Encapsulation of bioactive compounds for the formulation of functional animal feeds: the biofortification of derivate foods

Roberta Tolve[a], Fideline Tchuenbou-Magaia[b], Maria Di Cairano[a], Marisa Carmela Caruso[a], Teresa Scarpa[a], Fernanda Galgano[a*]

[a] School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Viale Dell'Ateneo Lucano 10, 85100, Potenza, Italy

[b] School of Engineering, Faculty of Science and Engineering, University of Wolverhampton, WV1 1LY, UK

*Corresponding author:
fernanda.galgano@unibas.it
Tel.: +39–0971–205570

Abstract

Microencapsulation is a technology that has increasing application in animal nutrition. This technology provides the possibility to protect sensitive compounds through the feed process and the storage conditions. Furthermore, the application of this technique affords the possibility to increase the bioavailability of compounds and to mask the unpalatable compounds taste. Different are the compounds to consider for microencapsulation include certain vitamins, amino acids, fatty acids, organic acids and live microorganisms. Understanding the interdependence between bioactive compound, coating, technology and environment is the key to successful application of microencapsulation also in animal nutrition. In this review a focus on the use of microencapsulated bioactive compounds for the feed supplementation and the related effects on the derivate animal product is given.

Keywords: animal-based products; biofortification; bioactive compounds; feed supplementation; encapsulation.

1. Introduction

There is a need to overcome the pressing challenge of meeting the nutritional demands of the growing population which is estimated to reach 9.7 billion in 2050 (United Nation, 2019). For example, over two billion people worldwide are micronutrient deficient with poorest regions been the most affected. Attractive approaches to tackle micronutrient deficiency include a targeted integration of nutrients directly into food (fortification) and increasing the concentration of the active compounds in edible crops (biofortification) (WHO, 2020). Micronutrients could also be administrated as medical supplements. The main disadvantage of this approach lies in the limited availability of particular compounds and shelf-life stability. Also, it has been suggested that micronutrients function better when consumed as part of a balanced diet, rather than as pills (Hunter, 2011). When using biofortification, the poor bioavailability of certain nutrients of plant-based foods must be considered (Willer and Aldridge, 2020) whereas the same nutrients in animal-based foods, like milk or meat, are generally easily absorbed by the human body (O'Keeffe, 2020). On this ground, the possibility to deliver micronutrients through animal products offers interesting additional benefits. Different studies have shown that feed fortified with nutrients can be used as dietary supplements in animal nutrition which contribute to improve the productivity of animals and the quality of the derived products (Grilli et al., 2013; Hu et al., 2015; Kim et al., 2020; Konkol and Wojnarowski, 2018; Natsir et al., 2019; Stamilla et al., 2020; Tao et al., 2020). Taking a holistic approach, micronutrients could be considered as part of a network of bioactive molecules within the food and the organism and as such, the same approach could be applied to other bioactive compounds.

Bioactive compounds or ingredients are substances which in small amounts have the ability to provide health benefits. These are usually natural occurring substances with specific properties that may preserve or improve the animal health condition and, at the same time, contribute to increasing their concentration in the products obtained from them (Korczynski et al., 2017). However, some of these bioactive compounds such as polyunsaturated fatty acids, essential oils, polyphenols and vitamins are prone to oxidation whereas plant extracts and essential oils are often difficult to process in addition to their unpleasant taste. Encapsulation has been proven as an efficient tool for improving functions and properties of bioactive ingredients and allowing the incorporation of even sensitive ingredients into products. Indeed, encapsulation allows a liquid, gaseous or solid substance to be protected into an envelope or coat material and has several other benefits. These include control and target release, taste masking, prevention of interaction with other incompatible ones in the product matrix as well as protection of bioactive compounds against oxidation promoters, such as air, light and metals (Piva et al., 2007; Tolve et al., 2016; Tolve et al., 2020). On the other hand, consumers' demand for natural, high-quality animal products produced with no negative effect or reduced environmental impact while protecting animal welfare reinforces the need for the development of more efficient and sustainable production methods. This article aims to review bioactive compounds used in animal feed formulations and different approaches for encapsulating them alongside potential effect on the derived products and animal welfare. In this review, the term "encapsulation" has been used if no size definition was given about the particles whereas "microencapsulation" was used for specified size ranged from 1 μm to 1 mm.

2. Encapsulation in animal feed and subsequent products biofortification

Although encapsulation is also used to improve formulation and safety of animal feed, this section focuses principally on its applications to improve product bioavailability, deliver nutrients and additives to the digestive tract or specific sites for enhancing animal performance and the quality of the resulting products. Animal farming industry is searching for new ways to improve the overall quality of animal-based products such as meat, milk and eggs. One of the recently developed approaches is the "biofortification" which utilizes animal feed supplemented with encapsulated bioactive compounds (Konkol and Wojnarowski, 2018). The choice of the encapsulation formulation and manufacturing processes depends upon the type of animal to be fed, the chemistry of the bioactive compound and the purpose of its encapsulation. As example, lipid based-shell material was proved effective to provide sustained release of organic acids along the swine intestine (Piva et al., 2007) thereby allowing to overcome any issue related to their rapid absorption and metabolization in the duodenum. It has also been demonstrated that lipid base-shell protects nutrients from ruminal degradation (Grilli et al., 2013), but may not be useful if the purpose of the encapsulation is to mask unpleasant taste such as in the case of tannins applications (Adejoro et al., 2019a; Tolve et al., 2021). In Figure 1 a schematic diagram showing the protecting effect and the site-specific release site of the encapsulated bioactive compounds has been reported. Indeed, different approaches have been employed for the encapsulation of bioactive compounds intended for animal feed as highlighted in Tables 1-7. These Tables also show diverse encapsulated commercial blends. Encapsulation methods used in animal feed have been reviewed by Temiz and Öztürk (2018) and each method presents different advantages and drawbacks. Coating materials include proteins (corn zein, wheat gluten, casein, gelatin, soy and whey proteins), polysaccharides (cellulose and other plant-based polymers), synthetic polymers (poly-2-vinylpyridine-co-styrene and polymethacrylate polymers) (Bribiesca et al., 2017; Temiz and Öztürk, 2018) and composites materials whereas the particle size varies from nano size to millimeter size. For example, particles diameter up to 6 mm has been suggested as optimum size of an encapsulated product for cattle (Sýkora et al., 2007). Based on the general values of rumen pH, 5.6 - 7.0, coating materials with ability to degrade a pH below 5 could potentially survive rumen degradation and thus candidate for release in small the intestine formulations if they survive microbial degradation. However, the mechanical stability of the shell material should also be considered. Bioactive compounds encapsulated for animal feed include aroma and fragrances, essential oils, omega fatty acids, plant extracts, enzymes, prebiotic and micro-organisms, vitamins, amino acids and minerals.

2.1 Encapsulation of minerals

Micro- and macro elements deficits have been observed for many years in human concerns about two billion people (Chojnacka et al., 2011). Generally, the animal feed supplementation with minerals allows for increasing animal production however, for specific minerals, the supplementation could also cause the increase of certain minerals in animal-based foods. This is usually expressed in terms of the level of incorporation of an element into the final animal-based product (Słupczyńska et al., 2014). In view of this, several authors (Bribiesca et al., 2017; Grilli et al., 2013) have evaluated the possibility to enhance the nutritional quality of animal-based product through the feed supplementation with encapsulated minerals (Table 1). Selenium is a trace element crucial for homeostasis and oxidative status in animals and essential component of about 25 selenoproteins in the body, including enzymes involved in cellular antioxidant protection and it is essential for thyroid hormone metabolism. Suboptimal Se status in humans has been associated with an increased risk of cancer, HIV/AIDS and other viral diseases (measles, hepatitis, influenza) including endemic cardiomyopathy namely Keshan Disease (Bribiesca et al., 2017). The recommended daily dosage (RDA) of Se per day for normal adults is 60-75 μg in Europe. However, there is an estimated 30-100 million people worldwide with Se deficiency which is mainly due to low Se concentrations in frequently consumed foods (Thavarajah et al., 2011). On the other hand, selenium supplements are partiality transformed in insoluble form by ruminal microorganisms and this process decreases its bioavailability in gastrointestinal system (Romero-Pérez et al., 2010). Therefore, Romero-Pérez et al. (2010) investigated the possibility of protecting Se from ruminal microbial complexation by encapsulation technology. The authors prepared microencapsulated sodium selenite by emulsion-evaporation and by nanoprecipitation methods and, by *in vitro* digestion study, found that the release of selenium from nanoparticles was higher at acid pH (less than 4) when compared to that at pH 6. This result indicates that a better bioavailability and absorption of Se would occur in the small intestine. Eudragit® RL and RS were used as shell materials because of their insolubility in aqueous environment with good permeability related to the presence of quaternary ammonium groups in their structure (Bribiesca et al., 2017). Since Eudragit® RL has high permeability to aqueous fluid than Eudragit® RS, the blend of the two polymers is usually used to tune the release kinetics to match the desired release profile. The *in vivo* study conducted by Grilli et al. (2013) confirmed the possibility to increase the bioavailability of Se via encapsulation by demonstrating an increase in Se concentration in milk after supplementing cows feed with microencapsulated Se in hydrogenated fat. Because the milk from yeast-supplemented feed did not differ from that of the control, the study also indicated that Se microencapsulated in lipid-based formulation of nanoparticles could be a promising route for protecting minerals from rumen degradability or complexation. Similarly, Bribiesca et al. (2017) reported an increase in both selenium and zinc content of meat from lambs fed with Se and Zn nanoparticles with Eudragit RL as coating material when compared to feed supplemented with nonencapsulated Se and Zn. The increased concentration of Se and Zn in meat was attributed to a better bioavailability of Se and Zn when they were supplemented in the form of nanoparticles as compared to sodium selenite and zinc carbonate. Moreover, the increase in Se concentration in meat could enhance its antioxidant properties preventing lipid peroxidation and consequently improving meat quality. The increase in Zn in the meat would also contribute to tackle deficiency induced clinical symptoms in children, such as undercurrent infections, body growth retardation, anorexia, dermatitis, impaired reproductive performance, skeletal abnormalities and diarrhea, alopecia, scarring defects and behavior disorders (Prasad, 2013).

2.2 Encapsulation of vitamins

The most common reason to encapsulate vitamins is their shelf-life extension, protection from damage during processing (e.g., heat and steam in the pelleting or extrusion process), storage and transport as well as delivery to the small intestine because of their rapid degradation in the rumen by ruminal microbes (Table 2). Albright and Kowarski (1994) demonstrated an improved stability of vitamin A encapsulated into a lipid bilayer containing lecithin, cholesterol and functionalized stearyls. Their hypothesis was that the lipid bilayer would physically separate vitamin A from the mineral components and reduce or inhibit hydrolytic degradation through water capture in a form of liposome when it penetrates the bilayer, thereby limiting water availability to interact with vitamin A and initiate its degradation.

Vitamins that are extensively metabolized in the rumen include choline and vitamin E (Santschi et. al., 2005). Most research with encapsulated vitamins has focused on choline however the use of rumen protected choline is generally related to the production of dairy cows. For example, rumen protected choline has been reported to be useful in increasing milk production during cow's first month of lactation and also to increase the milk choline concentration (Pinotti et al., 2003). This effect was confirmed by by Arshad et al. (2020) who through a meta-analysis showed an increased yield of milk, energy-corrected milk, fat, protein, and a tendency of a reduction of the risk of retained placenta and mastitis for parous dairy cows under rumen-protected choline supplementation. Similarly, Hu et al. (2015) evaluated the possibility of using 20 mg/kg of a commercial vitamin E encapsulated with sodium octenyl succinate in broiler chicken's diet for 42 days and found that vitamin E in both free and encapsulated form in the feed improved breast meat quality in broilers with a lower abdominal fat percentage. However, encapsulated-based feed resulted in better broiler growth performance with higher body gain when compared free vitamin E containing feed whereas higher pH and antioxidant enzyme activity was reported for boiler fed with vitamin when compared to those on basal diets without vitamin E. Since vitamin E easily oxidized, has poor water solubility and low bioavailability (Sagalowicz and Leser 2010), the encapsulated form effectively increased its stability and overall bio-accessibility. Vitamin E is an effective antioxidant that can protect cells from oxidative damage and numerous nutritional and physiological studies have shown that its supplementation is beneficial for growth performance in animals. In terms of biofortification, even more interesting are the results obtained by Lee et al. (2007) who assessed the effect of feeding with a rumen protected α-tocopheryl-acetate and sunflower oil, made using sodium caseinate on α-tocopherol concentrations of muscle in lambs over the 10-week. Results showed that lambs fed with encapsulated vitamin E and sunflower oil had higher α-tocopherol concentrations in their muscle when compared to those fed with non-encapsulated vitamin E. It is worth mentioning that no published work was found regarding the encapsulation of vitamin D for animal feed even though research increasingly suggests its role in stimulating immunity and protecting again viral and bacterial infection, its contribution to cardiovascular physiology, cells proliferation and differentiation alongside its principal to modulate calcium and phosphorus homeostasis. Fish meals and oils are good source of vitamin D_3 with 0.01 to 0.18 mg/kg (meals) and 0.03 to 3.9 mg/kg (oils) depending on the species and the season (EFSA, 2017). The increasing use of plant-based ingredients in aquaculture feeds has created more need for vitamin D fish farm feed supplementation as these formulations is expected to contain only 25–33% of the vitamin D_3 content of the conventional marine-based formulations (EFSA, 2017). Since fish has no ability to endogenous synthesize vitamin D_3 like other vertebrates such mammalian species and birds they have to be supplied through food (EFSA, 2017). Estimated vitamin D_3 transfer rate from feed to fish to be in the range of 0.1–0.15, thus given vitamin D_3 content in fish flesh ranging from 150 up to 220 μg/kg for a feed containing 1.5 mg/kg of vitamin D_3. This supplementation could help addressing low dietary vitamin D intake and consequently lowering the risk of vitamin D deficiency (Cashman, 2020). In this field, encapsulation could be useful to increase the shelf-life of vitamin D in formulations and for safety because vitamin D_3 is considered as irritant to skin and eyes as well as a dermal sensitizer (EFSA, 2014). Nowadays, it is possible to find on the market only calcifediol monohydrate containing nanoparticles used to protect the vitamin D analogue from oxidation (EFSA, 2021).

2.3 Encapsulation of amino acids and urea

The main source of amino acids for animal is the diet, though ruminants also produce them via their gut microflora during fermentative digestion. Amino acids are absorbed from the small intestine as free amino acids or as di- and tripeptides and released into the blood mostly as free amino acids but some peptides (Fuller, 2004). Protein and amino acid in the rumen are also degraded by rumen microbes leading to dietary protein conversion into ammonia, and excretion as urea. Sulphur containing amino acids are precursors for the others amino acids for ruminants (Gaillard et al., 2018). In this contest, methionine and lysine are limiting for milk and milk protein synthesis. In addition, histidine has been identified as first limiting amino acid for milk protein production when cows are fed with grass silage and barley or oats (Vanhatalo et al., 1999). Different approaches have been used to achieve delivery of amino acids at the absorption sites (Table 3). The first paper about the supplementation of animal feed with rumen protected amino acids was published in 1970 by Broderick et al. (1970), who used an encapsulated DL-methionine with kaolin and tristearin. The authors fed lactating cows with encapsulated methionine at 5, 15, and 45 g per day and observed no effect on yield of the milk or milk components, but instead an increase in plasma methionine and methionine/valine ratios in all cows at 15 g supplementation level. Although these results were confirmed in the same years by Williams et al. (1970), it could be postulated that the absence of improvement in milk or milk protein production using encapsulated methionine could have been due, to some extent, to the use of only methionine for the supplementation. Indeed, Rogers et al. (1987) demonstrated that rumen-protected methionine and lysine increased production of milk protein, whereas Sevi et al. (1998) reported a modification of the milk fatty acid composition in ewes with an increase in the ratio between long-chain and short chain fatty acids and the ratio of unsaturated/saturated fatty acids which is desirable from consumers' health perspective. Socha et al. (2005) obtained an enhanced milk quality when cows were fed with the rumen protected methionine and lysine as more true protein (1306 vs. 1221 g/d), and fat (1632 vs. 1550 g/d) were obtained, thus a better outcome than that previously reported by others researcher (Armentano et al., 1993). The authors supported the hypothesis that the greatest responses to improved lysine and methionine concentration occur during the earliest stages of lactation when the need for absorbed amino acids is the highest. On the other hand, different polymers have been used in order to ensure rumen bypass. Recently Carvalho Neto et al. (2019) demonstrated that a lipid matrix of carnauba wax with a core:shell 1:4 in combination with melt-emulsification method is effective as encapsulating system with high methionine protection through the rumen. It is well known that some lipids, particularly non-esterified saturated fatty acids, theoretically provide viable protection against ruminal digestion. In contrast, glycerol is converted to propionate without releasing carbon dioxide and short acids (acetic, propionic, and butyric acids), whereas unsaturated fatty acids undergo biohydrogenation by rumen microorganisms. Carnauba wax resistance to microorganism’s degradation has already been tested when used as microencapsulating agent for urea with good outcomes (de Medeiros et al., 2019). A recent research published by Albuquerque et al. (2020) corroborated with these results. The authors proposed the use of lipid nanoparticles as novel rumen-bypass systems to carry lysine. These nanoparticles were produced using different arachidic or stearic acids -based fatty acid and Tween 60 and resisted for up to 24 h ruminal digestion. Tao et al. (2020) investigated the effects of coated cysteamine on growth performance, carcass characteristics, meat quality, and lipid metabolism in finishing pigs and found an increase in carcass lean percentage and *longissmus muscle* area alongside higher total protein and free fatty acids but lower serum triacylglycerol and total cholesterol level. These results were explained by an increase in enzymatic activity related to lipid catabolism in adipose tissue and a decrease in activity of enzyme involved in the process of fat synthesis, and thus reducing the fat deposition in subcutaneous adipose tissue. Recently Zhang et al. (2019) demonstrated that the amino acid concentrations of plasma and muscle increased in response to incremental amounts of supplementation with the rumen protected amino acid. The same trend was observed for milk true protein yield. In detail, milk true protein yield increased to 0.98 kg/d when feeding cows with 246 g/d of rumen protected histidine.

Urea is the main source of nonprotein nitrogen used in the ruminant diet. It has a low cost per unit of nutrients and is a valuable alternative for partially replace the true protein found in a vegetable meal. However, when ingested urea is rapidly hydrolyzed in the rumen by microbial enzymes producing ammonia ($N–NH_3$) and carbon dioxide. The accumulation of ammonia in the bloodstream cause intoxication and could lead to animal death. Through the encapsulation processes, the degradation rate of urea in the rumen can be reduced and a slow-release could be obtained (Silva et al., 2013). This interest in slowly rumen released nitrogen compounds primarily stems from their potential to slow ammonia release post-feeding, thereby decreasing peak ammonia concentrations in the rumen. Slow-release urea compounds used to feed ruminants include biuret, starea, urea phosphate, coatings based on oil, formaldehyde-treated urea and polymer-coated urea. The development of products that slow down the ruminal release of ammonia without limiting the extent of urea degradation in the rumen has been challenging. To date, the coatings based on oil or polymers to control the release rate of ammonia from urea seems to be the most promising product (Joysowal et al., 2019). Several studies have been conducted to investigate the influence of feeding dairy cows with slow-release urea with particular attention to the feed intake and nutrient digestibility. A study from Puga et al. (2001) found that the ruminant feed with commercial controlled-release urea significantly increased dry matter intake above the level of the control diet. The authors ascribed the higher digestibility of the experimental diets to the better activity of fiber fermentation in the rumen. It indicates that the commercial controlled-release urea improves nutrient imbalance for rumen bacteria by increasing the availability of energy from simple carbohydrates. These results were in agreement with the findings from Xin et al. (2010), who found that polyurethane coated urea increased the ruminant dry matter intake and the nutrients digestibility compared to the uncoated urea.

2.4 Encapsulation of fatty acids

Generally, animal-based products such as meat and milk are characterized by high levels of saturated fatty acids and an unbalanced omega-6/omega-3 ratio. Therefore, increasing unsaturated fat concentration in animal-based products is one of the research priorities to respond to the concerns of the health-conscious consumers (Lee et al., 2004). High forage quality is certainly crucial in producing meat and milk with a healthy fatty acids profile (Heck et al., 2021). However, efforts to increase polyunsaturated fatty acids (PUFA) levels in ruminant meat and milk had very limited success due to their biohydrogenation in rumen (Francisco et al., 2015). Consequently, attempts to improve the proportion of beneficial fatty acids have been made by protecting dietary PUFA from biohydrogenation trough encapsulation techniques (Table 4). In the early 70' Cook et al. (1970) produced PUFA supplements protected with formaldehyde (a known carcinogen generally not allowed in animal feed additives) in order to prevent ruminal biohydrogenation of unsaturated dietary fat during digestion. Lee et al. (2004) encapsulated linoleic acid (LA) using casein treated with acetaldehyde or diacetyl, as an alternative to formaldehyde and determined the concentration of PUFA deposition in the body and muscle fats of lambs fed with the supplements. The results showed that fat of animals fed with protected LA had higher concentrations of both linoleic and linolenic acids (ALA), when compared with lambs fed with untreated supplements after 18 days feeding. Similarly, feeding lambs with LA and ALA encapsulated with formaldehyde-treated protein resulted in an increase in the concentration of LA and ALA in lamb muscle of about 247% and 57%, respectively, compared to those fed with unprotected lipids (Elmore et al., 2005). With the same aim, Alvarado-Gilis et al. (2015a) studied the possibility to protect PUFA from ruminal biohydrogenation thought three different methods. In method 1 a blend of ground flaxseed, calcium oxide, and molasses was processed through a dry extruder. For method 2 a blend of ground flaxseed, soybean meal, molasses, and baker's yeast were moistened and pre-warmed and the mixture was subsequently processed through a dry extruder whereas in method 3 ground flaxseed was encapsulated within a matrix of dolomitic lime hydrate (L-Flaxseed) as a protective barrier against biohydrogenation. Steers were fed for 12 days and jugular blood samples were taken for analysis of long-chain fatty acids. Results showed that method 1 and 2 failed to improve resistance of PUFA to biohydrogenation. In contrast, using microencapsulated ground flaxseed, the proportion of ALA resistant to ruminal biohydrogenation was approximately 4-fold greater when compared with ground flaxseed, suggesting that the dolomitic lime hydrate was effective as a protective barrier against biohydrogenation. In a complementary study, the same authors evaluated not only the plasma concentration of PUFA in steers fed with ground flaxseed and encapsulated ground flaxseeds, but also the concentration of fatty acids in the animal tissues (Alvarado-Gilis et al., 2015b). Supplementation with ground flaxseed significantly increased the concentration of ALA in meat and, even more interesting the fact that the ALA content of muscle tissue was 47% greater when flaxseed was encapsulated within the dolomitic lime hydrate matrix. The same trend was obtained by Lee et al. (2007), when they assessed the effect of feeding lambs with a rumen protected protein–oil supplement, made using sodium caseinate and containing α-tocopheryl-acetate and sunflower oil, on fatty acid composition of their muscles over the 10-week feeding trial. Lambs fed with encapsulated sunflower oil and vitamin E were characterized by a higher LA level in both *longissimus dorsi* and *psoas major muscles* lipids though a lower palmitic and oleic acid levels were observed when compared to the control. Recently, Kim et al. (2020), fed cows with encapsulated linseed oil coated with hydrogenated palm oil, in order to protect omega-3 in the rumen and found an increase in the omega-3 fatty acids in the milk. The authors performed an *in vitro* and *in vivo* evaluation on the use of a commercial product contained linseed oil, vitamin E, rosemary extract encapsulated in a hydrogenated palm oil shell on rumen fermentation, physiological profile, milk yield, and milk composition in dairy cows. Detailed information about the patented spray-cooling encapsulation process is not available. However, hydrogenated palm oil mostly consisted of molten saturated fatty acids was used as shell material and mixed with linseed oil and other additives. The mixture was atomized into a chilling chamber and cut to a size of 1000 to 1500 microns. The obtained microcapsules made of linseed oil (35%), vitamin E (0.5%), rosemary extract (0.3%), and hydrogenated palm oil (64.2%) were added at different concentration 0, 1, 2, 3, 4, and 5% to the diets and the ruminal fluids were collected. The results of the *in vitro* ruminal fermentation suggested that 2% of encapsulated linseed oil was an optimal dosage in cows' diets, therefore this level was chosen for the feed supplementation of 36 cows. Results of the *in vivo* experiment showed that the use of the commercial encapsulated linseed oil significantly increased the yield of total omega-3 fatty acids compared to the non-encapsulated linseed. Accordingly, the ratio of total omega-6 to omega-3 fatty acids significantly decreased when linseed oil was used in microencapsulated form. Encapsulation technology also seemed to improve meat content in conjugated linoleic acids (CLA). CLA are mixture of positional and geometric isomers of octadecadienoic acid containing a pair of double bonds in a conjugated configuration are widely recognized as a nutrient that exerts important physiological effects on human health. Numerous studies and reviews have investigated the supposed health benefits including anti-cancer, anti-atherogenic, anti-adipogenic, anti-diabetogenic, anti-inflammatory and effects on bone health (Lehnen et al., 2015; McCrorie et al., 2011; McGuire and McGuire, 2000). It has been reported that encapsulation of the CLA in a matrix of protein provided a protection of about 70% with a 30% hydrogenation of the CLA isomers (Gulati et al., 2000). Gulati et al. (2020) also showed that lactating goats fed with 80 g/animal per day of freeze dryer protected CLA-casein (1:1, w/w) led to an increase in the proportion of isomers 9-cis 11-trans and 10-trans 12-cis in milk fat. The total CLA levels were enhanced by about 10-fold above the control levels present in milk fat of goats fed with unprotected CLA, with a transfer efficiency of to the milk fat of 36-41% and 21-30% for the isomers 9-cis 11-trans and 10-trans 12-cis, respectively. The values observed in this research were higher than those obtained by Chouinard et al. (1999), with the CLA infusion directly into the abomasum of dairy cows. Interesting is also the possibility to use CLA in order to reduce the milk fat yield, as demonstrated by Peterson et al. (2002). The effectiveness of dietary supplements of CLA in animal feed is related to the extent to which their metabolism by rumen bacteria is avoided. On this background, Perfield et al. (2004) investigated the effects of rumen-protected CLA using lipid-based coating material on milk fat synthesis. A dosage of 138 g/d over the 7-day treatment period was able to reduce the milk fat yield of about the 22%. Encapsulation of oils has also proved useful in improvement of the nutritional characteristics of eggs. The current practice for producing omega-3 fatty acid-enriched eggs generally consists in feeding laying hens with flaxseed containing the omega-3 fatty acid ALA. Since laying hens have the ability to convert ALA to docosahexaenoic acid (DHA) as well as to eicosapentaenoic acid (EPA) to some extent, eggs obtained in this way are richer in these important fatty acids (Gonzalez-Esquerra and Leeson, 2001) The enrichment of eggs with EPA has been obtained through the use of marine oils (fish oil, fish meal) (Cachaldora et al., 2006). Here encapsulation is also used to mask the “fishy odors” and “fishy off-flavors” and to increase the feed consumption by laying hens. Indeed Lawlor et al. (2010) evaluated the effect of a diet supplemented with microencapsulated fish oil on DHA and EPA concentrations of table eggs, where 96 laying hens were randomly divided into 4 groups and fed with 0, 20, 40, or 60 g/kg for 21 days with a commercial product containing EPA and DHA from oily fish sources in a ratio of 18:12. The total omega-3 fatty acids significantly increased as the level of microencapsulated fish passed from 0 to 60 g/kg diet. Also, EPA and DHA were significantly higher with a 40-fold increase for the EPA and a 2.6-fold increase for DHA, when the laying hens were fed with 60 g/kg diet. Hard boiled and scrambled eggs were also evaluated from a sensorial point of view at the highest level of inclusion tested (60 g /kg diet) and only minor impact on the attributes “off-flavor” and “sulphur” was noted for boiled eggs. Conversely, there were no significant sensory differences between treatments for scrambled egg samples, demonstrating that the off-flavor perception was dependent on the method of preparation.

2.5 Encapsulation of organic acids

The ban on the use of antibiotic growth promoters in animals’ diet resulted in an increasing use of natural alternative substances that might be positive for both production performance and animal health. In this regard, probiotics, prebiotics, symbiotics and organic acids or their salts are promising alternatives. However, organic acids need to reach the intestine, without being absorbed too rapidly upon leaving the stomach. In fact, different research papers suggested that the effectiveness of unprotected organic acids may be limited, due to their prompt absorption and metabolism in the duodenum (Partenen, 2001, Upadhaya et al. 2014), which could cause bacterial resistance to acids and negative effects on gut mucosal membrane (Bearson et al. 1997). Therefore, many studies have been performed to investigate the effect of encapsulation as a tool to improve the delivery and efficacy of these compounds (Table 5). Piva et al. (2007) demonstrated that the microencapsulation of vanillin and sorbic acid in a hydrogenated vegetable lipid shell effectively allowed the slow-release of both active ingredients and prevented the immediate disappearance of such compounds upon exiting the stomach. Grilli et al. (2010) reported a significant antimicrobial effect of a commercial blend of citric and sorbic acids microencapsulated with hydrogenated vegetable lipids, when feeding pigs for 41 days with 3 kg/ton. They observed a reduction in volatile fatty acids and an enhanced fermentation in the large bowel with increased acids and tyramine production alongside an improved growth performance, with a higher average daily gain between 15 and 41 day and a higher body weight at 41 day for the treated group, when compared to control. Also, there was higher pig's feed intake throughout the study. Some studies harnessed the potential synergy between organic acids and plant-based essential oils (Gheisar et al., 2015; Tugnoli et al., 2020). Indeed, supplementing diets with microencapsulated blends of organic acids and essential oils (citric acid, 25%; sorbic acid, 16.7%; thymol, 1.7%; vanillin, 1.0%) loaded inside starch as a carrier (55.6%) showed a positive effect on growth performance broilers. The product used was a commercial microencapsulated organic acid added to the basal diet from 0 to 0.075%. The study showed an improvement of the broiler's growth performance and a decrease in drip loss percentage, without affecting other meat quality parameters, such as pH, color, and the relative organs weights. Similar results were reported by Cho et al. (2014), who demonstrated that the supplementation with the same microencapsulated commercial blend added at 0.05% to a finisher pigs' diet, significantly improved their growth performance. In addition, the supplementation was reflected in the increase in nutrient digestibility. Possible mechanisms for these effects include a reduction in the stomach pH and a direct action of the microencapsulated organic acids on the gastro-intestinal tract microbiota. This is because, the supplementation with the microencapsulated blend provokes changes in gut luminal metabolites, such as volatile fatty acids and polyamines. Volatile fatty acids deriving from the bacterial are rapidly absorbed by the epithelial cells of pigs, being metabolized to supply energy, and this could explain the increased growth and nutrient digestibility observed. However, dietary supplementation with the microencapsulated blend had no effect on meat color, sensory evaluation, cooking loss, meat pH and water holding capacity. The same commercial blend was used also by Stamilla et al. (2020), who fed chicken for the entire growing cycle at the level of 0.5%. Their dietary strategy led to a significant reduction in intramuscular fat content and an overall improvement in fatty acid profile. This dietary supplementation was also led to lipid oxidation reduction in the meat, which is one of the main problems related to chicken meat shelf life. Recently also Galli et al. (2020a) have dealt with this topic evaluating the effect encapsulated organic acid blend based on formic, phosphoric, lactic, acetic, butyric, and propionic acid with glycerol and silicon dioxide on the growth performance and meat quality of broilers. Results demonstrated that the encapsulated organic acid blend improves meat quality, with increased antioxidant levels and reduction of lipid peroxidation. Also, there was a decrease in total saturated fatty acid and an enhancement in monounsaturated/polyunsaturated fatty acid levels in broilers meat.

2.6 Encapsulation of essential oils and plant extracts

Phytogenic feed additives are commonly defined as plant secondary compounds and metabolites with beneficial effects on animal health and production, including animal products. Essential oils represent a major group of phytogenic feed additives. These compounds are usually added, at recommended levels, into animal feed and can have a broad range of bioactive properties, including feed palatability, digestive functions, animal intestinal microbiome structure, as well as improved production performance and quality of animal products in poultry, pigs, ruminant and aquaculture animals (Stevanović et al., 2018). However, their high reactivity represents an obstacle for direct application and incorporation into food and feed products. The main challenge in using these compounds is related to the preservation of their biological activity, as well as minimizing their impact on organoleptic properties of formulated feeds. The biological activity and the stability of essential oils could be compromised by temperature, light, metals, and water and oxygen (Turek and Stintzing

2013). For example, when a temperature of 58 °C is applied, which is the processing condition used for feed pelleting, the recovery of the essential oil indicator substances was low (Maenner et al., 2008). Secondly, the interaction between the essential oils the feed matrix could influence their activities (Śliwiński et al., 2002). Therefore, the encapsulation of essential oils is a reasonable choice for the supplementation of animal feed and, often, commercial blend containing different essential oils are used. The positive effect of encapsulated essential oils on the quality of ruminant, poultry and lamb meat is evidenced by several published works (Favaretto et al., 2020; Galli et al., 2020b; Spanghero et al., 2009), some of which summarized in Table 6. Recently, Galli et al. (2020b) explored the possibility of using a commercial phytogenic product with microencapsulated essential oil components (based on thymol, cinnamaldehyde and carvacrol) in the diet of 225 broiler chickens, in order to verify the effect on the meat composition with reference to the fatty acid profiles. There were significantly lower total saturated fatty acid levels with reduction of lauric and palmitic acid, which are undesirable as associated with the increase in LDL serum cholesterol. Moreover, a significantly greater monounsaturated/polyunsaturated fatty acid levels in broilers that consumed the commercial microencapsulated oil alone or in combination with curcumin was observed. With this research, the authors demonstrated that the fatty acids profile in broiler meat can be manipulated using various ingredients and/or additives. Additionally, Gomathi et al. (2018) reported that the reduction of the meat cholesterol content of broiler chicken supplemented with microencapsulated cinnamon oil and sodium butyrate had no effect on pH, cooking loss, water-holding capacity of meat. The sensorial attributes such as appearance, flavor, texture, mouth coating, juiciness and overall acceptability of meat were not influenced by the supplementation. Recently, Favaretto et al. (2020) used a commercial blend containing essential oils microencapsulated. According to the manufacturer, the product contains carvacrol, thymol and cinnamaldehyde. The objective of the study was to determine whether supplementation with these microencapsulated essential oils added to feed of growing lamb would affect their growth performance or produce antioxidants and immunological responses. Thirty female lambs were randomly distributed in three groups, as follows: control (basal feed), T500 (basal feed supplemented with 500 mg of microencapsulated essential oils /kg of concentrate) and T1000 (basal feed with 1000 mg of microencapsulated essential oils /kg of concentrate). Weights were measured and blood was collected on days 0, 15, 30 and 40 of the study. During the 40 days' trial, lambs belong to the T1000 group showed greater weight gain. Moreover, T1000 lambs had lower numbers of leukocytes and lymphocytes compared to control group. This supplementation changed the antioxidant profile without altering the levels of free radicals and lipid peroxidation, which could be a consequence of the high doses consumed by the animals. The activities of glutathione peroxidase and glutathione reductase were lower in the T1000 animals than in the control at day 30. In light of this, the authors concluded that supplementation with encapsulated essential oil in lambs stimulated antioxidant and anti-inflammatory responses and consequently increased weight gain in lambs. Similarly, a commercial blend of oregano, cinnamon, thyme and orange-peel essential oils was used to assess the influence of increasing concentration of the encapsulated mixture on milk yield and composition of primiparous dairy cows (Spanghero et al., 2009). In detail, the ruminant feed was fortified to provide 0, 40, 80 or 120 g/d of the microencapsulated essential oils and the ruminants were assigned to one of four dietary treatments. These authors reported a numerical trend for increased milk protein and fat concentration. The dietary supplementation with microencapsulated essential oils had no effect on dry matter intake, water consumption and milk production. Microencapsulated essential oils were also used to assess the possibility of modifying milk characteristics where Guasch et al. (2006) fed 40 cows for 56 days using 1.66 g/ kg DM with a combination of microencapsulated essential oil compounds containing coriander oil, geranylacetate, and eugenol. Their results showed that milk fat and milk protein were not affected by the supplementation with the microencapsulated essential oil and only a higher milk production between 25 and 56 day was observed. Different studies have disclosed that the use of encapsulated plant extracts could be useful to improve the animal body weight. As example, nanoencapsulation of aloe vera, dill, and nettle roots extracts using chitosan as shell material significantly improved the broiler chickens body weight gain after a 42-day experiment. Based on their results, Meimandipour et al. (2017) suggested that chitosan nanocapsules could be used as a substitute for antibiotics in the diet of broiler chickens. Likewise, the encapsulated form of garlic and *Phyllanthus niruri L.* mixture within an arabic gum shell revealed promising effects on live weight gain of broilers (Natsir et al., 2013). In another research, nanoencapsulated turmeric acid extract obtained thought bottom-up approach with ionic gelation method was able to reduced meat cholesterol of broiler chicken fed with supplemented diet. According to the obtained results, the authors conclude that 0.4% of nanocapsules was useful to improve the meat quality of broiler chicken (Sundari et al., 2014). Promising results were also obtained on rainbow trout (*Oncorhynchus mykiss*) juveniles fed with microencapsulated garlic extract especially in terms of growth performance. In addition, fish fed with 0.5% microcapsules had higher protein and lower lipid content when compared to the fish fed with the control diet (Adineh et al., 2020).

2.7 Encapsulation of phenolic compounds

The utilization of phenolic compounds and tannins has become very important in animal and specifically in ruminant feeding as a result of increasing studies that evidence their biological significance in animal nutrition. In general, the effects of tannins in ruminants' diet are related to their ability to form complexes mainly with proteins, polysaccharides whilst modulating ruminal fermentation which results in the improvement of the feed utilization and the final product quality (Costa et al., 2018, Patra and Saxena, 2011). They have been proposed as alternatives for antibiotic growth promoters in feed due to their antimicrobial, antioxidant, anti-inflammatory and gut health promoting when used at the appropriate concentration (Choi and Kim, 2020). Tannins can be broadly classified into hydrolysable and condensed tannins and their effect strongly depends on several factors including their molecular structure. The use of condensed tannin significantly reduces the enteric methane production (Costa et al., 2018) due to the direct inhibition of growth of the target methane-producing archaeal population (Animut et al., 2008). Condensed tannins also inhibit biohydrogenation (Vasta et al., 2019) and this would contribute to improve the quality of animal-based products, including the enrichment of conjugated linoleic acid (CLAs) in meat and milk (Patra and Saxena, 2011). However, the use of tannins in animal feed has important limitations mostly related to their astringency and bitter taste, which among other negative consequences, results in the reduction of voluntary dry matter intake in the animals (Dschaak et al., 2011). The administration of condensed tannin extracts could be thus facilitated by formulating them into products that could mask their taste, retain their structural integrity until consumption, increase their bioavailability, as well as release them precisely at the target site (Munin and Edwards-Lévy 2011). A careful selection of the appropriate encapsulation technology would pave the way for their integration into feeds. For example, the release of condensed tannins from the microcapsules must take place in the rumen where the modulation of biohydrogenation occurs (Tolve et al., 2021). Adejoro et al. (2019a) tested the ability to use encapsulated condensed tannins extract in ruminant feeding, using a water-soluble extract from the *Acacia mearnsii* tree as bioactive compound source and a combination of arabic gum and maltodextrin or native starch as shell material. The effect of the shell material and the core:shell ratio on the encapsulation efficiency, the loading and the *in vitro* kinetics of the tannin extract from microcapsules. According to their results, although starch and maltodextrin-arabic gum were successfully used in encapsulating the extract, the resulting encapsulates exhibited burst release profile under *in vitro* release in the digestive conditions. In order to overcome this problem, the same research group produced microencapsulated condensed tannins using the solid-in-oil-in-water and melt dispersion method choosing lard and palm oil as shell material. The results showed that both lard and palm oil could be used to encapsulate the condensed tannin extract with high encapsulation efficiencies, and good release of the tannin extract during the *in vitro* simulation of the digestive system. Moreover, the condensed tannins microencapsulated using lard or palm oil as shell materials also resulted in reduction in total gas and methane production evaluated trough *in vitro* tests (Adejoro et al., 2018). Adejoro et al. (2019b) evaluated the efficacy of encapsulated tannin extract in a lipid matrix using Africa Mutton Merino wethers as a model animal and found a higher nutrient digestibility (neutral detergent fiber digestibility) with better reduction in methane production for encapsulated extract when compared to non-encapsulated formulation. However, these researchers did not assess the effect of the encapsulated tannins on the fatty acids biohydrogenation and the derivate animal product. A recent study investigating the possibility of using microencapsulated anthocyanins as means to improve the production of natural pigments and provide natural pigments that enhance growth and pigmentation of fantail goldfish (Vanegas-Espinoza et al., 2019) is another compelling example of the broad application of encapsulation in animal feed. In this case, the technology allows the production of natural pigments and with enhanced stability which is known to be deeply influenced by environmental conditions such as light, oxygen, pH and temperature. In detail, Vanegas-Espinoza et al. (2019) microencapsulated Roselle calyx extract, (*Hibiscus sabdariffa*) via spray drying, using maltodextrins and sucrose as shell materials. The microcapsules were then used as supplement in Fantail goldfish (*Carassius auratus*) feed and the skin pigmentation of the fish was used as an indicator of the effectiveness of microencapsulation to protect anthocyanins and ensure their transport to the digestive system of the fantail goldfish where pigments are absorbed. It was observed an enhanced fish skin color with the increased supplementation level of microencapsulated anthocyanins from 150, 300, and 450 mg anthocyanin/kg of diet.

2.8 Encapsulation of probiotics and prebiotics

Probiotics are known as live microorganisms which when introduced into a host in adequate amounts confer health benefit whereas prebiotics act as fermentable substrates for probiotics and promote their growth. The positive effect of probiotics is now recognized with many reviews and studies suggesting that their addition into the feed improves the growth performance, population of microflora, intestinal microbiota, gut health, white blood cells and regulates the immune system of animals (Chen and Yu 2020; Abd El-Ghany, 2020). For example, lactating cows fed diets based on microbial ensiled straw resulted in increased milk production when compared with diets based on untreated agricultural by-products (Guo et al., 2002). *Lactobacillus* supplementation improved feed conversion rate, broiler chickens' productive performances and metabolic functions (De Cesare et al., 2017) and enhanced the fatty acid profile of meat even when broiler chickens were under heat stress (Jahromi et al., 2016). This microbial effect is related to their metabolites (postbiotics) and enzymes degrading fibrous compounds thereby increasing digestibility and nutrients utilization. Encapsulation technology increases probiotics stability during storage and processes, as well as their ability to pass unchanged through the mouth to the gizzard and intestines where they are most effective (Natsir et al., 2019) and therefore could enhance the effect of probiotics (Table 7). A study conducted on 192 hybrid ducks fed with non-encapsulated and encapsulated mixtures of natural acidifier (lactic acid and citric acid), phytobiotic (*Allium sativum* and *Phyllanthusiruri*) and probiotic (*Bacillus coagulans*) incorporated into the feed at different concentrations (0, 0.5, 1.0 and 1.5 %), showed that encapsulated formulations resulted in improved performance on production, intestinal morphometry and microflora with better body weight gain, feed conversion, number of *Lactic acid bacteria* and reduced number of *E. Coli* than non-encapsulated form of supplements (Natsir et al., 2019). The same authors suggested an optimum concentration of 1.5% which could partially explain reason behind the results obtained by Ardiansah et al. (2020) with no effect on dietary supplementation of either powdered or encapsulated probiotic (*Lactobacillus fermentum, Lacobacillus acidophilus, Bacilus spp*) on relative carcass, giblet weight and intestinal morphometry of duck since the concentration used was only up to 0.4%. Potential synergy between phytobiotics, probiotic and organic acids used could have also contributed to the difference in the results from the two studies. With a different approach, Park and Park (2011) reported that the use of microencapsulated inulin, a prebiotic with beneficial effects on animal health due to its ability to stimulate the selective growth of microorganism in the large intestine, in broiler chickens' diet significantly increases the breast weight.

3. Encapsulation of bioactive ingredients and animal welfare in a sustainable production context

Animal welfare is considered as a combination of both physical and mental well-being (Phillips, 2016) and could be affected by several factors, including thermal stress, disease, natural toxins and nutrient imbalances. This section discusses how the encapsulation of bioactive compounds could be used to improve animals' welfare, while increasing profitability for farmer and ensuring safety and quality through the food chains with reduced environmental impact. The use of the encapsulation to improve animal welfare is viewed through the five freedoms of animal welfare lenses according to the Farm Animal Welfare Committee (FAWC) (2014) presented in Table 8. A compelling example where encapsulation could have been used in this context is the study by Girard and Matte (2005), demonstrating an increase in the packed cell volume, blood hemoglobin and serum vitamin B_{12} of cows as well as the quantity of milk produced and its concentration in vitamin B_{12} after a weekly intramuscular injection of 10 mg vitamin B_{12} accompanied with folic acid and rumen-protected methionine supplemented feeds. Although the injection intramuscular avoided first-pass metabolism and achieved a precise plasma levels of the bioactive compound, it can cause severe tissue trauma and represent local portal of infection, even when correctly administrated (Abbate et al., 2018). Furthermore, the process is painful and may create a sense of fear, thus, could have potentially negatively affected four freedoms of animal welfare: freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behavior, and freedom from fear and distress (Table 8). The bioactive compound could have been encapsulated in a suitable shell designed to allow good stability and targeted delivery (see section with focus on vitamins) since oral administration is safer, non-invasive, simple and convenient for the animals. For example, it has been demonstrated that encapsulated vitamin E in feed resulted in the improvement of broilers growth and the quality of the meat whilst enhancing hepatic mitochondrial total antioxidant capacity and glutathione peroxidase enzyme activity (Hu et al., 2015), thus contributed to reinforcing the immune system of the broilers and their resistance to free radical’s attack. Similarly, acidification with various organic acids to diets such as fumaric, propionic, lactic and sorbic acid have been reported to decrease colonization of pathogen and production of toxic metabolites whilst improving digestibility of protein Ca, P, Mg and Zn as well as serving as substrate for the intermediary metabolism (Suiryanrayna and Ramana 2015; Swiatkiewicz and Arczewska-wlosek 2012). Choi et al. (2020) reported that the supplementation of microencapsulated organic acid and essential oil enhanced intestinal morphology and showed anti-diarrhea effects in weaned piglets challenged with enterotoxigenic *E. Coli* F4, and thus contribute to ensuring freedom from pain and disease. Zhang et al. (2015) showed an effective delivery of high concentration of a model essential oil, carvacrol, to the jejunum and ileum of chickens using a combination of food-grade biopolymers as shell material where alginate provided the gastric resistance microparticles, while whey protein modulated the intestinal release. This high release was sustained for more than 3 h after oral administration, though the *in vivo* release of carvacrol alginate-whey microparticles was faster when compared to the release from *in vitro* simulation. This work reinforced the point that *in vitro* simulation could only serve as a tool for formulation optimization and the need for *in vivo* study. Purba et al. (2020), after a systematic review and meta-analysis to predict and identify ways to increase conjugated linoleic acid formation in ruminant-derived products, reached the same conclusion that *in vivo* method was more suitable for the direct observation of fatty acid transformation than the *in vitro* method. This is even more important when come to assessing the effect of formulations of animal welfare as direct of observation of the behavior also come into play in addition to biological markers of their well-being. Encapsulation also creates opportunities for an integrated approach in the formulation and delivery of actives compounds for animal welfare by harnessing synergy between different actives to allow maximum efficacy, reduction potential risk of toxicity and to limit the production of greenhouse gas emissions. Indeed, it has been demonstrated that symbiotic formulations had enhanced beneficial impact on piglets' health especially during weaning period, when compared to commercial probiotics used individually (Chlebicz-Wójcik and Śliżewska 2020). Here one could also imagine formulations with symbiotic and other bioactive compounds such as polyphenols (Choi et al., 2020), omega-3 fatty acids (Baéza et al., 2017) to avoid energy excesses, digestive disorders and minimize inflammatory responses, thereby optimizing their immune system and contributing to ensure the five freedoms of animal welfare. In the context of sustainable production, where the use of renewable natural ingredients and available feed resources to their full potential alongside waste reduction are becoming necessary, the encapsulation of natural extracts containing mixed bioactive compounds from agricultural waste and by-product from food processing to improve their stability, delivery and efficacy for animal welfare and nutrition is important. In this regard, Mamvura et al. (2014) encapsulated nitrate with sesame gum and demonstrated *in vitro* the sustained release of nitrate and the microparticles potential for reduction methane production, thus enhancing sustainability of ruminant production while reducing the risk of nitrite toxicity. A preliminary *in vitro* study by Santos et al. (2020) showed calcium salts of long-chain fatty acids may accelerate propionate production and reduce methane production by ruminants. Hence, calcium salts of long-chain fatty acids could be a potential shell material for developing new multifunctional animal feeds loaded with different bioactive compounds Finally, to ensure welfare of animal when using encapsulated bioactive compounds, special attention should be paid to the toxicity aspect of such approach, especially when considering nano size materials. Factors affecting the toxicity include materials intrinsic toxicity, the size, shape and surface chemistry of the encapsulates. Although nanoparticles have been proving to improve the bioavailability and absorption of micronutrients and other bioactive compounds, it has been reported that epithelial cells of the small intestine are capable of absorbing nanoparticles less than 200 nm in size, whereas some literature suggested that smaller (<5 μm) are absorbed through the circulatory system (Bribiesca et al., 2017). Hence, nanoparticles have the potential to reach the circulatory system and be distributed anywhere in the body, principally in the liver, spleen and other organs of the reticuloendothelial (Rabanel et al., 2012). The toxicity of nanoparticles depends on their persistence in the organs, where they are deposited and the physiological response of the host tissue in maintaining or eliminating waste of the shell materials used in their manufacture.

Conclusion and outlook

This review has highlighted the potential of encapsulation technology to improve formulation of bioactive compounds into the feed matrix and their delivery for better animal production efficiency, animal welfare and quality of animal-based products. Different encapsulation approaches with a wide range of bioactive compounds are reported in the literature showing promising results for enriching animal-based product with feeds containing encapsulated live micro-organism and their metabolites, probiotics, phytobiotic in a form of a single type compound or blend of several compounds as growth promoter alternative to antibiotic for safe production across the food chain. Products such as eggs, meat, fish and milk could be enriched with selected micronutrients and other bioactive compounds through appropriate feeding, but to our knowledge, no literature is present dealing the effect of the encapsulated bioactive ingredients on dairy products. For example, it was observed an improvement in the content and profile of amino acids, fatty acids with increase in omega -3 fatty acid and conjugated linoleic acids while decreasing the content of cholesterol in some animals and subsequent food products, which are desirable attributes from consumers' health perspective. The review also showed how the encapsulated bioactive compound could contribute to support the five freedoms of animal welfare: freedom from malnutrition, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behavior, and freedom from fear and distress. However, there was limited number of publications that combined *in vitro* and *in vivo* studies of the effect of encapsulated bioactive compounds with focus on the animal welfare. *In vivo* studies are very important when come to assessing the effect of formulations of animal welfare as direct of observation of the behavior also come into play in addition to biological markers of their well-being. Further investigations should focus on addressing this apparent gap in this key knowledge. The development of simple, cheap, effective and environmentally friendly smart encapsulation system for delivery of bioactive compounds in animal feed deserves more research. Moreover, future research should give more consideration to factors that affect the mechanical disintegration of encapsulates during rumination, the effect of particles size on the amount lost from the rumen alongside gas via the belching process as such information would be vital for optimizing the encapsulation formulation and manufacturing process for better outcome in terms of profitability for farmers, animal welfare and the derived food product quality as well as green gas reduction during production.

Author Contributions: Conceptualization, F.G., M.C.C. and F.L.T.M. Writing—original draft preparation, R.T., F.L.T.M., T.S. and M.D.C.; Writing—review and editing, F.G, F.L.T.M. and R.T. supervision, F.G., M.C.C. and F.L.T.M. All authors have read and agreed to the published version of the manuscript.

Funding

The study was financially supported by MILK BIOACTINCAPS project "Use of microencapsulates of bioactive compounds from food industry waste as feed supplements for the improvement of the fermentative aptitude and nutraceutical value of milk" funded by the Italian Ministry of Agriculture, Food and Forestry Policies, Mipaaft (Ministry Decree n. 27443 of 25.09.2018).

Declaration of competing interest

The authors declare no conflict of interest.

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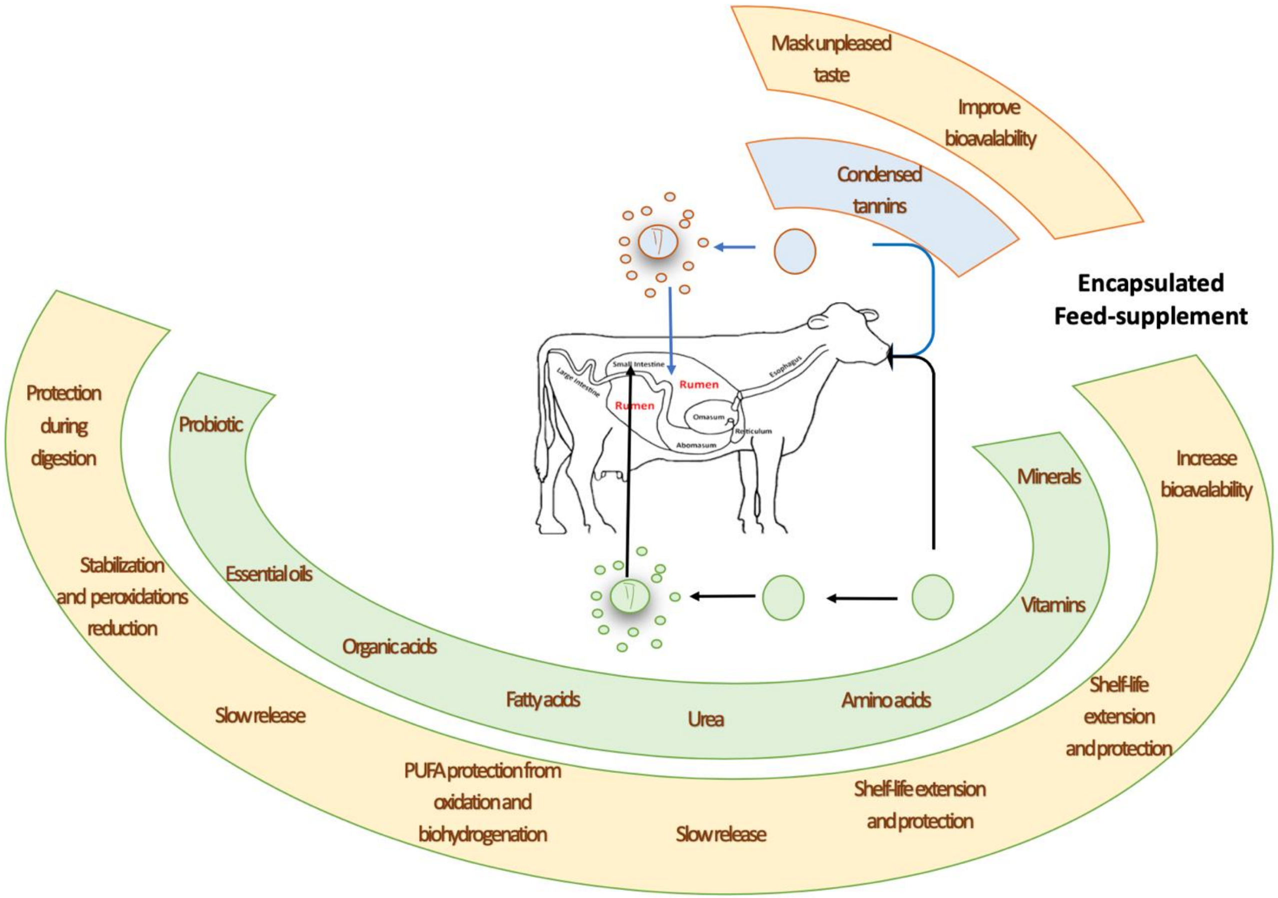

Figure 1. Schematic diagram showing the protecting effect and the site-specific release of the encapsulated bioactive compounds. Generally, the feed supplements that need to be protected from the environment and the microbial degradation that could occur in the rumen are protected by coating materials with the ability to degrade a pH below 5, thus are a candidate for release in small the intestine. The bioactive compounds characterized by unpleasant taste generally are encapsulated in coating materials that can mask the taste.

Table 1. Encapsulated minerals with potential in animal feed

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Encapsulation method	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference

Sodium selenite	Increase of the mineral content of milk	Protect minerals from the transformation in insoluble compounds in rumen	Spray-chilling	Hydrogenated fat	From 500 to 2000 µm	Microcapsules in order to provide 0.3 and 0.5 mg/kg DM of total Se	Cows fed with microencapsulated Se at 0.3 mg/kg had 38% higher milk Se content than control animals	Grilli et al. (2013)
Sodium selenite and zinc carbonate	Increase of the mineral content of meat	Protect minerals from the transformation in insoluble compounds in rumen	Emulsion-evaporation	Eudragit RL	From 500 to 2000 µm	0.6 µg g^{-1} Se + 30.0 µg g^{-1} Zn nanoparticles	Selenium and Zn nanoparticles increased the concentration of these minerals in lam's meat as compared with supplementation of raw Se and Zn	Bribiesca et al. (2017)
Sodium selenite	Increase of the mineral content of milk/meat	Protect minerals from the transformation in insoluble compounds in rumen	Emulsion-evaporation and nano-precipitation	Eudragit ® RL and RS	From 36.64 to 213.86 nm	No *in vivo* study	The release of selenium from nanoparticles was higher at acid pH (less than 4); this condition may represent a better availability of the mineral in the small intestine	Romero-Pérez. et al. (2010)

Table 2. Encapsulated vitamins with potential in animal feed

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Encapsulation method	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Vitamin E	Improvement of growth and performance	Increase of the overall bio-accessibility of vitamin E	NR	Sodium octenyl succinate	NR	20 mg/kg	Microencapsulated vitamin E was effective in increasing its bio-accessibility with a positive impact on stabilizing breast broiler and yielded better broiler growth performance (weight gain) when compared to non-encapsulated vitamin E fed groups	Hu et al. (2015)
Vitamin A	Beneficial effect on the immune, the central nervous system, reproductive performance, and normal bone growth	Vitamin protection	NR	Lecithin, cholesterol and functionalized stearyls	NR	NR	Retards of the vitamin A degradation	Albright and Kowarski (1994)
α-tocopheryl-acetate	Increase vitamin E concentration in meat	Vitamin protection	NR	Sodium caseinate	NR	250 IU	Higher α-tocopherol concentrations in psoas major muscle than lambs fed with nonencapsulated vitamin E	Lee et al. (2007)
Vitamin B (biotin, folic acid, pantothenic acid and pyridoxine)	Impact on health and milk production	Vitamin protection	NR	NR	NR	3 g/d	Supplying cows with a ruminally protected B vitamin blend increased milk and milk component yields, especially milk protein	Sacadura et al. (2008)

NR= commercial product/blend; information not reported

(continued)

Table 2. Continued

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Encapsulati-on method	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Vitamin B (niacin)	Increase of resistance to thermal stress	Increase vitamin absorption	NR	Lipidic shell	NR	12 g/d	Supplementation of lactating cows with encapsulated niacin reduced cow's body temperature	Zimbelma et al. (2013)

NR= commercial product/blend; information not reported

Table3. Continued

Table 3. Encapsulated amino acids and urea with potential in animal feed

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Technique of encapsulation	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Cysteamine	Beneficial effect on animal's growth performance	Cysteamine protection from the oxidation	NR	Palm stearin (vegetal)	NR	27, 54, or 108 mg/kg cysteamine	Microencapsulated cysteamine significantly increased carcass lean percentage and *longissmus muscle* area	Tao et al. (2020)
Methionine and Lysine	Improvement in milk or milk protein production	Delivery of amino acids in duodenal sites	NR	NR	NR	10.2 g/d of methionine and 16.0 g/d of lysine	Cows produced milk with more true protein (1306 vs. 1221 g/d), and fat (1632 vs. 1550 g/d)	Socha et al. (2005)
Methionine	Improvement in milk or milk protein production	Protection of methionine from the ruminal environment	Melt-emulsification technique	Carnauba wax	NR	No *in vivo* study	Efficient ruminal protection	Carvalho Neto et al. (2019)
Lysine	Improvement in milk or milk protein production	Protection of lysine from the ruminal environment	Organic solvent, free emulsifycation, sonication method	Stearic acid and SPAN 80	From 200 to 500 nm	No *in vivo* study	Solid lipid nanoparticles composed of arachidic or stearic acids and Tween 60 resisted ruminal digestion for up to 24 h	Albuquerque et al. (2020)

NR= commercial product/blend; information not reported

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Encapsulati-on method	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Histidine	Improvement in milk or milk protein production	Protection of histidine from the ruminal environment	NR	NR	NR	164 g/d	Supplementation of lactating cows with encapsulated histidine significantly increased the amino acid concentration in plasma and muscle. The milk true protein increased as well.	Zhang et al. (2019)
Urea	Urea is the main source of nonprotein nitrogen used in the ruminant diet	Slow release	NR	Polyurethane	NR	1.7%	Coated urea increased the ruminant dry matter intake and the nutrients digestibility compared to the uncoated urea.	Xin et al. (2010

NR= commercial product/blend; information not reported

Table 4. Encapsulated fatty acids with potential in animal feed

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Technique of encapsulation	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Sunflower oil	Reduce SFA and increase PUFA con-centration in animal products	PUFA protection from biohydrogenation	In situ polymerization	Sodium caseinate	<625 mm	2 kg/day	Lambs feed with encapsulated sunflower oil and vitamin E were characterized by a higher LA levels in both *longissimus dorsi* and *psoas major muscles*	Lee et al. (2007)
Ground whole soybeans	Reduce SFA and increase PUFA concentration in animal products	PUFA protection from biohydrogenation	In situ polymerization	Casein treated with acetaldehyde or diacetyl	<625 mm	10% of DM intake	Fat of lambs supplemented with encapsulated linoleic acid had higher concentrations of linoleic (LA) and linolenic acids (ALA)	Lee et al. (2004)
Oil high in linoleic and alfa-linolenic acid	Reduce SFA and increase PUFA concentration in animal products	PUFA protection from biohydrogenation	Spray-dryer	Formaldehyde-treated protein	NR	60 g of oil containing more than the 60% of PUFA	LA and ALA in lamb muscle of about 247% and 57%, respectively compa-red to those fed unprotec-ted lipids	Elmore et al. (2005)
Ground flaxseed	Reduce SFA and increase PUFA concentration in animal products	PUFA protection from biohydrogenation	High-speed turbulizer + dryer	Dolomitic lime hydrate	NR	2-4 or 6% of DM	ALA content of muscle tissue was 47% greater when flaxseed was encapsulated within the dolomitic lime hydrate matrix	Alvarado-Gilis et al. (2015b)

3 NR= commercial product/blend; information not reported

(continued)

Table 4. Continued

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Technique of encapsulation	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Commercial product contained linseed oil, vitamin E, rosemary extract	Reduce SFA and increase PUFA concentration in animal products	PUFA protection from biohydrogenation	Spray chilling	Hydrogenated palm oil	From 1000 to 1500 μm	2% of DM	The use of the commercial microencapsulated linseed oils significantly increased the yield of total omega-3 fatty acids in milk compared with non-encapsulated linseed	Kim et al. (2020)
Conjugated linoleic acid (CLA)	Increase conjugated linoleic acid concentration in milk	CLA protection from biohydrogenation	Freeze dryer	Formaldehyde-treated casein	NR	80 g/day	The total CLA levels were enhanced by about 10-fold above the control levels present in milk fat of goats fed with unprotected CLA	Gulati et al. (2000)
CLA	Reduces milk fat yield	CLA protection from biohydrogenation	NR	Lipidic coating	NR	138 g/day	Reduction of milk fat yield of about the 22%	Perfield et al. (2004)
Fish oil rich in eicosapentaenoic acid and docosahexaenoic acid	Increase eggs unsaturated fatty acids	Fish oil protection from the oxidation and fishy odors and flavors masking	NR	NR	NR	60 g/kg/day	Increase of 40-fold for the eicosapentaenoic acid and 2.6-fold for docosahexaenoic acid feeding the laying hens with microencapsulated fish oil	Lawlor et al. (2010)

4 NR= commercial product/blend; information not reported

Table 5. Encapsulated organic acids with potential in animal feed

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Technique of encapsulation	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Citric acid, sorbic acid, thymol, vanillin	Increase the growth performance	Allows a slow release	NR	NR	NR	0.5 % of DM	The addition of microencapsulated organic acid and essential oils to the chicken diet bring to a significant reduction in intramuscular fat content and an overall improvement in fatty acid profile. In addiction the reduction of the lipid oxidation in meat was observed	Stamilla et al. (2020)
Citric and sorbic acids	Increase the growth performance	Allows a slow release	NR	Hydrogenated vegetable lipids	NR	3 kg/ton/day	As well as exert an antimicrobial effect, microencapsulated organic acids significantly affect the growth performance due to a higher pig's feed intake throughout the study (+ 4.6%)	Grilli et al. (2010)
Citric acid, sorbic acid, thymol, vanillin	Increase the growth performance	Allows a slow release	NR	NR	NR	0.075 % of DM	The addition of microencapsulated organic acid and essential oils to the diet of broiler chickens improved their growth performance and decreased drip loss percentage	Gheisar et al. (2015)
Citric acid, sorbic acid, thymol, vanillin	Increase the growth performance	Allows a slow release	NR	NR	NR	0.05 % of DM	The addition of microencapsulated organic acid and essential oils to the diet of pigs significantly affect the growth performance	Cho et al. (2014)

NR= commercial product/blend; information not reported

(continued)

Table 5. Continued

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Technique of encapsulation	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Formic, phosphoric, lactic, acetic, butyric, and propionic acid	Increase the growth performance	Reduces the lipid peroxidation	NR	NR	NR	1-3 kg/ton/day	The addition of microencapsulated organic acid to the broilers significantly increased antioxidant levels and bring to a reduction of lipid peroxidation	Galli et al. (2020a)

NR= commercial product/blend; information not reported

Table 6. Encapsulated essential oils and plant extract with potential in animal feed

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Technique of encapsulation	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Carvacrol, thymol and cinnamaldehyde	Increase the growth performance	Reduces the lipid peroxidation	NR	NR	NR	1000 mg/kg/day	The supplementation with microencapsulated essential oil in lamb stimulated antioxidant and anti-inflammatory responses and consequently increased weight gain in lamb	Favaretto et al. (2020)
Thymol, cinnamaldehyde and carvacrol	Increase the growth performance	Stabilizes essential oils characterized by high reactivity	NR	NR	NR	100 mg/g/day	The addition of microencapsulated essential oils to broiler chicken diet significantly reduces total saturated fatty acid levels	Galli et al. (2020b)
Oregano, cinnamon, thyme and orange-peel essential oils	Increase the growth performance	Reduces the lipid peroxidation	NR	NR	NR	120 g/day	The addition of microencapsulated essential oil on dairy cows increased milk protein and fat concentration	Spanghero et al. (2009)
Cinnamon oil and sodium butyrate	Increase the growth performance	Reduces the lipid peroxidation	NR	Vegetable fatty acids	NR	250 mg/kg/day	Cinnamon microencapsulated significantly reduced the meat cholesterol of broiler chickens	Gomathi et al. (2018)

NR= commercial product/blend; information not reported
(*continued*)

Table 6. Continued

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Technique of encapsulation	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Coriander oil, geranylacetate, and eugenol	Modify milk characteristics	Reduce the lipid peroxidation	NR	NR	NR	1.66 g/ kg DM	The addition of microencapsulated essential oil had no effect on milk fat and milk protein	Guasch et al. (2006)
Aloe vera, dill, and nettle roots extracts	Improve animal body weight	Overcome the extract limitations related to the hydrophobic, highly active, reactive and volatile properties of bioactive compounds in the extracts	Ionic gelation	Chitosan	NR	0.02, 0.025 and 0.05 %	The addition of microencapsulated plant extract significantly improved the boiler chickens body weight gain	Meimandipour et al. (2017)
Garlic and *Phyllanthus niruri L.* mixture	Improve animal body weight	Overcome the extract limitations related to the hydrophobic, highly active, reactive and volatile properties of bioactive compounds in the extracts	NR	Arabic gum	NR	0.4%	The addition of microencapsulated plant extract had promising effects on live weight gain of broiler	Natsir et al. (2013)
Garlic extract	Improve meat quality	Overcome the extract limitations related to the hydrophobic, highly active, reactive and volatile properties of bioactive compounds in the extracts	Freeze dryer	Maltodextrine and arabic gum	NR	0.5%	Rainbow trout fed with microencapsulated garlic extract had higher protein and lower lipid content compared to the fish fed with a control diet	Adineh et al. (2020)

NR= commercial product/blend; information not reported

(continued)

Table 6. Continued

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Technique of encapsulation	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Acacia mearnsii extract	Increase the PUFA in milk	Masks polyphenols taste, retaining their structural integrity until consumption, increase their bioavailability, and release them precisely at the target site	Freeze dryer	Maltodextrine and arabic gum or native starch	From25 to 40 µm	No *in vivo* study	Starch and malto-dextrin- arabic gum were successsfully used in tannin extract encapsulation. Microparticles were smaller and homogenous	Adejoro et al. (2019a)
Roselle calyx extract (*Hibiscus sabdariffa*)	Improve skin pigmentation in fish	Masks polyphenols taste	Spray dryer	Maltodextrine and sucrose	From 1.0 to 16.0 µm	150, 300, and 450 mg antho-cyanin/kg of DM	It was observed that fish skin color increased, while the supplementation of microencapsulated anthocyanins increased	Vanegas-Espinoza et al. (2019)
Quebracho extract	Increase the PUFA in milk	Masks polyphenols taste, retaining their structural integrity until consumption, increase their bioavailability, and release them precisely at the target site	Spray dryer	Maltodextrine and arabic gum	< 100 µm.	No *in vivo* study	Maltodextrin- arabic gum were successsfully used in tannin extract encapsulation. Microparticles were smaller and a greater release occurred at the ruminal level	Tolve et al. (2021)

Table 7. Encapsulated probiotics with potential in animal feed

Bioactive compound	Putative beneficial biological effects	Potential advantage of encapsulation	Technique of encapsulation	Shell material	Particle size	Dose	Key Findings as Reported by Authors	Reference
Lactic acid and citric acid, *Allium sativum* and *Phyllanthu siruri* and *Bacillus coagulans*	Improve the performances, population of microflora, white blood cells and cholesterol content	Improves stability during processing transport and storage. Protection of pro-biotics during digestion	Microwave oven drying	Arabic gum and whey protein	NR	1.5%	The microencapsulated form of the mixture provided better results, in terms of body weight gain, than a non-encapsulated form	Natsir et al. (2019)
Lactobacillus fermentum, Lacobacillus acidophilus, Bacilus spp	Improve the performances, population of microflora, white blood cells and chole-sterol content	Improve stability during processing transport and storage Protect probiotics during digestion	NR	Arabic gum and whey protein	NR	0.2-0.4%	No significant effect of encapsulated probiotic on relative carcass, giblet weight, and intestinal morphometry of duck	Ardiansah et al. (2020)
Inulin	Positive effect on the productivity Antibacterial effect	Inulin is vulnerable to the air and has a low transit rate in caecum	Freeze dryer	Sureteric®	NR	200-250 g/ton	Significantly higher breast weights and thigh muscles and lower abdominal fat in broiler chickens fed with microencapsulated inulin compared to the control	Park and Park (2011)

Table 8. Five freedoms of animal welfare and their relation to nutrition alongside potential benefits of encapsulated ingredients-based feed with specific example (adapted from Phillips (2016) and FAWC, 2014).

Freedom	Relation to nutrition and environmental stress	Example of potential effect of feeds containing encapsulated bioactive ingredients
1. Freedom from thirst, hunger and malnutrition	Short-term malnutrition can result in the emotions of hunger in animals but long-term consequences of malnutrition are uncertain	Encapsulation results in increased nutrients bioavailability, thus reducing the risk of malnutrition at equivalent nutrients in the feed Fat coated cysteamine to mask its strong smell which may affect feed palatability an intake
2. Freedom from discomfort and exposure	Many consequences of unbalanced diet are likely to lead to discomfort such as bloating; acidosis, ketosis, deficiencies and toxicities. Some diseases causing discomfort have many predisposing factors including malnutrition	Encapsulation with slow-release of bioactive compounds to prevent their immediate absorption and reduce potential risk of toxicity. Increase of resistance to thermal stress with encapsulated vitamin B in the feed
3. Freedom from pain, injury and disease	Unbalanced diet often ends in a diseased state usually accompanied by pain	Encapsulated symbiotic and natural antioxidants such as polyphenols
4. Freedom to express normal behavior (both appetitive and consummatory)	Diets are often too concentrated in energy and other essential nutrients to ease distribution to the animals and accelerate production or reproduction rates. Because of the inverse relationship between energy and fiber concentration in forages, concentrated feeds are often deficient in fiber. Livestock that evolved to consume fibrous herbage or forage for a substantial portion of the day display abnormal oral behaviors in response to highly concentrated diets that can be rapidly eaten and digested	Sustained release of nutrients, modulate microbial flora with probiotic and prebiotic to increase fiber digestibility and conversion Use encapsulated tannins and polyphenol to increase mask taste, increase appetite and animal well-being
5. Freedom from fear and distress	Responses to repeated nutritional stress or environmental stress (heat) may invoke fear responses. Farmers should avoid or limit treatment which could cause mental suffering	Sustained and targeted delivery at desirable site using feed con-taining encapsulated of bioactive compounds to instead of frequent injections