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Microencapsulation of L-Lysine for Improving the Balance of Amino Acids in Ruminants

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L-Lysine was microencapsulated by the spray-dry method for improvement of the balance of amino acids in ruminants. The rumen-bypass microcapsule was prepared using zein or shellac as an outer coating agent for protection against microbial degradation in the rumen. L-Lysine was generated with a yield of 50% in case of zein microcapsules and 33% in shellac microcapsules and a mean particle diameter of 20–30 μm . Both rumen bypass microcapsules had high stability in a neutral solution that mimicked ruminal pH (pH 6.5), suggesting that L-lysine should not be susceptible to damage by ruminal microorganisms. Moreover, the efficiency of release of these microcapsules was 70% in the zein microcapsule and 85% in the shellac microcapsule within only 30 min in the abomasal environment (pH 3.0). In addition, about 90% of encapsulated L-lysine was released from zein microcapsules in pepsin solution.

INTRODUCTION

Post-ruminal supplements of amino acids and proteins may increase growth and productivity in ruminants. An increased emphasis on higher production per animal and improvement in efficiency of production has stimulated the use of high energy rations in feeding ruminants. However, the expected theoretical efficiencies of feed conversion often have not been achieved due to unknown factors which limit nutrient utilization. One of the many factors affecting energy conversion efficiencies is the amino acid profile of the diet. Ruminants synthesize microbial protein in the rumen as the major protein source instead of consuming the protein. Thus, the value of a protein source in improving ruminant performance is determined by its ability to supply limited amino acids, such as methionine or S-containing amino acids and lysine, to the small intestine and availability of nitrogen for use by ruminal microorganisms. Actually, ruminal microorganisms can supply the amino acids for growth and production in ruminants fed a purified diet containing only nonprotein nitrogen as the nitrogen source, but the amino acid supply is inadequate in animals whose protein requirements are high, *e. g.*, growing calves or high yielding milk cows. An insufficient supply of high protein feed seems to reduce the milk protein content, especially when the cow has a deficiency in the essential amino acids that form milk protein. Some studies have shown that abomasal or intraperitoneal infusion of methionine improved the wool growth of sheep (Wright, 1971) and milk production of dairy cows (Broderick *et al.*, 1974).

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Our interest has been focused on the preparation of a rumen-bypass microcapsule that protects contents against microbial degradation in the rumen (neutral pH), followed by their release in the abomasum (acidic pH). In a previous study (Yoshimaru *et al.*, 1997; 1999), we reported that the spray-dry microencapsulation method using porous starch allows us to prepare target-specific microcapsules with an appropriate coating agent. Nimrick *et al.* (1970) and Richardson and Hatfield (1978) indicated that the first limiting essential amino acid for microbial protein was methionine, followed by lysine and threonine. Therefore, we prepared rumen-bypass microcapsules of L-lysine to improve the balance of amino acids and increase synthesis of milk protein.

EXPERIMENTAL PROCEDURES

Materials

Porous starch was supplied by San-ei Surochemical Co., Ltd. (Aichi, Japan). Eudragit E100, a synthetic acrylic copolymer, was supplied by Röhm Pharma GmbH (Darmstadt, Germany), and AS-HF was from Shin-etsu Chemical Co., Ltd. (Tokyo, Japan). These are coating agents that are soluble under acidic and alkaline conditions, respectively. Zein from corn protein was obtained from Nacalai Tesque (Kyoto, Japan), and shellac, a thermoplastic resin obtained by purifying the resinous excreta of an Asian insect (*Laccifer lacca* Kerr), was from Gifu Shellac Co., Ltd. (Gifu, Japan). L-lysine was obtained from Nacalai Tesque. All other chemicals were obtained from Nacalai Tesque and were of analytical reagent grade.

Preparation of Rumen-Bypass Microcapsules

To protect the content of L-lysine from degradation in the rumen, we microencapsulated L-lysine by the spray-dry method because of its convenience and high degree of reproducibility. To prepare the microcapsules, 50 g of porous starch with numerous holes of a few μm in diameter in each particle (Suzuki, 1995) was added to 500 ml of a 10% solution (w/v) of L-lysine, and the mixture was stirred for 2 hr. The solution was then sonicated for 5 min to integrate the L-lysine into the porous starch. After filtration and freeze-drying, the freeze-dried materials were coated with Eudragit E100 by dissolving in 1000 ml of a 5% solution (w/v) of Eudragit E100 in ethanol. Further, the spray-dried microcapsules were coated with AS-HF to enhance stability in neutral conditions. Finally, these microcapsules were coated with zein from corn protein to protect L-lysine against microbial degradation in the rumen (zein microcapsule). The microencapsulated L-lysine was prepared using a CL-8 spray dryer (Ohgawara Kakouki Co., Tokyo, Japan) equipped with a rotary atomizer nozzle, a nozzle speed of 10,000 rpm and inlet and outlet air temperature of 105 and 62–79 °C, respectively. Similarly, L-lysine was microencapsulated with shellac (shellac microcapsule), which has an excellent reputation as a coating agent (Labhasetwar *et al.*, 1989), according to the same procedure.

Efficiency of Encapsulation of L-Lysine into Microcapsules

Fifty mg of microencapsulated L-lysine was ground in a mortar and a pestle and dissolved in 1 ml of a purified water. A suitable dilution of the resultant solution was

subjected to reverse-phase chromatography (Shimadzu LC-10 AD Instruments, Kyoto, Japan) in order to determine the amounts of L-lysine in the microcapsules. As the first step, each sample was derivatized by the Waters AccQ-Fluor™ Reagent Kit (Millipore Co., Milford, MA) (Cohen and Michaud, 1993). In brief, after drying under vacuum, each sample was resolubilized with 20 μ l of 20 mM HCl, the volume was brought to 80 μ l with AccQ-Fluor™ Borate Buffer, and the derivatives formed via the addition of 20 μ l of 10 mM AccQ-Fluor™ Reagent Powder in acetonitrile. Next, derivatized samples were applied to an AccQ/Tag™ amino acid analysis column (3.9 mm i. d. \times 150 mm, Japan Waters Co., Tokyo, Japan) that was operated at a flow rate of 1.0 ml/min. Gradient conditions: mobile phase A was 140 mM sodium acetate with 17 mM triethylamine at pH 5.04, mobile phase B was 60% acetonitrile in water (v/v); initial=100% A, 0.5 min=98% A, 15 min=93% A, 24 min=79% A, 27 min=78% A, 43 min=74% A (all segments linear), followed by a wash with 100% B for 5 min and reequilibration for 5 min at 100% A. Detection was by fluorescence with excitation at 250 nm and emission at 395 nm.

The encapsulation efficiency was calculated by expressing the amount of L-lysine encapsulated as a percentage of the initial amount of L-lysine used to prepare the rumen-bypass microcapsules.

Stability and Release Efficiency of Rumen-Bypass Microcapsules

The stability of microencapsulated L-lysine under pH and temperature conditions that are similar to those of the rumen was investigated. A 100 mg aliquot of the microencapsulated L-lysine was incubated at 39°C with reciprocal shaking (100 strokes/min) in 15 ml of 0.1 M phosphate buffer (pH 6.5). The microcapsules were then collected by filtration and washed with purified water. The collected microcapsules were dissolved completely in citrate buffer (pH 3.0), and the amount of L-lysine that was retained after treatment with the neutral solution was determined. The resistance of L-lysine was expressed as the amounts of L-lysine retained as a percentage of the amount of L-lysine encapsulated.

The release of L-lysine from the microcapsules was evaluated as follows. A 100 mg aliquot of the microencapsulated L-lysine was incubated at 39°C with reciprocal shaking (100 strokes/min) in 15 ml of 0.1 M citrate buffer (pH 3.0). At appropriate times, the solution was filtered and the amount of L-lysine in the filtrate was determined with the chromatographic systems described above.

RESULTS AND DISCUSSION

Efficiency of Encapsulation and Morphology of Microcapsule

Rumen bypass microencapsulation of L-lysine improves the balance of amino acids and increase the synthesis of milk protein. The microencapsulation method uses porous starch to prepare target-specific microcapsules, and the triple coating of Eudragit E100, AS-HF and zein or shellac allows the rumen to be bypassed.

First, we investigated the efficiency of encapsulation of L-lysine into the microcapsules. As shown in Table I, we found that L-lysine was generated with a yield of 49.8% in the case of zein microcapsules and 32.6% in shellac microcapsules. However, judging from the efficiency of integration of L-lysine into porous starch (55.6%), we had

Table 1. Encapsulation efficiency of L-lysine into the rumen-bypass microcapsules

condition of microcapsule	L-lysine contents encapsulated (g)	trap I ^a (%)	trap II ^b (%)
start	50.0	100.0	—
integration into porous starch	27.8	55.6	100.0
zein microcapsule	24.9	49.8	89.6
shellac microcapsule	16.3	32.6	58.6

^a Efficiency of encapsulation on the basis of L-lysine administered.

^b Efficiency of encapsulation on the basis of L-lysine integrated into porous starch.

assumed that a triple coating process with Eudragit E100, AS-HF, and zein or shellac by the spray-dry method would be efficient, without loss of L-lysine (*i. e.*, 89.6% or 58.6% in the efficiency of triple coating of porous starch, respectively). Thus, the relatively low efficiency of encapsulation in this study was probably due to the slightly reduced integration of L-lysine into the porous starch, indicating that low molecular substances, such as amino acids or peptides, have a tendency to reduce the integrating efficiency into porous starch, compared with macromolecules, proteins or enzymes. However, this defect can be overcome by reusing the filtrate of the inclusion substance that occurs in microencapsulation process. Therefore, our results suggest that most substances might be microencapsulated using porous starch and that this procedure is effective and applicable to the feed industry because of its economy, convenience and safety.

Microcapsules prepared by the spray-dry method are fine and homogeneous particles that are not expected to hinder absorption by the omasum. Both prepared rumen-bypass microcapsules had a mean particle diameter of 20–30 μm ; the microcapsules were spherical, and their surfaces were almost smooth. The appearance was similar to that of microcapsules previously reported (Yoshimaru *et al.*, 1997; 1999), indicating that this microencapsulation method is highly reproducible.

Evaluation of Rumen-Bypass Microcapsules for Resistance to Degradation in the Ruminal Environment

To investigate the resistance of rumen-bypass microcapsule to degradation in the ruminal environment, zein microcapsules and shellac microcapsules were treated with a phosphate buffer (pH 6.5) that mimicked the environmental pH of the rumen. As shown in Figure 1, we observed high stability of each microcapsule in the neutral solution, although the amount of L-lysine retained in the microcapsules was reduced 12% and 17% during a 48 hr incubation, respectively. Furthermore, L-lysine inside microcapsules was barely affected by various pH values around neutrality (pH 5.5–8.0) during the 48 hr incubation. In addition, we examined the resistance of zein microcapsules and shellac microcapsules to a solution of cellulase, as a substitute for rumen microorganisms (Goto

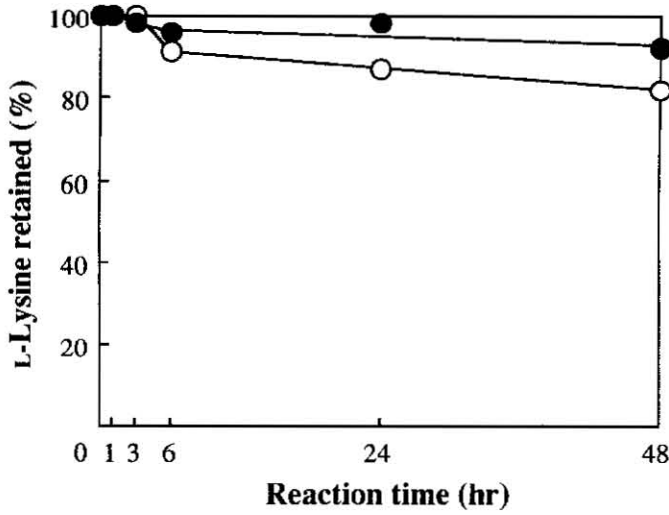


Fig. 1. Stability of rumen bypass microcapsules under conditions that mimic the rumen (39°C and pH 6.5); zein microcapsules (●); shellac microcapsules (○).

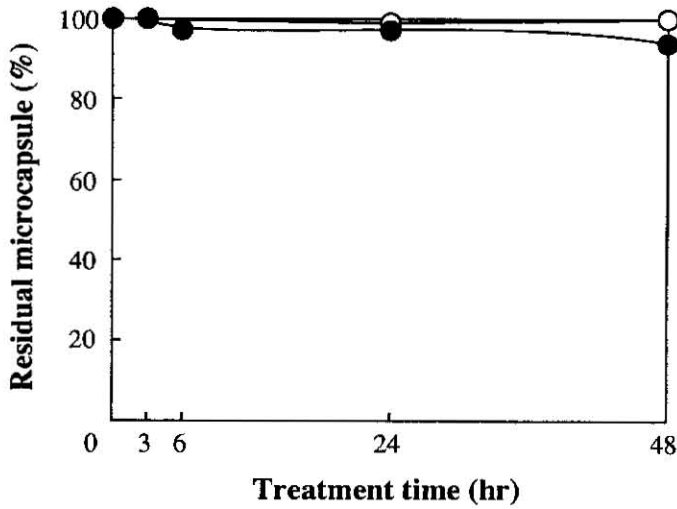


Fig. 2. Stability of rumen-bypass microcapsules in a solution of cellulase, as substitute for rumen microorganisms; zein microcapsules (●); shellac microcapsules (○).

and Minson, 1977). Figure 2 shows the variations in residual amounts of microcapsules after treatment with a 25% solution (w/v) of cellulase Onozuka SS (P-1500) from *Trichoderma viride* (Yakult Biochemicals Co., Tokyo, Japan). Little degradation of zein and of shellac microcapsules was observed in a 48 hr treatment, although 5% of zein microcapsules was degraded during a 48 hr treatment. That is, zein microcapsules and shellac microcapsules were highly protected from degradation by cellulase. Moreover, a previous study revealed that shellac used as the outer membrane might be useful and acceptable for protection from microbial degradation in the rumen by anaerobic cultures with rumen microorganisms (Yoshimaru et al., 1999). Consequently, these results and findings suggest that L-lysine should not be susceptible to damage in the environmental pH of rumen and rumen microorganisms and that it should pass through the rumen with little degradation.

Release Efficiency of Rumen-Bypass Microcapsule After Passage Through the Rumen

After passing through the rumen, these microcapsules must release their contents in an abomasal environment. We examined the release of L-lysine from the microcapsules in an acidic solution (pH 3.0, citrate buffer) that mimicked the abomasal environment. As shown in Figure 3, about 85% of encapsulated L-lysine in shellac microcapsules was released within 60 min in acidic fluid, and then the reaction proceeded only slowly. In zein microcapsules, about 70% of encapsulated L-lysine was released within 30 min. In

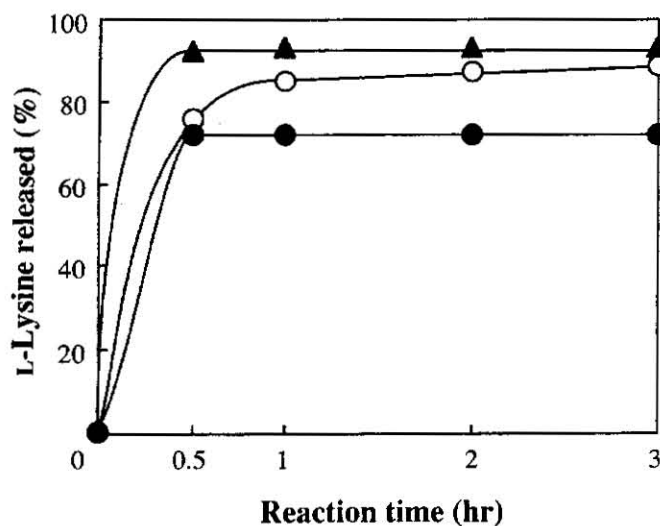


Fig. 3. Release of L-lysine from rumen-bypass microcapsules under conditions that mimic the abomasal environment. zein microcapsules: citrate buffer, pH 3.0 (●); pepsin solution (▲); shellac microcapsules: citrate buffer, pH 3.0 (○).

addition, we measured the release of L-lysine from zein microcapsules in a 0.2% solution of pepsin (pH 3.0). As a result, about 90% of encapsulated L-lysine was released within only 30 min. Consequently, these L-lysine microencapsules, which are fairly stable against digestion by ruminal microorganisms, can then be expected to improve the balance of amino acids and increase a synthesis of milk protein.

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