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Improvement of the Digestion in Pigs by Using Microencapsulated Asperase

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Asperase from *Aspergillus usamii mut. shiro–usamii* was microencapsulated by the spray–dry method in order to decrease fecal quantity and improve the digestion of pigs. Capsules were prepared with a yield of about 88% and a mean particle diameter of 10–20 μm. The microencapsulated asperase was very resistant to digestion in the gastric environment (pH 1.2), and the efficiency of release in intestinal fluids (pH 6.8) was about 100% within 30 min. A feeding study of 100–d–old pigs was performed to confirm the decrease of the fecal amount and the improvement in digestion due to microencapsulated asperase. In a group fed the diet that contained microencapsulated asperase, a significant reduction of 43% in the fecal quantity was observed ($p < 0.01$ vs. control). In addition, when pigs were fed the microencapsulated asperase, apparent digestion coefficients of dry matter, crude protein, nitrogen–free extract, ether extract, and crude fiber were observed a significant augmentation of 5.4%, 12.8%, 1.2%, 17.8%, and 13.1%, respectively, compared with those in the group of pigs fed the standard diet. These results suggest that the addition of microencapsulated asperase is the primary cause of decreased fecal amount and improved digestibility.

INTRODUCTION

The trend in the swine industry is younger weaning to increase sow productivity. Research has focused on minimizing the severity of the postweaning lag in pig performance. The early–weaned pig has been typically characterized by poor growth performance: low feed intake, poor feed conversion, intestinal malabsorption, and weight loss. A transient hypersensitivity to soy proteins is one possible explanation for the decreased postweaning growth performance (Friesen *et al.*, 1993). Reduced performance in pigs fed soybean proteins has been associated with reduced digestion (Wilson and Leibholz, 1981; Walker *et al.*, 1986). Also, newborn and postweaning pigs tend to suffer diarrhea because of changes in the morphology of the small intestine (Dunsford *et al.*, 1989). Finally, drying and fermentation treatment of the feces requires great labor and expense. It is, therefore, desirable to reduce fecal production and to improve the digestion of nutrients to save labor and cost and prevent spoiling the environment.

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However, attempts to improve the digestion in the pre- and postweaning pig by nutrient substitution (Lennon *et al.*, 1971) or improved nutrient processing (Walker *et al.*, 1986) have had limited success.

On the other hand, we found that asperase from *Aspergillus usamii mut. shiro-usamii*, which is complex enzyme of xylanase and pectinase, was very useful for the improvement of digestion and for the reduction of the fecal quantity in pig (Yamato *et al.*, 1997). That is, this enzyme has enabled the improvement of digestion and reduction of fecal quantity by degrading slightly digestive polysaccharides. However, despite improved performance and digestion of nutrients, the possibility of damage to the stomach and the microvillus is present due to the powerful saccharification activity of asperase. Therefore, it might be desirable to encapsulate asperase so it can act only in the small intestine. In a previous study (Yoshimaru *et al.*, 1997; 1999), we reported that a microencapsulation method (spray-dry method) using porous starch allowed us to prepare target-specific microcapsules by selection of appropriate coating agents. Therefore, we designed microencapsulated asperase, which will be supposed to disintegrate mainly in small intestine to further improve on digestion.

MATERIALS AND METHODS

Materials

Porous starch was kindly supplied by San-ei Sucrochemical Co., Ltd. (Aichi, Japan). Eudragit L100, a synthetic acrylic copolymer that is soluble at pH 6.0 and above, was kindly supplied by Röhm Pharma GmbH (Darmstadt, Germany). This coating agent readily dissolves under alkaline conditions. Asperase (complex enzyme of xylanase and pectinase) from *Aspergillus usamii mut. shiro-usamii*, which has an optimal pH of 5.0–7.0, an optimal temperature of 60 °C, was obtained from San-kyo Co., Ltd. (Tokyo, Japan). All other chemicals were obtained from Nacalai Tesque (Kyoto, Japan) and were of analytical reagent grade.

Preparation of Microcapsules

To prepare microcapsules that can act only in the small intestine, we microencapsulated asperase from *A. usamii mut. shiro-usamii* by the spray-dry method because of its convenience and high degree of reproducibility. One-hundred-fifty g of porous starch (about 5 μm in diameter) with numerous holes of a few μm in diameter were added to 350 ml of a 28.5% solution (w/v) of asperase (12,000 pectin-saccharification units/g), and the mixture was stirred for 2 hr. The solution was then sonicated for 5 min to integrate the asperase into the porous starch, which was then freeze dried and coated with Eudragit L100. The freeze-dried material was combined with 900 ml of a 5% solution (w/v) of Eudragit L100 in ethanol, as an enteric-coating copolymer. The microencapsulated asperase 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 temperatures of 105 °C and 62–79 °C, respectively.

Assay of Enzymatic Activity

Asperase activity was determined by a specific colorimetric method of galacturonic

acid with carbazole–sulfuric acid (Dische, 1947; 1950), using a calibration curve for different concentrations of asperase solution (0–200 mg/ml). One ml of asperase solution (suitably diluted so that the absorbance is in the range of the calibration curve) was incubated with 5 ml of a 0.01% solution (w/v) of pectin as substrate at 37°C for 10 min. The reaction mixture was filtered through filter paper (No. 5B, Advantec Toyo Co., Tokyo, Japan). Then, a 0.1 ml aliquot of the filtrate, which contained galacturonic acid produced by the reaction of pectin with asperase, was transfered into a test tube. The test tube was placed in an ice bath. 1.2 ml of a cold mixture of 1 part water and 7 parts concentrated sulfuric acid was pipetted into the tube and mixed; then, 40 μl of a 0.2% solution (w/v) of carbazole was added to the mixture under ice cooling. After being shaken vigorously, the test tube was immersed in a water bath at 75°C for 20 min and cooled in running tap water. Six ml of distilled water was then added, and the red color that developed was measured at 525 nm with a spectrophotometer (UV–1200, Shimadzu Co., Kyoto, Japan). The specific activity of the powdered asperase was determined separately. One pectin–saccharification unit was defined as the enzymatic activity that, under the present assay conditions, liberated the reducing power equivalent to 1 μmol of galacturonic acid in 1 hr.

Efficiency of Encapsulation of Asperase into Microcapsules

Before measurement, 50 mg of microencapsulated asperase was ground by a mortar and a pestle in 1 ml of 0.1 M phosphate buffer (pH 7.5) to dissolve the enteric–coating agent, Eudragit L100. A suitable dilution of the resultant solution was assayed for enzymatic activity as described above. The encapsulation efficiency was calculated by expressing the amounts of asperase encapsulated as a percentage of the initial amounts of asperase used to prepare the microencapsulated enzymes.

Stability and Release of Microencapsulated Asperase

The stability of microencapsulated asperase in simulated gastric fluids was investigated. A 100 mg aliquot of the microencapsulated asperase was incubated at 37°C with reciprocal shaking (100 strokes/min) in 15 ml of a solution of HCl containing 0.2% NaCl (pH 1.2) as described in the Japanese Pharmacopoeia (JP XII). The microcapsules were then collected by filtration and washed with distilled water. The collected microcapsules were dissolved completely in phosphate buffer (pH 7.5) and assayed for the asperase activity that was retained after treatment with the acidic solution. The resistance of the asperase was expressed as the percentage of initial asperase activity retained.

The release of asperase from the microcapsules was evaluated as follows. A 100 mg aliquot of the microencapsulated asperase was incubated at 37°C with reciprocal shaking (100 strokes/min) in 15 ml of phosphate buffer (pH 6.8) described in JP XII. At appropriate times, the solution was filtered, and asperase activity in the filtrate was assayed.

Feeding Experiment Protocols and Digestion Trials

The feeding test was performed using 100–d–old pigs for confirmation of effectiveness of this microencapsulation method. Three 100–d–old pigs of equal weight were used in a switchback method to study the digestibility of the three diets: the

Table 1. Percentage composition of experimental diets

ingredient	diet		
	standard	+ free asperase	+ microencapsulated asperase
soybean meal	31.10	31.10	31.10
dried whey	37.20	37.20	37.20
cornstarch	16.44	15.94	15.89
sugar	7.00	7.00	7.00
dextrose	1.00	1.00	1.00
corn oil	5.00	5.00	5.00
tricalcium phosphate	0.55	0.55	0.55
calcium carbonate	0.82	0.82	0.82
NaCl	0.20	0.20	0.20
L-lysine	0.19	0.19	0.19
D, L-methionine	0.30	0.30	0.30
premix of vitamin and mineral	0.20	0.20	0.20
asperase	–	0.50	–
microencapsulated asperase	–	–	0.55
total	100.00	100.00	100.00

standard diet, a diet containing free asperase, and a diet containing microencapsulated asperase. A switchback method means one which gives in turn each diet for one pig. Each pig was given a experimental diet shown in Table 1 and water *ad libitum*. The standard diet was composed of 14.5% crude protein, 3.1% ether extract, 3.2% crude fiber, and 61.6% nitrogen-free extract and served as control diet (TDN: 84.78%, DCP: 18.03%). Furthermore, the diet was supplemented with 10.0 g of 12,000 units/g free asperase or 11.1 g of 2700 units/g microencapsulated asperase per 2.0 kg/d of the standard diet, respectively. That is, free asperase was constituted 0.5% (w/w) of the standard diet, whereas the microencapsulated asperase added was 25% of activity of free asperase. If activity of encapsulated asperase is to match that of free asperase, four times the microencapsulated asperase must be used which, in addition to the microcapsules, is responsible for the higher cost. Also, preliminary experiments for feeding to pig had shown that effect of free asperase was equivalent to that of one-fourth of the microencapsulated asperase (data not shown). Therefore, we assumed that a limited addition of microencapsulated asperase would supplemented it effectively.

The conditions of the feeding study were as follows: a preliminary 5 d adjustment period was followed by a 4 d collection. Fecal samples were collected daily over 4 d and were immediately dried at 95 °C for 24 hr. The dry matter, crude protein, nitrogen-free extract, ether extract, and fiber fraction of feeds and fecal samples were determined according to the standard method of the Japan Oil Chemists' Society (1996): air oven method at 135 °C for 2 hr for dry matter, the Kjeldahl method for crude protein, the Soxhlet method extracting with diethyl ether for 8 hr for ether extract and filtration method subtracting the crude ash from a residue treated with H_2SO_4 and NaOH for crude fiber. And digestibility of each fraction was calculated.

Statistical analysis was performed using one-way analysis of variance ($p<0.01$ or $p<0.05$), followed by the Student's t-test for examination of the significance of differences.

RESULTS AND DISCUSSION

Efficiency of Encapsulation and Morphology of Microcapsules

We have already reported that microencapsulation using porous starch allowed preparation of target-specific microcapsules by selection of appropriate coating agents.

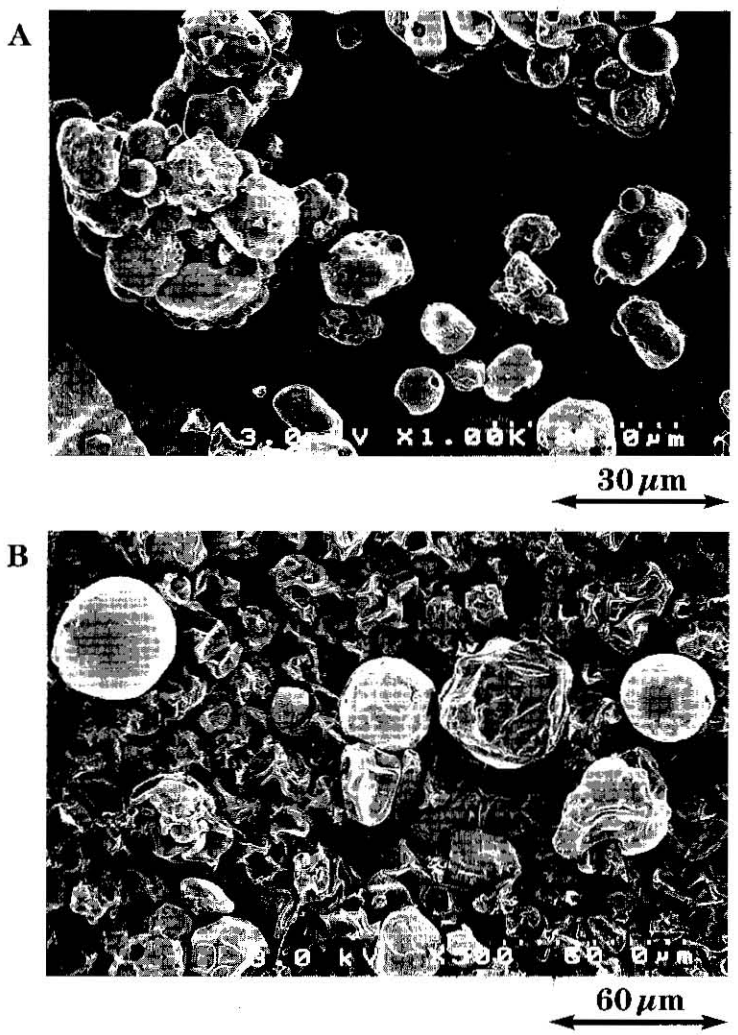

Fig. 1. SEM of freeze-dried material containing asperase in holes of porous starch (A) and microencapsulated asperase prepared by the spray-dry method (B).

Table 2. Encapsulation efficiency of asperase into microcapsules

condition of microcapsule	asperase activity[a] (U)	trap ratio (%)
start	1200000	100.0
integration into porous starch	1080000	90.0
microencapsulation	1059600	88.3

[a] One unit was defined as the enzymatic activity that liberated the reducing power equivalent to 1 μmol/h of galacturonic acid.

Hence, porous starch, which is produced by enzymatic degradation of corn starch (Suzuki, 1995), was used as the core material in this study for the purpose of preparing microcapsules that can release asperase only in the small intestine.

Typical scanning electron micrographs (SEM) of freeze-dried material and microencapsulated asperase are shown in Figure 1. The prepared capsules had a mean particle diameter of 10–20 μm. This miniscule size suggest that the quality of diets would be little affected by the addition of the microencapsulated asperase, in spite of uneven spheres in shape.

Next, we investigated the efficiency of encapsulation of asperase into the microcapsules. As shown in Table 2, we found that microencapsulated asperase was generated with a yield of about 88%. From the efficiency (90%) of integration of asperase into porous starch, we assumed that coating with Eudragit L100 by the spray-dry method was very efficient, without loss of asperase activity, and that the asperase activity was hardly affected by the ethanol used as the solvent for Eudragit L100. On the other hand, judging from the amount of asperase administered (100 g) and total microcapsules (392 g), activity per gram of free asperase and asperase encapsulated was 12,000 U/g and 2700 U/g, respectively, that is, asperase occupied in a unit weight of microcapsule was 22.5%. Therefore, the microencapsulation method using a spray-dryer seems effective and suitable to the swine industry because of its economy, safety, and convenience.

Resistance of Microencapsulated Asperase to Degradation in the Gastric Environment

To investigate the resistance of microencapsulated asperase to degradation in the gastric environment, microencapsulated asperase was treated with a gastric solution (pH 1.2, HCl solution containing 0.2% NaCl). As shown in Figure 2, we observed high stability of the microencapsulated asperase in the acidic solution. That is, even though the asperase activity retained in the microcapsules was rapidly reduced to about 80% in only 10-min incubation, the reduction then leveled off and the activity held constant. This indicates that the asperase should not be susceptible to much damage in the gastric environment and that it should pass through the stomach without a great loss of activity. Accordingly, it appeared that this microencapsulated asperase had the potential to pass

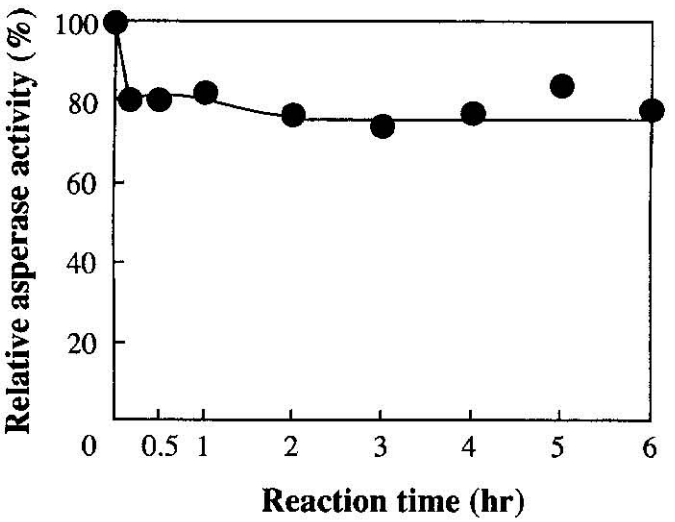

Fig. 2. Stability of microencapsulated asperase in artificial gastric juice (pH 1.2, HCl solution containing 0.2% NaCl) at 37°C.

through without negative effect of the environmental pH within its residence time (about 6 hr).

Release of Microcapsules in the Intestinal Tract

This microencapsulated asperase must be disintegrated in the intestinal tract after passage through the stomach, followed by release of asperase from the microcapsules. We next examined the release of asperase from the microcapsules under conditions that mimicked the intestinal tract (pH 6.8, phosphate buffer). As shown in Figure 3, all encapsulated asperase were released within only 30 min in simulated intestinal fluid, due to the ready solubility of Eudragit L100 used as a coating agent (Morishita *et al.*, 1992; Yoshimaru *et al.*, 1997). These findings suggest that asperase released from microcapsules has the potential to improve digestion within its residence time in the intestinal tract. Therefore, we concluded that these microcapsules permitted passage of asperase through the gastric tract with little degradation and offered the subsequent release of asperase in the intestinal tract.

Reduction of the Fecal Quantity and Improvement of the Digestion by Feeding the Diet Containing Microencapsulated Asperase

To evaluate the reduction of the fecal quantity and improvement in digestion by microencapsulated asperase *in vivo*, we performed a feeding test with 100-d-old pigs. As shown in Table 3, in the case of free asperase-fed pigs, the fecal amounts were to some extent smaller than in the standard diet-fed pigs ($p<0.05$ vs. control). Further, the fecal quantity from the groups fed the diet that contained microencapsulated asperase

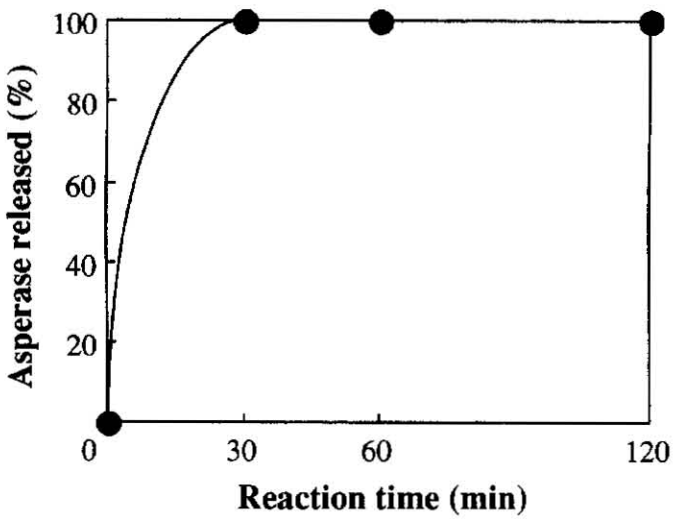

Fig. 3. Release of asperase from microcapsules under conditions that mimic the intestinal tract (37°C and pH 6.8, phosphate buffer).

Table 3. Fecal quantity of pigs given commercial diet, diet containing free asperase, and diet containing microencapsulated asperase

diet[a]	raw feces[b] (g/d)	dry matter (g/d)	water content (%)
control	923	292	68.4
free asperase	653[d]	223	65.8
microencapsulated asperase	523[c]	194[d]	62.9

[a] Experimental diets: control=standard diet; free asperase=control plus free asperase at 0.5% (w/w); microencapsulated asperase=control plus microencapsulated asperase at 0.55% (w/w) (*i.e.* 25% in activity of free asperase).

[b] The raw feces for each diet represent average per day of 3 pigs when pigs were given 2.0 kg/d of diet.

[c] Significantly different from the control diet at $p<0.01$.

[d] Significantly different from the control diet at $p<0.05$.

were significantly reduced, with a reduction of 43% for those of the commercial diet–fed groups, as a control ($p<0.01$), and 20% for those on the free asperase–fed groups. In addition, the intake of the diet containing microencapsulated asperase enabled reduction in dry matter of feces ($p<0.05$ vs. control). Therefore, fecal quantity and dry matter of feces were significantly diminished by administration of microencapsulated asperase, and this might aid treatment of excrement, which is accompanied by great labor and cost.

Table 4. Summary of the apparent digestibility of dry matter, crude protein, nitrogen-free extract, ether extract, and crude fiber in 100-d-old pigs given various diets

diet[a]	apparent digestibility (%)[b]				
	dry matter	protein	nitrogen-free extract	ether extract	fiber
control	84.0±2.1	78.1±3.2	91.8±1.0	62.8±6.6	34.5±9.7
free asperase	87.7±1.7	86.0±1.3[d]	92.8±1.0	75.9±5.8	42.0±6.4
microencapsulated asperase	89.4±0.7[d]	90.9±0.8[c]	93.0±0.3	80.6±0.7[c]	47.6±3.7

[a] For explanation of diet, see Table 3, footnote a.
[b] The apparent digestibility indicated for each diet represent averages±SE of 3 pigs.
[c] Significantly different from the control diet at $p<0.01$.
[d] Significantly different from the control diet at $p<0.05$.

Also, minimization of fecal quantity might improve the digestion in pigs, and induction of diarrhea might be depressed simultaneously.

Next, apparent digestibility of 100-d-old pigs before and after the intake of the diet containing microencapsulated asperase was determined. Table 4 shows a summary of digestion data for 100-d-old pigs fed various diets. Remarkable improvements in the apparent digestibility of the dry matter fraction, crude protein fraction, nitrogen-free extract fraction, ether extract fraction, and crude fiber fraction were observed. That is, the digestion coefficients of each nutrient were increased by feeding the diet containing free asperase, as compared with those of the standard diet-fed groups. Moreover, the intake of the diet containing microencapsulated asperase elicited augmentation of 1.7%, 4.9%, 0.2%, 4.7%, and 5.6% in the digestion coefficients, respectively, as compared with those of the free asperase-fed groups, and a significant augmentation of 5.4%, 12.8%, 1.2%, 17.8%, and 13.1% in the digestion coefficients, as compared with those of the standard diet-fed groups ($p<0.01$ in crude protein and ether extract, $p<0.05$ in dry matter). Therefore, microencapsulated asperase may improve digestion of various nutrients in the diet, even if the microcapsules added had only 25% of the enzymatic activity of free asperase. Lloyd *et al.* (1957) and Combs *et al.* (1963) have reported that the digestion in piglets improves with increasing age. However, the data reported here indicate a further improvement in the apparent digestion when 100-d-old pigs were fed the diets that contained microencapsulated asperase. Maner *et al.* (1962) reported that differences in gastric pH and rate of food passage might be factors in the efficiency of utilization of protein sources by the very young pig. Since this microencapsulated asperase was hardly affected by gastric pH, as shown in the preceding section, the addition of the microencapsulated asperase to the diet might also have the beneficial effects to young pigs, irrespective of gastric pH and rate of food passage. Accordingly, it appeared that adding microencapsulated asperase to the commercial mixing diet had efficiently improved digestibility.

In summary, we have examined the possibility of an improvement of digestion for

pigs. Microencapsulated asperase might be effective to minimize the fecal quantity and play a physiologically important role in nutrient absorption and utilization by pigs. At present, we are attempting to confirm the effectiveness of the microencapsulated asperase upon the growth of piglets that are characterized by poor growth performance.

This experiment was carried out under the control of the guideline for Animal Experiment in Faculty of Agriculture and the Graduate Course, Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government.

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