess the proper size of nanomaterials it is suggested that at least two methods are used, one being electron microscopy (EFSA, 2011). However, most of the retrieved studies relied only on one analytical technique for nanomaterial size characterisation, usually scanning electron microscopy (SEM) or transmission electron microscopy (TEM), with shape information often missing. Moreover, in some cases, the instrument adopted for characterisation was not mentioned even though the nanoparticle size was reported (see Tables 3, 4, 5). Few studies gave detailed characterisation based on two analytical approaches with size and shape being reported.

Assessment of research state of the art

Nanotechnology applications to animal farming

The selected studies drew a scenario where the application of nanotechnology in farming is mainly focused on two areas: dietary supplementation and vaccine development. In both these cases, the aim was to increase productivity through forcing animal growth performance and reducing the impact of diseases.

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The use of nanomaterials in animal feeding has been reported in several studies with a wide range of materials tested: gold (Pineda *et al.*, 2012a), silver (Fondevila *et al.*, 2009; Ahmadi, 2012; Pineda *et al.*, 2012b; Pineda *et al.*, 2012c), copper (Wang *et al.*, 2011; Mroczek-Sosnowska *et al.*, 2015; Nguyen *et al.*, 2015), zinc (Khah *et al.*, 2015; Mohammadi *et al.*, 2015; Nguyen *et al.*, 2015) and selenium (Selim*et al.*, 2015a, 2015b). The majority of studies were conducted on poultry (Table 3), where nano supplementation has been hypothesised to further increase growth performances beyond those currently achieved and the positive impact of the nano supplements on enrolled animals was evaluated by measuring different markers of performance such as metabolic rate, animal and/or carcasses weight, "livability" and/or feed intake. Moreover, the effect of nano supplements on gut microbiota was tested using materials known for their antimicrobial effect, such as silver and zinc.

The timing of administration of nano supplements in some retrieved studies included *in-ovo*, one-shot, administration (Pineda *et al.*, 2012a; Pineda *et al.*, 2012c; Mroczek-Sosnowska *et al.*, 2015), whereas most of them examined the effects of lifelong supplementation. Results did not always agree in terms of efficacy because of important differences in study designs and the nano materials examined. Negative or no effects were described in some studies (Ahmadi, 2012; Pineda, *et al.*, 2012b) whereas positive effects on growth performances were shown for zinc (Khah *et al.*, 2015) and zinc in forms such as zinc-nano-methionine and zinc-nano-max (obtained through nanochelating technology) (Mohammadi *et al.*, 2015). Copper in nano and salt forms both had positive effects on growth performances in poultry after *in-ovo* supplementation (Mroczek-Sosnowska *et al.*, 2015). Copper-loaded chitosan nanoparticles (NPs) led to an increase in broiler growth performances plus a better immune status and were suggested as

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potential substitutes for chlortetracycline in broilers (Wang *et al.*, 2011). *In-ovo* administered silver NPs exerted a positive effect on metabolic rate but did not influence embryo growth (Pineda *et al.*, 2012a). Silver NPs were also unable to influence growth performances of chickens when administered continuously during farming (Pineda *et al.*, 2012c). Supplementation with chitosan nanocapsules containing turmeric acid as an active extract was tested in broilers by Zuprizal and colleagues who found an improvement of the feed conversion rate and a reduction of total cholesterol without affecting growth performances (Zuprizal *et al.*, 2015). The same research group found also a positive effect of turmeric-laden chitosan nanocapsules in the reduction of subcutaneous fat deposition in broilers (Sundari *et al.*, 2014).

Fewer studies investigated the effect of nano supplementation in pigs. Nano silver administration in pigs led to an improvement in growth of farmed pigs also if one of three experiments did not agree on this result. Negative effects on gut bacteria were not observed whereas a low accumulation was described in liver but not in kidney and skeletal muscles (Fondevila *et al.*, 2009). Chromium-loaded chitosan NPs (Wang, *et al.*, 2012a) and trivalent chromium (Wang *et al.*, 2012b) were both effective in increasing the content of chromium in pig tested tissues, as suggested by pilot studies, and also had beneficial effects on growth, carcass characteristics, and pork quality, and positively affected lipid catabolism in finishing pigs (Wang *et al.*, 2014).

Several strategies have been applied for the reduction of disease impact on animals, relying on both prevention and therapy. In the context of prevention, new vaccination strategies with nanomaterials playing a major role have frequently been proposed as effective adjuvants due to their ability to effectively deliver antigenic components to target sites and to the possibility of increasing antigenic exposure (Viswanathan *et al.*, 2014). The potential of nanotechnology in vaccine development was focused on overcoming current limitations such as the level and the duration of immunisation (Jang *et al.*, 2011a; Jazayeri *et al.*, 2012) as well as the delivery effectiveness. Several materials have been tested as nanocarriers, including silver for avian influenza vaccine (Jazayeri *et al.*, 2012) and calcium phosphate for foot and mouth disease vaccine (Joyappa *et al.*, 2009) or Newcastle disease vaccine (Viswanathan *et al.*, 2014). In addition, specific formulations based on water-in-oil emulsions (Jang *et al.*, 2011a, , 2011b) allowed better delivery of sensible antigenic determinants such as genomic material in DNA-based vaccines, allowing the development of cross-effective vaccination strategies (Joyappa *et al.*, 2009; Jazayeri *et al.*, 2012). It has also been suggested that this technology could lead to the development of peptidic NPs able to confer cross-effective vaccination against important viruses such as low pathogenic avian influenza virus (Babapoor *et al.*, 2011). Finally, mesoporous silica NPs were effective as carriers of a subunit vaccine against bovine viral diarrhoea, providing stability during storage, effective delivery and balanced immunity (Mahony *et al.*, 2015). Nanomaterials were also applied against vectors responsible for animal diseases, and could have potential to overcome pest resistances, by reducing vector populations in the environment as suggested by studies performed in surrogate scenarios. Silver NPs obtained through a production method relying on plant as a chemical reduction agent, showed promising results against larvae of ticks of different species including *Hialomma anatolicum*, a bovine tropical theileriosis vector (Jayaseelan and Rahuman, 2012). Also, zinc (Kirthi *et al.*, 2011) and copper (Ramyadevi *et al.*, 2011) NPs showed promising results against *Rhipicephalus microplus* a cattle tick responsible for losses during animal farming.

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Nano-based therapies have been reported in a few studies mainly describing the antimicrobial effect of silver. Mordmuang and colleague described the antimicrobial effect of green synthesised silver NPs in an *ex vivo* bovine udder epidermal tissue model (Mordmuang *et al.*, 2015). Silver NPs were effective in reducing coccidian excretion (Chauke and Siebrits, 2012) in 40 day old male chicks.

Finally, nanosized material was also proposed as an effective tool for sterilisation treatments. Liu and colleagues tested in mice a novel strategy for male sterilisation based on single-layer $WO_{2.72}$ (tungsten oxide) nanosheet as an intelligent photo-responsive sterilant. These nanosheets, under appropriate UV stimulation, can target and destroy gonadic cells, allowing rapid, painless sterilisation without any side effect on blood parameters and with low tungsten bioaccumulation in liver and spleen (Liu *et al.*, 2015).

It is noteworthy that NPs could also reach the animal production chain due to their intentional use during feed production stages. Nano-based fertilisers (Liu and Lal, 2014; Adhikari *et al.*, 2015) have been described.

Nanotechnology applications to meat

Human health is another important target of nanotechnology application in the meat supply chain. The main benefits emerging from the retrieved studies were linked to the prevention of foodborne diseases and the reduction of chemical contamination of meat. The former goal was achieved through the development of effective packaging exerting antimicrobial activities or of intelligent packaging able to warn consumers of pathogen presence.

Meat poisoning and spoilage by microorganisms is of major concern in the industry. Synthetic compounds and biologically derived substances such as antibiotics or other naturally occurring compounds are widely used to control or to inhibit pathogenic and spoilage microbiota. Joe et al., 2012 formulated a surfactin-based nanoemulsion showing excellent antibacterial activity against foodborne bacterial species such as *Bacillus cereus, Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhi, *Staphylococcus aureus* and *Vibrio parahaemolyticus* on chicken meat (Joe *et al.*, 2012). Moreover, the sporicidal and antifungal activities exerted by the nanoemulsion suggested a possible use as a food preservative. Nanotechnology has also been applied to the formulation of innovative packaging solutions, exerting a functional antimicrobial role in slowing down meat spoilage, allowing both the maintenance of food safety criteria and the reduction of food waste. This was the case for: Olive oil-ZnO nanofibers mats (Amna *et al.*, 2015), found to be effective as packaging material against *S. aureus* and *S.* Typhimurium; cellulose nanocrystals conjugated with nisin, effective in inhibiting *L. monocytogenes* growth (Khan *et al.*, 2014); silver NPs in minced beef at 3°C on *E. coli*, *Sthaphylococcus aureus* and total bacterial count (Mahdi *et al.*, 2012); carboxymethyl cellulose (CMC) nanoparticulate films containing sinigrin hydrate, effective against *E. coli* O157:H7 on fresh beef (Herzallah and Holley, 2015).

Among packaging systems, active packaging is based on an innovative concept in which the package, the product, and the environment interact to prolong the shelf-life, enhance safety, or improve sensory properties, while maintaining the stability and quality of the product. In this context, the antibacterial activity of Ag and ZnO NPs, alone or incorporated into pullulan films, was effective against *S. aureus, L. monocytogenes, E. coli* O157:H7 and *Salmonella*

Typhimurium, *in vitro*, when associated with raw turkey breast, raw beef, or ready-to-eat (RTE) turkey (*in situ*), during long-term refrigerated storage (4°C) (Morsy *et al.*, 2014). Moreover, Panea et al. (2014) reached the same conclusions by testing nanocomposite packaging containing different proportions of ZnO and Ag on chicken breast meat safety (Panea *et al.*, 2014). Nanocomposite films also reduced the oxidation of beef displayed under modified atmosphere packaging (Picouet *et al.*, 2014). ZnO NPs were also film-embedded in active packaging in a challenge study on ready-to-eat poultry meat against *Salmonella* Typhimurium and *Staphylococcus aureus*, resulting in a substantial reduction in the number of inoculated target bacteria (Akbar and Anal, 2014).

One of the most important factors that influences the quality and acceptability of meat during refrigerated or frozen storage is lipid oxidation. Evidence for a role for chitosan-based nanocompounds as antioxidants in minced meat were found by several studies (Chantarasataporn *et al.*, 2014; Elbarbary *et al.*, 2015; Zimoch-Korzycka and Jarmoluk, 2015). Moreover, application of chitosan cellulose nanocomposites on minced meat decreased numbers of lactic acid bacteria (Dehnad *et al.*, 2014).

Nanotechnology applications to detection and diagnosis

One of the most studied areas in the context of detection is the use of nanomaterials in sensors to detect drug residues in food and feed. Illicit use of veterinary drugs such as growth promoters agents including β-agonists and antibiotics is still a current issue in animal husbandry and drug residue accumulation in food is a serious risk for consumer health (Kuiper *et al.*, 1998; Paige *et* *al.*, 1999; Ramos *et al.*, 2003; Han *et al.*, 2013). As a consequence, several studies have focused on the development of different drug-sensitive versatile "nano-diagnostic tools".

For example, gold NPs (AuNPs) have been successfully employed in devices for rapid detection of lean meat growth promoters: e.g. ractopamine in feed and pork meat (Lin *et al.*, 2012; Kong *et al.*, 2014), clenbuterol in swine urine (Li *et al.*, 2012), 3-amino-5-morpholino-2-oxazolidone (AMOZ) in meat and feed (Li *et al.*, 2014a), and sulfadimethoxine in chicken tissue (Peng *et al.*, 2013). Au in combination with platinum (Pt) was used to form Au-Pt bimetallic NPs to detect zeranol in bovine urine (Regiart *et al.*, 2014) and in combination with silver (Ag) in gold/silver core NPs to detect a new beta agonist, phenylethanolamine A, in pig urine (Li *et al.*, 2014b).

Also magnetic Iron (Fe)-based NPs have shown to be a promising material in diagnostic tools and have been adopted to detect enrofloxacin and its metabolite in chicken meat (Ahn and Lim, 2014), oxytetracycline in chicken meat (Lu *et al.*, 2015) and, in combination with multi-walled carbon nanotubes, to detect kanamycin in chicken and pig livers (Long *et al.*, 2015). The combination of Au and Fe magnetic NPs also allowed the development of a voltammetric immunosensor to detect, in bovine hair, the illicit use of clenbuterol (Regiart *et al.*, 2013).

Other studies suggest the promising application of lanthanide-sensitised luminescence of terbium oxide (Tb_4O_7) NPs in devices to detect lasalocid and salycilate in feeds (Castillo-Garcia *et al.*, 2012), ractopamine-tetraphenylborate NPs to detect ractopamine residue in pork meat (Zhang *et al.*, 2014), and ZnO NPs to detect the illicit use of dopamine in pork meat (Zhihua *et al.*, 2015).

Nanodevices have also been developed with other chemical purposes. This was the case for a titanium dioxide-graphene-platinum-palladium hybrid nanocomposite, specifically designed for

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nitrite detection in meat (Jiao *et al.*, 2015) and of a TiO_2-graphene-Pt-Pd hybrid nanocomposite used to estimate cholesterol content in meat (Cao *et al.*, 2013).

Nanodevices have been used to address microbiological issues. The rapid detection of pathogens in food can help to prevent foodborne illnesses that still significantly impact society and pose a threat to public health. For example AuNPs along with magnetic NPs were effectively used to speed up the culture steps to detect *Salmonella, L. monocytogenes, E. coli* and *Campylobacter* in beef, chicken, pork and turkey meat (Weidemaier *et al.*, 2015) and to develop a membrane-filter to selectively detect *E. coli* O157:H7 in ground beef (Cho *et al.*, 2015). AuNPs were also used in the design of a detection kit for *E.coli* O157:H7 in feed (Ali *et al.*, 2014), and *S.* Typhimurium *and S.* Enteritidis in chicken (Moongkarndi *et al.*, 2011).

Cadmium sulfide (CdS) NPs and lead sulphide (PbS) NPs proved to be effective in detecting *E.coli* O157:H7 and *S. aureus* in beef (Abdalhai *et al.*, 2014, 2015), silica NPs were adopted in *S.* Pullorum and *S.* Gallinarum detection in chicken serum, whereas Quantum dot NPs were used in a nanobiosensor to detect *S.* Typhimurium in different meat species (Kim *et al.*, 2015).

Food freshness and authenticity are of great importance within the food market and the development of rapid and sensitive tools able to monitor food spoilage and food adulteration have been explored. For example, AuNPs were used to develop a real-time monitoring chip able to detect amine formation in chicken meat (Abargues *et al.*, 2014), as well as changes in beef meat permittivity (Dang *et al.*, 2014), both markers of food spoilage.

AuNPs were also effective in easy-to-perform and cost-effective methods for the verification of pork adulteration in different kinds of processed meats (Ali *et al.*, 2011a., 2011b, 2012a, 2012b).

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The development of rapid assays could also play a key role in the livestock industry to detect diseases in their early phase, with a likely significant impact on animal health as well as public health and international trade. Encouraging results were obtained by Peled and colleagues who developed a rapid assay based on AuNPs to detect *Mycobacterium bovis* infection in cattle (Peled *et al.*, 2012). Karthik and colleagues used Fe NPs (Fe_2O_3) to develop a rapid, easy and sensitive bioelectronic sensor to diagnose Johne's disease in goats, through early in-serum detection of *Mycobacterium avium* subsp. paratuberculosis (Karthik *et al.*, 2013).

Feasibility, concerns and data gaps

The study of nanotechnology applications in the meat chain is well represented in the scientific literature, and the main concern expressed against the application of nanotechnologies in farming systems is uncertainty about the effects.

Nano supplementation was not always proved to be effective, and in some cases, for silver (Ahmadi, 2012) and selenium (Selim*et al.*, 2015a, 2015b), negative effects were described. Silver has been demonstrated to exert negative effects on blood parameters, oxidation, and the immune system (Ahmadi, 2012), whereas selenium NPs accumulated in poultry tissues (liver and kidney) to higher levels compared to organic forms of selenium (Selim *et al.*, 2015b). A strengthening of scientific evidence is needed for nanomaterials which could potentially be administered to animals. Good candidates, according to the literature, are zinc (Khah *et al.*, 2015) and copper (Wang *et al.*, 2011; Mroczek-Sosnowska *et al.*, 2015), but further research, especially on functional mechanisms, is warranted (Khah *et al.*, 2015). The potential for bioaccumulation should be better addressed due to potential concerns for chromium (Wang *et al.*, 2014) and selenium (Selim *et al.*, 2015a, 2015b).

It has been suggested that the use of nanomaterials as feed supplements could reduce the impact of chemicals on the environment due to the higher bioavailability of nutrients when they are in nano form. Higher bioavailability suggests nano forms could possibly be used at lower doses, with reductions of as much as 75% compared to non-nano forms (Nguyen *et al.*, 2015). Moreover supplementation strategies such as *in-ovo* administration would reduce release of chemicals into the environment (Mroczek-Sosnowska *et al.*, 2015). The subject of environmental release of NPs is of particular interest and is extensively described elsewhere (Ju-Nam and Lead, 2008; Bystrzejewska-Piotrowska *et al.*, 2009; Dwivedi *et al.*, 2015).

The long term effects and clinical application of nano based adjuvants such as Ag DNA vaccine (Jazayeri *et al.*, 2012) are yet to be considered. Some other nanomaterials, like calcium phosphate for example, may be of lesser concern due to their bioresorbable properties and safety (Viswanathan *et al.*, 2014).

Some nanotechnology applications have been tested only under *in vitro* conditions (Kirthi *et al.*, 2011; Ramyadevi *et al.*, 2011; Jayaseelan and Rahuman, 2012) or in laboratory animals (Liu *et al.*, 2015), and clearly, the results of these studies cannot be directly extrapolated to the human food chain. Therefore, these nanotechnology applications must be assessed in real meat chain scenarios. Indeed, most nanotechnology studies were not carried out under real meat chain scenarios, and moreover, both the study designs and their conclusions may be influenced by commercial interests. Some authors have claimed that the high hygienic standards applied in

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laboratory farming conditions could have an impact on the evaluation of the antimicrobial effects of nanotechnologies.

Commercial application will require a much better understanding of functional mechanisms, adsorption, translocation and interaction of NPs and nanomaterials with other elements or nutrients (Khah *et al.*, 2015). Once nanotechnology trial results are judged applicable in commercial farming, costs will have to be considered to guarantee feasibility of nano supplementation. Consideration of commercial costs is almost completely lacking in the scientific literature because of the difficulties in predicting costs in early stages of product development, but they probably would drive the choice of the best candidate metals to be used as NPs.

As regards health concerns, the main problem arising from the use of NPs in the meat chain is the potential accumulation in specific animal tissues, potentially producing animal health issues, human exposure via consumer ingestion, and negative impacts of nanomaterials eliminated from animals/humans or otherwise released into the environment. From a theoretical point of view, these problems are inversely proportional, as the higher the bioavailability and absorption of nanomaterials in the organism, the lower the excreted quota, and consequentially the environmental load. Other important factors are the amount of nanomaterial used (frequency of administration and dose) and the nature of the material being added to the meat chain. Concerns are also linked to the use of new materials like silver, the toxicity profile of which is still unclear, or to the nanoform itself, as the physico-chemical properties of NPs could potentially open new toxicity concerns even for materials previously considered safe for use in the human food chain.

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If the main concern for nanotechnology applications in animal farming is the potential bioaccumulation, the counterpart for application in food packaging is migration from materials to food. The scientifically proven lack of migration of nanomaterials or their constituents from packaging to food would mean lack of consumer exposure to the materials, and therefore, absence of concern for human health. In contrast, where such migration takes place, toxicity must be addressed. The few migration studies published to date have used food simulants, not real foods, so further studies are required. The complexity of food matrices (e.g. multi-component ready meals, meat products, differing and varying microbiota from different geographic locations and meat plants) and food chain conditions (e.g. changing atmospheres in packs over time, varying temperatures, which are not always under control) will need to be included in such studies. In EU legislation, where packaging components are intended to migrate into food to exert their activity (active packaging), such components have to be approved as additives following appropriate legal pathways demonstrating their safety.

Nanotechnology could also represent a great opportunity in terms of routine control along the meat chain. Official and routine controls along the agri-food chain to verify compliance with food and feed legislation often require expensive instruments as well as skilled personnel and time-consuming analysis (Kinsella *et al.*, 2009). Therefore the development of rapid, simple, cheap and accurate screening methods to be carried out on-site, by untrained people would be highly beneficial for consumer health protection as well as providing economic benefits due to the more effective and efficient methods. In this context, nanotechnology could be highly advantageous, and different versatile detection devices with “nano” components are currently under development by a number of research groups.

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These new analytical systems take advantage of the unique characteristics of NPs arising from their high surface/mass ratio (for example, involving new optical, electronic, chemical, catalytic or mechanical means) to improve existing analytical tests, as well as to create alternative assays by further lowering the limit of detection or speeding up the result output. However, despite the fact that low cost and rapid analysis make nanodevices extremely promising candidates in routine control along the whole agri-food chain, it is clear that appropriate characterisation of nano analytical methods is often still incomplete. In this context, validation approaches would be useful to better assess those parameters that characterise the performance of nano methods. This would give a better understanding of the suitability of the developing nano methods for the intended uses.

Parameters like detection limit and specificity were always defined in all the considered studies but only very few of them also focused on recovery (Castillo-Garcia *et al.*, 2012; Li *et al.*, 2012; Lin *et al.*, 2012; Peng *et al.*, 2013; Kong *et al.*, 2014; Li *et al.*, 2014b; Zhang *et al.*, 2014; Long *et al.*, 2015; Lu *et al.*, 2015), and reproducibility (Li *et al.*, 2012; Cao *et al.*, 2013; Kong *et al.*, 2014; Li *et al.*, 2014b; Jiao *et al.*, 2015; Long *et al.*, 2015; Lu *et al.*, 2015; Zhihua *et al.*, 2015). Moreover, few studies took into consideration the stability of the developed sensors over time (Cao *et al.*, 2013; Kong *et al.*, 2014; Li *et al.*, 2014a; Jiao *et al.*, 2015; Long *et al.*, 2015; Sun *et al.*, 2015; Zhihua *et al.*, 2015). This is a crucial aspect because stability is related both to the reliability of the results as well as to the actual cost of the designed system. Logically, any continuous maintenance required to guarantee good performance would negatively affect the final cost of the entire analysis and also the actual applicability of the method in routine analysis.

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Spiked samples were mainly employed to mimic real case scenarios, whereas only in a few cases were real contaminated samples tested (Ali *et al.,* 2011a, 2011b, 2012a, 2012b). Moreover, comparison of results among different analytical techniques have been rarely carried out (Cao *et al.*, 2013; Peng *et al.*, 2013; Regiart *et al.*, 2014; Lu *et al.*, 2015).

Characterisation of tested materials is another important issue transversally related to all the above discussed applications. Proper characterisation of nanomaterials is important to ensure reliable and reproducible results suitable for potential exposure assessment in the framework of risk assessment/management of nanomaterials (Stone *et al.*, 2010; EFSA, 2011). “Gold standard” characterisation methods are not still available and, often, the results obtained by different measurement techniques can differ because of the different physical principles applied in the different measurement methods (Domingos *et al.*, 2009; EFSA, 2011). As a consequence, it is deemed important to know the analytical methods adopted to characterise the nanomaterials, which would allow eventual comparison of results achieved in independent studies.

On the whole, as has already been highlighted in other studies (Domingos *et al.*, 2009; Dhawan and Sharma, 2010; EFSA, 2011), this review confirms that a large amount of uncertainty exists in the literature with respect to characterisation of NPs. This remains an important issue to overcome before scientific examination of the use of nanomaterials in the context of the agri-food chain will be able to be implemented.

Conclusions

Our analysis suggests that the application of nanotechnology in the meat chain is mainly aimed at the efficiency of animal production, the protection of human health, but also at enhancing food

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quality and authenticity. Despite the promising results obtained by the application of some nanomaterials in dietary supplementation and vaccine development, the use of nanotechnology in animal farming is at a very early stage and a clear picture about feasibility in terms of cost, safety and environmental impact is lacking.

On the other hand, nanotechnology could significantly contribute to human health protection and food quality with increasing research activities aiming at the development of novel technologies, instruments and methodologies. In this context, effective packaging containing nanomaterials which exert antimicrobial activities or intelligent nanomaterial packaging able to warn consumers of pathogen presence look promising areas to aid in guaranteeing the maintenance of food safety criteria and in reducing reduce food waste.

Finally, there are encouraging results for application of nanotechnology in the meat chain, where rapid, simple, cheap and accurate method to expedite chemical and microbiological analysis of food and feed are under development. Although further studies are required to better define nanodevice performance, this remains an innovative and promising application of nanotechnology, with potential to bring effective benefits both in terms of public health and cost.

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Table 1: Terms used to retrieve reviews on the applications of nanotechnologies in the agri-food sector

nanomaterial OR nanotechnology OR nanotechnologies OR nanoparticle	AND	food OR agri* OR crop	AND	Review* OR overview*

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Table 2: Terms used to retrieve primary research papers describing the application of nanotechnologies in the meat chain

Nanoparticle OR nanomaterial Nanotechnology OR nanocapsule OR nanocarrier OR nanoemulsion OR nanocomposite OR nanodelivery	AND	Meat OR Livestock OR Pig OR Pork OR Cattle OR Beef OR Poultry OR Chicken OR Hen OR Crop OR feed	AND	Farm OR Agrochemical OR Fertilizer OR pesticide OR sensor OR "Water decontamination" OR drug OR antimicrobial OR vaccine OR additive OR process OR processing OR nanosieve OR filtration OR supplement OR Nanocoating OR Packaging OR "Food contact material" OR Nanodetector OR Nanobarcode OR nutraceutical OR breeding

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Table 3: Summary of the main characteristics of studies dealing with nanotechnology applications during animal farming.

Reference	**Material**		**Size**		**Shape**	**Application**	**Function**	**Species**
		nm	**Method 1**	**Method 2**				
Adhikari et al., 2015	Zinc oxide (ZnO)	< 100	TEM	DLS	NR	Feed	Fertilizer	Maize
Ahmadi, 2011	Silver (Ag)	NR	NR	NR	NR	Feed	Supplement	Poultry
Babapoor et al., 2011	Peptide	22-35	TEM	DLS	Spherical	Vaccines	Antigene	Poultry
Chauke & Siebrits, 2012	Silver (Ag)	NR	NR	NR	NR	Drugs	Antimicrobial	Poultry
Fondevila et al., 2009	Silver (Ag)	60-100	NR	NR	NR	Feed	Additive	Pigs
Jang et al. 2011	WO emulsion	50	NR	NR	NR	Vaccines	Adjuvant	Poultry
Jang et al.	WO emulsion	50	NR	NR	NR	Vacci	Adjuva	Poul

2011b						nes	nt	try
Jayaseelan & Rahuman, 2012	Silver (Ag)	25-110	XRD, FTIR	SEM	Rod and cilinder	Pesticides	Against Vectors	Ticks from sheep-goats
Jazayeri et al., 2012	Silver (Ag)	<18	XRD, FTIR	TEM	Spherical	Vaccines	Adjuvant	Poultry
Joyappa et al., 2009	Calcium Phosphate ($Ca_3(PO_4)_2$)	50-100	TEM	NR	Spherical	Vaccines	Adjuvant	Mice/guinea pigs
Khah et al., 2015	Zinc (Zn)	NR	NR	NR	Spherical	Feed	Supplement	Poultry
Kirthi et al., 2011	Zinc (Zn)	60-120	TEM	NR	NR	Pesticides	Against Vectors	Collected

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								from animals
Liu et al., 2014	Apatite	10-40	TEM	XRD	Spherical	Feed	Fertilizer	Soybean
Liu et al., 2015	Tungsten (W)	1-20	NR	NR	Sheet	Drugs	Treatment	Mice
Mahony et al., 2015	Silicium (Si)	140-150	TEM	BET	Spherical	Vaccines	Adjuvant	Sheeps
Mohammadi et al., 2015	Zinc (Zn)	<50	TEM	NR	NR	Feed	Supplement	-
Mordmuang e al., 2015	Silver (Ag)	NR	NR	NR	NR	Drugs	Antimicrobial	*Ex vivo* bovine tissue
Mroczek-Sosnowska et al., 2015	Copper (Cu)	15-70	NR	NR	Spherical	Feed	Supplement	Poultry

Nguyen et al., 2015	Iron, Copper, Zinc, Selenium (Fe, Cu, Zn, Se)	20-90	NR	NR	NR	Feed	Supplement	Poultry
Pineda et al., 2012c	Silver (Ag)	2-35	TEM	NR	NR	Feed	Supplement	Poultry
Pineda et al., 2012a	Gold-Silver (Au-Ag)	2-35	TEM	NR	NR	Feed	Supplement	Poultry
Pineda et al., 2012b	Silver (Ag)	2-35	TEM	NR	NR	Feed	Supplement	Poultry
Ramyadevi et al., 2011	Copper (Cu)	35-80	SEM	XRD	Cubic	Pesticides	Against Vectors	*In vitro*
Selim et al., 2015a	Selenium (Se)	80	TEM	DLS	Spherical	Feed	Supplement	Poultry
Selim et al., 2015b	Selenium (Se)	80	TEM	DLS	Spherical	Feed	Supplement	Poultry
Sundari et al., 2014	Turmeric extract loaded chitosan	<100	TEM	NR	Spherical	Feed	Supplement	
Viswanathan et	Cyclodextrin-Calcium	<10	TEM	NR	Sph	Vacci	Adjuva	Poul

al., 2013	Chosphate ($Ca_3(PO_4)_2$)	0			eric al	nes	nt	try
Wang et al., 2012a	Chromium (Cr) loaded chitosan nanoparticles	90	NR	NR	NR	Feed	Supple ment	Pigs
Wang et al., 2012b	Chromium (Cr)	40-70	NR	NR	NR	Feed	Supple ment	Pigs
Wang et al., 2011	Copper (Cu) loaded chitosan	95	NR	NR	NR	Feed	Supple ment	Poul try
Wang et al., 2014	Chromium (Cr) loaded Chitosan	90	NR	NR	NR	Feed	Supple ment	Pigs
Zuprizal et al., 2015	Turmeric extract loaded chitosan	50	TEM	NR	Sph eric al	Feed	Supple ment	Poul try

WO=Water in Oil emulsion, TEM= Transmission Electron Microscopy, SEM= Scanning Electron Microscopy, BET= Brunauer, Emmett, Teller method to determine specific surface, XRD=X-ray Diffraction, FTIR= Fourier Transform Infrared, DLS=Dynamic Light Scattering, NR=Not Reported

Table 4: Summary of the main characteristics of studies dealing with nanotechnology applications during meat processing and storage

Reference	Material	Size			Shape	Application	Function
		nm	Method 1	Method 2			
Akbar & Anal, 2014	Zinc oxide (ZnO)	50	TEM	SEM	Spherical	Packaging	Antimicrobial
Amna et al., 2015	Olive oil/polyurethane nanocomposite with Zinc oxide (ZnO)	<100	SEM	TEM	fibrous	Packaging	Antimicrobial
Chantarasataporn et al., 2014	Chitosan	100-300	TEM	NR	Wiskers	Packaging	Food preservation
Dehnad et al., 2014	(Chitosan) nanocellulose	20-50	NR	NR	NR	Packaging	Antimicrobial
Elbarbary et al., 2015	Chitosan	23-82	TEM	NR	Spherical	Food additive	Antioxidant

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Herzallah et al. 2015	Nanoparticulate carboxymethyl cellulose containing sinigrin	50-100	NR	NR	NR	Packaging	Antimicrobial
Joe et al., 2012	Silver (Ag)	NR	NR	NR	NR	Packaging	Antimicrobial
Khan et al., 2014	Chitosan	NR	NR	NR	Rod	Packaging	Antimicrobial
Mahdi et al., 2012	Silver (Ag)	40-50	NR	NR	NR	Packaging	Antimicrobial
Morsy et al., 2014	Silver-Zinc (Ag-Zn)	40-130	NR	NR	NR	Packaging	Antimicrobial
Panea et al., 2014	Silver-Zinc Ag-Zn	NR	NR	NR	NR	Packaging	Antimicrobial
Picouet et al., 2014	Nanoclay	NR	NR	NR	NR	Packaging	Food preservation
Zimoch-Korzycka et al., 2014	Silver (Ag)	10-60	NR	NR	NR	Food additive	Antimicrobial

TEM= Transmission Electron Microscopy, SEM= Scanning Electron Microscopy, NR=Not Reported

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Table 5: Summary of the main characteristics of studies dealing with nanotechnology applications in detection/diagnostic tools applicable to the meat chain

Reference	Material	Size			Shape	Application	Function
		nm	Method 1	Method 2			
Abargues et al., 2014	Silver (Ag)	11	TEM	NR	Spherical	Storage detection	Detection of amine in chicken meat
Abdalhai et al., 2014	Lead sulfide (PbS)	6-May	TEM	NR	Spherical	Microbiological detection	Detection of *Staphylococcus aureus* in fresh beef
Abdalhai et al., 2015	Cadmium sulfide (CdS)	Jul-53	TEM	SEM	NR	Microbiological detection	Determination of *E. coli* O157:H7 in fresh beef
Ahn et al., 2014	Iron oxide (Fe_3O_4)	16	TEM	NR	NR	Chemical detection	Determination of enrofloxacin and metabolites in chicken meat
Ali et al., 2014a	Gold (Au)	40	TEM	NR	Spherical	Microbiological detection	Determination of *E. coli* O157:H7 in yellow corn
Ali et al., 2011a	Gold (Au)	40 ± 5	TEM	NR	NR	Authenticity detection	Determination of venison and shad meat adulteration with pork
Ali et al., 2011b	Gold (Au)	3 ± 0,2	TEM	NR	Spherical	Authenticity detection	Determination of meatball formulation adulteration with pork
Ali et al., 2012a	Gold (Au)	3 ± 0,2	TEM	NR	NR	Authenticity detection	Determination of meat adulteration with pork
Ali et al., 2012b	Gold (Au)	3 ± 0,2	TEM	NR	NR	Authenticity detection	Determination of processed meat product adulteration with pork
Cao et al., 2013	Gold (Au)	16n	TEM	NR	NR	Chemical detection	Detection of cholesterol in meat

Castillo-Garcia et al., 2012	Terbium oxide (Tb_4O_3)	<100 nm	NR	NR	NR	Chemical detection	Determination of lasalocid and salicylate in premix feed
Cho et al., 2015	Iron oxide and gold (Fe_3O_4 and Au)	30	TEM	NR	Spherical	Microbiological detection	Determination of *E. coli* O157:H7 in ground beef
Dang et al., 2014	Silver (Ag)	30	NR	NR	NR	Storage detection	Determination of dielectric permittivity in beef meat
Kim et al., 2015	Cadmium selenide/zinc sulfide (CdSe/ZnS)	20	NR	NR	NR	Microbiological detection	Determination of patogenic *Salmonella* in chicken extract
Jiao et al., 2015	Gold (Au)	20	SEM	NR	NR	Chemical detection	Determination of nitrite ion in meat
Khartik et al., 2013	Iron oxide (Fe_2O_3)	22-56	TEM	NR	NR	Disease detection	Diagnosis of Johne's Disease in goat serum
Kong et al., 2014	Gold (Au)	17	NR	NR	NR	Chemical detection	Determination of ractopamine residues in swine feed
Li.et al., 2014	Gold/silver core (Au/Ag)	35 ± 5	TEM	DLS	Spherical	Chemical detection	Determination of phenylethanolamine in pig urine
Li et al., 2014a	Gold (Au)	25-Oct	TEM	NR	NR	Chemical detection	Detection of 3-amino-5-morpholino-2-oxazolidone (AMOZ) in meat and feed
Li Z. et al., 2012	Gold (Au)	16	NR	NR	NR	Chemical detection	Detection of trace amount of clenbuterol in swine urine
Lin et al., 2012	Gold (Au)	NR	NR	NR	NR	Chemical detection	Determination of ractopamine, salbutamolo, clenbuterol in pig feed and meat products
Long et al., 2015	Iron oxide/muti-walled carbon	40-60	SEM	NR	NR	Chemical detection	Determination of kanamicin a in chicken and pig liver

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	nanotubes (Fe_3O_4/MWCNTs)						
Lu et al., 2015	Iron oxide (Fe_3O_4)	NR	TEM	NR	NR	Chemical detection	Determination of oxytetracycline in chicken meat
Moongkarndi et al., 2011	Gold (Au)	40	NR	NR	NR	Microbiological detection	Detection of *Salmonella enterica* serovars Typhimurium and Enteritidis in chicken serum
Peled et al., 2012	Gold (Au)	<50	TEM	NR	NR	Disease detection	Detection of *M. bovis*-infected cattle (cattle breath)
Peng et al., 2013	Gold (Au)	15	DLS	NR	NR	Chemical detection	Detection of sulfadimethoxine residues in chicken liver
Regiart et al., 2014	Gold-platinum (Au-Pt)	45 ± 5	SEM	NR	NR	Chemical detection	Detection of zeranol residues in ovine urine
Regiart et al., 2013	Gold (Au)	20-50	SEM	XRD	Spherical	Chemical detection	Determination of clenbuterol in bovine hair
Sun et al., 2015	Silica (Si)	50	SEM	NR	Spherical	Microbiological detection	Detection of *Salmonella* infection in chicken serum
Weidemaier et al .,2015	Gold (Au)	60	NR	NR	NR	Microbiological detection	Detection of *E. coli, Salmonella* or *Listeria* in raw ground beef, poultry and deli turkey
Zhang et al., 2014	Ractopamine-tetraphenylborate	40-50	SEM	NR	NR	Chemical detection	Determination of ractopamine residues in pork meat
Zhihua et al., 2015	Zinc oxide (ZnO)	NR	SEM	NR	NR	Chemical detection	Determination of dopamine in meat

TEM= Transmission Electron Microscopy, SEM= Scanning Electron Microscopy,

DLS=Dynamic Light Scattering, NR=Not Reported

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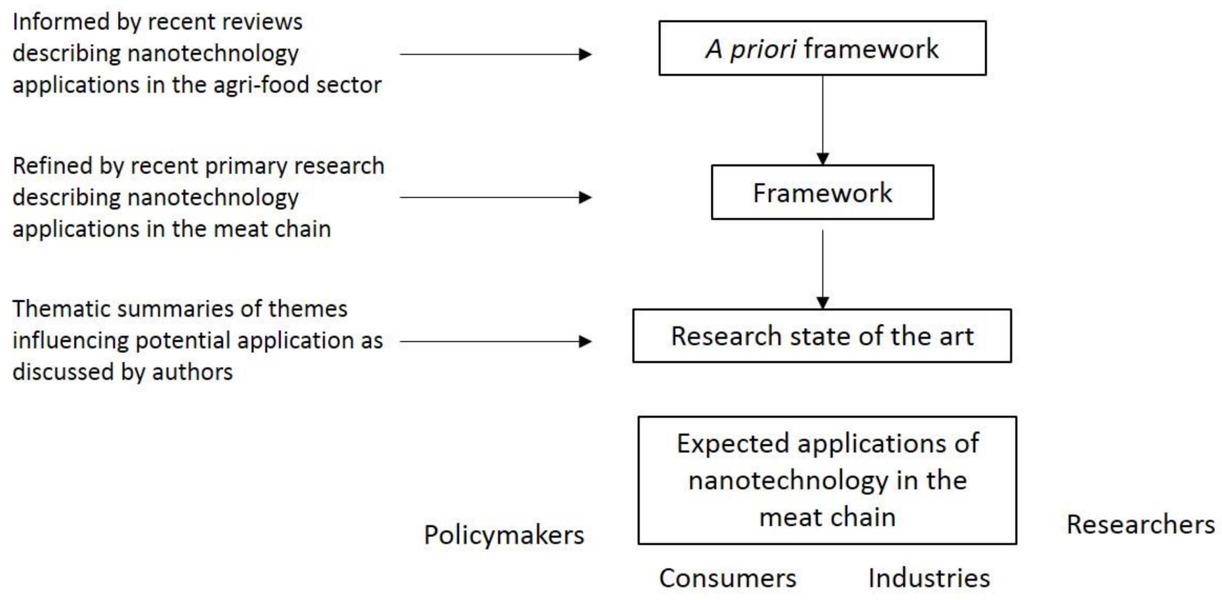

Figure 1: Study design and aims

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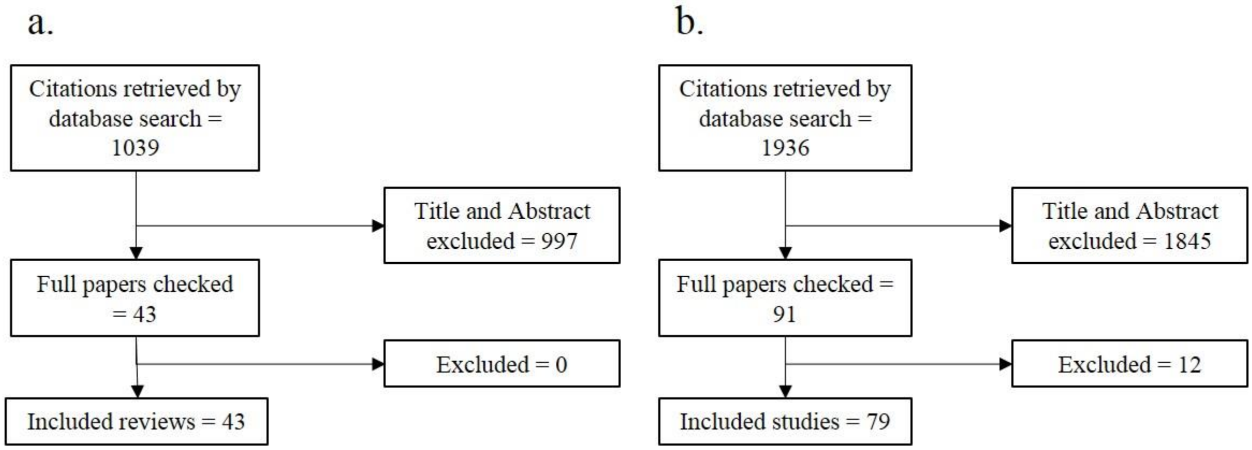

Figure 2: a. Flowchart of study selection for the definition of the *a priori* framework; b. flowchart of study selection for informing the final framework.

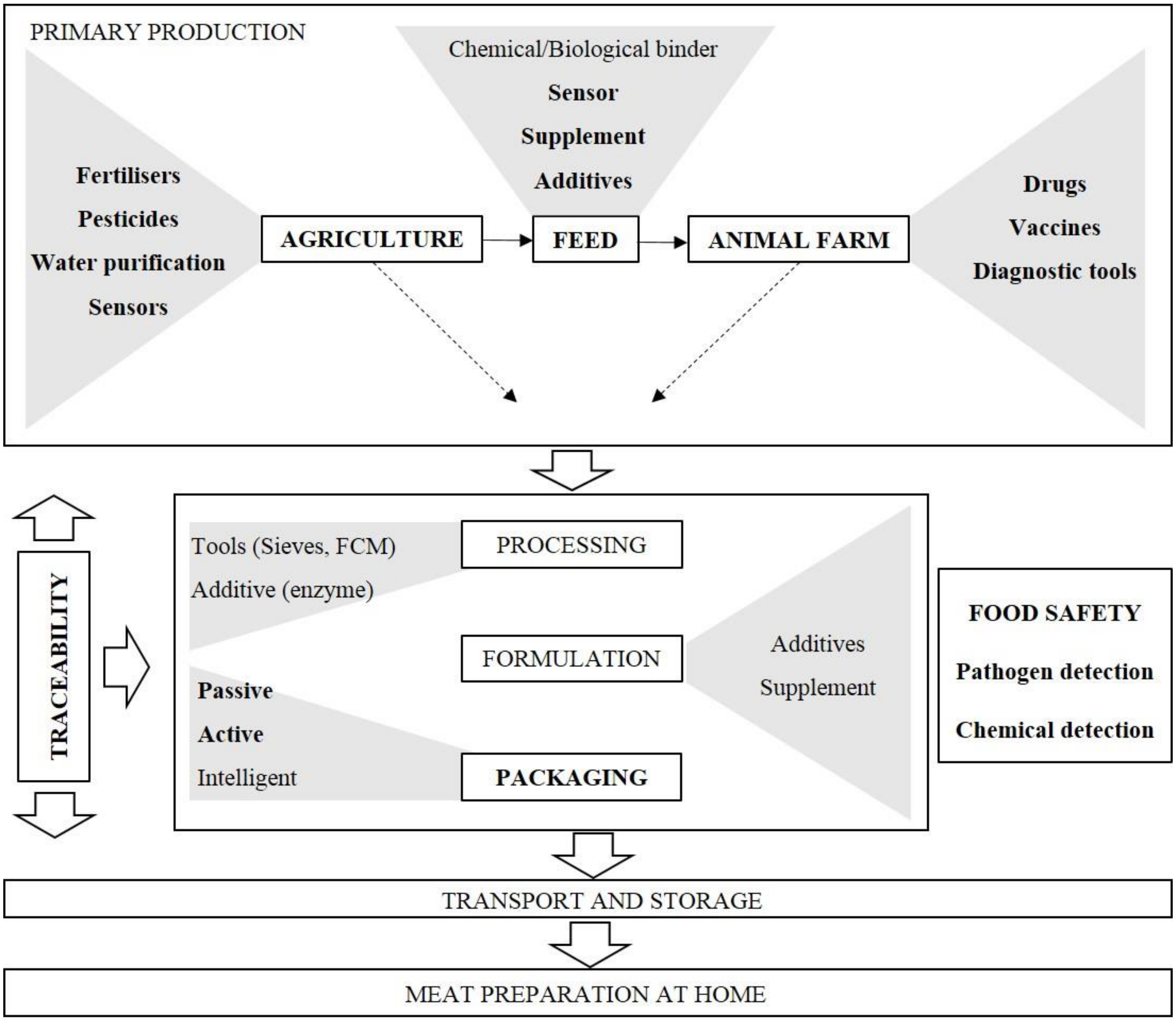

Figure 3: Framework describing nanotechnology application in the meat chain (bold) built upon the *a priori* framework describing applications in the whole agri-food sector (background).