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MICROENCAPSULATION OF FEED ADDITIVES WITH POTENTIAL IN LIVESTOCK AND POULTRY PRODUCTION: A SYSTEMATIC REVIEW

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ABSTRACT

The expected population growth will increase global food consumption, particularly meat consumption, which is estimated to increase 14% by 2030. Hence, the efficient utilization of all the resources involved in meat production, predominantly feed additives in livestock, is important due to economic costs and the high environmental in terms of gas production and ammonia excretion. Efforts have been made to increase efficiency in livestock production and improve the absorption and utilization of nutrients. Nevertheless, advances in technology in the chemical, pharmaceutical, and food industries have barely been used by the livestock and poultry industry. The micro/nano encapsulation process has been used in animal nutrition to protect bioactive compounds or to control the release of feed additives into the animal gastrointestinal tract, avoiding rumen microbes attack, or monogastric digestion in swine and poultry, to be available in the small intestine. However, not all the encapsulation techniques are suitable for applications in animal feeding. For example, spray drying, emulsion and coacervation can be used to control the release of feed additives in ruminants. In this sense, micro encapsulation of different feed additives such as amino acids, fatty acids, and probiotics may face enormous challenges to help improve livestock and poultry nutrition. The objective of this review is to highlight and discuss the techniques, compounds, and key aspects involved in the encapsulation of feed additives and nutrients with potential applications in the livestock and poultry production.

Keywords: Controlled release, protection, encapsulation, nutrients, additives.

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INTRODUCTION

Microencapsulation is related to physicochemical or mechanical processes by which a substance is embedded in another material (Yang et al., 2020). Environmental factors (i.e., oxygen, water, pH and interactions with other ingredients) can affect the stability of active compounds (Kumari et al., 2020). Hence, encapsulation processes have been used to protect these active compounds to deliver a controlled release, reduce adherence during storage and transport, or avoid changes in their physicochemical properties. Obtained products from the different encapsulation technologies can be classified according to the size of the final product. They are called capsules or macro-capsules when they are larger than 5,000 μm, microcapsules when the size ranges between 0.1 to 5,000 μm and nano-capsules when they are less than 0.1 μm (Murugesan and Orsat, 2012). In the last decades this technology became of a matter of interest due to its potential to protect active ingredients and allow their controlled release. For example, microencapsulation of particular nutrients (amino acids, fatty acids, and essential oils) has allowed a better synchronization of the ruminal degradation rate, favoring transport of feed additives into the small intestine. It is worth mentioning that there is still controversy regarding the size limits to define the micro and nano levels in encapsulation, including diameters between 1 to 1000 μm at the micro level and particles between 0.01 and 1 μm at the nano level. However, there is an overlap in the boundaries to define the micro and nano level, as the nano level often includes only smaller particles than 100 nm (0.1 μm). Several studies reviewed in this article do not provide details regarding size of encapsulation, since commercial and patented materials are evaluated, and trade secrecy does not allow wall materials or encapsulation techniques to be described. As in the food industry, the encapsulation process in animal nutrition has been introduced in the last few years to accomplish different objectives; for instance, to improve the intestinal delivery of bioactive molecules (*e.g.* fatty acids, amino acids, antioxidants, and enzymes) or living microorganisms such as probiotics (De Vos et al., 2010). This review focuses on the encapsulation of different feed additives aimed at improving livestock and poultry nutrition. Plant feeds supplied for animal production contain protein that can be digested by microbial fermentation in the rumen, including both essential amino acids (lysine, methionine, arginine, histidine, isoleucine, leucine, threonine, tryptophan, and valine) and non-essential amino acids. Therefore, animal production is subject to the passage of essential amino acids through the rumen so that they can be absorbed and bioavailable in the small intestine, and thus the protection of essential amino acids is necessary.

MATERIALS AND METHODS

Search strategy and selection criteria

The search for information was focused on studies reporting the use of microencapsulation processes in livestock and poultry production and the most widely used materials. The information was organized according to the following topics: microencapsulation techniques (the most widely used and easy to replicate), morphology (microcapsule and microsphere), wall materials, and the encapsulation of the most common feed additives.

The publications were obtained from databases such as Wiley Online Library, Springer Link, Elsevier, and Scopus, covering the years 2013-2022. Only full-length research articles and review papers were considered. The keywords used to search were microencapsulation techniques, protection, encapsulation, microparticles, feed, additives, ingredients, protein, minerals, probiotics, amino acids, essential oils, bovine, poultry, swine, and ruminant. The search terms within a string included "title, abstract, and keyword" searching, with the help of Boolean operators ("AND", "OR", and "NOT"). After that, 119 articles were included in the database shown in the PRISMA Flow Diagram (Fig. 1).

MICROCAPSULE AND MICROSPHERE COMPOSITION

Microcapsules consist of two components: the core material and the coating or envelope material. The core material contains an active ingredient, while the cladding or covering material covers or protects the core material (Fig. 2) (Jyothi et al., 2010). Microspheres and microcapsules are spherical microparticles with small diameters (microns or nanometers). They are generally made of a biodegradable or resorbable plastic polymer and filled with a substance (*i.e.* drug or food molecules) for controlled release (Wang et al., 2013). Microspheres are matrix type, characterized by the encapsulation of substance and polymers in a uniform mixture. In contrast, microcapsules are reservoir type, and the encapsulated substance is dispersed in the polymer cavity, forming agglomerates with a well-defined core (Fig. 2) (Wang et al., 2013; Dias et al., 2015).

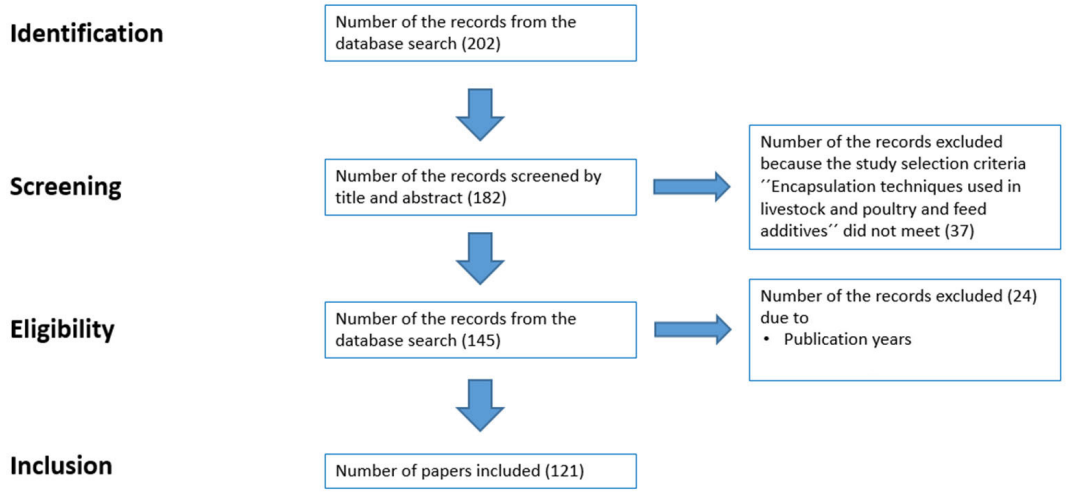

Fig. 1. PRISMA study flow of the selection process of the reviewed literature from initial search and screening to the final selection of publications.

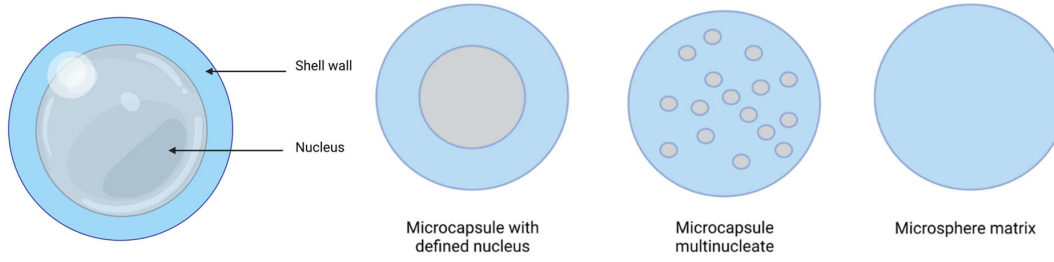

Fig. 2. Components and morphology of microcapsule and microsphere.

There is a wide variety of coating materials for microencapsulation. Numerous traditional coating materials seem to suit their use for protection in the gastrointestinal tract. These materials include inert and pH-sensitive polymers such as carboxylate and amino derivatives, which can be dissolved depending on the degree of crosslinking. Generally, hydrophilic and hydrophobic polymers or their combination are used for microencapsulation process. Several coating materials have been successfully used in the industry, such as gelatine, polyvinyl alcohol, ethyl cellulose, cellulose acetate phthalate, and styrene maleic anhydride (Singh et al., 2010). Coating materials must be applied to the core material to accomplish a specific purpose. In addition, the core material can be in solid form or in the form of liquid droplets and dispersions depending on the material application.

Different techniques for encapsulation have been developed to achieve the wanted shell and core characteristics, as well as to reduce the time and improve the bioavailability of the core.

MICROENCAPSULATION TECHNIQUES

Encapsulation techniques can be classified into chemical, physicochemical, and physical-mechanical methods, according to the basis for the synthesis technique (see Table 1) (Jyothi et al., 2010). One of the main techniques for the encapsulation of organic compounds is spray drying, which is a type of physical-mechanical technique. Spray drying, followed by emulsification, freeze drying and coacervation were the most widely used techniques between 2009 and 2019 (Yang et al., 2020).

According to capsule size, encapsulation is classified into two main types: a) microencapsulation; and b) nanoencapsulation.

Table 1. Classification of techniques used for microencapsulation modified from Ghosh (2006).

Chemical	Physicochemical	Physical-mechanical
Interfacial polymerization	Coacervation and phase separation	Spray drying and freezing
In situ polymerization	Sol-gel encapsulation	Fluidized Bed Coating
Polycondensation	Supercritical CO_2 assisted micro encapsulation	Solvent evaporation
Layer-by-layer Emulsification	Supercritical Electro-spraying Nanoprecipitation	Extrusion

Microencapsulation consists of coating micron-sized solid or liquid particles with a wall material. The particle size ranges between 1 to 1,000 μm (Ozkan et al., 2019). Examples of microencapsulation techniques are spray drying, emulsification, spray freezing, and coacervation. Besides, nanoencapsulation techniques for small-scale substances are considered bioactive packaging at the nano-scale level (between 10 to 1,000 nm). The most utilized techniques for nanoencapsulation are emulsification by solvent evaporation and nanoprecipitation (Pathak et al., 2019).

Spray drying

Spray drying is the most widely used technique for microencapsulation. It is a continuous process that produces dry particles of reliable quality, while the equipment required for the process is easily acquired (Akhavan Mahdavi et al., 2016). Spray drying is a low-cost, simple, and flexible commercial process mainly used for the encapsulation of fragrances, oils, and flavors. The particle size usually obtained by this method ranges between 10 and 100 μm. In general, the core particles are dispersed in a polymer solution and sprayed in a hot chamber (Jyothi et al., 2010). The most common wall materials in spray drying are polysaccharides (gums or starch) or proteins (gelatin and milk protein) (Timilsena et al., 2020).

Spray drying process consists of four stages. The first stage is preparing the feed fluid, basically by mixing the material to be encapsulated and the encapsulant. The second stage is the homogenization of the fluid, while the third stage consists of atomization by a nozzle or disk. Finally, stage four is the dehydration of the atomized particles. In other words, the droplets encounter the air at high temperature, and later the water or solvent evaporates and macroparticles are obtained. As example, hydrolyzed casein has been encapsulated in maltodextrin (ratio 40:60, m/m) using the spray drying technique. The hygroscopicity of casein was reduced after encapsulation, while the antioxidant properties remained within normal ranges after the spray drying process. The spray drying process was considered effective due to the reduction of off-taste in the hydrolyzed casein (Sarabandi et al., 2018).

'Bypass' protein is the protein portion of the diet that escapes the digestion of the rumen microorganisms in ruminants. It is of economical, physiological, and environmental relevance in ruminant nutrition, because it is required for achieving the animal's potential for growth, production, and reproductive performance. Furthermore, it is the protein that the animal would use to supply limiting amino acids such as lysine and methionine to the small intestine (Lee et al., 2012). An increase of bypass protein or bypass amino acids in ruminant diets may help to reduce nitrogen excretion by reducing crude protein inclusions (Broderick et al., 2008). Methionine, which is an essential amino acid in farm animals, has been encapsulated by spray drying using a 60 g mixture containing gelatin and sodium alginate (40 and 20 g, respectively). Evaluated rations of methionine:gelatin/alginate mix were 1:5, 1:1, and 1:1.5. The highest yield and efficiency of methionine spray drying microencapsulation was achieved with the 1:1 ratio, and the produced microcapsules had a regular spheric shape. In the *in vitro* release study, the microcapsules regulated the release of methionine and stimulated the absorption of other amino acids (Niu et al., 2015).

Rajam and Anandharamakrishnan (2014) microencapsulated *Lactobacillus platarum* (probiotic) using a mixture of fructo-oligosaccharides (FOS), whey protein isolate (WPI), and denatured whey protein isolate (DWPI). The studied rations of prebiotic:polymer

were 1: 1 and 1: 1.5. The FOS + WPI and FOS + DWPI combinations had higher encapsulation efficiency, lower residual moisture, and a shorter range of particle size (higher size homogeneity). However, 1:1.5 ratios increased the storage stability and tolerance of the probiotic to gastric and intestinal simulation. Maciel et al. (2014) microencapsulated *Lactobacillus acidophilus* using sweet whey and skimmed milk as coating materials by spray-drying. Both wall materials provided protection during the gastrointestinal simulation and, regardless of the material, viability was reduced after 90 d of storage.

Sarteshnizi et al. (2018) produced a feed additive from the waste of the ruminal fluid obtained after the slaughter of the animals with enzymatic potential to degrade carbohydrates. The ruminal content was encapsulated with sodium alginate (RA), guar gum (RG), chitosan (RC) and maltodextrin (RM), in proportions of 0.5 and 1% (m / v). Residual activities increased with cellulase, avicelase, amylase, and filter paperase, in comparison to those in fresh ruminal fluid. The addition of 1% maltodextrin showed the highest retention of enzyme activity.

As mentioned before, spray-drying encapsulation has shown a wide range of applications in animal feeding, varying from prebiotic to enzymatic functions. Additionally, it is a straight, full, and low-cost methodology that allows a quick and efficient way to encapsulate for industrial and research purposes. Besides, this technique confers thermic stability to biomaterials like proteins and amino acids. This is helpful in animal nutrition, particularly in ruminants, because it is required to increase the protein bypass and reduce the amount of protein in the diet at the same time. Other applications will be discussed later in this article.

Emulsification

An emulsion is a fine dispersion of two or more immiscible liquids, where one of the liquids contains a dispersion of small drops of the other liquid (McClements, 2015). In microencapsulation, a liquid core material is dispersed into an immiscible liquid phase that may contain a dissolved layer material. Later, a change is made in the two-phase system to induce layer formation around the drops of the dispersed phase (Fig. 3) (Arenas-Jal et al., 2020).

Microencapsulation by emulsification has been efficiently developed to encapsulate protein compounds. De Carvalho et al. (2019) microencapsulated methionine using carnauba wax as wall material, seeking to deposit the amino acid in the intestine after ruminal by-pass. Ruminal microorganisms did not attack microencapsulated methionine after *in situ* evaluation in sheep. Dry matter and crude protein had higher degradability when intact methionine was added in comparison to the wax microencapsulated treatments. The 4:1 ratio had the highest ruminal protection of methionine and lowest crude protein loss.

De Medeiros et al. (2019) succesfully microencapsulated urea with carnauba wax to obtain a system of slow release in the rumen, seeking to avoid possible toxicity and a more efficient use of ruminal nitrogen by

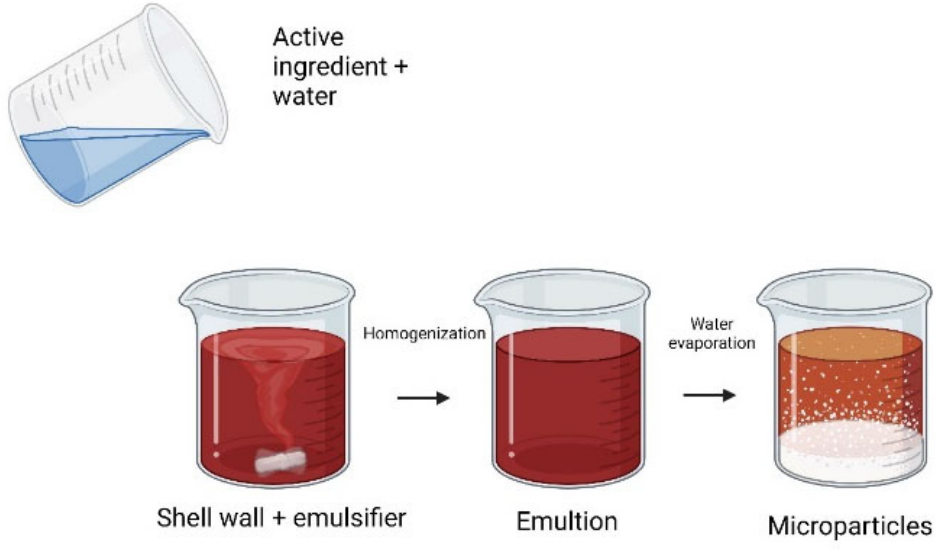

Fig. 3. Representation of the microencapsulation process by emulsification.

microorganisms. The 1:2 ratio had the best encapsulation yield, efficiency and the slower release of urea, and the 1:4 ratio had the best thermal stability.

Microencapsulation by emulsification has also been performed to protect lipophilic components such as polyphenols. Lupo et al. (2014) encapsulated polyphenols from cocoa by emulsification/internal gelling in alginate. Citrate and carbonate salts were used as sources of calcium and Span 80, Span 85, Span80-Tween 80, and polyglycerol polyricinoleate (PGPR) as surfactants. Smaller and more homogeneous microspheres were obtained with calcium citrate. Emulsions prepared with PGPR were more stable than with Span or Tween-Span mixture. Smaller and less polydisperse droplets produced smaller and less polydisperse microspheres with PGPR. Up to 60% of cocoa extract could be encapsulated for releasing into a suitable medium with internal emulsification/gelling. In another study, Davidov-Pardo and McClements (2015) encapsulated resveratrol in grape seed oil to be released after ingestion. Emulsions with a droplet size closer to the wavelength of UV light resulted in higher protection to resveratrol.

The emulsification has shown to be an efficient method to micro-encapsulate nitrogen and lipophilic components for application in the livestock and poultry industry, as well as its application in the food industry to achieve strategies for protection of functional ingredients in human nutrition. Emulsification also confers a protection to water-soluble compounds, achieving by consequence a better controlled release. Nevertheless, this technique has various disadvantages related to the stability of the emulsion, like the type and concentration of the emulsifier, and a high mobility of water droplets.

Freeze drying

Freeze drying is the process of solidifying an atomized liquid into particles in a cold chamber (Timilsena et al., 2020). This means, the core material is dissolved or dispersed into a molten carrier and the resulting mix is injected into a spray nozzle, to be sprayed in a cooling chamber (Alvim et al., 2016). The molten droplets that encounter cold air are solidified. This leads to microparticles formation with the core material uniformly distributed (Gavory et al., 2014). This technique is frequently used to encapsulate water-soluble core materials, such as water-soluble vitamins, proteins such as enzymes, chemical fertilizers, pharmaceutical ingredients, acidulants, and some flavors. Waxes, fatty acids, and polymers that are solid at room temperature but melt at a reasonable temperatures, can also be applicable to freeze drying (Jyothi et al., 2010).

Ma et al. (2014) used freeze drying to microencapsulate whey protein concentrate hydrolysate (WPCH) aiming to reduce the bitter taste and to increase its resistance to hygroscopicity, without affecting its immunoregulatory activity. Sodium alginate (WPC/SA) or a mix WPC/SA were used as a wall material. The bitter taste of encapsulated WPCH in WPC or WPC/SA was reduced. Freeze drying did not efficiently encapsulate WPCH compared to spray drying. Besides, Maity et al. (2017) synthesized nanocapsules of naringenin (flavonoid with anti-inflammatory, antimicrobial and antidiabetic activity) biopolymers of chitosan or alginate. Na_2SO_4 and $CaCl_2$ were used to produce dual cross-linked nanoparticles. The nano-formulations had a significant entrapment of naringenin (> 90%), whose slow and sustained release was pH dependent. A significant hypoglycemic effect was observed after oral administration of the nanoparticles in diabetic rats.

Xu et al. (2016) encapsulated of *Lactobacillus casei* ATCC 393 cells with a hydrogel matrix of isolate-alginate pea protein. They found compatibility of the technique, the matrix, and the probiotic *L. casei*, resulting in a yield of 85.69 ± 4.82%. The matrix did not show any protective effect compared to the non-encapsulated cells. *L. casei* encapsulated had the highest survival rate (59.9 ± 17.4%) after 84 d of storage (22, 4 and -15 ºC).

Quispe-Condori et al. (2011) microencapsulated by freeze and spray drying flax oil with zein as a coating material. The maximum microencapsulation efficiencies were 93.26 and 59.63 ± 0.36% for spray drying and freeze drying, respectively. However, the microcapsules had poor handling properties (Hausner ratio). Flax oil microcapsules produced by spray drying were more heterogeneous spheres with various sizes at high zein:flax oil ratios.

Freeze drying is a methodology with a wide potential application in the feed industry. It has a high efficiency to provide adequate physical structures in the capsules for protective/releasing activity, mainly for water-soluble compounds and microorganisms. Despite the heterogeneous shapes and sizes of the capsules produced by freeze drying, their bioactivity is reported as relevant. The result of the freeze-drying application is a powder, which is convenient in swine and poultry feeding because it can be easily mixed with the feed. Furthermore, its application can favor the storage time and improve the biological activity of bioactive compounds like prebiotics. However, the main disadvantage of freeze drying is its long drying period (24-48 h).

Coacervation

The encapsulation by coacervation technique is based on the concept of keeping the wall material separated of a polymeric solution in a homogeneous layer around the core particles suspended in a liquid phase (Fang and Bhandari, 2010). The core material is emulsified or suspended in the wall material solution, then another substance or solvent is added to reduce the solubility of the wall material. It is uniformly incorporated and surrounds the core material to form microcapsules (Yang et al., 2020). Coacervation can be divided into two processes: simple and complex coacervation. The microcapsule formation mechanism for both processes is identical, except for the development of the phase separation. In a simple coacervation, a desolvation agent is incorporated into the phase separation, while a complex coacervation involves an interaction between two oppositely charged polymers. The basic steps for complex coacervation are; 1) preparation of two polymers solution, 2) mixing the lipophilic core with a polymer solution to form an emulsion, 3) mixing another polymer solution, 4) change of pH and / or temperature to induce the formation of two immiscible phases, 5) deposition of the coacervates around the core, 6) rigidity of the coating by crosslinking or application of heat (Fig. 4) (Timilsena et al., 2020). Complex coacervation is a technique mainly used to imbibe fat-soluble food ingredients, but it is not limited to them (Yang et al., 2020). The complex coacervation encapsulation technique has been used for various active ingredients or agents, and for various purposes such as protection of heat sensitive ingredients, high encapsulation efficiency, and masking flavors.

Mendanha et al. (2009) used complex coacervation to microencapsulate casein hydrolysate, with soy protein / pectin isolate as wall material. They successfully attenuated the bitter taste by controlling the release via the incorporation of microcapsules into food products, produced by complex coacervation. In other study, Eratte et al. (2015) successfully co-encapsulated *Lactobacillus casei* 431 alone or in combination with tuna oil (omega-3 fatty acids) and a complex coacervated whey protein isolate (WPI)-gum arabic (GA). The viability of *L. casei* was significantly higher ($p < 0.05$) when it was co-encapsulated with tuna oil in coacervates of the WPI-GA complex, compared to individual encapsulation. The oxidative stability of tuna oil notably improved in the coacervates of the WPI-GA complex, regardless of the presence of *L. casei*.

Butstraen and Salaün (2014) synthesized GA-chitosan microcapsules using complex coacervation technique. The microcapsules contained a commercial mixture of triglycerides (55% C8 triglycerides and 45% C10 triglycerides; Miglyol 812 N®) in the core. The optimal phase volume ratio was 0.10, for 15 min at 11,000 rpm for the emulsion stage. Calderón-Oliver et al. (2017) evaluated the effect of two matrix-wall systems (collagen-alginate and collagen-pectin), two drying methods (freezing and spray-drying), and two core dispersion systems (water in oil emulsion or in suspension) in complex coacervation to encapsulate a mixture of nisin (peptide) and avocado skin extract (antioxidant). The core dispersion method and the drying method were the two most important variables for the final characteristics of the microencapsulated material. The interaction of the three factors had an effect only on the humidity of the microcapsules. The drying methods modified the morphology and final structure of the microcapsules.

Coacervation can be useful for adding antioxidants (sensible to the light, heat, and

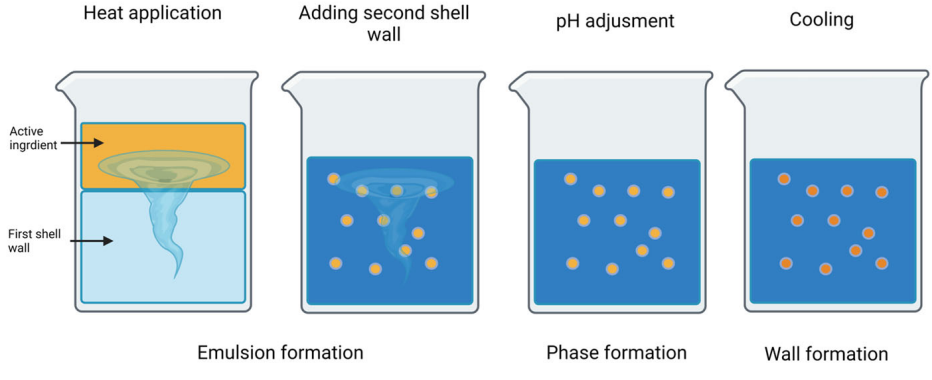

Fig. 4. Phases of microencapsulation by complex coacervation.

oxygen) in animal feeding. For example, essential oils have gained popularity in research due to their beneficial properties on animal health, but their sensibility to environmental factors has limited their use. The formation of coacervates can have as disadvantage the dependence of factors such as, pH, ionic strength, and heat treatment.

Emulsification by solvent evaporation

This consists of a polymer solution in an aqueous phase and the evaporation of the solvent, by inducing the precipitation of the polymer as nanospheres (Ghaderi et al., 2014). Nanocapsules are spherical and the size is determined by several factors, such as the viscosity of the organic/aqueous phase, rate of agitation, temperature, and type and amount of dispersing agent (Walia et al., 2019). The phases of this technique are: 1) formation of the organic phase by mixing the polymer with the organic solvent (ethanol or acetone); 2) formation of the aqueous phase by mixing the active ingredient and the surfactant; and 3) mixing of the two phases and heating to evaporate the solvent. Examples of polymers commonly used in this method are polylactic acid (PLA), poly(lactic-co-glycolic) acid (PLGA), cellulose acetate phthalate, ethyl cellulose, β-hydroxybutyrate, and polycaprolactone (Ezhilarasi et al., 2013; Cavallaro et al., 2015; Fornaguera et al., 2015).

Cao et al. (2016) developed nanoparticles as delivery systems to improve water dispersion and bio-accessibility of phytosterols (PS), using the emulsification-evaporation method. They tested soy protein isolate (SPI), whey protein concentrate (WPC), and sodium caseinate (SC). Sodium caseinate was the most suitable protein for the nano-formulation of phytosterols. Lyophilized nanoparticles of phytosterols based on sodium caseinate showed a high redispersion in water and low crystallinity of phytosterols. Phytosterol in nanoparticles showed better bio-accessibility compared to free phytosterol.

Wei et al. (2018) produced nanoparticles with propylene glycol alginate (PGA) and zein to study their potential as β-carotene delivery vehicle. The PGA:zein ratio affected structural characteristics, physicochemical stability, and *in vitro* gastrointestinal digestion of β-carotene-loaded. The nanoparticle-entrapped was amorphous but the improved stability and sustained release of β-carotene makes it a potential vehicle to deliver the vitamin into the food system.

Lira-Casas et al. (2019) encapsulated urea with Eudragit RS100® + calcium silicate (CS) and activated carbon (AC) as wall material, by solvent evaporation emulsification. Sixty-nine % of urea was encapsulated with CSU (calcium silicate + urea + Eudragit RS100® + dichloromethane) combination, and 71% with ACU (activated carbon + urea + Eudragit RS100® + dichloromethane). Unprotected urea reached a maximum peak of release kinetic after 6 h, while CSU and ACU needed more than 24 h to reach the maximum release of ammonia-N.

Ospina-Villa et al. (2019) encapsulated two proteins LpanUA.27.1860 and LpanUA.22.1260 for a Leishmaniasis treatment into PLGA polymer using the single emulsion-solvent evaporation method. They evaluated size distribution, morphology, encapsulation efficiency and release capacity. For this, different concentrations (50, 100, 150, 200, 250, 500, and 750 μg/mL) of both proteins were used. The results showed the best encapsulation efficiency in both proteins LpanUA.22.1260 (94.66% ± 4.86) and LpanUA.27.1860 (89.03% ± 4.91) at 750 μg/ml concentration. To test protective efficacy, mice were inoculated with three doses of PLGp-rLpanUA.27.1860 and then infected with *L. panamensis* promastigotes. Eight weeks later the mice did not show lesions of the parasite.

Emulsification by solvent evaporation has proven a wider potential for encapsulation despite its higher complexity when compared with the previously described techniques. Encapsulation of proteins, non-protein nitrogen, precursors of vitamins, and minerals has been achieved with an improvement of the morphology of the capsules and the delivery in gastrointestinal conditions. This technique, when being a micro spherical system, presents some disadvantages in animal feeding. For example, the produced microspheres cannot be chewed as these may deteriorate the morphology and physical properties. However, when preparing nano spherical systems, the nanospheres can be administered to the animal through various routes.

Nanoprecipitation

Nanoprecipitation is also known as solvent displacement. The method is based on the spontaneous emulsification of the internal organic phase, which contains the polymer, the active ingredient and the organic solvent dissolved within the external aqueous phase. The nanoprecipitation technique involves the precipitation of the polymer from the organic solution and the diffusion of the organic solvent in the aqueous medium. This encapsulation method is able to produce both, nanocapsules and nanospheres (Ezhilarasi et al., 2013). Biodegradable polymers such as polycaprolactone (PCL), polylactic acid (PLA) and poly (lactic-co-glycolic acid, PLGA), Eudragit and poly (alkyl

cyanoacrylate, PACA) are commonly used in this method (Bacinello et al., 2015; Cauteruccio et al., 2015; Mahalingam and Krishnamoorthy, 2015). The nanoprecipitation method includes the formation of the organic phase, and thus the polymer and the organic solvent (ethanol or acetone) are mixed using ultrasonication; the formation of the aqueous phase by mixing the active ingredient and the surfactant; and the mixture of the two phases.

Romero-Pérez et al. (2010) encapsulated sodium selenite within polymeric nanoparticles by nanoprecipitation. A 40:60 ratio of Eudragit RL:RS polymer was evaluated using ethanol as solvent. Selenium entrapment was 78% and the nanoparticles produced by nanoprecipitation were spherical with much variation in size. The release of selenium from the nanoparticles was higher at pH lower than 4.0, making its availability in the intestine feasible. Noronha et al. (2013) produced α-tocopherol-containing polycaprolactone (PCL) nano-capsules. They used three independent variables (α-tocopherol 200, 300, or 400 mg; lecithin 1.5, 2.5 or 3.5 mg/mL, and Pluronic F68® 0.5, 1 or 1.5% w/v). The optimal conditions for the encapsulation of α-tocopherol were with 200 mg of α-tocopherol, 2.5 mg/mL of lecithin and 1% of Pluronic F68, predicting 95.08% of encapsulation efficiency, 187.91 nm for particle size and 0.114 of polydispersity index. Nanocapsules of tocopherol and selenite may have a wide application in foods as natural antioxidants.

Finally, Hedayati et al. (2020) prepared tapioca starch nanoparticles (SNPs) by acetone nanoprecipitation and sonication. The ultrasonication increased yield and decreased acetone consumption. The tapioca starch nanoparticles were spherical in shape with heterogeneity in particle size. The nanoparticles synthesized using 3 g of starch and sonication had relatively similar particle sizes to those prepared with 1 g of starch without ultrasonication. The increase in starch concentration increased particle size. The crystal structure of native starch was destroyed by nanoprecipitation and sonication. Additionally, the thermal properties of the nanoparticles were lower than those of native starch.

Nowadays, a wide range of techniques to encapsulate pharmaceutical and nutritional components have been used in human and animal medicine and nutrition. The simplest techniques are relatively easy, low-cost, and fast. Nevertheless, they have shown relatively minor disadvantages such as heterogeneous sizes, heterogeneous shapes, and limited range of components to be encapsulated for efficient release. On the other hand, encapsulation techniques with a wide range of components potentially encapsulated, and a stable production of shape and size of capsules are complex and time-consuming processes and require expensive equipment. The challenge of the industry or the capsules developers is to develop techniques to reduce the inconveniences of time and expensive materials, involving the selection of easy-to-manage chemicals/materials for encapsulation or the replacement of equipment components. Otherwise, better components of the biological processes need to be adopted, which implies the use of low-cost-and fast techniques. Future research will continue exploring encapsulating techniques and chemical components, particularly those with potential applications in the food and pharmaceutical industries. Nonetheless, the seek for alternative components or chemicals to encapsulate is another growing concern for different industries.

MATERIALS FOR ENCAPSULATION

Fundamental knowledge of the chemical and physicochemical properties of materials is necessary to select an appropriate material and achieve successful product development. Hence, the physicochemical properties of the material for encapsulation is critical to accomplish a proper functionality of the microcapsule systems (Sarkar et al., 2013). Product characteristics such as stabilization, low volatility, and release characteristics should also be considered when choosing the material to constitute a polymer. Additionally, the polymer must have the ability to form a cohesive film with the specific core material (Goda and Arora, 2012). Moreover, materials used for the design of the protective layer of packages (coating, membrane, capsule, carrier material, external phase, or matrix) must be food-safe, biodegradable, and able to form a barrier between the internal phase and the environment. Different types of materials have been used as walls for microencapsulation, including polysaccharides (starches, maltodextrins, corn starch, and GA), lipids (stearic acid, mono- and diglycerides), and proteins (gelatin, casein, whey, soy, and wheat). The most extensively used materials for encapsulation of food ingredients with potential application in the livestock and poultry industry are listed and described below.

Carbohydrates

Among carbohydrates, starches and derivatives such as maltodextrin, gums (GA), and

cellulose derivatives (carboxymethyl cellulose) are the most frequently used wall materials for encapsulation. Carbohydrates have a wide availability in the market, and they also provide a mild flavor and an excellent protection ability. Due to that, carbohydrates have been widely used to encapsulate food ingredients, such as oils, vitamins, proteins, and enzymes (Timilsena et al., 2020).

Maltodextrins (MD) are acid- or enzyme-hydrolyzed starches extensively used for encapsulation of food ingredients (Carneiro et al., 2013). MD have relatively low cost, sensory benefits due to their neutral aroma and flavor, low viscosity at high solids concentrations, and good protection against oxidation. However, the main disadvantage of MD is their low emulsifying capacity (Carneiro et al., 2013). A single carbohydrate encapsulation matrix may not provide all the required characteristics and other strategies need to be developed to improve encapsulation, such as mix of carbohydrates, proteins, and polysaccharides. GA is an exudate gum originated by the *Acacia senegal* tree. Due to its low viscosity, good emulsion properties, high stabilization, and film formation, it is one of the most commercial wall materials used for spray-drying microencapsulation (Carneiro et al., 2013; Sarkar et al., 2013). In addition, GA is a natural colorless plant polysaccharide, which is widely accepted by consumers (Hosseini et al., 2015).

Proteins

Soybeans, milk proteins from whey, egg proteins, and hydrolysates are proteins commonly used for encapsulation (Đorđević et al., 2016). Physicochemical and functional characteristics of the proteins make them an excellent encapsulating material in the food industry. Protein wall materials are also considered nutrient-rich systems that may provide essential amino acids supply, versatility in solubility, gelation, film formation, and emulsification (Ye et al., 2018).

Gelatin is the most widely used wall matrix for making highly stable gels of omega-3 fatty acids, vitamin D, and fish oil. Milk proteins such as whey protein isolate and sodium caseinate, along with other vegetable proteins (soy protein and pea protein) have been used as wall materials for several years. Gelatin is a mixture of peptides and proteins obtained by partial hydrolysis of collagen found in skin, bone, and connective tissue of animals. Due to its biodegradability, biocompatibility, zero toxicity, low cost, water solubility, film formation and emulsification, gelatin has been ideal to encapsulate essential oils (Sutaphanit and Chitprasert, 2014).

Other natural-origin proteins have been used for encapsulation. Whey protein is an excellent material for encapsulating PUFA-rich oils and sensitive flavors compounds (Timilsena et al., 2020). Besides, milk proteins have excellent functional (emulsion preparation and stabilization, water, and fat binding, thickening and gelation) and nutritional properties. They can be used as probiotic cell delivery systems, due to their described properties (Livney, 2010). Therefore, milk proteins encapsulating probiotic cells have been applied in cookies, vegetables, and frozen cranberry juice (Heidebach et al., 2009). Milk proteins also possess the ability to supply functional ingredients, bind small molecules, and interact with other polymers to form complexes. Because of that, milk proteins have been successfully used in combination with polysaccharides, such as GA, xanthan gum, and carboxymethylcellulose in food emulsion systems (Livney, 2010).

Lipids

Lipids are hydrophobic materials used to encapsulate mainly hydrophilic substances. Various types of lipids, including glycerides, fatty acids, waxes, and phospholipids have been investigated due to their ability to encapsulate active food ingredients. Lipids have been used to encapsulate nitrogenous compounds such as urea with fat or bees wax (Carvalho et al., 2019), arginine with fatty acids (Meyer et al., 2018), and Lysine with a lipid mix (Prandini et al., 2013).

Lipid-based encapsulation technology is relatively recent compared to other ingredients for encapsulation. Hence, it may be considered as an emerging field, which is becoming very popular for delivering bioactive food, pharmaceutical, and nutraceutical ingredients (Timilsena et al., 2020). For example, the Carnauba wax is already used in medicine in capsule coatings, as dental wax and products for skin, being an inert compound in the rumen and stomach and harmless to animal health (Lim et al., 2017).

Despite the wide range of materials for encapsulation, a few of them have been utilized in the livestock and poultry industry. Due to the nature of the applications, these materials are usually the lowest-cost, wide availability, and good ability to protect-release the inner components. Nevertheless, they are not necessarily the ideals for emulsification, sensorial, solubility, or fast encapsulation production or their capacities have not been fully evaluated in animal applications. Hence, extensive research of materials and their applications in the livestock and poultry industry resulted compulsory to take advantages of the results found in pharmaceutical and food claims.

ENCAPSULATION OF FOOD/FEED INGREDIENTS

A vast amount of functional ingredients is used in the food industry to improve properties such as flavor, color, and texture, as well as to extend the shelf life of food. Natural ingredients with functional health benefits such as antioxidants and probiotics are nowadays of great interest for the industry and for consumers. The technological advantages of encapsulation include protection of food and feed ingredients from chemical degradation (caused by oxidation or hydrolysis) or reaction with other ingredients. Additionally, it also protects the food and components from unwanted changes of the environment (Fang and Bhandari, 2010).

The characterization of the microcapsules produced by the different technologies considered in this review includes mainly the study of physical properties, such as moisture content and water activity, bulk density, particle yield, microencapsulation efficiency, quantification of core material (encapsulated ingredient), flow properties, and powder solubility and hygroscopicity (Xue et al., 2013). Specific determinations of the encapsulated ingredient may be included according to its chemical properties (*i.e.* antioxidant activity, total content of the ingredient or compound, and the chemical structure by Fourier Transform Infrared Spectrometry, FTIR). (Ahmad et al., 2019). Those analyses are relevant when the objective is to study the role of the encapsulating material for releasing activity in the intestine, supporting the conditions of the upper gastrointestinal tract, such is the case of probiotics (Mun et al., 2015).

Hydrophobic compounds for encapsulation of food/feed are recent. Li et al. (2020) developed a microgel delivery system (lysozyme in starch nanoparticles) to enhance the controlled release of quercetin under intestinal conditions. Yang et al. (2020) developed a conjugated linolenic acid (CLA) carrier complex (oil phase) consisted of starch modified with octenyl succinic anhydride and xanthan gum. CLA is poorly soluble in water and highly sensitive to oxidation, which results in very low bioactivity. They achieved an encapsulation efficiency higher than 97%, trapping the CLA within the internal structure of the nanoparticle. Furthermore, the *in vivo* study indicated that nanoparticles were rarely released in the stomach of the rats, and highly released after entering the small intestine. Shao et al. (2018) also successfully used an emulsion stabilized with malanga gelatinized starch granules to encapsulate and protect polyphenols. Polyphenols normally have low bioavailability due to gastric degradation. Finally, Mehran et al. (2020) efficiently encapsulated anthocyanins (from Iranian borage extract) combining maltodextrin and modified corn starch. The microencapsulated powders obtained by spraying showed an encapsulation efficiency higher than 90%, high antioxidant stability during storage and controlled release in the gut.

Most of the reviewed articles in this work (> 90%) performed encapsulation with patented commercial products. Hence, details about encapsulation techniques and materials are not described. Consequently, capsule sizes and characteristics are unknown to perform repeatability, and thus new studies have limited information to generate new capsules and explore their potential in animal production. Table 2 summarizes recent studies using different encapsulation technologies and molecules of agri-food interest (proteins, peptides and amino acids, oils, and prebiotics).

CONCLUSIONS AND FUTURE PERSPECTIVES

Encapsulation has played an important role in innovation for the delivery of bioactive ingredients, including food supplements and pharmaceuticals. Recent research on the encapsulation of bioactive ingredients has focused on controlled release, protection, and nutritional enrichment. A vast amount of encapsulation components such as carbohydrates, proteins and lipids have been successfully applied for those purposes. In addition, a substantial amount of bio-ingredients has been encapsulated and tested, from vitamins to essentials oils for nutritional benefits. The use of hydrocolloids such as sodium alginate, guar gum, chitosan, and maltodextrin, as well as carnauba wax and copolymers for the encapsulation of urea and ruminal fluid, constitute a first approach for the implementation of micro and nanotechnology in animal nutrition. Nevertheless, most studies have been based on simulated conditions, and thus further *in vivo* studies are needed in order to validate the results under real conditions. Furthermore, the severe gastrointestinal/ruminal conditions for the dietetic nutrients remain the biggest challenge for animal nutritionist and researchers. Therefore, there is reduced research on encapsulation of ingredients to improve nutrition and controlled release of nutrients (*i.e.,* minerals and amino acids) whose effect can be evaluated in the quality of animal origin food products. Future research may focus on the microencapsulation of live microorganisms (probiotics) and encapsulation of proteins with

Table 2. Selection of studies on encapsulation of feed and food ingredients using different technologies.

Core material	Wall material	Technology applied/size/ others	Results	Reference
Proteins, peptides, amino acids y nitrógeno no protéico				
Ruminants				
Arginine and lysine	Hydrogenated soy oil, lecithin (AjiPro®-L, Japan)	Not reported (AjiPro®)	Average daily gain and dry matter intake were not affected. Likewise, serum concentrations of arginine and lysine were not affected. Lysine supplementation decreased fat thickness and increased Longissimus muscle area, supplementation with arginine tended to increase choice carcasses.	Teixeira et al., 2019
Arginine	Fatty acids and glycerides	Not reported (patent request USA No 61/321.604) Ruminal protection: 50%	Rumen protected arginine resulted in a better ruminal disappearance (%) compared with injected arginine. In addition, rumen protected arginine had the highest duodenal flow and small intestinal disappearance compared with injected arginine.	Meyer et al., 2018
Calcium and ammonium nitrates	Cardanol and walnut anacardic acid	Not reported (patent GRASP Ind&Com. LTDA®, Brazil) Controlled release (50-100% for 4-30 h)	Dry matter and crude protein intakes and digestibility of encapsulated nitrate (EN) and encapsulated nitrate with cashew nutshell liquid (EN + CNSL) treatments were similar. No differences were observed in any carcass characteristics among treatments.	(El-Zaiat et al., 2020
Methionine	MetiPEARLTM, Kemin®, USA.	Spray drying Size < 2 mm, DL-methionine 48%	None of the live performance parameters had significant differences (average daily gain and dry matter intake) among treatments. There was no effect in the hot carcass weight across treatments. However, the Longissimus muscle area increased as methionine levels increased.	Baggerman et al., 2021
Calcium and ammonium nitrates	Cardanol and walnut anacardic acid	Not reported (patent GRASP Ind&Com. LTDA®, Brazil) Controlled release (50-100% at 4-30 h)	The inclusion of encapsulated nitrate (EN) reduced the final body weight (BW), but it was not affected by microencapsulated blend essential oils (MBEO). Dry matter intake and methane (CH4) emission were reduced when feeding EN, while MBEO increased CH4 emission.	Alemu et al., 2019
Methionine	Carnauba wax	Melt-emulsification No evidence of microcapsules	Lower in situ degradability in encapsulated methionine compared to pure methionine.	De Carvalho et al., 2019
Urea	Fats (NitroShure, Balchem Encapsulates, USA.)	Controlled release, 87-91% urea	Addition of encapsulated urea in early lactation cows increased the content of fat and protein in milk. No differences were observed in milk production and in urinary N components.	Highstreet et al., 2010
Poultry				
Lysine and methionine	Stearic acid	49.5% of methionine and lysine at encapsulates. Additional information not reported.	BW decreased at the starter phase, while the fed-to-gain ratio increased in the encapsulated methionine-lysine treatment (60CLM). Breast muscle weight was reduced at 42 d in 60CLM compared to the control.	Sun et al., 2020

Swine				
Lysine	Hydrogenated lipid matrix	Slow release Commercial lysine (Vetagro®)	Microencapsulated lysine (-80 MLys) tended to reduce feed intake (FI) and BW gain compared to the other treatments. Fat thickness was the highest in -80 MLys across treatments.	Prandini et al., 2013
		Oils, fatty acids, essential oils		
Ruminants				
Eugenol, thymoland vanillin	No described (Safeeds®)	Not reported (Safeeds®, Brazil)	The essential oils did not affect the chemical composition of the meat (moisture, ash, crude protein, and total lipids). No differences were observed in the collagen content in muscle samples among treatments. Type III collagen fibers were lower in BRC (eugenol, thymol, vanillin, rosemary) than CON (control); sarcomere length was larger in BRC compared with CON.	Monteschio et al., 2019
Poultry				
Butyric acid	Lipids (ButipearlTM, Kemin®, USA)	Spray drying	The addition of butyric acid in the starter phase did not affect BW gain or FI. BW increased in broilers fed with encapsulated butyric acid (300g) compared to the control.	Levy et al., 2015
Cinnamaldehyde, citral	Soy protein-polysaccharide Maillard reaction product	Oil-in-water emulsion, 400-1000 nm	BW increased among encapsulated essential oils treatments compared to the control. Non-vaccinated birds had lower oocyst counts compared with vaccinated birds.	Yang et al., 2020
Thymol, carvacrol, B-cymene, borneol, myrcene and organic acids (formic, acetic, and butyric)	Triglyceride matrix (GallinatTM, Jefont®, Canada)	Not reported (GallinatTM, Jefont®, Canada)	Inclusion of Gallant 600 g/ton decreased cholesterol level compared to the control. Besides, the triglyceride level had a decrease using Gallant 600 g/ton and 900 g/ton. Final BW and total BW gain increased when Gallant 300 g/ton was added.	Maty and Hassan, 2020
Mint, thyme, cinnamon essential oils	Chitosan	Ionic gelation	FI increased, including encapsulated mint compared to non-encapsulated mint. BW gain was higher when including encapsulated thyme.	Nouri, 2019
Swine				
Palm and coconut oil	Dried casein and whey powder	Spray drying, 20-50 µm	Addition of encapsulated fat increased BW, average daily gain and gain-to-feed ratio compared to the control.	Yang et al., 2018
Formic and citric acid, essential oils (cinnamon, oregano, thyme, capsicum)	Not reported (FormaXOLTM, Kemon®, USA)	Coacervation and fluid bed	The encapsulated essential oils decreased Salmonella shedding at 14 d, and by 28 d. There was a tendency for a decrease in the prevalence of fecal Salmonella compared to the control. Average daily gain was not affected across treatments.	Kavita et al., 2016

Carvacrol	Alginate-whey protein	Extrusion, 250-800 μm	Microcapsules with small size showed a faster released of carvacrol than those with large size. Total release of carvacrol was reached at 4 h in both treatments.	Zhang et al., 2016
Tea tree oil	Arabic gum and maltodextrin	Spray drying	Average daily gain and average daily FI increased and tended to reduce the diarrhea rate when using encapsulated tea tree (Encp TTO). Encp TTO reduced the abundance of *Escherichia–Shigella* in cecal and colonic digesta.	Wang et al., 2021
		Probiotics		
Ruminants				
Lactobacillus plantarum	Three layer (phosphatidyl choline and vitamin E, lactose and gum arabic, maltodextrin)	Freeze drying	Addition of encapsulated probiotics reduced methane production, but also increased total gas production.	Abdelbagi et al., 2021
Poultry				
Lactobacillus lactis and *Bifidobacterium bifidum*	Sodium alginate and chitosan	Extrusion	Encapsulated *L. lactis*, encapsulated *B. bifidum*, and encapsulated *L. lactis* and *B. bifidum* produced higher plasma total protein and plasma globulin values compared with treatment without probiotic. Encapsulated *L. lactis* and *B. bifidum* had higher plasma albumin across all the treatments. Besides, encapsulated treatments reduced plasma cholesterol.	Yazhini et al., 2018
Swine				
Additive Enzymesporine™ (Fermlab®, Russia): strains of *Bacillus subtilis* and *Bacillus licheniformis*, trypsin crystalline	Sodium alginate	Non-solvent addition (acetone and calcium chloride), 80-150 μm	Encapsulated Enzymesporin increased average daily BW.	Trubnikov et al., 2020
		Minerals		
Poultry				
Ionic trace minerals (Cu, Zn, I, Se, Fe and Mn)	Bio-protective matrix of plant origin (carbohydrates) (MinCo®, SynoBioTech, Singapore)	Not reported (MinCo®, SynoBioTech, Singapore)	Encapsulated mineral M-350 promoted higher growth than inorganic minerals (ITM). The inclusion of M-350 resulted in better feed conversion ratio (FCR) than organic minerals. BW, FI and FCR was not affected across treatments for the entire growth cycle.	Ramirez et al., 2022

Sodium butyrate (30% of content)	Plant triglycerides (vg. Vegetable oil)	Embedded granulation	Cumulative BW gain was higher by adding encapsulated sodium butyrate (CMA, 2 H releasing time), both encapsulated treatment CMA and CMP (4 H releasing time) had lower mortality and lesion score in middle intestine compared to the control.	Liu et al., 2019
Swine				
Heme and nonheme iron	Maltodextrin	Spray drying	Most of the time was occupied for resting and suckling in animals with parenteral and encapsulated iron. The addition of encapsulated iron had lower resting time and an increase in exploration than parenteral.	Valenzuela et al., 2016
Zinc oxide	Lipids (Shield Zn®)	Not reported (patented commercial product Shield Zn®, Korea)	In the first 14 d the ADG was higher with the encapsulated zinc (Shield-Zn-100). Shield-Zn-100 had lower average daily FI compared to the control (Zno-100). Znp-2500 treatment had lower fecal consistency score than Zno-100 and Shield-Zn-100. Concentration of zinc in serum and liver were higher in Zno-2500 compared to the others.	Park et al., 2015
		Enzymes		
Ruminal liquid	Hydrocolloids (sodium alginate, guar gum, chitosan, and maltodextrin	Spray drying	The addition of maltodextrin retains the highest enzyme activities after spray drying. Higher digestibility of dry matter with fresh ruminal fluid at 1%.	Sarteshnizi et al., 2018

potential uses as vaccines or nutrients for *in vivo* supplementation of animals and its impact on food and consumer health.

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