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THE DIAGNOSIS OF PANCREATIC DEGENERATIVE ATROPHY IN DOGS — A PRACTICAL METHOD

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WESTERMARCK, E.: *The diagnosis of pancreatic degenerative atrophy in dogs — a practical method.* Acta vet. scand. 1982, 23, 197—203. — A convenient method was developed to diagnose pancreatic degenerative atrophy (PDA) in small animal practice based on the observations, that feeding crude soybean increases faecal protease activity in dogs with normal pancreatic function, while PDA-dogs remain completely negative in faecal protease activity.

When PDA is suspected, 1 or 2 faecal samples should be investigated with the X-ray film method based on incubation for 2 h at 37°C. If any activity is seen the dog should be considered non-PDA. If no activity is seen the dog should be given food supplemented with 1 g crude soybean/kg body weight twice daily for a few days and a new faecal sample should be collected. If the activity remains negative the diagnosis of PDA is reliable.

As the amount of crude soybean in the food increased, a dose dependent increase of faecal protease and amylase was seen as measured with the radial enzyme diffusion method (RED).

pancreatic degenerative atrophy; soybean stimulation test; X-ray film digestion test.

Several screening tests have been used to diagnose pancreatic degenerative atrophy (PDA) in dogs. Many of these methods are based on the detection and measurement of the proteolytic enzyme activity in the duodenal fluid or stools (*Haverback et al.* 1963, *Bush* 1975, *Säteri* 1975, *Strombeck* 1978, *Batt et al.* 1979). However, these tests have not been fully reliable as many normal dogs do not continuously secrete proteases into the duodenal fluid and faeces. It has been described that crude soybean stimulates faeces excretion of pancreatic hydrolasis in the rat and chicken (*Hewitt et al.* 1973, *Kakade et al.* 1973). In the dog a reliable method to diagnose PDA was developed: the food of the

dogs is supplemented with crude soybean for a few days and faecal protease activities is then determined with radial enzyme diffusion (RED) in agar gel containing Ca-paracaseinate (*Westermarck & Sandholm* 1980). This provided distinct separation between PDA and non PDA-dogs. Adding crude soybean to the food significantly increased the faecal protease activity in healthy dogs but in the PDA-dogs the protease activity remained completely negative. However, the RED method requires rather advanced laboratory facilities to prepare the plates and is therefore not practical for the average small animal practice.

The present paper introduces the soybean stimulation test where the protease activity is determined with the simple X-ray film digestion test. The optimal dose of crude soybean supplement for the soybean stimulation test is also determined.

MATERIAL AND METHODS

Soybean stimulation test

Two healthy German shepherd dogs which had low faecal hydrolase activities were selected for a study of the dose-effect of crude soybean. The food of these 2 dogs was supplemented with crude soybean powder at a level of 125, 250, 500 and 1000 mg/kg body weight respectively for the last 3 days of 4 consecutive periods of 6 days each. The dogs were fed the supplemented diet twice daily. The faecal samples were collected daily and the amylase, lipase and protease activities were analysed with the RED method in substrate containing agar gels.

The faecal protease activity was determined from a further 57 dogs of different breeds of which 15 had been classified as PDA dogs. The food of the 57 dogs was supplemented with crude soybean powder at dose level of 1 g crude soybean/kg body weight twice daily for 4 days. Before and on the third, fourth and fifth day after starting the supplemented feeding faecal samples were collected and analysed for faecal proteases. The samples were analysed with the RED method, the X-ray film method (22°C) (*Bush* 1975) and with the X-ray film method at 37°C.

Radial enzyme diffusion (RED)

Canine faecal samples were diluted 1 to 10 in Phosphate buffered saline (PBS) to diffuse from 6 mm ∅ wells in 2 mm thick agar gel containing a substrate (Ca-paracaseinate, starch, corn

oil). The diameter of the circular zones after 24 h incubation correlates linearly with the log of the enzyme concentration (*Westermarck & Sandholm* 1980).

X-ray film digestion test

One gram of the faecal sample was homogenised using a household food mixer in 9 ml of 5 % $NaHCO_3$. A 1×10 cm strip of unexposed X-ray film was dipped half way into the faecal suspension and allowed to incubate for 2.5 h at room temperature (22°C). Alternately the incubation time was 2 h at 37°C. The strip was washed under running tap water and deemed positive if the gelatinous surface of the film had been digested.

RESULTS

As the amount of crude soybean was increased in the food of 2 healthy German shepherd dogs a dose dependent increase of faecal protease and amylase was seen as measured by RED (Fig. 1). The maximum increase in faecal protease and amylase activity was obtained by a dose of 1000 mg/kg body weight. This dose was chosen for further studies in the soybean stimulation test. After the feedings of crude soybean had been disrupted the faecal hydrolase activities quickly returned to pre-test low levels in both dogs. The effect of crude soybean feedings on the faecal lipase activity was not clear as the activities showed no correlation with the level of crude soybean supplementation.

Fifteen of the 57 investigated dogs proved to be PDA dogs as indicated by completely negative faecal protease activities before and during soybean stimulation measured with the RED method as well as with the X-ray film digestion tests. Twelve of these dogs were later autopsied and the diagnosis of PDA was confirmed. In the healthy dogs ($n = 42$) the faecal protease activities measured with the RED method increased significantly during the crude soybean feeding. The X-ray film test (22°C) was negative in 42.9 % of the faecal samples of the healthy dogs before and in 15.1 % during the soybean feeding. In 3 dogs the test remained negative in every sample collected during soybean feeding. When the faecal samples of the healthy dogs were examined with the X-ray film method at 37°C, 9.5 % of the samples were negative before but only 1 sample showed negative result when the dogs' food was supplemented with crude soybean (Table 1).

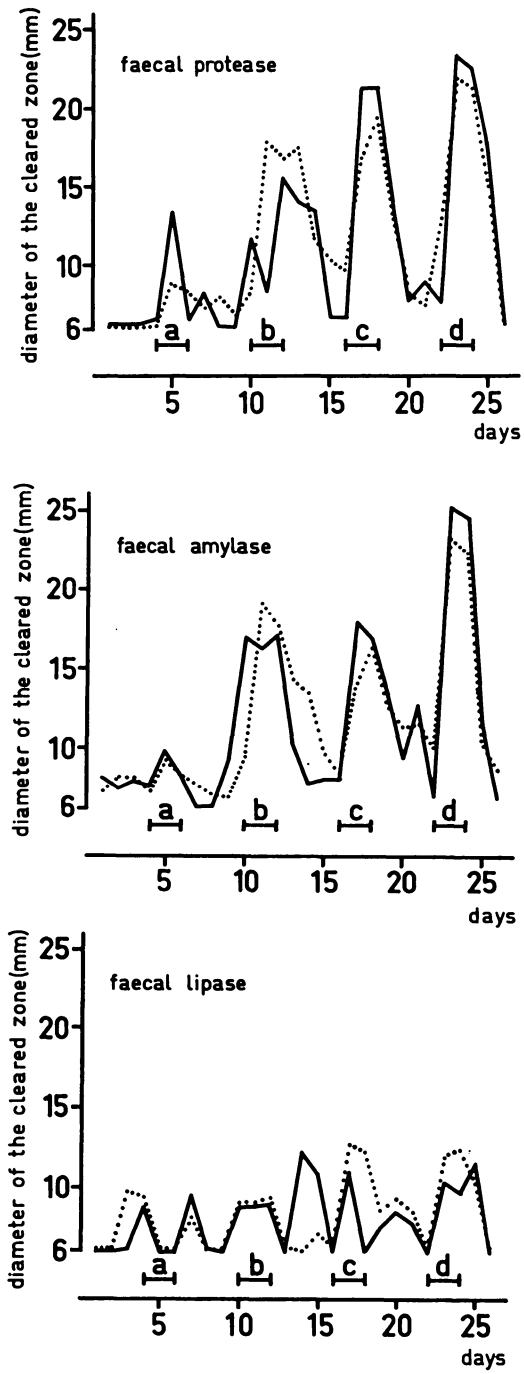

Figure 1. Effect of feeding crude soybean powder on faecal hydrolase activities in 2 normal dogs as determined by the enzyme diffusion in agar gel technique. The food was supplemented by crude soybean powder at a level of 125 (a), 250 (b), 500 (c) and 1000 mg (d)/kg body weight/twice daily for the last 3 days of 4 consecutive periods of 6 days each.

Table 1. Faecal protease activity of 57 dogs. One faecal sample from each dog was taken before and 3 samples during the soybean supplementation with a dose of 1 g crude soybean/kg body weight.

	Number of faecal samples tested	Digestion on RED; diameter of the cleared zone (mm)*)	X-ray film digestion % negative results (no protease activity)	
		$\bar{x} \pm s$	2.5 h/22°C	2 h/37°C
Healthy dogs ($n = 42$)				
before soybean stimulation	42	11.7 ± 4.8	42.9	9.5
during soybean stimulation	126	17.7 ± 3.5	15.1	0.8
PDA dogs ($n = 15$)				
before soybean stimulation	15	6.0 ± 0	100	100
during soybean stimulation	45	6.0 ± 0	100	100

*) The diameter of the well was 6.0 mm.
6.0 means no digestion.

DISCUSSION

The faecal protease activities of the PDA-dogs remained completely negative after soybean stimulation as measured by any of the tests used. This would indicate that the faecal protease activity could be measured by any of the methods used. However, X-ray film digestion test performed at room temperature (22°C) did not completely distinguish between PDA- and non PDA-dogs but if the incubation was performed at 37°C the separation was good. When performing X-ray film test at 37°C the possibility that a healthy dog might give negative results during soybean feeding was very small (1 out of 126 samples). Earlier investigations have described that it might also be possible to obtain a positive result in the X-ray film digestion test in PDA dogs. This would be due to the bacterial proteolytic activity in the faeces which may digest the gelatinous surface of the film (*Strombeck* 1978). In the present investigation such cases were not observed. It can be suggested that in an average small animal practice whenever PDA is suspected 1 or 2 faecal samples (without soybean feeding) should be investigated with the X-ray film method at 37°C. If any activity is seen, the dog should be considered non-PDA. If no activity is seen the dog should be given food supplemented with soybean for a few days and repeated faecal samples should be collected. If the activity remains negative with the

X-ray film method at 37°C, a reliable diagnosis of PDA can be made.

It is important that the faecal samples are well homogenized. In the present study the homogenization was performed with a house-hold food mixer. In larger clinics with laboratory facilities the faecal protease determination can be done by the RED method as previously described. This method gives quantitative information about the protease activity. The X-ray film test should be considered a qualitative test. There were a few samples in which the gelatinous surface of the X-ray film was not completely digested. In such cases the test was repeated and if the result was similar the test was deemed negative.

There were not much difference in faecal protease activities whether the feeds were supplemented with 500 mg or 1000 mg crude soybean/kg body weight. Because crude soybean does not seem to cause any harm to the dogs a dosage of 1000 mg crude soybean/kg body weight can be used in the stimulation test.

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SAMMENDRAG

En praktisk metod att diagnostisera bukspottkörtelns atrofi hos hund.

En behändig metod att diagnostisera degenerativ atrofi i bukspottskörteln inom ramen för vanlig smådjurspraktik har utvecklats.

Metoden baserar sig på observationen att när hundar med normal funktion i bukspottskörteln matas med rå soja, så ökar proteas aktiviteten i avföringen, men PDA-hundarnas proteas aktivitet i avföringen förblev fultständigt negativ.

När PDA misstänks, skall en eller två avföringsprov undersökas med rtg-film metoden med inkubationstiden två timmar i 37°C. Om aktivitet kan konstateras, kan hunden anses vara en ej-PDA hund. Om däremot ingen aktivitet kan konstateras, bör hundens mat kompleteras med 1 g rå soja/kg kroppsvikt två gånger dagligen under några dagar, varefter nya avföringsprov tas. Om aktiviteten fortfarande är negativ, kan diagnosen PDA anses vara pålitlig.

När mängden av rå soja ökades i maten, steg proteas och amylas avtiviteten i avföringen vid mätning med radial entzym diffusion (RED).

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