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Rumen bypass and biodistribution of L-carnitine from dual-layered coated pellets in cows, *in vitro* and *in vivo*

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ABSTRACT

A ruman bypass delivery system was investigated to improve the delivery efficiency of L-carnitine in biological samples of cows. Highly water-soluble L-carnitine used for dietary supplement in ruminants was chosen. L-Carnitine-loaded compact pellets were prepared by extrusion method and then coated with various coating materials such as ethylcellulose (EC), Eudragit E100 (E100), Eudragit RS100 (RS100), stearyl alcohol and glyceryl monostearate, for single-layered coated pellets (SCP). Two types of dual-layered coated pellets (DCP) were also designed as DCP-A (inner E100/outer EC) or DCP-B (inner EC/outer E100). Preparation of compact pellet and methods of polymeric coatings are the most important strategies for modulated release and rumen bypass efficiency based on chewing behaviors and physiology of veterinary species. DCPs were more efficient in retarding L-carnitine release in rumen fluid (pH 6.8) than the SCP but DCP-B gave much faster release in abomasums fluid (pH 1.2). Both DCP-A and DCP-B showed high *in vivo* rumen bypass efficiency in cows compared with the nonprotected preparation and most of L-carnitine was readily absorbed. DCP-B was also efficient for delivering L-carnitine in biological samples of cows, mainly in muscle but no statistical differences were observed among the tested preparations after the multiple oral feeding to cows for 3 months. Interestingly, DCP-B produced higher L-carnitine levels in milk in a dose-dependent manner. However, delivery efficiency of L-carnitine preparations in biological samples of cows would rather be more dependent on feeding schedules.

1. Introduction

Ruminants have a distinct digestive system involving cooperation between the host animal and the predominant anaerobic rumen bacteria and protozoa. Post-ruminal supplements of proteins, amino acids and nutrients may increase the growth and productivity as well as treat diseases in ruminants (LaCount et al., 1996a; Bunting et al., 2002). However, the rumen, which is the largest compartment of the four-compartment stomach, may act as the main site of rumination and fermentation that would destroy dietary ingredients severely limiting direct applications (LaCount et al., 1996a; Wu and Papas, 1997; Koenig and Rode, 2001).

The formulation approaches to bypass the rumen focused on simple heat or chemical treatments of proteins, the use of low-soluble peptides or amino acid analogues, and the use of lipids as a protective matrix for proteins (Sklan and Tinsky, 1993; Yoshimaru et al., 1999; Wu and Papas, 1997; Rossi et al., 1999). A bi-layered compressed matrix tablet based on polymers was also reported to be a controlled release system for ruminants (Sanna et al., 2004). However, these approaches showed low rumen bypass efficiency for a broad spectrum of active ingredients. The ideal rumen bypass delivery systems must resist the physiological factors in rumen such as a neutral pH, microflora and digestive components, and then release the dietary nutrients in the abomasum and intestine (Yoshimaru et al., 1999; Wu and Papas, 1997).

Although some rumen bypass delivery systems had been attempted, their efficiency in veterinary medicine was in question. Most of the oral dosage forms in veterinary medicine are unique in terms of physicochemical properties of active ingredients, animal behaviors of rumination and husbandry practices (Martinez et al., 2002). One of the simplest methods for a rumen bypass delivery system in veterinary medicine is to coat microparticulate pellets or granules by using various pH-dependent polymers in a controlled release manner. For rumen bypass and delivery efficiency, these coated pellets must resist the rumen fluid but deliver the active ingredients at the low pH of the abomasums for intestinal absorption. The release profiles of dietary ingredients in ruminants can be strongly dependent on types and levels of coating materials and their structures. The solubility of the active ingredients is also a factor (Yoshimaru et al., 1999; Wu and Papas, 1997; Sanna et al., 2004).

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L-Carnitine was selected as the model ingredient. Currently, it is used clinically for the treatment of a carnitine deficiency or as a dietary supplement for various chronic diseases in ruminants and humans (LaCount et al., 1996a; Bunting et al., 2002). However, its usefulness is very limited due to the potential degradation in rumen before post-luminal absorption. Furthermore, high water solubility and low molecular weight of L-carnitine may limit the formulation approaches. However, no advanced rumen bypass delivery systems for L-carnitine is investigated until now based on chewing behaviors and physiology of veterinary species.

The aim of this study was to investigate a new rumen bypass delivery system containing L-carnitine for improving the delivery efficiency of L-carnitine in biological samples of cows. The L-carnitine-loaded pellets were prepared by extrusion and spheronization. The SCP were obtained by coatings with EC, E100, RS100, stearyl alcohol (SA) and glyceryl monostearate (GM). The EC and E100 were used for DCP. The *in vitro* release profiles of the coated pellets were studied in simulated rumen (pH 6.8) and abomasum fluid (pH 2.0), respectively. The *in vivo* rumen bypass efficiency of the DCP was evaluated in cows and compared with nonprotected commercial product (Canipass®). Finally, biodistributions of L-carnitine in biological samples of cows such as plasma, muscle and milk were evaluated after the multiple oral feeding of L-carnitine preparations for 90 days.

2. Materials and methods

2.1. Materials

Commercially available Carniking® powders containing 50% L-carnitine were obtained from Lonza Ltd. (Basel, Czech). The methanol (HPLC grade) and corn starch were purchased from Duksan Chemicals Co. (Seoul, Korea). The hydroxypropylcellulose (HPC) was obtained from Richwood (Seoul, Korea). The Eudragit® E100 (E100) and Eudragit® RS 100 (RS100) were acquired from Rohm GmbH (Damstadt, Germany). Silicone dioxide and lactose monohydrate (Pharmatose®, DMV) were obtained from Degussa (Seoul, Korea). The ethylcellulose (EC, 14 cps), dibutyl sebacate (DBS), talc ethanol, acetone and isopropyl alcohol (IPA) were purchased from Sigma (St. Louis, MO, USA). Stearyl alcohol (SA) and glyceryl monostearate (GM) were acquired from the Junsei Chemical Co. (Tokyo, Japan). The commercial L-carnitine preparation (Canipass®), a fat matrix of vegetable fats and fatty acids, supplied by Lonza Ltd. (Basel, Czech) was purchased for *in vitro* and *in vivo* comparison. All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of core pellets

Initially, 500 g of Carniking® and 10 g silicon dioxide were blended in a V-mixer for 5 min. 20 g corn starch was then added and mixed for 10 min. 10 g of a HPC solution (10%) as a binder was then added slowly to the dry blends. The resulting wet masses were passed through a single-screw type extruder fitted with a 2.0 mm screen from the hopper at a constant speed of 50 rpm. The wet pellets were extruded three times. The extrudated pellets were processed immediately with a spheronizer at the speed of 850 rpm for 10 min. It has been reported that the extrusion and spheronization method are the best for preparing compact pellets for controlled release of drugs (Chatchawalsaisin et al., 2005). The resulting core pellets were then dried at 40 °C in an oven for 24 h. The dried core pellets were then sieved using a sifter (Model 35-VSS-300, Kukje Sci., Seoul, Korea) that was equipped with a series of four standard stainless steel sieves (10, 12, 14 and 16 mesh). The 10–12 mesh size pellets were collected for the subsequent polymeric film coating. The fraction of core pellets with a 10–12 mesh size was 73.4% with a narrow size distribution.

2.3. Coating process of core pellets

The E100 and EC were used to coat the core pellets. Table 1 shows the compositions of the coating materials. Each composition was dispersed in the each solvent and stirred continuously with a mechanical stirrer for 2 h until a uniform coating solution had been formed. DBS was then added as a plasticizer. Talc was finally dispersed in the resulting coating solution and mixed for 30 min. The resulting coating solution was left overnight to allow the air bubbles to escape.

200 g of the L-carnitine-loaded core pellets with a 10–12 mesh size were preheated for 10 min and then coated using a laboratory-scale fluidized bed coater (Model Strea 1, Aeromatic AG, Switzerland). The inlet and outlet air temperature were set 50 and 46 °C but was 40 and 35 °C in the case of the E100 coating. The pneumatic spraying pressure was 1.6 bar and the airflow rate was 80 m^3/h. The coating solutions (EC, E100, RS100, SA/GM) were delivered for single-layered coated pellets (SCP) at a pump rate of 4.0 ml/min with a nozzle diameter of 0.8 mm until a desirable coating level had been achieved. The coated pellets were further aged in the drying chamber for 30 min to completely evaporate the residual solvent content. The nozzle and spraying tube were washed carefully when the subsequent coating solution was switched.

The DCP preparations were obtained by applying coating solution of EC or E100 onto the SCP subsequently, giving DCP-A (inner E100/outer EC) or DCP-B (inner EC/outer E100).

2.4. Release studies

The release of the L-carnitine from L-carnitine-loaded pellets was carried out according to the USP dissolution I basket method at a rotation speed of 100 rpm in 900 ml of the simulated rumen fluid (pH 6.8) or abomasum fluid (pH 2.0) at 37 ± 0.5 °C using a DST-600A dissolution tester (Labfine, Seoul, Korea). It is well known that L-carnitine is unstable and easily destroyed by the bacteria and protozoa in rumen environment. LaCount et al. (1996a) also determined that L-carnitine was degraded to some extent in ruminal fluid; however, the degree of degradation appeared to have been dependent on type of diet and adaptation of microbes to dietary L-carnitine. In order to minimize the degradation effect during *in vitro* release, release characteristics of L-carnitine from each preparation were just carried out in simulated rumen fluid (0.05 mol/l K_2HPO_4 buffer adjusted with NaOH to pH 6.8) and abomasum fluid (0.03 mol/l NaCl buffer adjusted with HCl to pH 2.0) instead of rumen and abomasum fluids, respectively. An aliquot (1 ml) of the samples was withdrawn at 0.5, 1, 1.5, 2, 3, 4 and 6 h with the replacement of an equal volume of fresh test medium. The collected sample solution was filtered through a 0.45 μm Millipore filter.

2.5. HPLC analysis of L-carnitine

The L-carnitine concentration in *in vitro* samples was analyzed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a Waters 600E system controller, a Waters 717 autosampler, a Waters 486 tunable absorbance detector at 205 nm and a Degasser Model DG-4400 (Phenomenex®). The mobile phase was a 0.05 M phosphate buffer (pH 2.4)/methanol 8:2 (v/v) mixture and the flow rate was 1.2 ml/min. The 0.05 M phosphate buffer was prepared by dissolving 5.7 ml phosphoric acid in 1000 ml of water and adjusting the pH to 2.4 with 1N NaOH after first dissolving 555 mg sodium 1-heptanesulfonate. A reverse-phased column

(Phenomenex® Synergi, Hydro-RP 80A 150 mm × 4.60 mm, 4 μm) was used. *p*-Aminobenzoic acid was used as the internal standard.

On the other hand, L-carnitine concentrations in *in vivo* biological samples such as plasma, milk and muscle samples of cows were analyzed by the HPLC method with fluorescent derivatization. The detailed analytical HPLC condition was established previously by our studies (Cao et al., 2007).

2.6. Scanning electron microscope

The surface and cross-sectional morphology of the dual-layered coated pellet were examined using scanning electron microscopy (JEOL, JSM-5410). The sample was cross-sectioned using a micro-cutter after dipping the pellet in liquid nitrogen for 10 min. The sample was then coated with gold under an argon atmosphere using a JEOL JFC-1100 sputter coater (Tokyo, Japan) for approximately 2 min to obtain a coating thickness of approximately 200 Å. The micrographs were taken using a Cambridge Stereo Scan 200 (London, England) at an accelerating voltage of 15 kV.

2.7. In vivo rumen bypass efficiency in cows

The nylon bag technique was used to evaluate the bypass efficiency of the dual coated pellets from digestion in cows (De Boer et al., 1987; Cone et al., 2002). The rumen and abomasum were sampled from the cows through surgically operated holes. The 12 cows were randomly divided into four groups each containing 3 cows.

A commercial product (Carnipass®, Lonza Ltd.) and two DCP preparations (DCP-A and DCP-B) equivalent to 6 g of pure L-carnitine were placed into the nylon bag, respectively. DCP-A and DCP-B consisted of inner 10% E100/outer 15% EC and inner 20% EC/outer 30% E100, respectively. The nylon bag was incubated in the rumen for 9 h or in the abomasum for 3 h. The nylon bags were then withdrawn for the analysis of the L-carnitine content. On the other hand, the nylon bag was placed in the rumen and allowed to pass through the whole intestine. The nylon bag was recovered in the feces. After washing with water to remove the feces, the nylon bag was dried under an ambient temperature. The residual amount of L-carnitine retained in the nylon bag was determined by HPLC. The rumen bypass efficiency of the L-carnitine from the tested preparations is expressed as the percentage of the amount of L-carnitine retained relative to the initial L-carnitine content.

2.8. Biodistribution of L-carnitine in biological samples

The 20 cows were randomly divided into four groups each containing five cows. One group was as a control and other three groups were treated as raw L-carnitine, commercial product (Carnipass®) and DCP-B equivalent to 12 g L-carnitine, respectively. Fresh biological samples of plasma and muscle were collected after the multiple oral administrations for 3 months daily to each cow. In order to investigate the effect of L-carnitine feeding amounts on the biodistribution of L-carnitine in milk, 20 cows were also randomly divided into four groups, each containing five cows. One group was as a control. Three treatment groups were daily fed with DCP-B containing 4, 8 or 12 g L-carnitine for 3 months, respectively. The L-carnitine in biological samples was determined by the HPLC method with fluorescent derivatization as reported previously (Cao et al., 2007).

The reason for selecting 3 months as administration schedule should be explained in more detail. Because of shorter half-life of L-carnitine, it is expected to reach steady-state concentration in plasma after the multiple oral administrations daily for one or two weeks (Harper et al., 1988). However, we dosed for 3 months to investigate the difference of rumen bypass systems by maximizing the concentration of L-carnitine in biological samples based on *in vitro* and *in vivo* bypass efficiency (see Figs. 3–5, 7). High variations of endogenous levels of L-carnitine are also a reason. L-Carnitine concentration in plasma was not significantly increased than that of control group, when 12 g/day dose of raw L-carnitine was fed to cows for 1 week in our previous work (data not shown).

2.9. Statistical analysis

The statistical significance between each treatment was assessed by analysis of variance using the SPSS™ software program (Statgraphics, USA). A p-value of <0.05 was considered significant.

3. Results and discussion

3.1. In vitro release characteristics of coated pellets

An important point in developing modified release oral formulations in veterinary species is to consider the impact of chewing behaviors on *in vivo* bioavailability (Martinez et al., 2002). The preparation of compact pellet using extruder is prerequisite for rumen bypass delivery of highly water-soluble L-carnitine. The various coating materials were then tested for their resistance to the simulated rumen fluid after being coated on the L-carnitine-loaded core pellets. Fig. 1 shows the L-carnitine release profiles of SCP in the simulated rumen fluid (pH 6.8). Due to its high solubility of L-carnitine, the lipophilic SA/GM coating materials were not sufficient to reduce the L-carnitine release rate. The RS100 decreased the initial release rate but the release rate increased rapidly at 6 h. The EC was more efficient in modifying the release rate. The release rate gradually decreased with increasing coating levels. The pH-dependent E100, which is readily soluble at low pH but relatively insoluble at high pH, could also retard the release rate as a function of coating levels.

For rumen bypass delivery, the retarded coated pellet is able to release L-carnitine rapidly for subsequent intestinal absorption when switched to abomasum fluid (Yoshimaru et al., 1999; Wu and Papas, 1997). Fig. 2 shows the release profiles of SCP in the simulated abomasums fluid (pH 2.0). The SCP with E100 and SA/GM showed rapid release but the other coating materials were not efficient in the abomasums fluid. From this result, the EC and E100 were chosen as the coating polymers and used to prepare the DCP to efficiently control the release rate of L-carnitine.

The coated structures of DCP after applying the pH-dependent E100 and pH-independent EC either in the inner or outer layer were investigated. Fig. 3 shows the release profiles of DCP-A (inner E100 and outer EC) at various coating levels in the simulated rumen fluid. The release rate decreased significantly with increasing total coat-

Table 1
Compositions of the coating materials (g)

Type	Coating materials	DBS (g)	Talc (g)	Solvent
pH-dependent	E100 (10 g)	2.0	0.5	Acetone/IPA = 1:2 (200 ml)
pH-independent	EC (10 g)	2.0	0.5	Acetone/ethanol = 7:3 (200 ml)
	RS100 (10 g)	1.2	0.5	Water/ethanol = 1:3 (150 ml)
	SA (7 g)/GM (3 g)	–	0.5	Water/ethanol = 7:5 (130 ml)

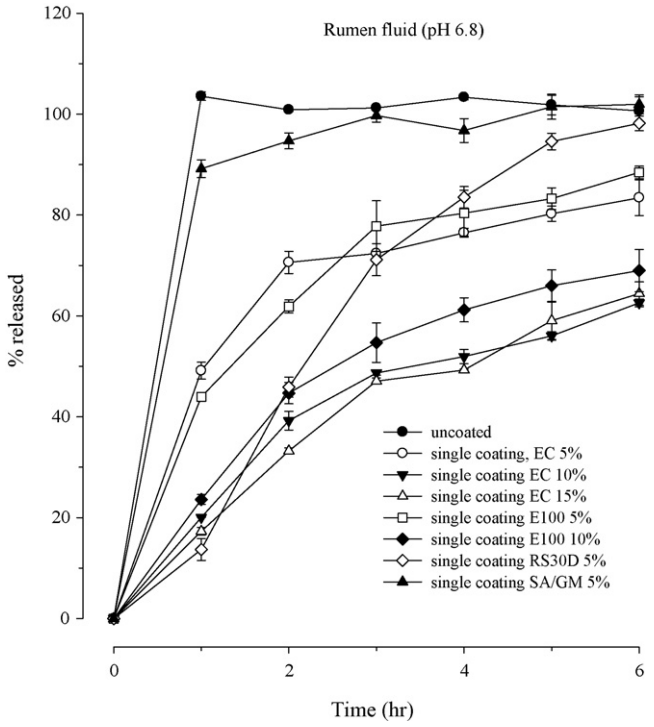

Fig. 1. Release profiles of SCP in the simulated rumen fluid (pH 6.8).

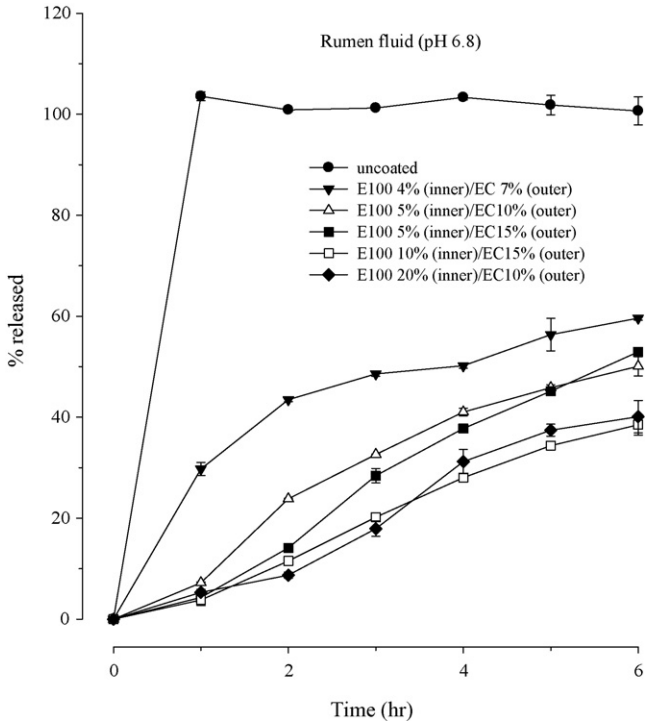

Fig. 3. Release profiles of DCP-A (inner E100 and outer EC) at various coating levels in the simulated rumen fluid (pH 6.8).

ing levels of the E100 and EC due to the fewer flaws or cracks on the coated films. Excessively high coating levels did not further decrease the release rate. The EC and E100 were further switched for the coatings. Fig. 4 shows the release profiles of DCP-B (inner EC and outer E100) at various coating levels in simulated rumen fluid. Likewise, the release rate decreased with increasing coating level. However, the coating order of both E100 and EC did not alter the rumen resistance at the same coating levels. Generally, total coating levels of the two hydrophobic polymers could decide the release profiles of DCP.

The release profiles of the DCP-A and DCP-B at various coating levels were also compared in the simulated abomasum fluid (Fig. 5). In case of DCP-A with outer EC coating, the release rate was

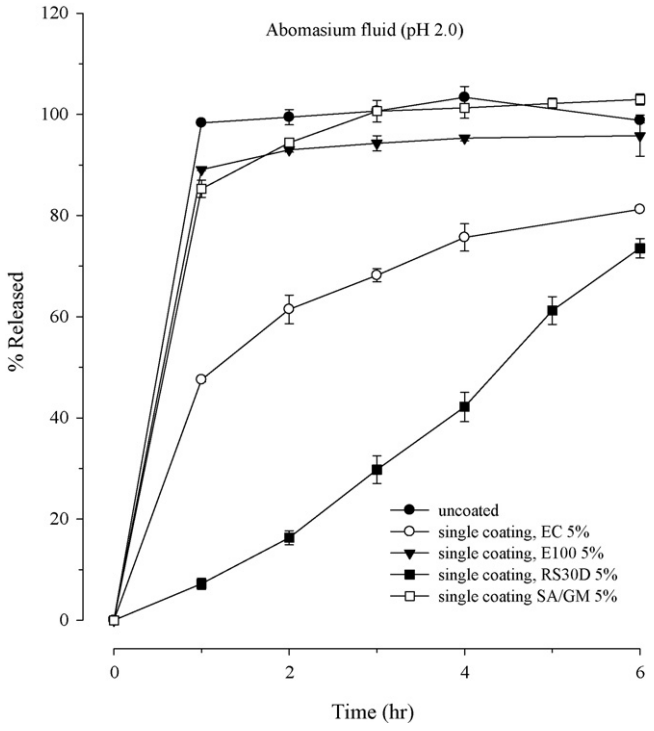

Fig. 2. Release profiles of SCPs in the simulated abomasums fluid (pH 2.0).

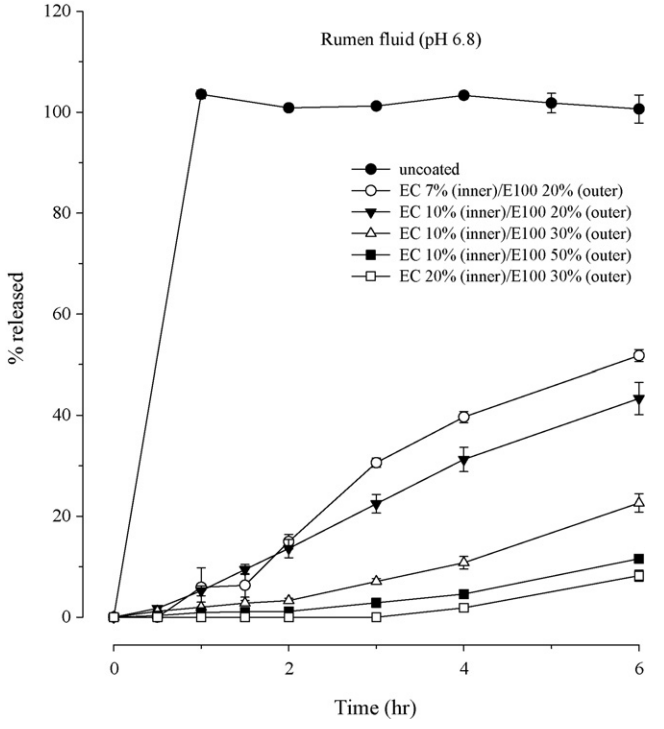

Fig. 4. Release profiles of DCP-B (inner EC and outer E100) at various coating levels in the simulated rumen fluid (pH 6.8).

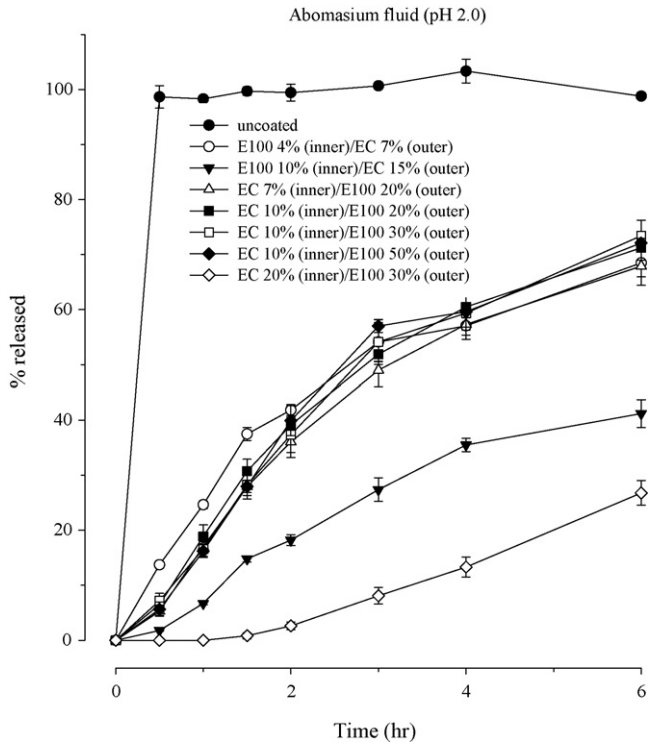

Fig. 5. Release profiles of DCP-A and DCP-B at various coating levels in the simulated abomasum fluid (pH 2.0).

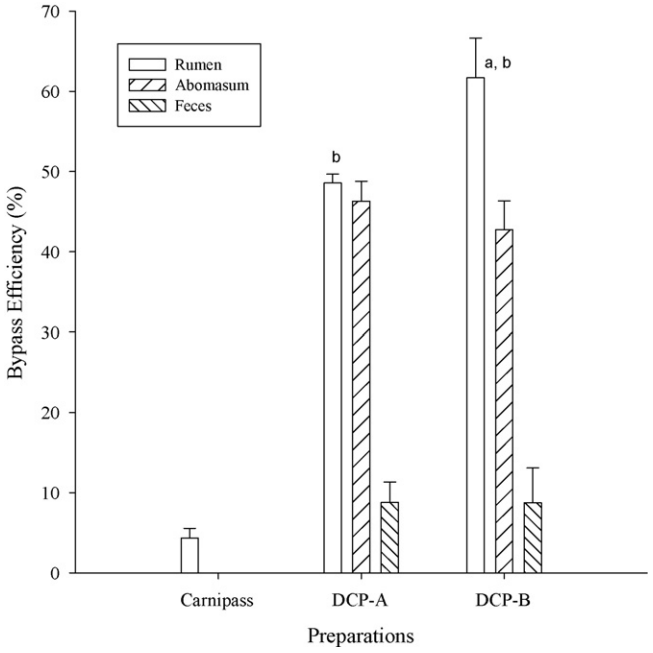

Fig. 7. Comparison of the *in vivo* rumen bypass efficiency (%) of L-carnitine preparations in cows. [a]Significantly different from DCP-A, $p < 0.05$, [b]Significantly different from Carnipass®, $p < 0.05$. The statistical comparison of DCPs in abomasums and feces was not done because no L-carnitine was detected.

slowly increased in abomasum fluid while the DCP-B was significantly increased even at the higher coating levels. Although the EC and E100 resisted to the rumen fluid to the same extent, the DCP-B with the outer E100 film was more efficient in increasing the release rate due to the high solubility of the E100 films at the low pH of abomasum fluid. It was evident that the coating order of both E100 and EC in DCP was important for rumen bypass delivery. For this reason, DCP-B with the inner EC and outer E100 can be further used for *in vivo* rumen bypass delivery.

3.2. Surface morphology of the DCP

Fig. 6 shows SEM images of the surface and cross-sections of DCP-B. The surface of the DCP was homogenous and smooth without any pores and cracks. Distinct dual-layers were also present although some spaces were observed between the two layers.

3.3. In vivo rumen bypass efficiency in cows

Rumen bypass efficiency of different preparations in cows using a nylon bag technique is compared in Fig. 7. The DCP preparations showed higher rumen bypass efficiency for 9 h compared with the commercial Carnipass® as a result of the high resistance to the rumen fluid of cows. The unprotected Carnipass® showed very low rumen bypass efficiency. Most of L-carnitine was lost in rumen. No L-carnitine was detected in abomasum fluid and in feces. Among the three preparations, DCP-B (50% total coatings) showed significantly higher *in vivo* rumen bypass efficiency than DCP-A (25% total coatings) and commercial preparation ($p < 0.05$). Bypass rate was also influenced by the structural difference of the coated films. DCP-B with the outer E100 coatings was slightly lower bypass rate in abomasum fluid as compared to DCP-A. The outer E100 film of DCP-B could readily dissolve in the abomasum of cows even though the DCP-B had a much higher coating levels (50% total coatings).

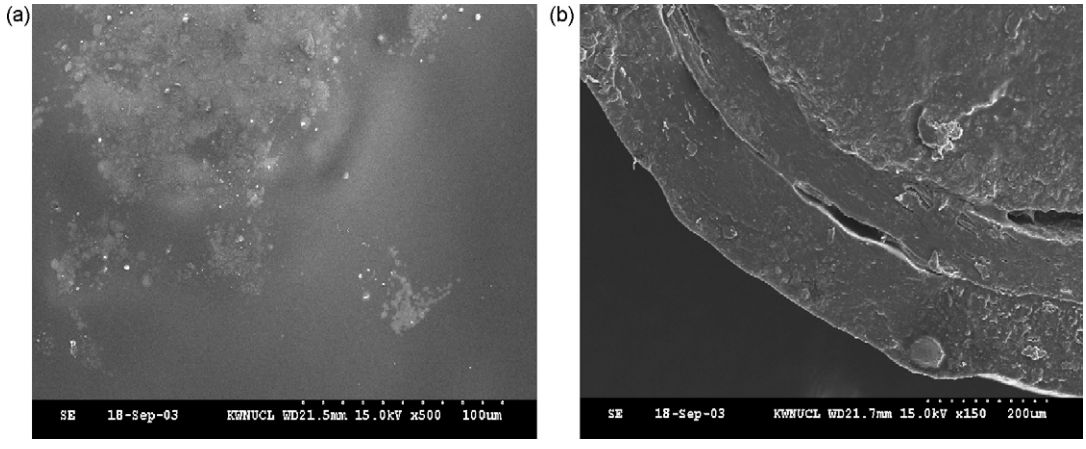

Fig. 6. Scanning electron micrographs of the surface (A) and cross-section of DCP7, core: IR, coating: 20% EC(inner)/30% E100(outer).

Most of L-carnitine was absorbed in cows but approximately 10% L-carnitine from the DCP preparations (DCP-A and DCP-B) was recovered in feces. There was no significant difference of DCP-A and DCP-B in the absorption. It was evident that structures of coated films and coating levels of DCPs influenced the *in vivo* rumen bypass efficiency. However, extremely high rumen resistance might also hinder the intestinal absorption of L-carnitine due to the retarded release rate in cows.

3.4. Biodistribution of L-carnitine in biological samples of cows

L-Carnitine levels of biological samples can be an index of ruminal degradation and delivery efficiency of L-carnitine in dairy cows (LaCount et al., 1996a; Koenig and Rode, 2001). Fig. 8 shows the concentration of L-carnitine in cow plasma (μg/ml) and muscle (mg/g) after the multiple oral feeding of different types of products (raw L-carnitine powder, Carnipass®, and DCP-A and DCP-B) containing 12 g L-carnitine daily for 3 months to cows. Control group was not treated with L-carnitine. Plasma concentrations of L-carnitine in cows treated giving L-carnitine preparations were much higher than that of the endogenous L-carnitine concentration from the control group. There was no statistical difference between the types of products containing L-carnitine in plasma level of cow although *in vitro* release rate and *in vivo* rumen bypass efficiency were quite different. It was evident that plasma concentration reached the steady-state levels by the multiple oral administrations of L-carnitine preparations for 3 months because the half-life of L-carnitine was rather short (Harper et al., 1988). As demonstrated by LaCount et al. (1996b), the concentrations of carnitine in plasma and milk appeared to be maximized when 6 g/day of carnitine were infused just for 2 weeks. This is the reason why DCP of L-carnitine prepared in our research could not show significantly higher concentration in plasma samples of cows than raw L-carnitine and Carnipass®, when 12 g/day dose of preparation was fed for 3 months, respectively. The feeding schedule should be more important for studying delivery efficiency of L-carnitine from a new rumen bypass delivery system.

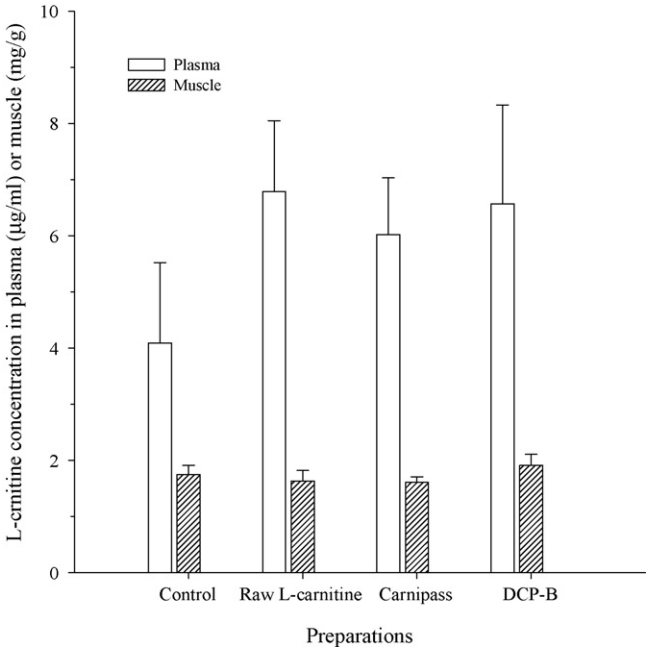

Fig. 8. Concentration of L-carnitine in plasma (μg/ml) and muscle (mg/g) of cows after the multiple oral feeding of L-carnitine preparations containing 12 g L-carnitine for 3 months.

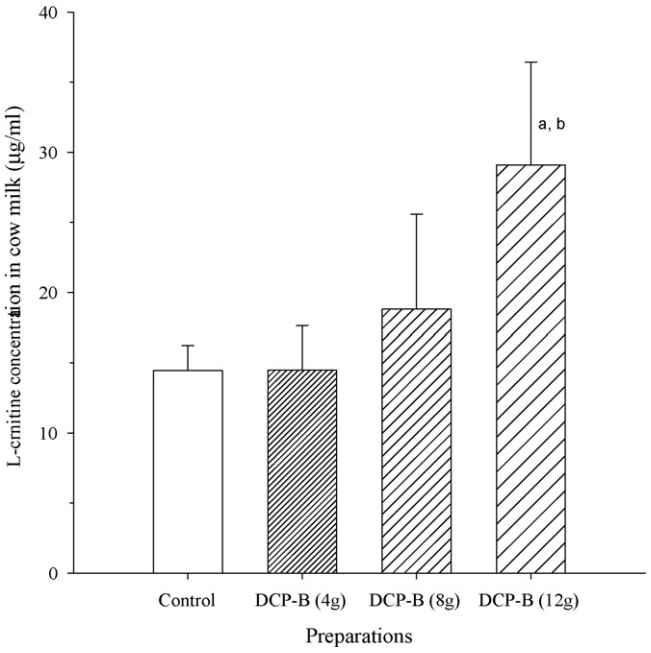

Fig. 9. Concentration of L-carnitine in milk (μg/ml) of cows after the multiple oral feeding of L-carnitine preparations containing 4, 8 or 12 g L-carnitine for 3 months. Significant different from control group, $^{a}p < 0.05$. Significant different from DCP-B (4 g) group, $^{b}p < 0.05$.

Interestingly, a relatively higher amount of L-carnitine in muscle of cow was observed in DCP-B group when compared with non-protected preparations, possibly via slow accumulation in muscle. However, no significant difference was observed among L-carnitine preparations tested.

Fig. 9 shows the effect of feeding amount of DCP-B on concentration of L-carnitine in cow milk after the multiple oral feeding for 3 months. Concentration of L-carnitine in cow milk increased as the feeding dose of a rumen bypass system increased. Oral feeding of 12 g DCP-B was almost twice higher than 4 g DCP-B or endogenous levels (control group). Lower doses of DCP-B were not so efficient for increasing concentration of L-carnitine in cow milk. These results suggested that accumulation of L-carnitine in biological samples could be more dependent on daily feeding schedules rather than L-carnitine delivery systems. Effect of more detailed feeding schedules (period or amount) of different rumen bypass delivery preparations on the concentration of L-carnitine in biological samples should be investigated in near future.

4. Conclusions

Types of coating materials, coating levels and structures of coated films were the most important strategies to modulate release rate and *in vivo* rumen bypass efficiency. Preparation of compact pellet was also required based on chewing behaviors and physiology of veterinary species. Mostly, the DCP with inner EC and outer E100 coatings retarded release rate in rumen fluid and showed higher *in vivo* rumen bypass efficiency in cows. DCP was also efficient for delivering L-carnitine in cows. However, the delivery efficiency of L-carnitine preparations in biological samples of cows would rather be more dependent on feeding schedules. The current DCP was applicable in delivering L-carnitine and other highly soluble nutrients in ruminants.

Acknowledgments

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References

Bunting, L.D., Yabuz, M., Fernandez, J.M., Solaiman, S.G., 2002. Growth and metabolic responses of Holstein calves fed broiler litter-based diets supplemented with L-carnitine. Anim. Feed Sci. Technol. 98, 61–71.

Cao, Q.-R., Ren, S., Park, M.-J., Yun-Jaie Choi, Y.-J., Lee, B.-J., 2007. Determination of highly soluble L-carnitine in biological samples by reverse phase high performance liquid chromatography with fluorescent derivatization. Arch. Pharm. Res. 30, 1041–1046.

Chatchawalsaisin, J., Podczeck, F., Newton, J.M., 2005. The preparation by extrusion/spheronization and the properties of pellets containing drugs, microcrystalline cellulose and glyceryl monostearate. Eur. J. Pharm. Sci. 24, 35–48.

Cone, J.W., Kamman, A.A., Van Gelder, A.H., Hindle, V.A., 2002. Rumen escape protein in concentrate ingredients determined with the nylon bag and enzymatic techniques. Anim. Feed Sci. Technol. 97, 247–254.

De Boer, G., Murphy, J.J., Kennely, J.J., 1987. Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. J. Dairy Sci. 70, 977–982.

Harper, P., Elwin, C.E., Cederblad, G., 1988. Pharmacokinetics of intravenous and oral bolus doses of L-carnitine in healthy subjects. Eur. J. Clin. Pharmacol. 35, 1041–1432.

Koenig, K.M., Rode, L.M., 2001. Ruminal degradability, intestinal disappearance and plasma methionine response of rumen-protected methionine in dairy cows. J. Dairy Sci. 84, 1480–1487.

LaCount, D.W., Ruppert, L.D., Drackley, J.K., 1996a. Ruminal degradation and dose response of dairy cows to dietary L-carnitine. J. Dairy Sci. 79, 260–269.

LaCount, D.W., Emmert, L.S., Drackley, J.K., 1996b. Dose response of dairy cows to abomasal administration of four amounts of L-carnitine. J. Dairy Sci. 79, 591–602.

Martinez, M., Augsburger, L., Johnston, T., Jones, W.W., 2002. Applying the biopharmaceutics classification system to veterinary pharmaceutical products. Part I: Biopharmaceutics and formulation considerations. Adv. Drug. Del. Rev. 54, 805–824.

Rossi, F., Fiorentini, L., Masoero, F., Piva, G., 1999. Effect of fat coating on rumen degradation and intestinal digestibility of soybean meal. Animal Feed Sci. Technol. 81, 309–318.

Sanna, V., Gavini, E., Giunchedi, P., 2004. Bilayer tablets based on poly (ε-caprolactone) and polymethylmethacrilates as controlled-release system for ruminants. Pharm. Dev. Tech. 9, 321–328.

Sklan, D., Tinsky, M., 1993. Production and reproduction responses by dairy cow fed varying undegradable protein coated with rumen bypass fat. J. Dairy Sci. 76, 216–223.

Wu, S.H.W., Papas, A., 1997. Rumen-stable delivery systems. Adv. Drug Del. Rev. 28, 323–334.

Yoshimaru, T., Shibata, M., Fukugomori, T., Matsumoto, K., 1999. Preparation and characteristics of rumen-bypass microcapsules for improvement of productivity in ruminants. J. Agric. Food Chem. 47, 554–557.