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REVIEW ARTICLE

Use of nanoparticles as feed additives to improve digestion and absorption in livestock

Surej Joseph Bunglavan[1]*, AK Garg, RS Dass and Sameer Shrivastava

CAFT in Animal Nutrition, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, 243122.
[1]Present Address: Assistant Professor, KVASU, Base Farm, Kolahalamedu, Idukki, Kerala, India, 685501.

*Corresponding Author

Surej Joseph Bunglavan

Email: surej138@gmail.com

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Abstract

The word nano technology is derived from the Latin *nanus*, meaning *dwarf*). Nanoparticles are of different types based on their ability to carry different ingredients and react to different environmental conditions. Nanotechnology will play a major role in the future areas of research in animal nutrition. The particle size of minerals as feed additives in nanoparticle form is claimed to be smaller than 100 nanometer, so they can pass through the stomach wall and into body cells more quickly than ordinary minerals with larger particle size. Nanoadditives can also be incorporated in micelles or capsules of protein or another natural food/feed ingredient. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Manipulation of matter at the nanolevel also opens up possibilities for improving the functionality of food/feed molecules to the benefit of product quality.

Key words: Nano-particles, minerals, feed additives, livestock, nutrition.

Introduction

Nanotechnology is defined as the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications" according to National Nanotechnology Initiative (2013), USA. The concept of nanotechnology was first introduced by Nobel Laureate Richard Feynman's (1959) talk entitled "There's plenty of room at the bottom". He is regarded as the father of nanotechnology. A nanometer is one billionth of a meter. The typical nanotechnology utilizes structures under 100 nanometer in size, more than 1000 times narrower than the diameter of a human hair.

Classification of nanoparticles

Nanoparticles can be broadly divided into inorganic, organic, emulsions, dispersions and nano clays based on the chemical characteristics of the nanoparticles (Table 1). Various nano products available for commercial feeding are listed in Table 2.
Organic nanoparticles are likely to be used to enhance the nutrient value of feed systems through improvement or alteration of feed functionality. Organic nanoparticles also referred to as nanocapsules have been designed to deliver vitamins or other nutrients in feed without affecting the taste or appearance. These nanoparticles encapsulate the nutrients and carry them via the gastrointestinal tract (GIT) into the bloodstream, increasing their bioavailability. Also, several types of nanomaterials are considered relevant for applications in feed. Organic nano-materials include proteins, fat and sugar molecules. Nutraceuticals consisting of feed additives derived from plants are also organic nanomaterials used in feed. Nanoparticles already reported to be incorporated into foods/feeds include those engineered to provide encapsulation systems, e.g. micelles, liposomes, for delivery of food/feed ingredients, and those tailored for use in food/feed packaging such as biosensors, identification markers, shelf-life extenders and antimicrobials (FSAI, 2008).

Inorganic nanoparticles are inorganic ingredients manufactured at the nanoscale are variations of additives already approved for use in feed, e.g. titanium dioxide, a feed colorant, can be used as a UV protection barrier in feed packaging when used as a nanoparticle. The most common application is the use of nanoparticles of silver as an antimicrobial. Applications for nanosilver include use in fridge panels, storage boxes, packaging lines and other surfaces which come into contact with feed during -

Table 1: Types of Nano materials used in Animal Nutrition Research (FSAI, 2008)

Category	Example Materials	Example Application
1. Nanoparticles		
Inorganic	Iron	Food / Feed supplement
	Silver	Food / Feed supplements, antimicrobial agent used in food/Feed contact surfaces (cutlery, storage containers, fridges and worktops)
	Iridium	Food / Feed supplement
	Platinuim	Food / Feed supplement
	Zinc	Food / Feed supplement/colourant
Organic	Liposomes	Encapsulation and targeted delivery of food / feed components
	Protein	Re-micellised calcium caseinate from dairy protein. Increased functionality (gelation, heat stability and other properties)
	Polymeric	Non-degradable: polystyrene Biodegradable: PGLA, gelatin, collagen
2. Nanoemulsions/ dispersions		
Emulsions	Oil in water	Stabilisation of biologically active ingredients; delivery of active compounds; extended shelf-life; flavour release; low fat products
Dispersions	Calcium Carbonate	Increased solubility of calcium carbonate–can be used at higher addition levels.
3. Nanoclays	Clay composites	Used in packaging materials to extend shelf-life, durability, and thermal properties (includes nano-laminates)

Table 2: Nano products available for Commercial Feeding (FSAI, 2008)

Product Example	Nanomaterial or Technology Used	Technology Used
MesoSilver MesoGold MesoCopper MesoPlatinum MesoPalladium MesoIridium MesoTitanium MesoZinc	Nanoparticles of silver or gold Copper Platinum Palladium Iridium Titanium Zinc	Food / feed supplements
OilFresh	Zeolite	OilFresh is a device to keep frying oil fresh. OilFresh uses zeolite, a mineral, in the form of beads with an average diameter of 20 nanometers across, coated with an undisclosed material.
Aerosil	Silica Nanoparticles	Used to increase flowability of powdered ingredients.
Titanium dioxide Silicon dioxide Silver	Nanoparticles	Packaging materials containing nanoparticles of titanium dioxide, silicon dioxide or silver, which increase shelf-life of food/feed by modifying mechanical and heat resistance properties, and developing antimicrobial and antifungal surfaces.
MultiSal™	Nanoparticles	Delivery system for active ingredients (water and fat soluble).
Fabuless™	Emulsion	A nanoemulsion that delays digestion until lower regions of the small intestine, stimulating satiety and reduce food/feed intake.
Nutralease	Nanoparticles	Enhances the solubilisation and bioavailability of nutrients.
Canola Active Oil	Nanoclusters	Slimming drink using nanoclusters to enhance flavour without the need for added sugar.
Durethan	Nanoclay	Transparent plastic film (called Durethan) containing nanoparticles of clay to block oxygen, carbon dioxide and moisture from reaching the food/feed.
NovaSOL	Miceller organic nanocapsules	Delivery system for hydrophobic substances.
Encapsome™	liposomes (Micellar)	Nanoparticles capable of carrying both water-soluble and oil- or fat-soluble compounds within a single particle.

manufacture. Feed storage bins are being produced with silver nanoparticles embedded in the plastic, killing bacteria from any feed that was previously stored in the bins and minimising health risks. Inorganic nanomaterials for applications in feed, feed additives, food packaging or storage include nano-clay platelets for feed packaging, minerals such as silicon dioxide, calcium and magnesium and silver nanoparticles for water purification or antimicrobial packaging or feed storage.

Differences between nanomaterials and larger materials

The physical, chemical, electrical, optical, mechanical, and magnetic properties at an atomic scale are quite different from those present at a larger scale, even when compared with those present at a scale of microns (10-6) (Buzea *et al*., 2007). Nanomaterials are different from larger ones because of two effects:

1. **Surface effects:** The atoms of nanomaterials are less stable than those of larger structures since the energy required to join adjacent atoms is less. As a consequence of this, the fusion point of a given element changes. For example, the fusion point of a gold particle measuring 2.5 nm is about 930K (≈ 657°C), which is much lower than 1336K (≈ 1,063°C), the normal fusion point of this metal at greater volumes. Cao (2004) mentioned that this phenomenon is characteristic in metals, inert gases, semiconductors and molecular crystals when the size of the particle is less than 100 nm.

2. **Quantum effects:** Quantum points are a type of nanostructures, just a few nanometers in size that show a behavior similar to a single atom. Their spatial arrangement allows them to have properties not proper to the element, such as magnetism in metals like gold or platinum when they are in the form of nanoparticles.

Preparation and design of nano particles

There are different methods for the preparation of nanoparticles. The selection of any of these methods depends on the particular objectives and conditions for where and how they obtained particles are meant to be used. Thus, it is necessary to consider the physical and chemical stability of the active agent, as well as its toxicity, its liberation profile, among many other considerations. Agnihotri *et al*. (2004) specifies some common methods for the preparation of nanoparticles, such as:

1. **Cross-linking emulsion**: In this method, a water-oil (w/o) emulsion is prepared through emulsification of a watery solution in an oily phase, which when shaken vigorously separates and hardens the particles. It requires the use of agents that facilitate the union of the involved agents.

2. **Precipitation/coacervation:** In this case, the particles are produced by "blowing" the interest agent in an alkaline solution. The separation and purification of the particles is done through filtration and centrifugation, followed by rinsing with hot and cold water.

3. **Spray-drying**: This is one of the best-known techniques used to produce dusts, granules, or agglomerates, besides being an easy and quick way to do it. It is based on the drying of droplets sprayed into compressed hot air. It requires the use of a solvent (for example, a solution of acetic acid), which is instantly evaporated, allowing the formation of particles.

The shape of the nanoparticles strongly influences its biological behavior. These are spherical, rectangular discs, cones, canes, "worms", elliptical or circular discs, "rolls", among many others. All these can come up in the 1st, 2nd or 3rd dimension, depending on the preparation method and the materials used. The viscosity and thickness of the material used determines whether the particle will show sharp or flattened endings. It is even possible that the nanoparticles will show regions with different curvature, texture, concavity, and other characteristics (Champion *et al*., 2007).

Besides capsules, other nanostructured materials can be used, which have the potential of changing the structures of other particles. Some specific examples of these are **fullerenes** (structures made up of 60-80 carbon atoms arranged in spherical shapes, used for the controlled liberation of medication), **dendrimers** (branched structures which, due to their structure, can serve as vehicles for medication, liberating it in a specific location), and **quantum dots** (nanometric crystals designed for optical and electronic applications. When a quantum dot is stimulated, it emits a fluorescence of varying intensity) (Scott, 2005).

Mechanism of action of nanoparticles

The mechanism of action of the nanoparticles were enumerated by (Chen *et al.,* 2006) below:

- Increase the surface area available to interact with biological support
- Prolong compound residence time in GIT
- Decrease influence of intestinal clearance mechanisms
- Penetrate deeply into tissues through fine capillaries
- Cross epithelial lining fenestration (e.g. liver)
- Enable efficient uptake by cells
- Efficient delivery of active compounds to target sites in the body

Benefits

Nanoparticles improve the bioavailability of nutraceutical compounds, carotenoids, ώ- 3 fatty acids, natural antioxidants and trace minerals.

Application of nanotechnology in Animal Nutrition

The various applications of nanotechnology in animal nutrition are depicted in Fig 2. There are mainly four possible applications of nanotechnology in animals: 1) administration of medication, nutrients, probiotics, supplements and other substances, 2) diagnosis and treatment of diseases with nanoparticles that allow the detection and elimination of the cause of the disease without the need for surgery, 3) identity registry that allows a follow up on the history of an animal and its products (meat, milk, eggs, mainly) and 4) management of reproduction with hormonal immunosensors.

Digestion and Absorption

Nanoparticles can enter the gastrointestinal tract (GIT) in many ways such as ingestion directly from food and water and from administration of therapeutic nano-drugs (*Ingestion or swallow pathway*). Inhaled nanoparticles can also be swallowed and enter the GIT following clearance from the respiratory tract (*Inhalation pathway*) (Hoet *et al.,* 2004). Also, oral or smart delivery into GIT (*Oral pathway*), particle uptake in the GIT depends on diffusion and accessibility through mucus and contact with the cells of the GIT. The smaller particle diameter the faster is the diffusion through GIT mucus to reach the cells of the intestinal lining, followed by uptake through the GIT barrier to reach the blood.

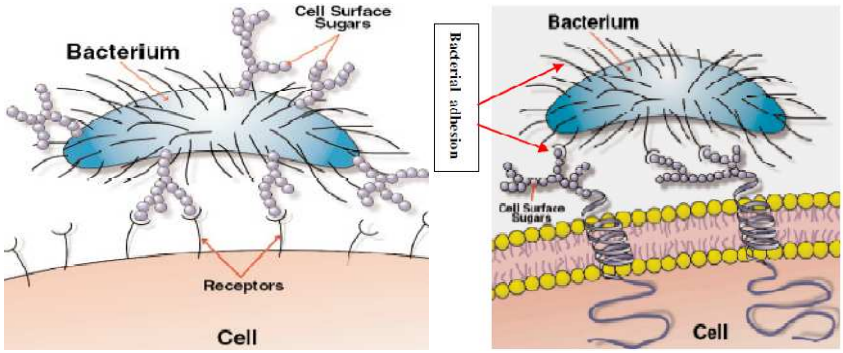
Ingestion or swallow pathway

Here the absorption is through the intestinal tract and little information is currently available concerning the uptake of nanoparticles from the intestinal tract. Uptake occurs variously by passive diffusion across the mucosal cells, via active transport mechanisms and intercellularly (O' Hagan, 1996), nanoparticles that are swallowed will sooner or later end up in the intestinal tract. The particles of under some 300 nm reach the bloodstream, while particles that are smaller than 100 nm are also absorbed in various tissues and organs (Hett, 2004). As a general rule, the smaller the particles are, the more of them are absorbed and the deeper into the body they can go. Insoluble nanoparticles (primarily inorganic, but also including man-made polymeric materials such as polystyrene and carbon-based nanoparticles such as fullerenes) can be predicted to have a more restricted distribution. Following uptake from the GIT, nanoparticles can translocate *via* the lymph system to the liver and spleen, as demonstrated for polystyrene nanoparticles of 100 nm or less (Jani *et al.,* 1990). Smaller particles that are capable of being taken up by the villus epithelium (Hillery *et al.,* 1994) may directly enter the bloodstream, and are then predominantly scavenged by the liver and the spleen. Organic nanoparticles such as casein micelles are likely to behave similarly to their micro or macro equivalents and can be predicted to be readily absorbed and highly bio-available. Insulin encapsulated in vitamin B_{12}-dextran nanoparticles has recently been shown to be taken up from the GIT without degradation (Florence and Hussain, 2001).

Latour and co-workers at Clemson University have recently developed biofunctionalized nanoparticles (BN) (Taylor *et al.,* 2004). BN have attracted interest as a treatment for enteric infection, serving as pathogen purging agents prior to transporting and processing. Adherence to intestinal wall epithelial tissues is facilitated by adhesins, or surface molecules, on a bacterial cell which recognize the receptor sites on the epithelium and is illustrated in Fig 1a-b. The objective of BN development is to create an affinity for these bacterial adhesins. In addition, reports have shown that the presence of D-mannose inhibits the adherence of bacteria to both animal and human intestinal cells (Stanley *et al*., 2000). The attachment of *Campylobacter jejuni* to epithelial cells is also mediated by mannose-specific, lectin-like adhesins present on the bacterial surface which bind to mannose receptor sites. Thus, the BN are hypothesized to be adhesion-specific to the enteropathogen *C. jejuni* (Cinco *et al.,* 1984). Preliminary research has shown that the BN have an affinity for the mannose receptor sites on the *Campylobacter* cell surface and that cell aggregation or attachment between the bacteria and BN

may occur. The ability of these BN to adhere to *Campylobacter* cell surfaces will enable BN to compete with host cell receptors to reduce or eliminate the extent of bacterial colonization on the poultry intestinal wall. BN synthesis is based on the self-assembly of organic polymers structured with intra-polymer binary pseudo-phase separation characteristics (McSweegan and Walker, 1986). The nanoparticle structure consists of a hydrophobic polystyrene core, a hydrophilic tether such as polyethylene glycol and sugars or peptides that are functionally identical to the host cell receptor groups that are bound by the pathogen's surface adhesions. By presenting these same functional groups from the surface of the nanoparticles, the BN should effectively compete with the mucosal lining of the gastrointestinal tract for microbial attachment. Furthermore, because the BN contain a relatively large surface density of functional sites, they should be able to agglutinate large numbers of pathogens, prevent them from binding to their poultry host and enable them to be expelled from the gastrointestinal system.

Nanoparticles diffuse more easily than solid particles and behave more like gas molecules in the air and like large molecules in solutions, being less subject to sedimentation than bigger particles. This may have implications also for the movement of nanoparticles in tissue. Whether nanoparticles enter and transfer within the body to different organs can have a significance importance for the impacts of nanoparticles on human



a b

Fig 1a-b: Cells express various receptors that bind mannose or other sugars expressed on the surface of other cells or bacteria. (Stanley *et al.*, 2000)

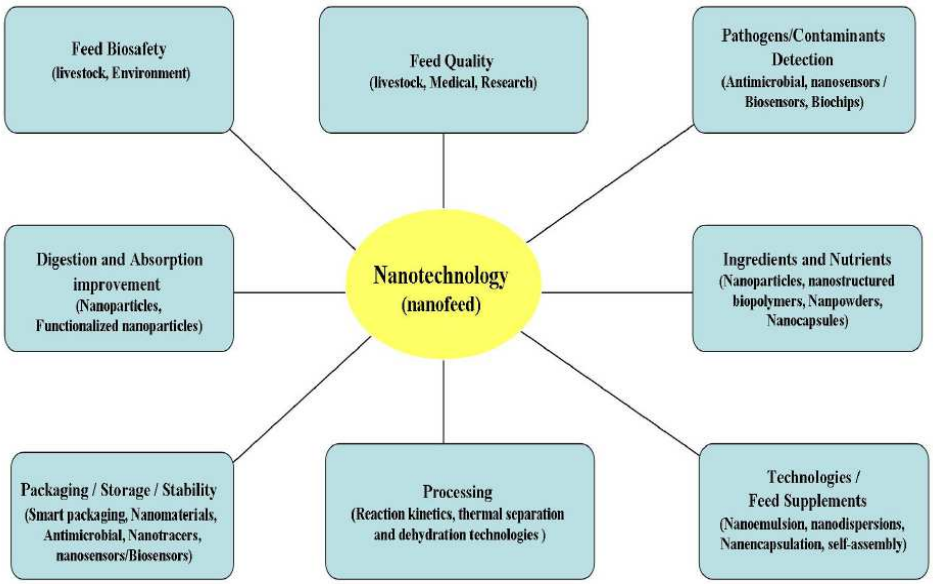


Fig 2: Application of Nanotechnology in Animal Nutrition (FSAI, 2008)

health and in the environment. In a particle translocation experiment (Jani *et al.,* 1990), polystyrene spheres (50 nm-3 μm) were fed by gavage to female Sprague Dawley rats for 10 days, and the results demonstrated that about 34 and 26% of nanoparticles (50 and 100 nm, respectively) were absorbed while particles larger than 300 nm were absent from blood, heart or lung tissue. The smaller is the particle, the faster would be the penetration action across the mucus barrier.

Depending on size, nanoparticles either pass through the GIT without uptake into the body and are eliminated rapidly (Oberdörster *et al.,* 2005), or they cross the lining of the GIT and enter the blood stream, from whence they relocate to other organs. Following uptake by the GIT, gold nanoparticles of less than 50nm translocated to the blood stream and distributed all over the body**.** De Jong *et al.* (2008) demonstrated that tissue distribution of gold nanoparticles after intravenous (IV) administration was size dependent with the smallest (10 nm) particles showing widespread organ distribution. As with absorption, the distribution, breakdown and excretion of nanoparticles in the body will be dependent on physicochemical characteristics such as solubility, charge and size. Distribution/translocation of nanoparticles to body organs is expected to mirror that of their micro or macro equivalents, with small water-soluble or fat soluble organic nanomaterials such as lipid nanoparticles being widely distributed throughout the body. It can be anticipated that distribution and breakdown of such nanoparticles into constituent molecules occurs rapidly and efficiently, with subsequent clearance from the body.

Inhalation pathway

Inhaled ultrafine particles are depending on the particle size, deposited in the nose region and upper and lower level of the respiratory system. Oberdorster *et al*. (2004) concluded that the central nerve system and the brain can be targeted by airborne solid ultrafine particles and that the most likely mechanism is from deposits in the nose region. The study furthermore concluded that, the nose region could provide a portal of entry into the central nerve system for solid ultrafine particles, evading the tight blood-brain barrier. The blood-brain barrier represents a challenging obstacle for a large number of drugs, including antibiotics, antineoplastic agents and a variety of central nervous system-active drugs, especially neuropeptides and scientists have successfully transported drugs through this barrier of drug delivery to the brain by using nanoparticles (Kreuter, 2001). There are a number of studies done that supports the hypothesis that ultrafine particles are able to translocate from the lung into the systemic circulation and reach organs like the liver. The large surface area of nanoparticles also enables to bind, absorb and carry compounds such as drugs, probes and proteins (Bormand Kreyling, 2004), but also other substances like metals or toxic substances. This increasing reactivity with other substances can have consequences both for human health and the environment.

Oral or smart pathway

Nanoparticles are used for oral delivery of peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration (Damge *et al.,* 1990). The gastrointestinal tract provides a variety of physiological and morphological barriers against protein or peptide delivery, e.g. (a) proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin, (b) proteolytic enzymes at the brush border membrane (endopeptidases), (c) bacterial gut flora and (d) mucus layer and epithelial cell lining itself (Lee and Yamamoto, 1990). One important strategy to overcome the gastrointestinal barrier is to deliver the drug in a colloidal carrier system, such as nanoparticles, which is capable of enhancing the interaction mechanisms of the drug delivery system and the epithelia cells in the GIT. Targeting of nanoparticles to epithelial cells in the GI tract using ligands, targeting strategies to improve the interaction of nanoparticles with adsorptive enterocytes and mononuclear cell (M-cells) of Peyer's patches in the GI tract can be classified into those utilizing specific binding to ligands or receptors and those based on nonspecific adsorptive mechanism. The surface of enterocytes and M cells display cell-specific carbohydrates, which may serve as binding sites to colloidal drug carriers containing appropriate ligands. Certain glycoproteins and lectins bind selectively to this type of surface structure by specific receptor-mediated mechanism. Different lectins, such as bean lectin and tomato lectin, have been studied to enhance oral peptide adsorption (Hussain *et al.,* 1997). Vitamin B_{12} absorption from the gut under physiological conditions occurs via receptor-mediated endocytosis. The ability to increase oral bioavailability of various

peptides (e.g., granulocyte colony stimulating factor, erythropoietin) and particles by covalent coupling to vitamin B_{12} has been studied by Russell-Jones *et al.* (2001). For this intrinsic process, mucoprotein is required, which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin. The mucoprotein completely reaches the ileum where resorption is mediated by specific receptors. Absorption enhancement using non-specific interactions, in general, the gastrointestinal absorption of macromolecules and particulate materials involves either paracellular route or endocytotic pathway. The paracellular route of absorption of nanoparticles utilizes less than 1% of mucosal surface area. Using polymers such as chitosan (Schipper *et al.,* 1999), starch (Lehr *et al.,* 1990) or poly (acrylate) (Bjork *et al.,* 1995) can increase the paracellular permeability of macromolecules.

Endocytotic pathway for absorption of nanoparticles is either by receptor-mediated endocytosis, that is, active targeting, or adsorptive endocytosis which does not need any ligands. This process is initiated by an unspecific physical adsorption of material to the cell surface by electrostatic forces such as hydrogen bonding or hydrophobic interactions (Florence and Hussain, 2001). Adsorptive endocytosis depends primarily on the size and surface properties of the material. If the surface charge of the nanoparticles is positive or uncharged, it will provide an affinity to adsorptive enterocytes though hydrophobic, whereas if it is negatively charged and hydrophilic, it shows greater affinity to adsorptive enterocytes and M cells. This shows that a combination of size, surface charge and hydrophilicity play a major role in affinity. This is demonstrated with poly (styrene) nanoparticles and when it is carboxylated (Jani *et al.,* 1989).

Feed additives

Minute micelles (nanocapsules) are used as carriers for essential oils, flavor, antioxidant, coenzyme Q10 and vitamins, minerals and phytochemicals to improve their bioavailability (ElAmin, 2006). Encapsulating the nanoparticles of active ingredients (e.g. polyphenols, minerals and micronutrients) to protect them from oxidation and getting to the taste receptor site, thus to reduce their undesirable off-tastes in the finished application (Heller, 2006). In food industry application of liposomal nanovesicles for the encapsulation and delivery of nutrients and functional ingredients such as proteins, enzymes, flavors and antimicrobial compounds were conducted (Wen *et al.,* 2006).

The particle size of minerals as feed additives in nanoparticle form is claimed to be smaller than 100 nanometre so, they can pass through the stomach wall and into body cells more quickly than ordinary minerals with larger particle size. Nano-additives can also be incorporated in micelles or capsules of protein or another natural food/feed ingredient. Micelles are tiny spheres of oil or fat coated with a thin layer of bipolar molecules of which one end is soluble in fat and the other in water. The micelles are suspended in water, or conversely, water is encapsulated in micelles and suspended in oil. Such nanocapsules can contain healthy omega 3 fish oil (ώ3 fatty acids) which has a strong and unpleasant taste and only release it in the stomach.

The wet milling of inexpensive feedstock and silicon nanoparticle consolidation as food additive releases orthosilicic acid in the gut, the bioavailable form of silicon for which proposed beneficial roles are under increasing scrutiny for the prevention of osteoporosis (Canham, 2007). Vitamin E is sensitive to light, heat and oxygen. Its stability can be affected when stored in food for relatively short periods of time. Natural sources of vitamin E include vegetable and soybean oils. Synthetic versions are less expensive, but have lower biological activity. Incorporation of the high viscosity liquid form (vitamin E 97%) was assessed gravimetrically by weight increase. Mesoporous silicon segments of 65% porosity and 158 μm thickness were immersed for varying times at room temperature, and residual liquid on the outer surface removed by pressure onto filter paper. The capillary forces are sufficiently large to achieve partial loading in short periods (Canham, 2007).

Polyunsaturated fatty acids are increasingly being considered for improving cardiovascular parameters. A commercial formulation (Vertese™-Omega 3, 6 and 9) was used for incorporation. The oil blend contributes alphalinoleic acid, linoleic acid, oleic acid, palmitic acid and stearic acid. Capsules were broken and the oils extracted and pooled. Immersion of the silicon membranes was carried out for 1 h and at room temperature. After excess oil removal by filter paper, the weight increase was equivalent to an average of 41 wt% loading throughout the silicon. Cross-sectional EDX spectra confirmed the uniformity throughout and high level of loading into the silicon structure, with a very high carbon to oxygen ratio consistent with the chemical composition of such oils (Canham, 2007).

Upon Nano-Se supplementation in sheep at rate of 3ppm in basal diet ruminal pH (range of 6.68-6.80) and ammonia N concentration (range of 9.95-12.49 mg/100 mL) was decreased ($p<0.01$), and total VFA concentration (range of 73.63-77.72mM) was increased linearly ($p<0.01$) and quadratically ($p<0.01$) with increasing nano-Se supplementation (Shi *et al.,* 2011a). The ratio of acetate to propionate was linearly ($p<0.01$)

and quadratically (p<0.01) decreased due to the increasing of propionate concentration. *In situ* ruminal neutral detergent fiber (NDF) degradation of *Leymuschinensis* and crude protein (CP) of soybean meal were linearly (p<0.01) and quadratically (p<0.01) improved by feeding nano-Se (Shi *et al.,* 2011a). Similarly, nutrients digestibility in the total tract and urinary excretion of purine derivatives were also quadratically (p<0.01) changed by increasing nano-Se supplementation (Shi *et al.,* 2011a).

Dietary supplementation of nanoselenium in male goats at the rate of 0.3ppm showed that the final body weight was increased (p<0.05) in bucks supplemented with Se compared to the controls, and ADG in nanoselenium and seleno-yeast supplemented groups were greater (p<0.05) than sodium selenite or control bucks (Shi *et al.,* 2011b). Whole blood, serum and tissue Se concentration, serum antioxidant enzymes activity were also affected by dietary Se supplementation. Serum GSH-Px, SOD and CAT in nanoselenium supplemented group were higher (p<0.05) than those in sodium selenite and selenoyeast supplementation groups and Se retention of whole blood, serum and some organs in nanoselenium were also higher than sodium selenite and selenoyeast supplementation groups (p<0.05) (Shi *et al.*, 2011b).

Dietary supplementation of chromium (Cr) as chromium nanocomposite (CrNano) at the rate of 200µg in finishing pigs significantly reduced serum levels of glucose, urea nitrogen, triglyceride, cholesterol and non-esterified fatty acid. In contrast, serum levels of total protein, high density lipoprotein and lipase activity were significantly increased in pigs offered the diets supplemented with CrNano. Supplementation of the diet with CrNano also increased serum insulin-like growth factor I and reduced serum insulin and cortisol levels significantly. In addition, supplemental CrNano resulted in significant increments of immunoglobulin M, immunoglobulin G contents in plasma (Wang *et al.,* 2007).

Also, dietary supplementation of chromium (Cr) as chromium nanocomposite (CrNano) at the rate of 200µg in finishing pigs resulted in higher (p<0.05) carcass lean percentage, 19.96% (p<0.05) larger *Longissimus* muscle area and 25.53% lower (p<0.05) carcass fat percentage, 18.22% lower (p<0.05) backfat thickness. Drip loss in chops from pigs fed CrNano was decreased by 21.48% (p<0.05) and weights of *Longissimus* muscle and *Semimembranosus* were increased by 16.33% (p<0.05) and 14.87% (p<0.05) respectively (Wang and Xu, 2004). In addition, supplemental CrNano resulted in 184.11% (p<0.05), 144.99% (p<0.05), 88.13% (p<0.05) and 52.60% (p<0.05) increment of Cr concentration in *Longissimus* muscle, liver, kidney and heart, respectively. These results suggest that supplemental CrNano has beneficial effects on carcass characteristics, pork quality and individual skeletal muscle weight, increase tissue chromium concentration in selected muscle and organs (Wang and Xu, 2004).

The supplementation of nano copper (Cu) in piglets at the rate of 50ppm produced statistically significant improvements in growth performance of the piglets when copper was supplemented at nanosize. Copper availability was significantly improved and fecal copper level was reduced in the nanoCu supplemented group, as compared to the copper sulphate ($CuSO_4$) group. Significant differences were observed in the improvement of the digestibility of crude fat and energy in pigs under nanoCu diet. The serum copper level and serum cholesterol concentrations, as well as hematology traits *viz.* RBC, WBC, MCV, HGB, HCT, PLT and RDW, were not affected by nanoCu supplementation. Statistically significant improvements were observed in the IgG, γ-globulin and total globulin protein levels, and in the SOD activity of the nanoCu group (Eguia *et al.,* 2009). Coliform reduction in ileal contents was observed *in vivo* by Fondevila *et al.* (2009) when 20 and 40 ppm of metallic silver nanoparticles were given to weaned piglets as metallic silver adsorbed in a sepiolite matrix as antimicrobial and growth promoter for weaned pigs during their transition phase (from 5 to 20 kg weight). Besides, although concentration of major bacterial groups in the ileum of pigs were not markedly affected, the concentration of the pathogen *Clostridium perfringens/ Cl. histolyticum* group was reduced with 20 ppm silver ($p = 0.012$).

Effect on feed quality and nutritional values

Liquid droplet technology named 'Nano-sized Self-assembled Liquid Structures (NSLS) involves encapsulation and release particles in cells. The micellar particles are used to encapsulate nutraceuticals (beta-carotene, CoQ10, docosahexaenoic acid/eicosapentaenoic acid (DHA/EPA) and other compounds) into 30nm diameter self assembled spheres. Micelles are organic nanoparticulates that can be assembled by the thermodynamically driven process known as self-assembly. Micelles made in this way have the ability to encapsulate non-polar molecules such as lipids, flavours, antimicrobials, antioxidants and vitamins (Chen *et al.,* 2006). Compounds that are normally insoluble or only sparingly soluble in water can be made water soluble, extending their use in foods/feeds and potentially changing their bioavailability once ingested. The micelles are essentially made from lipid molecules and have a unique hydrophobic interior. The NSLS particles are

reported to act as vehicles for compounds to be absorbed into the bloodstream from the gut more readily, increasing their bioavailability.

Liposomes are another example of micelles and can be used to encapsulate both water and lipid soluble compounds (Taylor *et al.,* 2005). The dissolution of fat-soluble nutrients in water-based drinks is one of the key applications of liposomes. Liposomes can be produced to differing sizes (10-500nm) and engineered to have different stability and/or surface charge under different environmental conditions. Liposome technology can be used potentially to target specific sites within a food/feed product for enzymatic degradation.

Nanoemulsions are emulsions which are thermodynamically stable compared to conventional emulsions under a range of different conditions. This is due to their small size (typically 50 to 500nm compared to 1200nm) and monodispersivity. They can be diluted with water without changing the droplet size distribution. The type of surfactant used to formulate a nanoemulsion is critical to the stability of the final emulsion. Preparations of nanoemulsions can be used to encapsulate functional food/feed components at oil/water interfaces, or throughout the continuous phase of the system (Weiss *et al.,* 2006). The applications of nanoemulsions include: delivery of active compounds in the body, stabilization of biologically active ingredients, extended shelf-life due to increased stability and increased viscosity at lower concentrations of oil phase.

Research has shown that stabilized mono-dispersed oil-in-water (O/W) or water-in-oil (W/O) nanoemulsion systems can be used for controlled release of nutraceutical and other bioactive components in food/feed (Weiss *et al*., 2006). The technology has been combined with advanced processing technologies to develop novel microencapsulated products that allow controlled release of food/feed bioactives in the gastrointestinal tract. These products may be either ready-to-drink or powdered formulations fortified with functional ingredients from a wide range of sources. The physical properties of nanoemulsions such as rheological and micro structural properties, phase separation behavior and stability in food/feed products is significantly different to those found in emulsions manufactured using standard emulsification techniques. Because of their small particle size, nano emulsions may be used in the future in the development of low fat products due to the viscosity that is imparted at low oil droplet concentrations.

Manipulation of matter at the nano level also opens up possibilities for improving the functionality of food/feed molecules, to the benefit of product quality. Dziechciarek *et al*. (1998) have developed starch-based nanoparticles that behave like colloids in aqueous solution, and can be used in food/feed applications such as mixing, emulsification and imparting specific rheology to foods/feeds.

Another aspect to be considered is the characterization of the nanoparticles under a range of relevant biological conditions, such as in complex food/feed products, where they can interact with proteins, lipids, sugars or other biomolecules. This may have consequences for the surface composition of the nanoparticles and their aggregation behaviour, as the adsorbed proteins and biomolecules may have different hydrophobicity, charge and charge distribution than the as-synthesised nanoparticles. The adsorbed proteins and other biomolecules confer a "biological identity" (Lynch *et al.,* 2007) to the nanoparticles, as it is these adsorbed molecules that are responsible for the primary interaction with living systems. Additionally, the nanoparticles can alter the functioning of the adsorbed biological molecules, e.g. enzyme activity, degradation and other properties, making them more or less active than the unbound form (Palocci *et al*., 2007).

An additional aspect of the adsorption of biomolecules to the surface of nanoparticles is the effect on the conformation of proteins such as enzymes, and also on their function, stability, activity and aggregation state, among other properties. There are a number of examples of enhanced enzyme stability and function following adsorption to nanoparticles, e.g. the lifetime of the enzymes trypsin and peroxidase was shown to increase dramatically, from a few hours to weeks, by attaching them to magnetic iron nanoparticles (Sharma *et al*., 2007). This ability to enhance protein stability by interfacing them with nanomaterials may impact numerous biological processes such as digestion, metabolism and nutrient uptake.

Feed processing

The ultrafine dimensions of nanoparticles, and consequently their very large surface area, enable them to function more effectively than conventional macro-scale structures. New types of membranes including micro and nano-sieves can be applied in food/feed processing. The pores of the sieves are in the micrometer and nanometer range. They can also be used for encapsulating valuable food/feed ingredients such as minerals in a coating of another ingredient to boost take up by the body or to avoid these ingredients being lost during processing. Nanotechnology is already making an impact on the development of functional or interactive foods/feeds, which respond to the body's requirements and can deliver nutrients more efficiently. Various research groups are also working to develop new "on demand" foods/feeds, which will remain dormant in the body and deliver nutrients to

cells when needed. A key element in this sector is the development of nanocapsules that can be incorporated into food/feed to deliver nutrients. Other developments in food/feed processing include the addition of nanoparticles to existing foods to enable increased absorption of nutrients. Nanocochelates, which are 50 nm coiled nanoparticles and can be used to deliver nutrients such as vitamins, lycopene and omega fatty acids more efficiently to cells, without affecting the colour or taste of food/feed.

Risks and hazards

Risk assessment consists of four components: hazard identification, hazard characterization, exposure assessment, and risk characterization. All four of these stages are essential to the process of risk assessment. A substance may be extremely hazardous, but have a small exposure potential, and the risk may be small, whereas something that is of limited hazard but to which exposure is high and/or over long periods may present a much greater risk. It is essential to characterize both the nature of the hazard and the exposure (FSAI, 2008).

The specific hazard issues relating to feeding of nanoparticles include (i) the increased bioavailability of nanoparticles compared with the macro-forms of the same material, (ii) the potential role of nanoparticles induced ROS in inflammatory digestive diseases (ii) the potential effects of nanoparticles on protein and enzyme stability and functionality whereby the metabolic processes may be disrupted, or nutrient bioavailability may be altered, (iii) the potential effects of storage, heating/and ageing on nanoparticles biomolecule complexes in feed (FSAI, 2008).

Conclusion

Nanotechnology can be applied in the production of nanoparticles which can be used in improving the digestion and absorption in livestock both as novel food ingredients or additives and for improving food safety and quality control. Nanotechnology is in constant development and its applications are ever more varied and specific, with a high potential for improving livestock production and animals in general. The study of nanotechnology in these areas is still very limited. Nanoparticle incorporation in animal nutrition studies which can greatly enhance the efficiency of growth and production of livestock should be conducted at a lower risk to consumers. However, a great amount of research is still required to support the effectiveness, and mainly the safety of nanotechnology, avoiding any harm to the livestock, environment and to human beings.

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