Comparison of Digestive Function Among Rabbits, Guinea-Pigs, Rats and Hamsters. II. Digestive Enzymes and Hindgut Fermentation

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ABSTRACT : The aim of this trial was to study the response of laboratory animals including omnivores (rats) and herbivores (rabbits, guinea pigs and hamsters) to the same level of dietary fiber on their digestive enzymes and hindgut fermentation. Ten weanling animals of each of four species, rabbits, guinea-pigs, rats and Syrian hamster, were fed a basal diet of 18% crude protein and 10% crude fiber for six weeks. The digesta and tissue of each intestinal segment were collected to measure the activity of digestive enzymes. Rabbits contained the highest secreted pepsin activity in the stomach, whereas rats contained the highest protease and α-amylase activity in the small intestine, and lower fibrous hydrolases in the hindgut than rabbits, guinea pigs and hamsters. The total VFA productions in the caecum and colon were highest in rats, followed by hamsters and rabbits, while the guinea pigs contained the lowest VFA and a different pattern of VFA molar ratio from the other laboratory animals. The degree of hindgut fermentation in these laboratory animals was in reverse to the trend for their fiber digestion. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 11 : 1508-1513)*

Key Words : Laboratory Animals, Hindgut Fermentation, Digestive Enzymes, Volatile Fatty Acids

INTRODUCTION

Rabbits, guinea pigs and hamsters are small herbivores, depending upon forage and agricultural by-products for survival. They utilize crude fiber less efficiently as compared to the large herbivores, i.e., cattle, sheep, goats and horses (Maynard et al., 1979). Makkar and Singh (1987) indicated that domesticated rabbits so-called pseudo-ruminants, possess a caecum which contains higher protease and amylase and less cellulase as compared to the rumen in the large ruminants. Low crude fiber digestion capability in domesticated rabbits may be due to the low cellulase, inulinase and β-glucosidase, but high pectinase in their GI tract (Marounek and Vovk, 1995; Dojana et al., 1998). Yu and Tsen (1996) indicated an increase in cellobiose digestion by β-glucosidase as domesticated rabbits grow from suckling to maturity, indicating an increase in fiber utilization as the rabbits grow. Laplace (1978) suggested a high correlation among food, digestive enzymes and diarrhea occurrence, indicating that different animals with different digestive characteristics, utilize different food compositions with varying degrees of efficiency. Although rats can tolerate high levels of diverse fiber in their diet, Demigne and Rémésy (1985) pointed out that a large amount of undigested fiber is fermented in their caecum. Rabbits on the other hand, experienced excessive caecal fermentation on a low fiber diet (6% crude fiber) (Cheeke and Patton, 1980). Hasdai et al. (1989) fed a raw soybean flour diet to guinea pigs which caused depression in the secretion of pancreatic and intestinal digestive enzymes. Feeding the same raw soybean flour to rats, hamsters or chicks caused excessive secretion of the digestive enzymes (Hasdai and Liener, 1983). Borel et al. (1991) suggested an adaptation period for the lipase secretion. They found an increase in lipase activity after lipid supplementation of the diet for two weeks. Rabbits, guinea pigs and hamsters are herbivores whereas rats are omnivores with high tolerance for dietary fiber. Dietary fiber is a negative nutrient factor in nutrient digestion, but it provides a substrate for microbial fermentation in the GI tract. The aim of this trial was to study the response of the digestive enzymes and hindgut fermentation in these laboratory animals to a common diet with same level of dietary fiber.

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METERIAL AND METHODS

Animals and diets

The experimental animal, diet and condition of housing were described in part (I) (Chiou et al., 2000). Ten weanlings of each animal species with equal numbers of each sex and a mean live-weight of 1021 g for the California rabbit, 175 g for the Hartley guinea pig, 165 g for the Wistar rat, and 71 g for the Syrian hamster were selected and fed a common diet. This diet was formulated to have 18% crude protein and 10% crude fiber according the nutrients requirement for rabbits (NRC, 1977), and laboratory animals (NRC, 1995; Sakaguchi et al., 1987). Composition of diet and husbandry details are described earlier (Chiou et al., 2000).

Sample preparation

At the end of six weeks feeding period, rabbits,

guinea pigs, rats and hamsters were sacrificed after 12 hours fasting. The intestinal tracts with the contents in different segments were then removed to measure enzyme activities. These gut segments included stomach, duodenum (20% proximal end), jejunum (60% of the middle section) and ileum (20% distal end) of the small intestine, caecum, colon and rectum. The crude enzymes from the intestinal tissues with digesta were prepared according to the method of Kidder and Manners (1980). Samples of the intestine were weighed and homogenized at 4℃ in a 9 g/kg NaCl solution that was 4 times the volume of sample (v/w). The supernatants, after centrifuging at 2000 g for 30 min, were stored at -20℃. The analysis of crude enzyme activities was performed within three months of storage. The portions of caecal and colonic digesta samples (5-10 g fresh matter) were stored at -20℃ for further analyses of volatile fatty acids (VFA).

Analysis

The activity of pepsin (EC 3.4.4.1) in the stomach was assayed by the method of Rick and Fritch (1974) using hemoglobin as a substrate. The activity of one unit of pepsin was expressed as an increase in absorbance of 0.001 value per min. under 280 nm of TCA (trichloroacetic acid) soluble products hydrolyzed from the substrate at assay condition of 37℃ and pH 2.5. The protease activity in the small intestine was assayed according to the method of Walter (1981) using hemoglobin as the substrate. The activity of one unit protease was expressed as 1 μmole tyrosine released from the substrate per hour at an assay condition of 37℃ and pH 7.5. The activity of α-amylase (EC 3.2.1.1) was assayed using the method of Rick and Stegbauer (1974) using soluble starch as a substrate. The activity of one unit of α-amylase was expressed as 1 mg reducing sugar (maltose as a standard sugar) released per hour at 37℃, pH 6.9 assay condition. The activity of maltase (EC 3.2.1.20, α-glucosidase) was assayed using maltose as a substrate. Maltase activity was expressed as 1 mg glucose released per hour at 37℃, pH 4.9 assay condition.

The activities of fiber related hydrolases, namely endoglucanase (cellulase, EC 3.2.1.4), cellubiohydrolase (EC 3.2.1.91) and cellobioase (EC 3.2.1.21), were assayed according to Onodera et al. (1988) using carboxymethylcellulose, avicel and cellobiose as substrate, respectively. Activities of each unit were expressed as 1 μg of reducing sugar (glucose as standard sugar) released per hour at assay condition of 50℃ and pH 5.0. The total concentration of volatile fatty acids was determined according to Parker and McMillan (1976).

Statistical analysis

A completely randomized design was applied to find the species effect. Analysis of variance was calculated with the General Linear Model (GLM) procedure of the Statistical Analysis System (1985).

RESULTS

Digestive enzyme activity

Figure 1 (A, B) presents the activity of protein hydrolases in the stomach and the segments of small intestine of rabbits, guinea pigs, rats and hamsters. The activity of pepsin in the stomach of rabbits was highest followed by guinea-pigs and rats, while hamsters were lowest ($p<0.05$). Rats had the highest protease activity in the small intestine among these laboratory animals ($p<0.05$). Hamsters however, had a higher protease activity in the duodenum than rabbits ($p<0.05$).

The enzyme distribution of α-amylase and maltase in the segments of small intestine, as shown in figure 1 (C, D), indicated a high activity for α-amylase in the duodenum and a gradual decrease in enzyme activity along the tract. On the contrary, the maltase activities gradually increase along the GI tract in the small intestine since it is secreted from the enterocytes of the intestine. The distribution pattern of carbohydrate hydrolases activity in the segments of the GI tract of these rodent species is similar to that in chickens (Nitsan et al., 1991). The activity of α-amylase in the small intestine was higher in the rats than in other three species of animals ($p<0.05$). The activity of maltase was highest in the hamsters ($p<0.05$), followed by rats, while rabbits and guinea pigs were significantly lower than hamsters and rats ($p<0.05$).

Figure 2 presents the fiber hydrolases activity in the caecum and colon-rectum of the four species. Enzyme activity was higher in the caecum than the colon-rectum. The caecum and colon-rectum are the major site for fibrous hydrolases in all species of animals including both herbivores and omnivores. Except for the endoglucanase activity in colon-rectum, rats contain lower fibrous hydrolases per gram tissue, especially in the caecum, as compared to the herbivores ($p<0.05$).

Hindgut fermentation

Table 1 presents the total VFA concentration and molar proportions in the caecal and colonic-rectal contents of the four species. The concentration was higher in the caecum than in the colon-rectum. The total VFA production in the caecum or colon-rectum was highest in rats ($p<0.05$), followed by hamsters and rabbits, while guinea pigs contained the lowest VFA

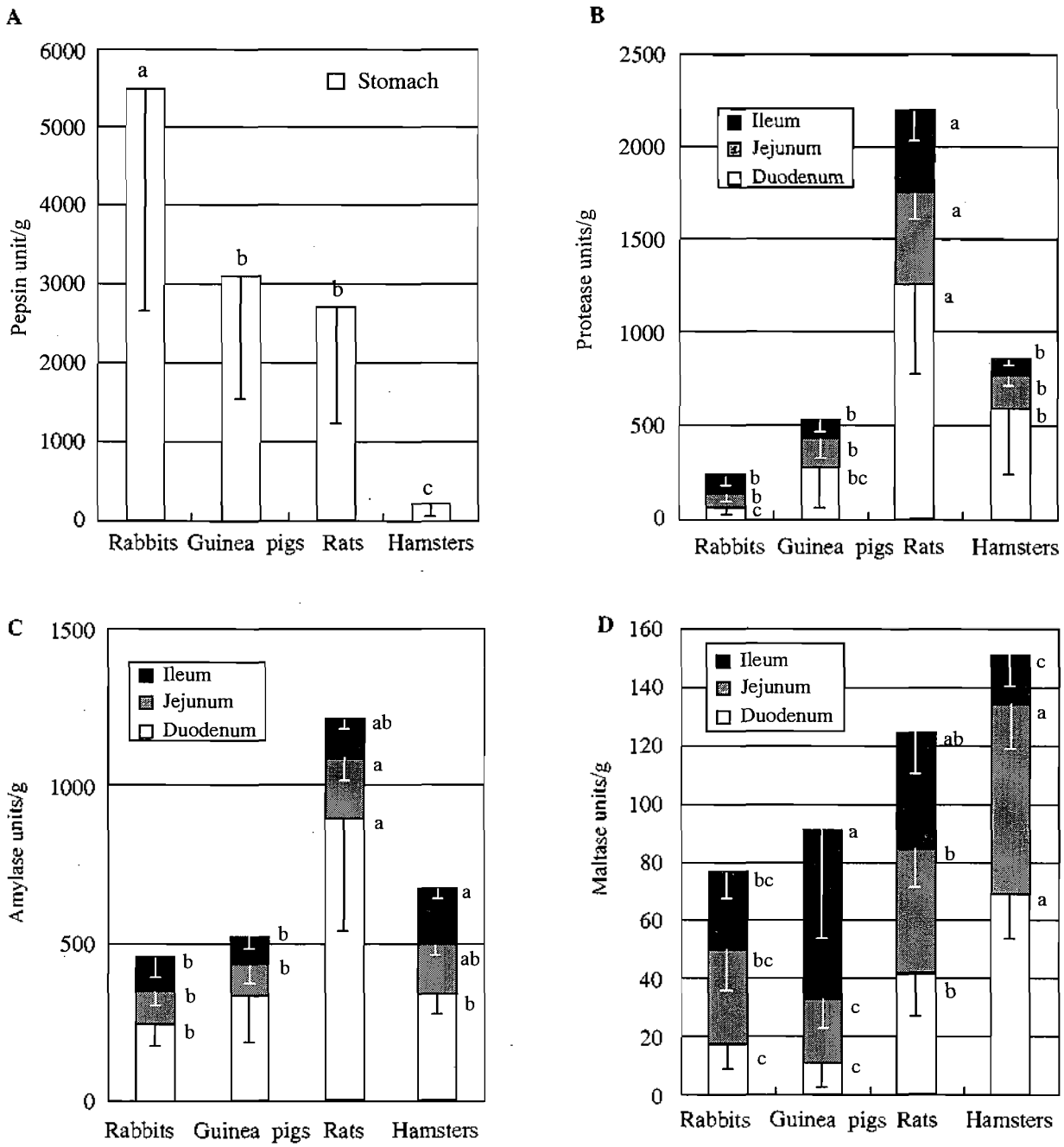

Figure 1. The activities of pepsin (A), protease (B), α-amylase (C) and maltase (D) in the various segments of GI tract of rabbits, guinea-pigs, rats and hamsters. a,b,c means within the same segments under different species without the same superscripts are significantly different ($p<0.05$). The definition of enzyme activities was described in the material and methods.

among the four species. The pattern of the VFA molar ratio in caecal and colon-rectal contents in the guinea pigs was similar to than in the rumen contents of ruminants, being highest in acetic acid, followed by propionic, and butyric acid the lowest. However, butyric acid was higher than propionic acid in rats, hamsters and rabbits. Data in this trial agreed with the results obtained by Marty and Vernay (1984) for the VFA ratios in the caecal content of rabbits.

DISCUSSION

Since the stomach of rabbits was the major storage site of digesta, it not only possessed greater space, about 21-34% of the GI tract (Chiou et al., 2000), but also secreted the highest pepsin activity among these small herbivores, indicating the significance of protein digestion in the stomach of rabbits. The protease in the stomach of newborn rabbits was rennin, and

gradually changed to pepsin within the first week after birth. The major protease in the stomach of guinea pigs however, was pepsin at birth (Henschel, 1973). The stomach of hamsters on the other hand contained keratinized epithelial cells with a glandless fore-stomach that was structurally and functionally similar to the rumen, and also contained the lowest pepsin activity among the rodent species.

Amylase is the enzyme that initiates starch hydrolyzation. It showed a high activity in duodenum. since the enzyme was secreted from the pancreas via the duodenum and reached the distal end of the small intestine. The dilution effect of the digesta and the digestive secretions gradually decreased the enzyme activity along the tract. Young suckling rabbits, 15 day-old, possess amylase activity in their pancreas even without starting to consume starch feed (Dojana et al., 1998). The α-amylase activity increases from the introduction of starch with the change to solid food in weaning rabbits. Maltase is the enzyme produced from brush border secretion that showed a similar trend with increasing age as the increase in α-amylase secretion (Dojana et al., 1998). Data from this trial showed the same trend toward the distribution of α-amylase and maltase activities in the intestine of the four species. Rats and hamsters possessed higher activities in both amylase and maltase over rabbits and guinea pigs. The characteristics of the feed that animals consume influence the secretion and the activities of digestive enzyme. The activities of proteases and carbohydrases, however, were significantly different among the four species even when they were fed the same common diet as in this trial, indicating that the enzyme secretion is dependent upon the feed habitually consumed. Animals can adapt volumetrically in the digestive tract to the dietary composition, especially to a bulky fibrous diet. The effects of dietary components upon the capability of enzyme secretion in the intestinal mucosal cells have not yet been determined.

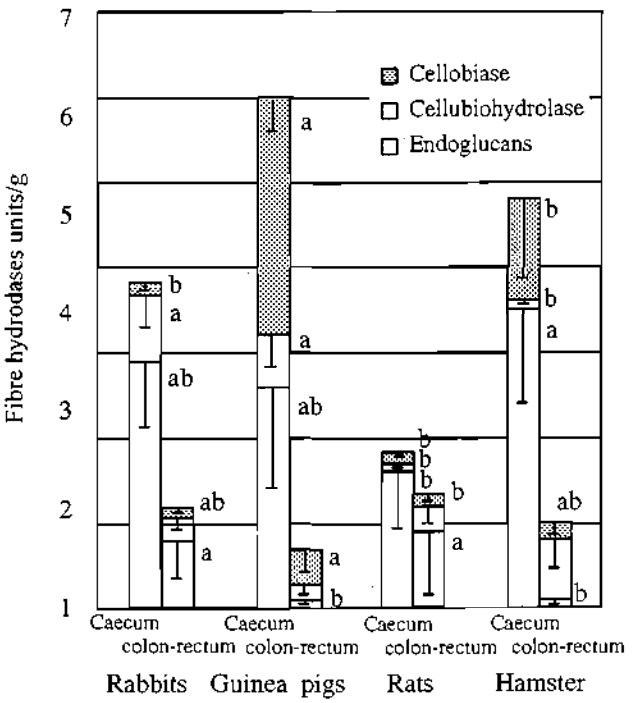

Figure 2. The activities of fiber related hydrolases in the caecum and colonic-rectum of rabbits, guinea-pigs, rats and hamsters. a,b,c means within the same segments under different species without the same superscripts are significantly different ($p<0.05$). The definition of enzyme activities was described in the material and methods.

Plant cell walls depend upon the synchronous action of the three fiber hydrolases enzymes, especially endoglucanase and cellobiase, for hydrolysis. When comparing the activities of the fiber hydrolases, endoglucanase and cellobiase in the caecum, guinea pigs contain the highest activity, hamsters the next, and rabbits and rats the lowest enzyme activities. Lower utilization efficiency of fibrous diet components in rabbits might be attributed to the low fibrous hydrolases activities in the GI tract as suggested both by Marounek and Vovk (1995) and Makkar and Singh (1987). On the contrary, rabbits possess high pectinase and xylanase activities with better digestive capability (73%) in soluble fiber, i.e. pectin and xylan (Gidenne et al., 1991). We also found that rabbits and rats both possessed lower activity of fiber hydrolases and also showed lower digestibility of crude fiber, neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Chiou et al., 2000).

Although the digestive enzyme activities were highly correlated to the nutrient digestion, dry matter and protein digestion in rats were inferior to the herbivorous guinea pigs and hamsters in our previous study (Chiou et al., 2000). This may be attributed to the high crude fiber content (10%) in the basal diet of this trial. The negative nutrient effect of dietary fiber adversely affected the digestive function of rats.

The trend of total VFA production in the caecum and colon-rectum of four laboratory animals were in reverse to the trend for fiber digestion shown in our previous trial (Chiou et al., 2000). Rats showed the highest VFA production and least fiber digestibility among the four species of animals. VFA can be an indicator of the degree of fermentation in the hindgut, since these are the end products of anaerobic microbial fermentation (Marty and Vernay, 1984). In addition to the microbial flora in the hindgut, the dietary composition was also one of the major factors that affected VFA concentration. Soluble carbohydrates are more susceptible to a high degree of fermentation than the insoluble fiber components. Feeding a high fibrous diet to rats decreased not only the digestion of the fiber per se, but also the other nutrients, leading

Table 1. The total VFA concentration and molar proportions in the hindgut of rabbits, guinea pigs, rats and hamsters

Items	Rabbits	Guinea pigs	Rats	Hamsters
Caecum				
Total volatile fatty acid, u mole g^{-1}	73.0 ± 11.13^{1b}	40.9 ± 6.50^{c}	179.4 ± 12.22^{a}	85.0 ± 15.85^{b}
Volatile fatty acid composition, mole %				
Acetic acid	48.1 ± 7.8^{b}	57.1 ± 3.4^{a}	41.2 ± 3.8^{b}	59.8 ± 6.1^{a}
Propionic acid	6.4 ± 0.9^{c}	24.3 ± 3.2^{a}	10.9 ± 0.9^{b}	12.5 ± 1.2^{b}
n-Butyric acid	43.1 ± 7.9^{a}	16.1 ± 1.9^{c}	46.3 ± 3.6^{a}	25.1 ± 5.2^{b}
Iso-Butyric acid	0.1 ± 0.05	0.1 ± 0.04	0.3 ± 0.17	0.2 ± 0.2
n-Valeric acid	2.1 ± 0.39^{a}	2.1 ± 0.35^{a}	1.1 ± 0.17^{b}	1.8 ± 0.44^{a}
Iso-Valeric acid	0.2 ± 0.09^{b}	0.3 ± 0.10^{ab}	0.3 ± 0.14^{ab}	0.6 ± 0.31^{a}
Colonic-rectum				
Total volatile fatty acid, u mole g^{-1}	64.4 ± 19.15^{b}	35.2 ± 7.09^{c}	149.2 ± 20.68^{a}	60.3 ± 14.77^{b}
Volatile fatty acid composition, mole %				
Acetic acid	64.5 ± 9.6^{ab}	58.3 ± 10.10^{ab}	50.0 ± 6.4^{b}	74.7 ± 13.6^{a}
Propionic acid	5.2 ± 1.2	20.9 ± 4.80^{a}	14.6 ± 2.4^{b}	8.4 ± 6.1^{bc}
n-Butyric acid	27.4 ± 8.55^{ab}	17.8 ± 7.72^{ab}	33.5 ± 8.15^{a}	12.5 ± 14.7^{b}
Iso-Butyric acid	0.1 ± 0.05	0.1 ± 0.11	0.3 ± 0.23	0.5 ± 0.61
n-Valeric acid	2.2 ± 0.62^{ab}	2.7 ± 0.51^{a}	1.3 ± 0.19^{b}	2.7 ± 1.5^{a}
Iso-Valeric acid	0.6 ± 0.55	0.2 ± 0.25	0.4 ± 0.05	1.3 ± 1.17

[a,b,c] Means within the same row without the same superscripts are significantly different ($p<0.05$).

[1] Mean ± SD (n=10).

to more undigested residue in addition to fiber retained in the caecum and colon (Remesy et al., 1992). This undigested residue provided additional substrate for increasing the degree of fermentation, hence the increase in the concentration of VFA. On the other hand, hamsters, with the best capability for nutrient digestion provided less undigested residue as a fermentation substrate, hence the decrease in VFA production in the hindgut.

CONCLUSION

The activities of protease and carbohydrases were significantly different among the four species even when they were fed the same common diet, indicating that the enzyme secretion is depend upon the feed habitually consumed. Low utilization efficiency of a fibrous diet in rats or rabbits might be attributed to the low fibrous hydrolases activity in the GI tract.

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