Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits

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Activities of twelve hydrolytic enzymes in the digestive tract of young rabbits before weaning (4 weeks old) and adult rabbits (3 months old) were measured. The principal digestive enzymes in both groups of rabbits appeared to be amylase (*EC* 3.2.1.1), maltase (*EC* 3.2.1.20), pectinase (*EC* 3.2.1.15) and proteinases. The stomach of young rabbits contained most of the lipolytic activity and 45·7 % of the total proteolytic activity of the digestive tract. The highest specific activities (per g digesta) of amylase, maltase and proteinase in young rabbits were found in the small intestine. Total activities (per segment) of amylase and maltase in the small intestine and the caecum were similar. Activities of cellulase (*EC* 3.2.1.4), inulinase (*EC* 3.2.1.7) and β-glucosidase (*EC* 3.2.1.21) were low and activity of pectinase was fairly high in all segments of the digestive tract. The highest activity of urease (*EC* 3.5.1.5) was found in the caecum. Enzymic profiles of the colonic chymus resembled those of the caecum. Total hydrolytic activity was lower in the colon than in the caecum. Specific activities of amylase and invertase (*EC* 3.2.1.26) were lower and those of inulinase and lactase (*EC* 3.2.1.23) higher in 4-week-old rabbits than in 3-month-old rabbits. Gastric proteinase represented almost half of the total proteolytic activity of the digestive tract, whereas lipolytic activity of gastric contents was not found in measurable quantities in adult rabbits. The caecal contents of adult rabbits contained most of the total activity of lipase (*EC* 3.1.1.3), cellulase, xylanase (*EC* 3.2.1.32), pectinase, lactase, invertase, β-glucosidase and urease present in the digestive tract. The presence of microbial enzymes (pectinase, cellulase, xylanase, inulinase and urease) in non-fermentative segments of the digestive tract reflects the reingestion of caecal contents during coprophagy.

Hydrolytic enyzmes: Digestive tract: Rabbit

Rabbits can be successfully raised on various diets composed of forages, agricultural by-products, concentrates and protein supplements. The digestibility of organic matter of feeds is lower in rabbits than in other herbivorous animals, primarily due to lower digestion of crude fibre (Slade & Hintz, 1969; Sakaguchi *et al.* 1987). The degradation of organic matter in the digestive tract of rabbits involves a number of hydrolytic reactions which are catalysed by enzymes of endogenous and/or microbial origin. It is generally assumed that hydrolytic activity and digestive volume correlate positively with the digestion efficiency. The nutritive value of rabbit diets, thus, can be improved by enzyme supplementation as suggested by Yu & Tsen (1993), and by use of other dietary additives influencing the digesta enzyme activity. Several studies have attempted to measure activities of hydrolytic enzymes in the digestive tract of rabbits. Griffiths & Davies (1963) demonstrated a high activity of

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amylase (EC 3.2.1.1) in rabbit soft faeces. Forsythe & Parker (1985) found a high activity of urease (EC 3.5.1.5) in the rabbit caecum and a low level in the stomach. Makkar & Singh (1987) compared enzymic profiles of the rabbit caecum and bovine rumen contents. The activities of urease, amylase and protease were higher and activity of cellulase (EC 3.2.1.4) lower in the rabbit caecum than in the rumen. Moreau *et al.* (1988), Borel *et al.* (1991) and Levy *et al.* (1991) described rabbit gastric lipase (EC 3.1.1.3). Alus & Edwards (1977) studied the postnatal development of intestinal disaccharidases, amylase and proteases in rabbits. Their results indicate that activities of invertase (EC 3.2.1.26), maltase (EC 3.2.1.20) and amylase increase from birth to weaning, whereas activity of lactase (EC 3.2.1.23) decreases in this time interval. Information on activity of other hydrolytic enzymes is scarce. A high digestibility of pectin reported by Gidenne *et al.* (1991*a*) suggests a high activity of pectinolytic enzymes in the rabbit digestive tract. A similar general conclusion can be drawn on the basis of the high digestibilities of protein (Slade & Hintz, 1969; Gidenne, 1992) and starch (Gidenne & Perez, 1993) and the low digestibility of cellulose (Gidenne, 1992). We have not found reports on activity of hemicellulases in rabbits in the available literature. Xylose, which is the principal monomeric unit of hemicelluloses, was the least-degraded neutral sugar in the rabbit digestive tract (Gidenne *et al.* 1991*a*; Gidenne, 1992). Low digestibility of hemicellulose (10·7–25·4%) was reported by Gidenne *et al.* (1991*b*). In contrast, Sakaguchi *et al.* (1992) found that 57·9% of hemicellulose was digested in rabbits.

The purpose of the present study was to measure the digesta weight and activities of twelve hydrolytic enzymes in the rabbit stomach, small intestine, caecum and colon. The study aimed (1) to evaluate the hydrolytic activity of these digestive tract segments, and (2) to compare adult rabbits and rabbits before weaning in this respect. A potential benefit of enzyme supplementation of rabbit rations will be briefly discussed.

MATERIALS AND METHODS
Animals and diets

All rabbits were New Zealand × Californian hybrids, obtained from a rabbitry with the capacity of 250 does. Rabbits were kept in stainless steel mesh cages (400 mm × 650 mm × 320 mm) in a room with natural lighting at a temperature of $16 \pm 2°$ and relative humidity of about 70%. Does with their young were housed individually in cages with nest boxes; other rabbits were housed four per cage. Seven young rabbits from one litter remained continuously with the non-pregnant dam until day 28 when they were killed. Rabbits had free access to a commercial granulated feed containing wheat, barley, oats, maize, soya-bean meal, dehydrated lucerne (*Medicago sativa*) and a vitamin–mineral supplement. The feed contained (g/kg): crude protein 176, fat 22 and crude fibre 126. The feed did not contain any coccidiostat. Eleven rabbits of the same breed, 3 months old, were fed on similar granulated concentrate feed, differing in the proportions of the components mentioned above. The feed contained (g/kg): crude protein 147, fat 22 and crude fibre 132. No attempt to prevent coprophagy was made.

Sampling of digesta

Rabbits were killed approximately 4 h after the morning feed, weighed and segments of their digestive tract (stomach, small intestine, caecum and colon) were emptied by gentle squeezing. The pH of the digesta was measured immediately. Contents of individual segments were weighed and then stored under CO_2 in hermetically sealed bottles at $-40°$ until analysed.

Table 1. *Weight, dry matter* (*DM*) *content and pH of digesta from different segments of the digestive tract of rabbits*†

(Mean values and standard deviations for seven 4-week-old rabbits and eleven 3-month-old rabbits)

	Stomach		Small intestine		Caecum		Colon	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4-week-old rabbits								
Weight (g)	38·0	6·4	10·1	3·8	28·2	4·1	7·4	1·2
DM (g/kg)	234	16	117	22	213	58	248	23
pH	2·88	0·73	6·50	0·23	5·52	0·19	5·95	0·56
3-month-old rabbits								
Weight (g)	96·9**	12·2	16·9**	3·2	116·7**	27·1	29·8**	7·6
DM (g/kg)	198	34	80**	20	208	65	277**	14
pH	2·58	0·48	7·09**	0·17	6·05**	0·19	6·73**	0·22

** Values were significantly different from those of 4-week-old rabbits: $P < 0.01$.
† Rabbits were killed 4 h after the morning feed. For diets see p. 464.

Analyses

Activities of amylase, cellulase, xylanase (*EC* 3.2.1.32), pectinase (*EC* 3.2.1.15), inulinase (*EC* 3.2.1.7), urease and proteinases were assayed according to Kopečný & Bartoš (1990) using soluble starch, carboxymethylcellulose, oat-spelt xylan, citrus pectin, inulin, urea and azocasein as substrates respectively. Contents of the stomach were diluted with a citrate–phosphate buffer (pH 2·5), whereas contents of other parts of the digestive tract were diluted with a phosphate buffer (pH 7·0). Liberated reducing sugars were determined by reaction with Somogyi reagent (Somogyi, 1952), ammonia with Nessler solution, and azocasein fragments colorimetrically at 440 nm. Activities of lactase, invertase and maltase were measured in the same way, using lactose, sucrose and maltose as substrates. Glucose liberated from disaccharides was measured by the glucose oxidase – peroxidase method. A commercial kit from Lachema (Brno, Czech Republic) was used. Activity of β-glucosidase (*EC* 3.2.1.21) was estimated by measuring the liberation of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucopyranoside (Williams & Withers, 1981). Activity of lipase was measured by the modified method of Bier (1955). Samples of digesta (0·1 g) were incubated with 0·0025 M-Tris-HCl buffer containing 0·025 M-CaCl$_2$ (0·8 ml), 0·2 M-Na$_2$HPO$_4$ (0·1 ml) and emulsified tributyrin (1·0 ml). The emulsion was prepared from tributyrin (10 ml), Tween 80 (1 ml) and water (100 ml). The reaction mixture was incubated (37°, 1 h) and then centrifuged. Liberated butyrate was determined by titration, after steam distillation in the Markham apparatus. Again, the estimation of the gastric lipolytic activity was performed at low pH.

Dry matter of digesta was determined by heating at 105° for 16 h. The significance of differences was evaluated by Student's *t* test.

RESULTS

Table 1 presents weights, DM contents and pH values of digesta from the stomach, small intestine, caecum and colon of rabbits. Digesta represented on average 120 g/kg body weight of young rabbits (before weaning) and 93 g/kg body weight of adult rabbits (3 months old).

Table 2. *Distribution of activity of hydrolytic enzymes in the digestive tract of 4-week-old rabbits*

(Mean values and standard deviations for seven rabbits)

Enzyme	Stomach		Small intestine		Caecum		Colon	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Specific activity (per g digesta)								
Lipase† (*EC* 3.1.1.3)	2·2	1·3	1·6	0·3	1·6	0·3	2·6	0·2
Amylase‡ (*EC* 3.2.1.1)	0	—	55·5	4·8	20·7	4·4	22·5	7·2
Cellulase‡ (*EC* 3.2.1.4)	0	—	2·4	0·3	3·5	0·3	2·9	0·2
Xylanase‡ (*EC* 3.2.1.32)	0	—	8·4	0·8	13·2	2·9	15·9	1·5
Pectinase‡ (*EC* 3.2.1.15)	6·7	1·1	18·6	2·0	18·7	1·8	33·7	4·4
Inulinase‡ (*EC* 3.2.1.7)	0	—	6·1	0·8	4·5	0·9	6·3	1·1
Lactase§ (*EC* 3.2.1.23)	0	—	8·0	0·6	6·0	1·1	6·2	0·7
Invertase§ (*EC* 3.2.1.26)	0·6	0·1	7·6	1·2	3·2	0·5	3·2	1·1
Maltase§ (*EC* 3.2.1.20)	0	—	45·4	10·2	18·9	5·6	21·3	9·0
β-Glucosidase§ (*EC* 3.2.1.21)	0	—	0·8	0·2	2·8	0·2	3·5	0·6
Urease‖ (*EC* 3.5.1.5)	0	—	1·4	0·3	64·8	8·6	58·9	9·6
Proteinases¶	40·8	1·9	65·6	27·8	32·2	2·0	36·7	6·3
Total activity (per segment)								
Lipase†	84	21	16	2	45	3	19	2
Amylase‡	0	—	560	47	584	48	166	24
Cellulase‡	0	—	24	2	99	3	21	2
Xylanase‡	0	—	85	8	372	11	118	5
Pectinase‡	255	24	188	18	527	19	249	13
Inulinase‡	0	—	62	8	127	10	47	2
Lactase§	0	—	81	5	169	11	46	3
Invertase§	23	3	78	13	90	5	24	3
Maltase§	0	—	458	99	533	61	157	29
β-Glucosidase§	0	—	8	2	79	4	26	3
Urease‖	0	—	14	2	1827	93	436	32
Proteinases¶	1550	32	662	278	908	21	272	21

† Expressed as mmol butyrate liberated from tributyrine/h.
‡ The assay was based on the increase in reducing power, calibrated by corresponding monomeric sugars. The activity was expressed as mg sugar liberated/h.
§ Expressed as mg glucose liberated/h.
‖ Expressed as mg urea hydrolysed/h.
¶ Expressed as mg azocasein decomposed/h.

Table 2 summarizes data on the activity of twelve hydrolytic enzymes in the contents of the digestive tract of rabbits before weaning. The gastric lipase in young rabbits represented most of the lipolytic activity of the whole digestive tract. The stomach of young rabbits contained almost half of the total proteolytic activity, 20·9% of the total pectinolytic activity and a low but measurable activity of invertase. The small intestine and caecum of young rabbits contained highly active amylase and maltase. Specific activity of proteinases (per g digesta) was higher in the small intestine than that in other parts of the digestive tract. The caecum contained most of the activity of cellulase, xylanase, β-glucosidase and urease. The colonic contents exhibited the highest specific activities of lipase, xylanase, pectinase, inulinase and β-glucosidase. Total activities of hydrolytic enzymes were, however, lower in the colon than in the caecum.

Table 3 presents data on the activity of hydrolytic enzymes in the contents of digestive tract segments of adult rabbits. In the stomach, activities of hydrolytic enzymes other than proteinases, pectinase and invertase were absent or below the detection limit of a method at pH 2·5. When the analyses were done at pH 7·0, enzymic profiles of gastric contents

Table 3. *Distribution of activity of hydrolytic enzymes in the digestive tract of 3-month-old rabbits*

(Mean values and standard deviations for eleven rabbits)

	Stomach		Small intestine		Caecum		Colon	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Specific activity (per g digesta)								
Lipase† (*EC* 3.1.1.3)	0	—	1·3	0·3	1·7	0·2	3·3**	0·3
Amylase‡ (*EC* 3.2.1.1)	0	—	105·7**	11·2	26·4**	3·2	25·9	5·8
Cellulase‡ (*EC* 3.2.1.4)	0	—	1·7**	0·4	2·6**	0·4	1·9**	0·3
Xylanase‡ (*EC* 3.2.1.32)	0	—	8·7	0·8	12·9	2·3	12·4**	1·4
Pectinase‡ (*EC* 3.2.1.15)	10·1**	1·9	22·7	3·6	26·1**	4·2	24·3	9·8
Inulinase‡ (*EC* 3.2.1.7)	0	—	2·3**	0·7	2·2**	0·7	2·0**	0·7
Lactase§ (*EC* 3.2.1.23)	0	—	3·4**	1·0	4·5**	0·5	2·8**	0·9
Invertase§ (*EC* 3.2.1.26)	2·5**	0·7	15·7**	1·9	7·7**	1·5	7·5**	1·5
Maltase§ (*EC* 3.2.1.20)	0	—	69·1**	14·0	24·2	8·4	19·0	6·1
β-Glucosidase§ (*EC* 3.2.1.21)	0	—	0·9	0·3	3·9**	0·5	3·4	1·8
Urease‖ (*EC* 3.5.1.5)	0	—	3·7**	1·4	18·0**	8·0	12·9**	5·5
Proteinases¶	44·6	6·8	69·6	7·6	25·9	8·3	27·0**	8·5
Total activity (per segment)								
Lipase†	0	—	22**	3	198**	20	98**	10
Amylase‡	0	—	1786**	119	3081**	282	772**	149
Cellulase‡	0	—	29**	3	303**	33	57**	7
Xylanase‡	0	—	147**	10	1505**	209	370**	33
Pectinase‡	979**	96	384**	36	3046**	375	724**	249
Inulinase‡	0	—	39**	6	257**	63	60	17
Lactase§	0	—	57**	10	525**	46	83**	23
Invertase§	242**	30	265**	20	899**	129	223**	36
Maltase§	0	—	1168**	149	2824**	756	566**	152
β-Glucosidase§	0	—	15**	5	455**	47	101**	46
Urease‖	0	—	63**	13	2100	713	384	139
Proteinases¶	4322**	272	1176**	80	3023**	743	805**	216

** Values were significantly different from those of 4-week-old rabbits: $P < 0.01$.

† Expressed as mmol butyrate liberated from tributyrine/h.

‡ The assay was based on the increase in reducing power, calibrated against corresponding monomeric sugars. The activity was expressed as mg sugar liberated/h.

§ Expressed as mg glucose liberated/h.

‖ Expressed as mg urea hydrolysed/h.

¶ Expressed as mg azocasein decomposed/h.

resembled those of the caecum (results not shown). The gastric proteolytic activity represented 46·3 % of the total proteolytic activity of the digestive tract. Highly active amylase, maltase and proteinases were present in the small-intestinal digesta. Total activities of these enzymes were lower in the small intestine than in the caecum, but higher than those in the colon. The caecal digesta contained 62·3, 77·9, 74·4, 59·3, 78·9, 55·2, 79·7 and 82·4 % of the total activity of lipase, cellulase, xylanase, pectinase, lactase, invertase, β-glucosidase and urease respectively. Enzymic profiles of colonic contents resembled those

of the caecum. Total hydrolytic activities were 2–6 times lower in the colon than in the caecum. Most of the differences found in specific and total activities of enzymes between young and adult rabbits were statistically significant ($P < 0.01$). Total activities (per segment) were, however, more or less influenced by extensive enlargement of digesta volume in all four digestive organs sampled.

DISCUSSION

It is obvious from our results that all hydrolases which are necessary for enzymic conversion of complex nutrients into available forms are present in the rabbit digestive tract. In addition, mucosal cells are able to split some low-molecular-weight substrates by the action of brush-border enzymes, but this was not investigated in the present study. The activity of cellulase was low in all segments of the digestive tract. This finding is consistent with a rather low digestibility of crude fibre, observed in rabbits by several authors. Slade & Hintz (1969) found that in rabbits only 16·2–18·1 % crude fibre was digested. A similar efficiency of fibre digestion was reported by Sakaguchi *et al.* (1987). Very low digestibility of crude fibre (2·8 %) was observed by Stephens (1977). The supplementation of rabbit diets with an acid-tolerant fungal cellulase, which has been suggested by Yu & Tsen (1993), thus may improve the productive value of feeds. Also, the activities of inulinase (a fructan-splitting enzyme) and β-glucosidase were low in comparison with the activities of other saccharolytic enzymes. On the other hand, the high digestibility of pectic substances (73 %), found by Gidenne *et al.* (1991 *a*), corresponds with the presence of highly active pectinase in all segments of the rabbit digestive tract. The activity of xylanase was relatively high in both groups of rabbits, despite the fact that digestibility of hemicelluloses *in vivo* seems to be low (Gidenne *et al.* 1991 *b*; Gidenne, 1992). In our opinion the direct association between hemicelluloses and lignin (Jung & Vogel, 1986), rather than lack of activity of hemicellulolytic enzymes, limits the digestibility of hemicelluloses in the rabbit digestive tract. A suitable alkali treatment of feeds might increase the digestibility of hemicelluloses more than supplementation of rabbit rations with hemicellulolytic enzymes. Purified hemicelluloses were fermented very easily by rabbit caecal contents *in vitro* in our preliminary experiments (results not shown).

Specific activities of amylase and invertase were lower and those of inulinase and lactase higher in young rabbits than in adult rabbits. Young rabbits at this age (4 weeks) consume both milk and solids and actively practise coprophagy (Alus & Edwards, 1977). Age-related differences also include the decrease of gastric lipase and urease activity in the caecum and colon in adult rabbits. The gastric lipase activity was positively correlated with the daily fat intake in rabbits in the experiments of Borel *et al.* (1991). The high activity of gastric lipase in suckling rabbits enables them to utilize milk fat, which accounts for 105–167 g/kg whole milk (Altman & Dittmer, 1961). The digestive potential of the caecum increased with age.

Symbiotic micro-organisms inhabiting the rabbit hindgut synthesize all hydrolytic enzymes measured in the present study. Lipase, amylase, proteinases and disaccharidases are synthesized also by the host. The presence of pectinase in the stomach and cellulase, xylanase, pectinase, inulinase and urease in the small intestine can be explained by coprophagy. The coprophagy, which must be taken into account in any study on the rabbit digestive physiology, thus compensates faecal losses of microbial hydrolytic enzymes and increases the efficiency of digestion of non-starch polysaccharides and other plant constituents. The low gastric pH, however, limits the hydrolytic action of ingested enzymes. The presence of microbial pectinase in the stomach and small intestine is consistent with the finding of Gidenne (1992) that about 50 % digestible pectin disappeared before the caecum.

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