POTENTIAL FOR APPLICATION OF FEED ADDITIVES ENCAPSULATION TECHNOLOGY IN ANIMAL NUTRITION

POTENCIJAL ZA PRIMJENU TEHNOLOGIJE INKAPSULACIJE DODATKA HRANI U HRANIDBI ŽIVOTINJA

Sara Kolar, S. Jurić, Kristina Vlahoviček-Kahlina, M. Vincaković

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SUMMARY

Demands for higher production and consumer demand for healthier food have encouraged intensive research for alternative animal growth promoters in recent years. Research trends are focused on the development of new products enriched with feed additives to improve animal health and enhancing production. Many of these compounds are unstable in the presence of light, air, water, or high temperatures and need to be protected during processing, storage, and application. By encapsulated in microparticles, feed additives are protected from harmful external influences, and their stability and functionality are not diminished.

Microencapsulation technology is used to protect payload and improve bioavailability by controlled and targeted delivery to the digestive tract. It is particularly suitable for the addition of feed additives in ruminant's nutrition, because the correct choice of microparticle material allows the release of the feed additives in the small intestine, and not in the rumen. With proper use, microparticle formulations are an effective tool in animal nutrition that delivers nutrients and/or drugs to a specific site at the desired rate.

The paper summarizes laboratory studies on the application of microencapsulation technology in feeding ruminants and monogastric animals. Numerous results of the application of encapsulated feed additives have shown a positive effect on animal health, increased productivity without negative effects on the final product, and environmental protection.

Keywords: encapsulation, nutrition, targeted release, feed additives, animals

INTRODUCTION

In the last few decades, there has been exponential population growth globally, accompanied by increased demands in food production. Since the 1960s, global milk production has doubled while meat production tripled, reaching the amount of 340 million tons in 2018 (Ritchie, 2019). An increase of 400% was also reported in grain production (Kopittke et al., 2019). According to Rös et al., (2017) by 2050, the number of Earth’s population is estimated to be between 9 and 11 billion. For the growing population in the world to be qualitatively and quantitatively supplied with food, it is necessary to increase production in the agricultural sector by 60% (Fróna et al., 2019). Apart from producing enough food for a growing population with a lack of

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rural working population (Alexandrotos and Bruinisma, 2012), agriculture is facing many other challenges like conservation of plant and animal genetic resources as the basis of selection (FAO, 2016), reduction of environmental pollution and decreases in climate change by applying sustainable, efficient and environmentally friendly production methods (Herrero and Thornton, 2013; FAO, 2016). Livestock breeding, as an exceptionally important part of agriculture, aims to increase feed conversion, milk and meat production per animal, egg production, the earlier achievement of target weight, reduction of greenhouse gas emissions in ruminants, etc. (Hume et al., 2011). Mostly, success has been achieved through breeding selection and the formation of adequate breeding programs (Hume et al., 2011), genetic and genomic advances (Chowanadisai et al., 2020), alongside precise and sustainable animal nutrition and feeding (Muck et al., 2018; Morais et al., 2020).

The demands faced by food production (increased production efficiency, health promotion, and prevention of animal diseases by diet, reduction, or prohibition of chemical additives use) have prompted numerous studies of finding alternative growth promoters in animal nutrition. Various synthetic compounds (antibiotics, antiparasitics, coccidiostats, fungicides, or anti-inflammatory drugs) are used for better animal health and growth. However, certain chemicals that until recently served as growth promoters have caused additional problems for humans and animals. This primarily refers to antibiotics and increasing the resistance of pathogenic organisms, to the non-selective action of antibiotics in the digestive system of animals and harmful residues in products for human consumption. Globally, the use of antibiotics and chemicals in the diet is increasingly being abandoned (e.g., Europe completely banned in 2006 (Regulation 1831/2003 / EC), the USA partially restricted, etc.) and alternative additives are being used.

The animal feed industry is increasingly focusing on safer and more cost-effective supplements (phytochemicals, lipids, vitamins, peptides, fatty acids, antioxidants, minerals, probiotics) that are important for the metabolic needs of domestic animals. Phytochemicals are compounds of natural origin (whole plant or parts, essential oils), contain a broad-spectrum of bioactive ingredients (antimicrobial, antioxidant, antiviral, antifungal, etc.), have a positive effect on appetite, digestion, and animal health, and increase production (Syed, 2015; Upadhaya and Kim, 2017). Phytagenic additives are proven to be less toxic compared to the synthetically derived antibiotics and could successfully replace the latter as animal growth promoters (Hengel et al., 2011; Gregačević et al., 2014).

Most feed additives are chemically unstable and decompose in the presence of air, light, moisture, and/or high temperatures. Losses in production and metabolic disorders caused by environmental factors, reduction of greenhouse emissions in ruminants can be minimized by implementation of technologies such as encapsulation of feed additives. Encapsulation protects additives without compromising their stability and functionality. An additional advantage is the possibility of controlled release and targeted action, and therefore encapsulation technology is an important tool for adding feed additives to food products.

Significant results were achieved by the application of the encapsulated ingredients in the agriculture, pharmacy, medicine, veterinary medicine, biotechnology, textile, and food industry (Vinceković et al., 2016, 2017; Ozturk and Temiz, 2018; Fathi et al., 2019; Jurić et al., 2020a, 2020b, 2020c). Therefore, the application of encapsulation in animal nutrition to manipulate the rumen microflora, controlled release of ingredients in the gastrointestinal tract, and reduction of digestible energy loss is intensively investigated. This review aims to summarize current knowledge on the use and potential application of feed additives microencapsulation in the feeding of ruminants and monogastric animals.
Encapsulation technology

Encapsulation is a process of incorporating solid, liquid, and gaseous ingredients into particles of various sizes (Desai and Jin Park, 2005; Ozturk and Temiz, 2018; Lengyel et al., 2019) to preserve the functional properties of the encapsulated ingredient. Particles larger than 5000 μm are defined as macro-particles, 0.2 – 5000 μm as microparticles, and particles smaller than 0.2 μm as nanoparticles (Texeira da Silva et al., 2014). We distinguish two basic types of encapsulation particles, matrix particles, and capsules (Figure 1). Matrix particles are systems in which the active ingredient is uniformly dispersed or dissolved in the matrix. They can be homogeneous or heterogeneous, depending on whether the active ingredient is in the molecular state (dissolved) or in the form of particles (suspended) (Shahidi and Han, 1993). Capsules consist of an inner core that contains the active substance and is covered with a polymer layer (membrane). Regarding the shape, regular and irregular particles can be distinguished (Vasihi, 2014; Ribeiro et al., 2019), and structurally and morphologically, the particles can be classified as mononuclear, poly-nuclear, microspheres, aggregates, and multilayer particles (Desai and Jin Park, 2005; Jabeen et al., 2017; Ozturk and Temiz, 2018; Ribeiro et al., 2019; Jurić et al., 2020d). Preferably, particles of spherical shapes and micron dimensions are prepared and are called microspheres or microcapsules, and with the encapsulated active ingredient (payload), microsphere formulations, or microcapsule formulations.

The choice of material for a particle depends on the physical and chemical properties of the active ingredient, the desired characteristics of the particle, and its final application (Desai and Jin Park, 2005; Jurić et al., 2019). The particle, as a physical barrier between the active ingredient and the environment, must be chemically inert to the payload (Jabeen et al., 2017) and stable to the action of environmental factors (heat, light, moisture, temperature) (Desai and Jin Park, 2005). Retention within the particle during storage protects the active ingredient from oxidation or any other degradation processes, i.e. stability, strength, and flexibility are maintained (Jabeen et al., 2017). The basic requirements for material selection are that: (i) it is permitted for food production, (ii) it protects the payload from interaction with other food ingredients, (iii) it ensures bioavailability after consumption, (iv) it releases a payload

Table 1 Commonly used biopolymers for encapsulation of payload used in the food industry

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in a controlled manner, and (v) easy application in industrial production. No material could meet all the requirements (depending on the application of the particle) and capsules with two or more shells are often used.

For example, encapsulation with lipid materials is suitable for use in animal nutrition. When designing a microparticle, the active ingredient is incorporated into a lipid matrix or prepared in small beads and then coated with a lipid material (Gadeye et al., 2017). Lipids protect the particle from rumen enzymes, and feed additives are released in the small intestine by the action of intestinal enzymes.

Table 1 lists the most commonly used encapsulation materials in food products (proteins, lipids, and polysaccharides) (Doppalapudi et al., 2014; Namdeo, 2014; Sobel et al., 2014; Teixeira da Silva et al., 2014; Rastogi et al. Samyn, 2015; Gadeye et al., 2017; Leyva-Gómez et al., 2018).

Besides particle properties, physical and chemical properties of the payload, the particle efficiency also depends on the method of encapsulation. Methods can be classified into chemical (interfacial and in situ polymerization), physical (spray drying, centrifugal extrusion, supercritical – assisted encapsulation), and physicochemical (ionic gelation, spray cooling, coacervation, solvent evaporation) (Sobel et al., 2014; Teixeira da Silva et al., 2014; Jabeen et al., 2017). The spray encapsulation method is most often used for food products because it is flexible, continuous, and economical (Nedović et al., 2011).

Implementation of encapsulation technology in pharmacy, medicine, agronomy, biotechnology, food, textile, and cosmetics industry has demonstrated significant results so far. Encapsulation protects the active ingredient from degradation due to the action of external (water, light, temperature, pH, metals) and gastrointestinal influences (Desai and Jin Park, 2005; Sobel et al., 2014; Vasisht, 2014; Jurić et al., 2020a); does not lose functionality, increases its bioavailability, stability, and extends durability (Sobel et al., 2014). At the same time, volatility, flammability (Sobel et al., 2014), and evaporation (Ozturk and Temiz, 2018) are reduced and interaction of the active ingredient with other components is prevented. Besides, undesirable tastes and scents are masked (Desai and Jin Park, 2005; Sobel et al., 2014; Ozturk and Temiz, 2018; Fathi et al., 2019).

and liquid components are converted into solids (Sobel et al., 2014). The most important feature of encapsulation is its ability of targeted and controlled release (at a certain time and place) of the encapsulated ingredient. The process of releasing payload from particles occurs by diffusion, dissolution of coating material, and by the action of osmotic pressure initiated by physical, chemical, or microbiological stimulants (Lengyel et al., 2019).

Application of microencapsulation in the feeding of ruminants and monogastric animals

In animal nutrition, there are several objectives to be achieved by microencapsulation: protection of sensitive components during the process of feeding and storage (mixing, pelleting, etc.), prevention of oxidation of the encapsulated payload and its targeted release in the gastrointestinal tract (Emameu and Putnam, 2006, Jurić et al., 2020a). Depending on the encapsulated active ingredient and particle material, a targeted release may be achieved in the rumen or small intestine. Hence, to protect the encapsulated ingredient from microbial degradation in the rumen, low pH in the abomasum, and to achieve its release in the small intestine, the coating material should meet several conditions: (i) insolubility in the rumen (pH greater than 6), (ii) insolubility in the abomasum (pH 1.5–2), (iii) resistance to microbial degradation and (iv) density from 1.2 to 1.7 g/cm³ (to avoid “swimming” of microparticles at the top of rumen fluid) (Sahraei Belvedery et al., 2019). One of the main challenges, which microencapsulation technology in animal nutrition is facing, is the limitation of particle materials. Due to the enzymatic activity of rumen microorganisms (proteolytic, cellulolytic, pectinolytic bacteria, protozoa, etc.) a degradation of particle materials made of proteins and carbohydrates occurs. Therefore, lipids that undergo degradation in the small intestine are often used. Combining various coating materials is also common, e.g. zein or caseinate as an inner coating material, while the outer coating is made of delayed-release materials such as Arabic gum and gelatin (Sahraei Belvedery et al., 2019).

Numerous studies have demonstrated that urea, as a source of non-protein nitrogen, is used by microorganisms to synthesize their protein, to improve exploitation, and reduce toxicity. The aim of scientific research conducted by de Medeiros et al. (2018) was to evaluate the efficiency of the release mechanism in situ from microparticles of different
carnauba ratio in the shell. The carnauba to urea ratios in the tested formulations were 2:1, 3:1, and 4:1, respectively. Results of degradation kinetics showed that increasing the carnauba content of the shell reduces the release of urea, thereby reducing toxicity and preventing the occurrence of alkalosis. Application of honey bee wax in the urea microencapsulation process and sheep feeding confirmed the prevention of ruminal alkalosis by slow release of urea, and increase in digestibility of neutral detergent fiber (NDF) (hemicellulose, cellulose, lignin) and acid detergent fiber (ADF) (cellulose and lignin) (Carvalho et al., 2019). Effective results in the gradual release of urea in vitro, using a combination of various envelopes, were demonstrated in the study of Lira-Casas et al. (2018). Two formulations were developed using an evaporation method. The first formulation contained 69 % urea and calcium silicate, polymer Eudragit RS100® and dichloromethane. The second formulation contained 71 % urea, activated coal, Eudragit RS100®, and dichloromethane. The results of in vitro ruminal fluid release kinetics were compared with unencapsulated urea which increased ammonia concentration after 30 min, therefore the highest concentration was observed after 6 hours (−11 mg/dl). Both formulations showed an increase in ammonia concentration (during the first hour) of 4 mg/dl, and after gradual release, maximum ammonia concentration was recorded after 12 hours (6 mg/dl). These results suggest that Eudragit RS100® (a polymer that consists of ethyl acrylate, methyl methacrylate, and a smaller proportion of methacrylic acid) has proven to be an effective coating material in a gradual release of urea.

Although microbial proteins are the main source of proteins to ruminants, highly productive animals require much higher quantities to meet their production needs. The aim of the Neto et al. (2019) research was to develop microparticles that will protect methionine from ruminal degradation and thus enable its absorption in the small intestine of sheep (bypass amino acids). Two formulations were developed in which the ratios of carnauba (shell) and the active ingredient (methionine) were 2:1 and 4:1. The results were compared with exposure of unencapsulated methionine to a rumen environment. The first formulation (2:1) demonstrated intactness of methionine in the amount of 91 % (at exposure in the rumen), and the formulation with a higher carnauba content (4:1) resulted in the intactness of 95.8 %. The photos obtained by the SEM microscope confirmed experimental data, therefore the unencapsulated methionine was completely degraded in the rumen. Significant resistance to microbial ruminal degradation, under in vitro conditions, was achieved by Yoshimara et al. (2000). Both formulations were developed by spray drying method and contained encapsulated L-lysine. Eudragit E100 and AS-HF, which had been proven effective in previous studies, were used as coating materials. The first formulation contained, except the stated, zein and the second formulation contained shellac. Exposing the first formulation (with zein) to simulated rumen conditions (pH 6.5 and temperature of 39 °C) for 48 hours resulted in a reduction of L-lysine content by 12 %, while a reduction of 17 % was detected for the second formulation (with shellac). Exposing both formulations to cellulase solution (substitution for microorganisms), less than 5 % microcrystal degradation was observed. After passing through the rumen, microcrystals were exposed to acidic conditions of the abomasum. 85 % of the encapsulated L-lysine in a shellac formulation was released during the first 60 minutes, while 70 % of L-lysine was released during the first 30 minutes from the zein formulation. From these studies, it can be concluded that Eudragit E100, AS-HF, shellac, and zein are effective microencapsulation materials for protection against microbial degradation of the rumen.

Looking for a more innovative solution, Yoshimara et al. (1999) developed microcrystals by spray drying method and tested the resistance of selected coating materials to microbial degradation in the rumen and release mechanism in the abomasum. Starch was selected as a model payload and was encapsulated in a triple coating material (Eudragit E100, AS-HF (hypermellose - acetate - succinate), and shellac) and incubated in the presence of rumen microorganisms (ruminal fluid) which resulted in 65 % microcrystal resistance. Exposing microcrystals to acidic conditions in the abomasum, within 30 minutes, an effective release of starch (85 %) was achieved.

Methane emission, as one of the challenges which livestock production is facing, has a significant impact on global warming and represents a loss of 2 % to 12 % of gross feed energy (Mamvura et al., 2014). Wood et al. (2009) state that anthropogenic sources (including livestock production) contribute to total global warming of 14 %, while 90 % of this amount is contributed by ruminants. According to previous studies, the addition of nitrate (encapsulated and
non-encapsulated), as a source of non-protein nitrogen, in case of too low intake of rumen degradable crude protein significantly decreases methane production without symptoms of intoxication (Mamvura et al., 2014; Silveira et al., 2018; Alemu et al., 2019). Furthermore, in the study conducted by Silveira et al. (2018) reduction in methane emission of 0.21 grams per gram of microencapsulated calcium nitrate in Saarens goats was detected. The usage of sesame gum, as an economically and ecologically sustainable coating material in nitrate microencapsulation, confirmed a 19% reduction of methane production in Hanwoo ox under in vitro conditions (Mamvura et al., 2014). Moreover, indigestion of encapsulated and unencapsulated nitrate reduced this emission by 76% compared to the control group. Although the difference between microencapsulated and unencapsulated nitrate was only 3%, microencapsulation allows gradual release of nitrate that protects the animal from intoxication caused by its rapid and total reduction to ammonia. Furthermore, the decrease in the total concentration of volatile fatty acids (acetic, propionic, butyric, and valeric acid) in the rumen was observed. In addition to nitrate, the potential in reduction of methane emission had also been shown by fumaric acid due to the reduction of hydrogen to succinate and its conversion to propionate. A study by Wood et al. (2009) showed that microencapsulation of fumaric acid in coconut and palm oil (coating materials) reduced methane emission by 12% to 20% during 24 hours in vitro conditions. Regardless of coating material (coconut and palm oil), there was a 45–46% increase in propionate concentration, feed conversion, and consumption in Dorset and Suffolk sheep cross-breeds. Secondary plant metabolites, as alternative antibiotic growth promoters and potential fermentation modulators in the rumen, are the subject of interest in ruminant nutrition because of their binding to proteins of feed, saliva, enzymes, tissues and microbes resulting in a reduction of ruminal protein degradation and methane emission. Microencapsulating tannins of Acaciae meannii extract in palm oil (coating material) by double emulsification and using these microparticles in feeding Merino sheep resulted in 19% methane emission reduction (compared to the control group) (Adejoro et al., 2019).

Microencapsulation of vitamins and minerals has also been proved effective in feeding pigs. Vitamin A, essential for growth and reproduction, is sensitive to heat, light, moisture, and oxygen. Thereby, its optimal absorption and bioavailability in the gastrointestinal tract can be achieved by selecting an adequate coating material. The aim of the Hua et al. (2020) study was to determine the effect of encapsulated vitamin A in the microparticles of gelatin and starch on growth and the immune status of suckling piglets. In the group fed with microencapsulated vitamin A in starch, significantly higher final weight was observed and higher daily gain than in the control group and those fed with microencapsulated vitamin A in gelatin. Both formulations (gelatin and starch) increased IgA and IgM levels. The most commonly used method of preventing anemia in suckling piglets is the intramuscular application of iron which is not effective enough (Churio et al., 2018). Studies by Churio et al. (2018) and Valenzuela et al. (2016) point to microencapsulated iron in maltodextrin as a possible solution.

The implementation of essential oils in the swine diet has a positive effect on the growth and digestibility of nutrients (Cho and Kim, 2014). Due to its sensitivity to temperature, pressure, acidity, and digestive enzymes, the bioavailability of essential oils decreases by passing through the gastrointestinal tract (Zhang et al., 2015). Microencapsulation of carvacrol (the main component of essential oil of oregano, thyme, pepper, etc.) by extrusion method into microparticles coated with alginate and whey proteins and their exposure in the porcine gastrointestinal tract resulted in a targeted and gradual release of carvacrol in the small intestine (Zhang et al., 2015). Cho and Kim’s (2014) research also highlights the positive effect of microencapsulated essential oils on the daily growth of piglets. Furthermore, the gradual release of thymol (essential oil) and its stability was achieved by its microencapsulation in the lipid matrix (Choi et al., 2019). The authors stated (in vivo conditions in piglets) that the detected release of thymol in the stomach is 15.5% and in the small intestine 41.85%.

Stamilla et al. (2020) investigated the effect of dietary supplementation based on a blend of microencapsulated organic acids (sorbic and citric) and essential oils (thymol and vanillin) on chicken meat quality. Commercial microcapsules (AviPlus® P) manufactured by Vetagro S.p.A. (Reggio Emilia, Italy) were used. Results revealed microencapsulated organic acids and essential oils as additives in poultry feeding could represent a valid dietary strategy to improve the quality of poultry meat. The
profile of intramuscular fatty acid was improved, atherogenic and thrombogenic indices were reduced, and enhanced the oxidative stability of lipids in cooked meat, with very small changes in the color of the meat.

CONCLUSIONS

Research trends of alternative growth promoters in animal nutrition are focused on the application of feed additives. The main problem in the application of additives is their chemical instability in the presence of air, light, moisture and high temperatures leading to degradation and loss of functionality. Encapsulation is a technology that protects feed additives and produces a stable and effective product, a microparticle formulation. The advantages of using microparticle formulations with feed additives are multiple from preserving the stability and functionality of the feed additives during processing and storage to the possibility of controlled and targeted delivery to the digestive system of animals. Formulations of microparticles with nitrate, fumaric acid, or secondary plant metabolites (phytochemicals) have shown great potential in environmental protection because they reduce methane emissions in livestock production.

According to the published research, it can be concluded that microparticle formulations with feed additives have a positive effect on the health and growth characteristics of animals, that is, their effectiveness in using them as growth promoters has been confirmed. Numerous and positive results of laboratory research on the impact on animal health, increasing the quality and quantity of products, and environmental protection are a good foundation for the industrial production and commercialization of encapsulated feed additives. An important factor in the transition from laboratory to industrial level is the assumption of cost-effectiveness, that the economic effects are greater than the cost of production. The preparation of microparticle formulations is relatively expensive, and an additional limitation could be the choice of materials for the microparticle.

For example, given the complexity of the digestive system of ruminants (microbial degradation in the rumen) and the limited use of coating materials, it is a great challenge to create microparticles with a targeted and controlled release in a certain part of the gastrointestinal tract. Research conducted so far on microencapsulation sources of non-protein nitrogen, vitamins, minerals, and essential oils in ruminants, pigs and poultry has shown great potential, however, further research is needed on their impact on production.

Leading animal feed companies such as BioMin Holding GmbH (Austria), Vetagro S.p.A. (Italy), and Kemin Industries (USA) have marketed formulations of microparticles loaded with feed additives (essential oils, methionine, choline chloride, lysine, etc.) to ensure better and safer animal nutrition. Like other food additives, microparticle formulations must meet strict registration guidelines in terms of safety and efficacy for animals, consumers, and the environment. The risk of residues in animal tissues and the development of bacterial resistance associated with the use of feed additives is considered to be significantly lower compared to the use of conventional antibiotics. As the exact mechanism of action of payload and the possible effects of overdose have not yet been elucidated, further research is needed to safely and productively use and exploit the full potential of microencapsulation of feed additives in animal nutrition.

REFERENCES


SAŽETAK

Zahtjevi za većom proizvodnjom i potražnja potrošača za zdravijom hranom potaknuli su posljednjih godina intenzivna istraživanja alternativnih promotora rasta životinja. Trendovi istraživanja su orijentirani na razvoj novih proizvoda obogaćenih dodacima stočnoj hrani s ciljem poboljšanja zdravlja životinja i boljeg prirasta. Mnogi od tih spojeva su nestabilni u prisutnosti svjetla, zraka, vode ili visokih temperaturi te ih je potrebno zaštititi tijekom procesiranja, skladištenja i primjene. Inkapsulacijom u mikročestice dodaci stočnoj hrani se štite od štetnih vanjskih utjecaja, a ne unajuju im se stabilnost i funkcionalnost. Tehnologija mikroinkapsulacije se koristi za zaštitu dodataka i poboljšanje biodostupnosti kontroliranom i ciljanom isporukom u probavni traktu. Posebno je pogodna za dodavanje u hranidbi preživača, jer se pravilnim izborom materijala mikročestice omogućava oslobađanje dodataka u tankom crijevu, a ne u burgu. Uz pravilno korištenje, formulacije mikročestica su učinkoviti alat u hranidbi životinja koja isporučuje hranjive sastojke i/ili lijekove na određeno mjesto željenom brzinom. U radu su sažeta laboratorijarska istraživanja primjene tehnologije mikroinkapsulacije dodataka stočnoj hrani u hranidbi preživača i monogastričnih životinja. Brojni rezultati primjene inkapsuliranih bioaktivnih spojeva pokazali su pozitivne učinke na zdravlje životinja, povećanje produktivnosti bez štetnih učinaka na konačni proizvod i zaštitu okoliša.

Ključne riječi: inkapsulacija, hranidba, ciljana isporuka, dodaci stočnoj hrani, životinje