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Enhancing Synergistic Roles of Stakeholders for Development of Sustainable Livestock Production

Batu, Indonesia, October 19-21, 2016

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Microencapsulation of Anti Escherichia coli Enterotoxigenic Colostrums for Passive Immunity Against Diarrhea caused by Colibacillosis in Calves

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Abstract

Oral administration of IgG facing a serious constrain since IgG is very sensitive to gastrointestinal tract environment. Microencapsulation technique may protect colostral IgG against peptic and trypsin digestion and acidicity (low pH) in the stomach. The objective of this experiment was to evaluate the morphology of microcapsule coated by chitosan-alginate for passive immunity against diarrhea caused by colibacillosis in calves. Pregnant cows were injected subcutaneously with *E. coli* vaccine consist of whole cell ETEC. Colostrum samples were collected immediately after parturition. Bovine colostrum samples were prepared for defatting and decaseinated. Purification of IgG was done by salt presipitation method. The microcapsules were made by extruction method. The microcapsules obtained were freeze-dried. The particle size and the surface morphology of the microcapsules were analyzed using scanning electron microscope (SEM). Results of this experiment indicated that the diameter of IgG-loaded chitosan-alginate microcapsules were approximately 2000 μm, larger than blank chitosan-alginate microcapsules (1000 μm) and no microphore at the surface of microcapsules.

Keywords: *bovine colostrums, chitosan-alginate, ETEC K-99, microencapsulation*

Introduction

Enterotoxigenic Escherichia coli (ETEC) K-99 is by far the most common cause of enteric colibacillosis in neonatal calves (1 week of age). The use of antibiotic for treatment of diarrhea due to colibacillosis in calves remained unsuccesful. The case of diarrhea to contribute to calves mortality is still very high. Various experiments reported that *Escherichia coli* K-99 from calves show a ressistence to antibiotic used in the field (Supar 1986). Therefore the approach through passive immunization of calves using hyperimmune colostrum could be an alternative way out in controlling diarrhea due to ETEC.

Oral administration of colostrums facing a serious constrain since colostral IgG is very sensitive to gastrointestinal tract environment in neonatal calves (1 week of

age). The activity of IgG may be destroyed by stomach environment condition, particularly due to such enzymes (pepsin and trypsin) and low pH (Esfandiari *et al.* 2014; Kovacs-Nolan and Mine 2005; Murtini *et al.* 2014), so colostral antibody may not be effective in controlling diarrhea in calves (1 week of age). Therefore, it is necessary to find a method to preserve the therapeutic value of IgG during gastric passage. Chitosan-alginate microencapsulation may effectively protect colostral IgG from gastrointestinal tract environment, so colostral IgG may perform its function effectively. The objective of this experiment was to evaluate the morphology of microcapsule coated by chitosan-alginate for passive immunity against diarrhea caused by colibacillosis in calves.

Methodology

Hiperimmune colostrum was produced by vaccinated pregnant cows in the last trimester of pregnancy. The cows were injected by whole cells of *Escherichia coli enterotoxigenic* (ETEC) K-99 emulsified with an equal volume of complete Freund's adjuvant. Bovine colostrum samples were prepared by modification of Zarrilli *et al* (2003) methods, for removing the lipid and casein fraction to produce whey. The supernatant (whey) were collected for IgG purification. Precipitation method was used to concentrate IgG using 40% ammonium sulfate. The precipitate was dissolved and dialysis.

Blank chitosan-alginate microcapsules (BCAM) and IgG-loaded chitosan-alginate microcapsules (IgG-CAM) were prepared by modification of Li *et al* (2007) methods. The microcapsules obtained were filtered and rinsed with distilled water and were freeze-dried. The particle size and the surface morphology of the microcapsules were examined using a scanning electron microscope (SEM) (HITACHI S-4300SE/N).

Results and Discussion

The microcapsules formed obtained were transparent rounded mass (spherical), smooth with jelly-like consistency. The particle size and the surface morphology of the microcapsules were examined using a scanning electron microscope (SEM).

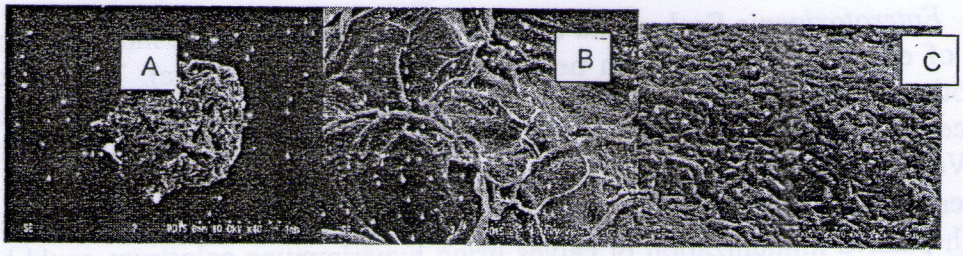

Figure 1. Whole blank microcapsules (A), surface of blank microcapsules (B and C)

According to the SEM analysis, the mean diameter of BCAM was approximately 1000 μm. However, upon freeze-drying, the spherical structure of microcapsules was disrupted as visualized using SEM (Figure 1A). The SEM micrographs of fine surface structures of BCAM formed were shown in Figure 1 (A-C). The outer surface of microcapsules formed was apparent, where microphores showed a smooth and wrinkled surface.

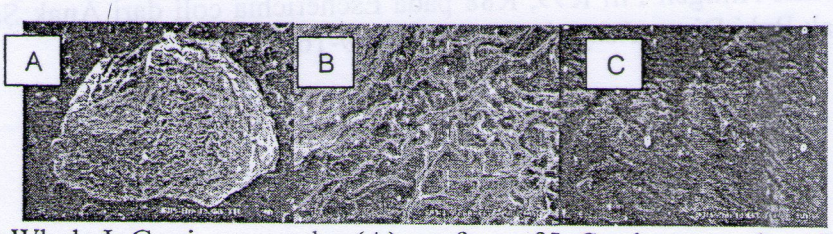

Figure 2 Whole IgG microcapsules (A), surface of IgG microcapsules (B and C)

After loading with IgG, the size of the microcapsules retained was changed as compared to BCAM. The diameter of microcapsules were approximately 2000 μm. The BCAM size were generally smaller if compare to of those microcapsules consist of IgG (IgG-CAM). The microcapsules size depended on nozzle diameter and the distance between the needle and encapsulation medium (Krasaekoopt *et al.* 2003). The SEM micrographs of fine surface structures of IgG-CAM formed were shown in Figure 2 (A-C). It was observed that the IgG blended into the alginate and there were no microphories in microcapsules (IgG-CAM) as compared to corresponding the BCAM.

Conclusion

This study demonstrated that the diameter of IgG-loaded chitosan-alginate microcapsules (IgG-CAM) were approximately 2000 μm, larger than blank chitosan-alginate microcapsules (1000 μm), and no microphore at the surface of microcapsules.

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