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PROTEINASE, LIPASE AND AMYLASE ACTIVITIES IN THE SMALL INTESTINE OF PIGS SUFFERING FROM COLIENTEROTOXAEMIA

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LIVEN, EIVIND: *Proteinase, lipase and amylase activities in the small intestine of pigs suffering from colienterotoxaemia.* Acta vet. scand. 1978, *19*, 184—191. — The activities of proteinases, lipases, amylases and the activities of proteinase inhibitors, as well as the numbers of Escherichia coli in the contents from the small intestine were examined for pigs suffering from colienterotoxaemia and for healthy pigs. Enzyme activities were determined using an agar diffusion test.

Compared with healthy animals the activities of proteinases and amylases in diseased animals were reduced while lipases showed increased activity. In pathologically changed contents showing large numbers of E. coli, proteinases could not be demonstrated; however, proteinase inhibitors were found in these contents. In healthy animals, proteinase inhibitors were not demonstrated in ingesta-containing contents.

In diseased animals, E. coli were found in large numbers in all parts of the small intestine. In healthy animals, E. coli was demonstrated especially in the posterior part of the small intestine and regularly in small numbers.

The possible influence of digestive enzymes, especially proteinases and their inhibitors, on enterotoxins from E. coli is discussed.

digestive enzymes; intestinal contents; enterotoxaemia; pig.

Bacteriological findings in connection with colienterotoxaemia among pigs are well documented (*Sojka* 1971, *Kenworthy* 1973). Information about the activities of digestive enzymes in the small intestine, both in healthy and in diseased animals is, however, scarce.

The effect of trypsin on enterotoxins from Clostridium perfringens and other clostridia is relatively well documented (*Smith* 1975). *Mèszàros et al.* (1967) claimed that, in pigs, accumulation of toxins from Clostridium perfringens type C was due to a decrease or cessation of proteolytic enzyme activity. As

reviewed by *Banwell & Sherr* (1973) little information exists on the possible effect of proteinases on enterotoxins from enteropathogenic strains of Escherichia coli. *Juhász et al.* in 1967 studied the activity of proteinases, lipases and amylases both of pancreatic and intestinal origin in pigs suffering from infectious gastroenteritis and "arrival" diarrhoea and concluded that these animals had marked reductions in the activities of pancreatic amylase and lipase as well as in jejunal proteinase and amylase.

The intention of the present work was to examine the activities of proteinases, lipases and amylases as well as the activities of proteinase inhibitors in contents from the small intestine of pigs suffering from colienterotoxaemia, and, to some extent, to compare the enzymatic patterns in the small intestines in diseased and in healthy animals.

MATERIAL AND METHODS

Animals

Pigs, which died with symptoms of colienterotoxaemia at the age of 6—8 weeks, were taken to the laboratory shortly after death. The animals were untreated and originated from 4 ordinary husbandry herds. The elapse of time between death and post-mortem examination was limited to a maximum of 12 hrs. Only pigs with the diagnosis colienterotoxaemia, based on necropsy and bacteriological examinations, were included in the investigation. Out of 12 animals, 9 fulfilled such criteria.

Six normally reared pigs, weaned at the age of 8 weeks, served as controls. Control animals which originated from 3 different litters were stunned with a bolt pistol and bled. These animals had been fed an antibiotic free diet composed for fattening purposes.

Determination of enzyme activities and proteinase inhibitors

The sampling procedure of contents from the small intestine and the measurements of the activities of proteinases, amylases and lipases, as well as the demonstration of proteinase inhibitors in the contents were performed as described by *Fossum & Liven* (1974). Enzyme activities were recorded in diffusion units applying an agar-diffusion test. Diffusion units refer to the activities in 0.05 ml of intestinal contents previously diluted 10^{-2} in saline.

Bacteriological examinations

With the object of determining the number of Escherichia coli in contents from the small intestine, qualitative and quantitative bacteriological examinations were carried out according to standard bacteriological techniques.

RESULTS

Intestinal contents

The contents in the small intestine from the diseased animals had a watery consistency and were usually mixed with blood. In a few animals, these pathologically changed contents (PCC) were found only in parts of the small intestine. In the healthy animals, the consistency of the contents varied considerably usually being more solid in the posterior regions of the small intestine.

In healthy animals, regions of the small intestine in contraction, with no ingesta, and containing only mucus of a mayonnaise-like consistency, were regularly found. Such regions were also found in intestines from the diseased animals, but the extension and frequency of these regions were markedly reduced compared to healthy animals.

Enzymological and bacteriological investigations

Fig. 1 presents the distribution of proteinase, lipase, and amylase activities in diseased and healthy animals. Proteinases and amylases showed decreased, and lipases increased, activities in diseased animals compared to control. In PCC, which constituted more than 50 % of the samples from diseased animals, proteinase activity was not detectable. In the other type of contents, proteinase activity was also markedly reduced compared to healthy animals. Inhibitors against proteolytic enzymes were consistently found in PCC. In the contracted regions with no ingesta, the simultaneous presence of proteinase inhibitors and absence of proteinase activity was demonstrated in both groups of animals.

Lipase and amylase activities were demonstrated in all types of intestinal contents. In the mayonnaise-like contents no differences between the 2 groups of animals were recorded in activities of these enzymes.

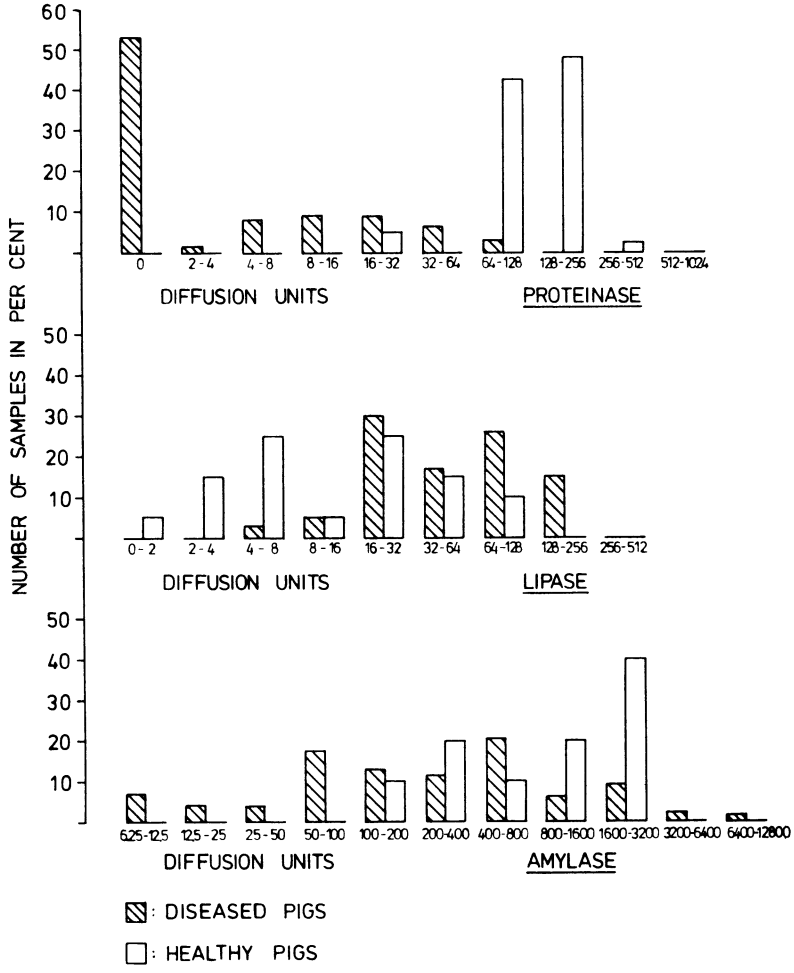

Figure 1. The number of samples in per cent showing various levels of proteinase, lipase and amylase activities from diseased and healthy animals. Enzyme activities are expressed in diffusion units.

In Table 1, statistical evaluations of the enzyme activities are given, showing that significant differences are found for all 3 categories of digestive enzymes. The enzyme activities in the contracted regions are not taken into account when doing the statistical analyses.

The number of E. coli usually amounted to several billions per g contents in the diseased animals. In the contracted regions, E. coli was found in smaller numbers regularly less than 1 mil-

Table 1. Mean ± standard deviation for proteinase, lipase and amylase activities in contents from the small intestine originating from pigs suffering from colienterotoxaemia and from healthy pigs. Enzyme activities are expressed in diffusion units.

	Proteinase	Lipase	Amylase
Diseased animals	9.4 ± 6.7	66.4 ± 11.3	688.1 ± 192.7
Healthy animals	125.3 ± 8.3	20.5 ± 13.8	1382.5 ± 236
Significance	$P < 0.001$	$P < 0.03$	$P < 0.04$

lion per g contents. In a few cases, E. coli was not detected in this type of contents.

In healthy animals, E. coli was seldom demonstrated in duodenum and the upper jejunum. In the lower part of the small intestine the number of E. coli often amounted to several millions per g contents. In healthy animals, E. coli was never demonstrated in the contracted regions containing no ingesta.

DISCUSSION

Pigs suffering from colienterotoxaemia showed reduced activities of proteinases and amylases and increased activities of lipases in contents from the small intestine. In PCC, proteolytic activity could not be demonstrated whereas inhibitors against proteinases were consistently found in these contents.

Regarding the relationship between digestive enzymes, proteinase inhibitors and the counts of E. coli, marked differences were found in diseased and healthy animals. In diseased animals the number of E. coli was high, the activities of proteinases and amylases were reduced, while lipases had increased activity. Proteinase inhibitors were present in diseased animals in large parts of the small intestines, where also large numbers of E. coli were found. In healthy animals, the numbers of E. coli were low, proteinases and amylases had increased and lipases decreased activities compared to diseased animals, while proteinase inhibitors were found only in contracted regions with no ingesta without detectable numbers of E. coli.

Initially it should be emphasized that the animals may differ due to their different origins. Consequently, there is no exact knowledge about diet composition or number of feedings each

day. The time elapsing between death and last uptake of feed is also unknown.

Based on the differentiated reduction in proteinase and amylase activities and the increased activity of lipase found in this investigation, the suggestion that the decreased actvity of proteinases and amylases could be based on a dilution effect, due to the actual accumulation of liquid in the intestinal lumen, seems unlikely. In the experiment of *Juhász et al.* (1967) in which enzyme activities were related both to liquid (1 ml) and dry (1 g) material of the contents results similar to those found in the present work concerning intestinal amylases and proteinases, were found.

The elevated lipase activity in the experiment of *Juhász et al.* was not significant. The present investigation, however, showed significantly increased differences ($P < 0.03$) in the activities of lipases. The cause of the raised levels of lipase activity seems not clear. However, the possibility of leakage of serum lipases into the intestinal lumen pre- and post mortem seems probable.

Generally little information exists on the role of the altered enzymatic pattern in initiation of colienterotoxaemia in pigs. *Larivière* (1971) found in in vitro experiments that lipases inactivated the LT-enterotoxin and that amylases inactivated ST-enterotoxin of E. coli. *Larivière & Lallier* (1975) also discussed a possible effect of amylases on ST-enterotoxin in in vivo experiments. Consequently, it is reasonable to consider whether the reduced amylase activity may influence the effect on ST-enterotoxin which is usually produced by porcine enteropathogenic strains of E. coli.

The present investigation shows an obvious correlation between the absence of proteinase activity, the presence of proteinase inhibitors and the occurrence and distribution of regions with PCC showing large numbers of E. coli, and it is reasonable to suggest that these regions represent places in the intestine where the organism is exerting its most damaging effect upon the host. Consequently there seems to be a relationship between toxin production, or the effects of toxins, and the activities of digestive enzymes. The effect of proteinases and proteinase inhibitors on enterotoxins from E. coli is of particular interest. Proteinases may have both an activating and inactivating effect on enterotoxins from Clostrodium perfringens. Similarly, as both the LT and ST E. coli enterotoxins are of protein nature, it seems

logical to suggest that proteolytic enzymes may influence the activity of these toxins. The low activities of proteinases in the intestines in the diseased animals, which might be explained to some extent by the presence of proteinase inhibitors, may thus be of importance in the development of colienterotoxaemia.

REFERENCES

Banwell, J. G. & H. Sherr: Effect of bacterial enterotoxins on the gastrointestinal tract. Progr. Gastroenterol. 1973, *65*, 467—497.

Fossum, K. & E. Liven: The distribution of enzymes and bacteria in the small intestines of slaughter pigs. Acta path. microbiol. scand. Sect. B 1974, *82*, 644—652.

Juhász, S., G. Tamási & L. Pesti: Studies on some digestive enzymes in swine gastroenteritis. Acta vet. Acad. Sci. hung. 1967, *17*, 413—421.

Kenworthy, R.: Intestinal microbial flora of the pig. Advanc. appl. Microbiol. 1973, *16*, 31—54.

Larivière, S.: Physical and biological characterization of Escherichia coli enterotoxin. Thesis. University of Guelph, Ontario 1971.

Larivière, S. & R. Lallier: Relationships of intestinal enzymes and serum antitoxin to the pig response to Escherichia coli enterotoxin. Canad. J. comp. Med. 1975, *39*, 371—376.

Mèszáros, J., L. Pesti, B. Lomniczi & S. Juhász: Study of the role of Clostridia in swine gastroenteritis. Bull. Off. int. Epiz. 1967, *67*, 1307—1318.

Smith, L. DS.: The pathogenic anaerobic bacteria. 2nd Ed., Charles C. Thomas, USA 1975.

Sojka, W. J.: Enteric diseases in new-born piglets, calves and lambs due to Escherichia coli infections. Vet. Bull. 1971, *41*, 509—522.

SAMMENDRAG

Aktivitet av proteinaser, lipaser og amylaser i tynntarmen hos griser angrepet av kolienterotoksemi.

Aktiviteten av proteinaser, lipaser og amylaser og aktiviteten av proteinase inhibitorer samt antallet av Escherichia coli ble undersøkt i tynntarminnhold fra griser angrepet av kolienterotoksemi og sammenholdt med tilsvarende forhold hos friske griser. Enzymaktiviteten ble bestemt ved å benytte en agar diffusjonstest.

Sammenlignet med forholdet hos de friske dyrene var aktiviteten av proteinaser og amylaser redusert hos de syke dyrene, mens lipasene, derimot, hadde høyere aktivitet. I innhold som var patologisk forandret og hvor det var et høyt antall av E. coli kunne proteinaser ikke påvises. Proteinase inhibitorer ble imidlertid funnet i slikt innhold. Fra de friske dyrene ble proteinase inhibitorer ikke påvist i innhold som bestod av fórbestanddeler.

Fra syke dyr ble E. coli funnet i et stort antall i alle deler av tynntarmen. Fra friske dyr ble E. coli påvist særlig fra de bakre deler av tynntarmen og regelmessig i et lite antall.

En mulig effekt av fordøyelsesenzymer, spesielt proteinaser og deres inhibitorer, på enterotoksiner fra E. coli blir diskutert.

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