FEEDING FIDO

Development, validation and application of a dynamic, *in vitro* model of the gastrointestinal tract of the dog

Promotoren: Dr. Ir. M.W. A. Verstegen
Hoogleraar op het vakgebied van de Veevoeding, in het bijzonder de voeding van de eenmagigen

Dr. Ir. G.J. Schaafsma
Hoogleraar op het vakgebied van Voeding en Levensmiddelen

Co-promotor: Dr. R. Havenaar
Product manager *in vitro* GI research
TNO Voeding

FEEDING FIDO

Development, validation and application of a dynamic, *in vitro* model of the gastrointestinal tract of the dog

Marianne Smeets-Peeters

Proefschrift

Ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
dr. ir. L. Speelman,
in het openbaar te verdedigen
op vrijdag 8 september 2000
des namiddags te vier uur in de Aula

ISBN: 90-5808-259-8

Cover design: Mira Bückmann en Daan Zeydner

Printing: Universal Press, Veendendaal

Stellingen

1. Door te stellen dat *in vitro* methoden gevalideerd moeten worden ten opzichte van *in vivo* methoden, wordt er te gemakkelijk vanuit gegaan dat de *in vivo* methode de gouden standaard is.

2. Hoewel wordt aangenomen dat bindmiddelen een effect kunnen hebben op de beschikbaarheid van nutriënten, blijkt dit niet het geval voor de concentratie die gebruikt wordt in hondenvoeding.

3. Bij het samenstellen van een hoog kwalitatieve hondenvoeding, moet naast de beschikbaarheid van de nutriënten, ook rekening gehouden worden met de invloed van de voeding op de passagetijd door het maag-darmkanaal.

4. Zolang de beschikbaarheid voor absorptie van nutriënten uit voedingsmiddelen niet bekend is, spreken voedingsmiddelentabellen slechts de halve waarheid.

5. Het 'vrij' kunnen uitlaten van honden en katten staat in schril contrast met het mestbeleid bij landbouwhuisdieren en de rioolheffing voor de burger.

6. Het volgen van trends in de humane voeding is voor honden minder functioneel dan het volgen van trends in de hondenvoeding voor mensen.

7. De mate waarin een onderzoeker zich kritisch opstelt ten opzichte van zijn resultaten, wordt bepaald door zijn verwachtingspatroon.

8. De kunst van het weglaten is zeker van toepassing op FIDO.

9. Het openbaar vervoer wordt steeds minder openbaar.

10. De meest eenvoudige manier om FIDO uit te laten, is om hem niet aan te zetten.

Stellingen behorend bij het proefschrift: 'Feeding FIDO. Development, validation and application of a dynamic, *in vitro* model of the gastrointestinal tract of the dog'.
Marianne Smeets-Peeters. Wageningen, 8 september 2000.

Aan pap en mam

CONTENTS

1 INTRODUCTION

INTRODUCTION

Globally, petfood is a more than USD 21 billion industry. The market grew annually by 4.5% in value and 2.7% in volume between 1994 and 1998 (Datamonitor, 1998). In both wet cat and dog food segments, volumes are under increasing pressure from dry food although, up to date, manufacturers have been largely able to maintain value growth by launching premium and super premium products. Table 1 gives an overview of total sales in different European countries, Canada, USA, Australia and Japan until 1998.

Table 1 Pet food, total value sales (million USD), 1994–1998 (Datamonitor, 1998)

	1994	1995	1996	1997	1998
Belgium	192	208	200	174	202
Denmark	88	99	95	85	94
France	1354	1578	1487	1327	1416
Germany	1476	1647	1775	1627	1937
Italy	588	651	726	683	792
Netherlands	293	318	307	269	313
Portugal	43	51	56	55	62
Spain	128	149	157	148	175
Sweden	272	309	321	338	355
UK	1911	1937	2038	2237	2172
Czech Republic	23	28	31	28	35
Hungary	47	518	52	50	52
Poland	72	868	106	113	120
Canada	583	607	624	637	672
USA	7677	7918	8145	8773	9896
Australia	553	545	580	555	492
Japan	2390	2356	2224	2056	2291
Overall	17688	18539	18923	19155	21076

This grow in value sales will continue in the next few years. The expected growth (1999–2003) will be 3.0% in the Netherlands, 2.8% in the whole of Western Europe, and 4.1% worldwide.

The petfood market follows trends of the human food industry. Health promotion, safety, indulgence and convenience are important topics, and pet owners want the best for their pets. To fulfil the wishes of the owners and the needs of pets, new and approved products are being launched on the market. Before launching new products or to confirm the quality of existing products, research on products or ingredients is necessary. Quality is one of the key factors in new product development. This has led to a marked shift away form low-cost, low-quality economy products in favor of premium and, increasingly, super premium foods.

The chemical composition of diets must be known, but the digestibility and availability of nutrients may be even more important. The chemical composition of a diet can (easily) be determined with different methods of analysis. Digestibility and availability, however, can be influenced by the composition of the diet (e.g. selected ingredients and interactions among ingredients) but also by processing. Besides, the value of digestibility is influenced by how digestibility is defined and by the method of determination (Boisen and Moughan, 1996). Apparent, true and real digestibility are distinct concepts. 'Apparent' values relate directly to the feed. 'True' digestibility values have traditionally been obtained by correcting ileal digest flows for endogenous components (e.g. protein by feeding a N-free diet). 'Real' digestibility is a direct measure of the digestibility of a feed/food component, and is not influenced by endogenous losses (Boisen and Moughan, 1996). Besides ileal digestibility, also total tract digestibility is measured. However, the total tract digestibility does not correlate with the availability of nutrients to the animal. Part of the food is fermented by microflora in the large intestine, and for amino acids this does not have any nutritional value for the host animal. In contrast, short-chain fatty acids can be absorbed and used for energy provision. For that reason, it is important to study ileal digestibility for protein. This is more complicated because invasive studies (ileal cannulas) are needed to get more insight into ileal digestibility. In the past such *in vivo* studies were performed with dogs and cats and ethics was not a big issue in performing these studies. However, within the European Community more and more regulations have been and will be introduced with respect to the performance of *in vivo* studies and the use of alternatives. Examples of such regulations, adopted by the European Convention, are:

– the choice of species shall be carefully considered and, where required, be explained to the responsible authority; in a choice between procedures, those should be selected which use a minimum number of animals, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results (Part III: conduct of procedure, article 7).

– Where it is planned to subject an animal to a procedure in which it will or may experience severe pain which is likely to endure, that procedure must be specifically declared and justified to, or specifically authorized by, the responsible authority (Part III: conduct of procedure, article 9.1).

Besides, the members have agreed that scientific research into the development of methods that could provide similar information as that obtained in studies with animals is to be encouraged (European Convention, 1996). Similar statements are used in the regulations of other countries, such as the USA, Australia and New Zealand (Altweb). Besides regulations and agreements, petfood manufacturers are also restricted in doing (invasive) studies with dogs or cats due to the public opinion about *in vivo* studies. Therefore, it is important to develop alternatives for doing research on petfood. Besides the advantage of not imposing ethical problems, *in vitro* models have other advantages over *in vivo* studies: lower costs, the possibility to study underlying mechanisms, and less time needed for testing (new) products. *In vitro* models also enable to easily distinguish apparent digestibility from true digestibility. In developing new products shorter test periods are interesting because this reduces the time to market. When developing an *in vitro* model for the gastrointestinal tract of the dog it should fulfil certain criteria. It should simulate (1) a sequential use of digestive enzymes in physiological amounts, (2) appropriate pHs at different sites for enzyme activities and addition of relevant co-factors such as bile salts and co-enzymes, (3) appropriate mixing at each digestion step, (4) a physiological transit time for each digestion step, and (5) removal of digested products (Longland, 1991).

In the past several *in vitro* procedures have been developed to estimate digestibility, including the pH-drop and pH-stat method, the filtration method and the dialysis cell method (Boisen and Eggum, 1991). The objectives of each of these methods were different. The pH-drop and pH-stat methods predict the digestibility in foods but the method cannot be considered to be applicable for all types of food. The filtration method can be used to predict the nutrient digestibility but the products of digestion are not removed from the system. With the dialysis cell method, the influence of various factors on the digestion of nutrients can be studied when end-product inhibition is prevented by a dialysis system. However, transit

times are not taken into account in this system. The choice of a method depends, of course, on the objectives of a study.

From the evaluation performed by Boisen and Eggum (1991) it can be concluded that most of the *in vitro* models are not able to mimic the physiological conditions as closely as necessary. These models do not meet all the criteria described by Longland (1991). An *in vitro* model that does fulfil these criteria has been developed by Minekus *et al.* (1995; 1998). This dynamic model simulates multi-enzyme (and bile) digestion, absorption of digested products, and physiological pH values in different parts of the gastrointestinal tract combined with physiological transit times. This model has been validated for human subjects, pigs and pre-ruminant calves by Minekus (1998).

Because the petfood industry is bound to restrictions for doing (invasive) studies, this model is very useful in evaluating existing and new pet foods. Therefore, the aim of the study was to develop an *in vitro* model simulating the gastrointestinal tract (GI tract) of the dog that can be used as an alternative to *in vivo* studies. The model should simulate the physiological circumstances in the GI tract of the dog as closely as possible. In that way luminal processes as well as physical and chemical properties of diets can be investigated.

The objectives of the studies presented in this thesis were :

1) development of a dynamic *in vitro* model of the gastrointestinal tract of the dog
2) validation of the model
3) application of the model.

For the development of the model a literature study was performed to study the physiological parameters of the dog to meet the criteria described by Longland (1991; Chapter 1). Based on the data found in the literature, experiments were performed with canned and dry dog foods. After these exploratory experiments, a 'technical' validation was performed to study the possibilities of using canned and dry dog foods under different conditions in the model (Chapter 2). After this 'technical' validation, studies were performed to prove the value of the model in comparison to *in vivo* studies in dogs (Chapter 3). In Chapters 4, 5 and 6 experiments are described aimed at studying the digestibility and availability for absorption of some macronutrients (proteins and carbohydrates) and micronutrients (calcium and phosphorus). These components were used as nutritional model compounds because they require different digestive processes in the dog. Also the mode of absorption varies among these components (active and passive absorption). These studies were used for both a further

validation of the model (with respect to the *in vivo* data, reproducibility and sensitivity) and a proof that the model can be used for applied research.

The model mimics the availability for absorption of nutrients, whether it is absorbed in an active or passive fashion *in vivo*. This gives the opportunity to study the characteristics of a diet without interference of the physiological state of an animal. By changing parameters, like pH or enzyme concentration, the effect of these parameters on digestibility and availability for absorption of nutrients can be studied. In Chapter 6 results for effects of physical parameters, such as viscosity and buffering capacity, on digestion are presented. Because it is not possible to distinguish between passive and active transport in the intestine, experiments were also performed in intestinal segment chambers; these data are compared with *in vivo* data (Chapter 7). The results of the literature review and the experiments involved in the thesis are discussed in Chapter 8.

2 A REVIEW OF THE PHYSIOLOGY OF THE CANINE DIGESTIVE TRACT RELATED TO THE DEVELOPMENT OF *IN VITRO* SYSTEMS

Marianne Smeets-Peeters[1,2], Tim Watson[3], Mans Minekus[1] and Robert Havenaar[1]

1 TNO Nutrition and Food Research, PO Box 360, 3700 AJ Zeist, The Netherlands
2 Wageningen University, Department of Animal Nutrition, Marijkeweg 40, 6709 PG Wageningen, The Netherlands
3 Waltham Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, Leicestershire LE14 4 RT, United Kingdom

Nutrition Research Reviews 11 (1), 45-69, 1998

ABSTRACT

Food and nutrition studies in animals and human beings often meet with technical difficulties and sometimes with ethical questions. An alternative to research in living animals is the dynamic multi compartmental *in vitro* model for the gastrointestinal tract described by Minekus *et al.* (1995) and Havenaar and Minekus (1996). The dynamic conditions that are simulated in this model are peristaltic movements, transit times, pH responses, secretion of enzymes and electrolytes, and absorption of nutrients and water. To obtain data for an *in vitro* model of the dog gastrointestinal tract, the literature was surveyed for physiological responses to different types of dog food. These included: values of enzyme activities, electrolyte concentrations, gastric emptying and intestinal transit times, pH values, secretion and composition of bile, and absorption rate in different parts of the dog gastrointestinal tract. The review focuses in research carried out on healthy, adult dogs of 10-20 kg and on parameters related to the oral cavity, stomach and small intestine. This literature research gives sufficient data on the physiology of the canine digestive tract or the development of an *in vitro* dynamic model that adequately simulates the functions of the stomach and small intestine of the dog.

INTRODUCTION

In vivo studies on the physiology of food in the gastrointestinal tract both in living animals and men do meet with serious technical difficulties and sometimes ethical questions. Therefore much attention is given recent years to the development of *in vitro* models which mimic metabolic processes of the gastrointestinal tract. Such models can lead to information on and prediction of food digestion in the gastrointestinal tract.

An interesting *in vitro* model of the stomach and small intestine has been described in literature (Havenaar and Minekus, 1996; Minekus *et al.*, 1995; Minekus and Havenaar, 1996). The model is a multi-compartmental laboratory system which is made of glass and silicon and which simulates the kinetics in the successive parts of the gastrointestinal tract of humans, calves and pigs (Figures 1a and 1b).

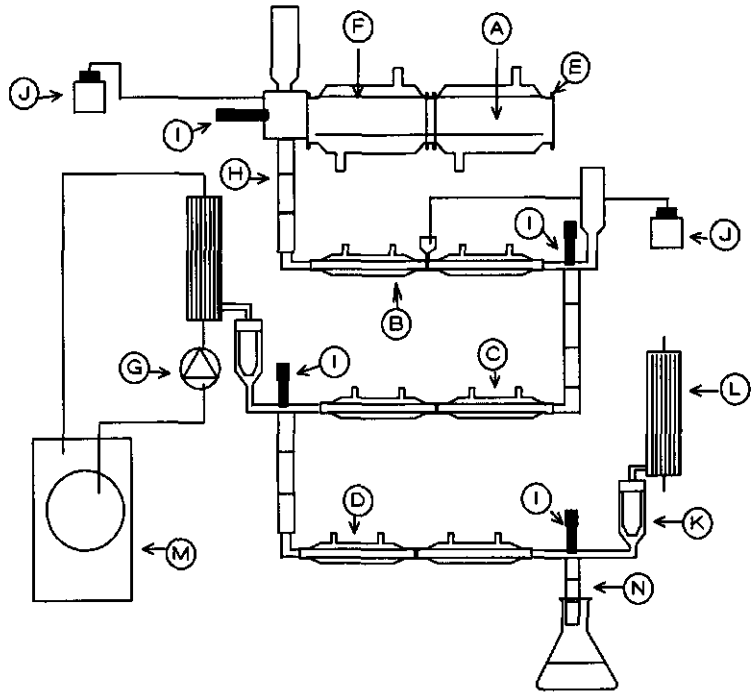

Figure 1a Schematic view of TIM (TNO gastro-intestinal model) (Minekus *et al.*, 1995): a) gastric compartment; b) duodenal compartment; c) jejunal compartment; d) ileal compartment; e) glass jacket; f) flexible wall; g) rotary pump; h) pyloric valve; i) pH electrodes; j) secretion pump; k) pre-filter; l) hollow-fibre device; m) dialysis system; n) ileo-caecal valve

Parameters, such as peristaltic movements, transit time, pH and secretion are included in this model. Different aspects can be studied in this model such as digestion of food components (Minekus, 1996), survival of bacteria (Marteau *et al.*, 1997), availability for absorption of minerals (Larsson *et al.*, 1997) and pharmacokinetics.

This dynamic model enables simulating of the physical conditions that occur in the gastrointestinal tract of a dog. It can thus be used to study the hydrolyses of food components into nutrients as part of the digestive processes. For such a dynamic *in vitro* model to function it is of significance that the *in vivo* conditions regarding the physiology of the canine gastrointestinal tract are properly simulated.

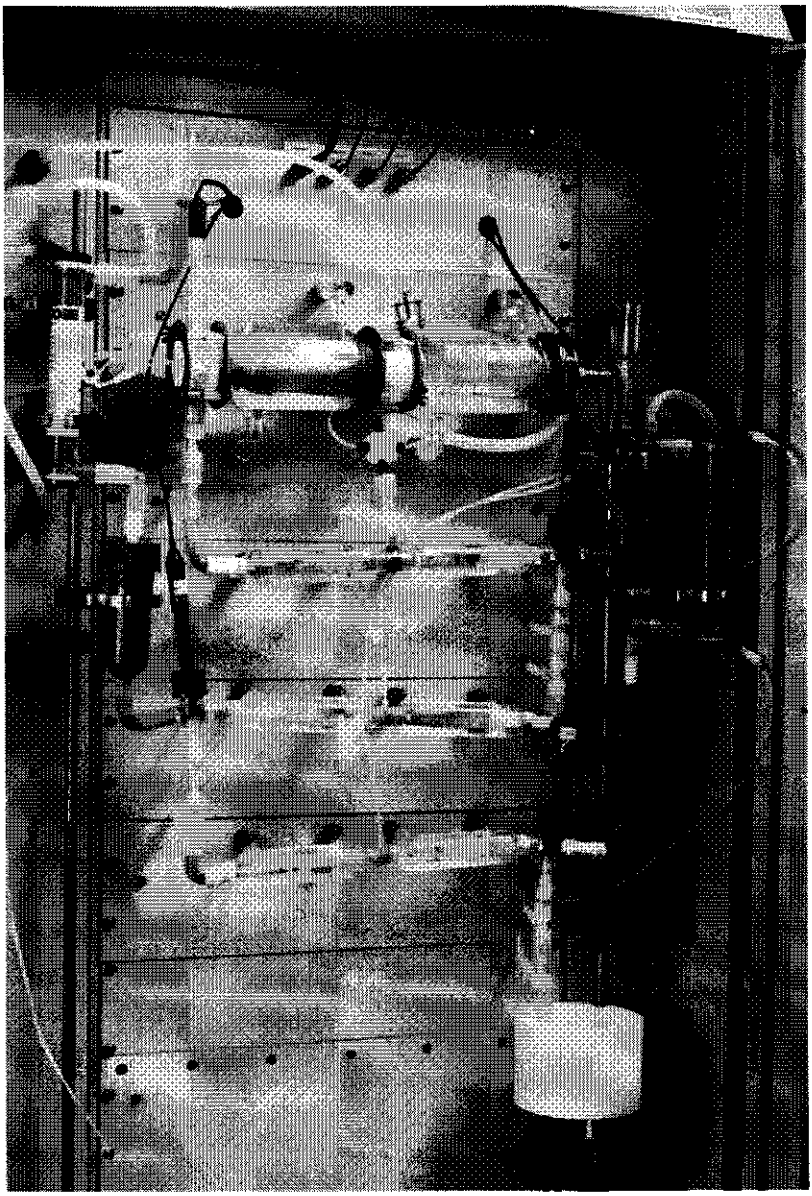

Figure 1b Photograph of TIM

The objective of this review is to make an inventory of the published data on diet and canine digestive tract to get a standard which can be used for the design and interpretations of *in vitro* studies from the model as described above. The review focusses on research carried out on healthy, adult dogs with a body weight between 10 and 20 kg, and on those parameters which are of special importance for the setup of the dynamic *in vitro* model simulating the stomach and small intestine. Although the large intestine is important for further (microbial) breakdown of food components, data on the large intestine are not included in this review.

PHYSIOLOGICAL DATA

Physiological data of the mouth, stomach, small intestine, pancreas and gall bladder are discussed in response to different types of dog food.

Mouth

The first step in the digestion of food is the secretion of saliva during mastication. Amount and composition of the saliva are dependent on the type of food (especially the water content) ingested. Enzyme activity levels are usually not influenced by the rate of secretion. The dog produces saliva from the parotid gland (0.14 to 1.40 ml/min; mean = 0.55) and from the submaxillary gland (range 0.20 to 3.84 ml/min; mean = 1.31) (Chauncey *et al.* 1963). Saliva consists of ca. 99% water, the remaining 1% comprises mucus, inorganic salts and enzymes (Maskell and Johnson, 1993). Table 1 gives a survey of the amounts of various components of saliva (Altman and Dittmer, 1968; Larmas and Scheinin, 1971). The pH of the saliva varies between 7.34 and 7.80 (Gurtler, 1967; Altman and Dittmer, 1968). These results are comparable with the results of Larmas and Scheinin (1971) who found a pH varying between 7.2 and 8.1 (mean 7.7). The pH of the saliva can be influenced by food. In a study of Larmas and Scheinin (1971) the pH felt rapidly about 0.5 pH units after administration of sugar.

The dog misses the starch digesting enzyme α-amylase in its saliva. The lack of this enzyme is reflected in the eating behaviour of dogs, which tend to bolt all but the toughest foods (Maskell and Johnson, 1993).

Table 1 Amounts of electrolytes and amylase in saliva of dogs

constituent	source of saliva	mean mmol/l	range	reference†
calcium	mixed	n.m.*	1.45-3.3	1
	mixed	1.85	1.0-2.75	2
	parotid	4.3	2.5-5.2	1
chloride	mixed	n.m.	16.3-69.3	1
	parotid	81.9	37.5-103.7	1
potassium	mixed	n.m.	12.3-23.7	1
	mixed	20.2	14.1-24.8	2
	parotid	11.4	4.3-12.6	1
sodium	mixed	74.1	42.0-99.8	2
	parotid	108	48.8-132.9	1
bicarbonate	parotid	55	34.7-69.1	1
amylase	submaxillary	$< 0.010 \times 10^2$ mg/ml		1
	parotid	not demonstrable		1

* n.m. = not mentioned

† 1 = Alltman and Dittmer (1968) ; 2 = Larmas and Scheinin (1971)

Stomach

Digestion in the stomach is determined by physical and chemical properties of ingested food and by the concentrations of electrolytes and activity of enzymes. Gastric emptying and pH are of major importance because they play a role in the activity of enzymes. Also the contact time of the food with the enzymes is determined by these factors.

Electrolytes and enzymes

The concentrations of electrolytes in gastric juice (Table 2) reported in the literature vary widely. The major enzymes present in the lumen of the stomach are lipase and pepsin.

Table 2 Electrolyte composition of gastric juice in dogs

constituent	mean ± S.D. (range) mmol/l	reference*
bicarbonate	5.0 ± 0.7	1†
	3	2‡
potassium	28.0 ± 3.7	1
	7.0	2
	7.2	3
	15.2 (10.3-22.0)	3§
sodium	58.0 ± 9.1	1
	155	2
	22	3
	64 (46.3-79.0)	3§
chloride	149.0 ± 5.3	1
	133	2
	172.9	3
	123 (98-143)	3§
calcium	4.0	2
	0.5 -1.7	3
phosphate	12.0 ± 2.7	1
	0.25 mg/100 ml	3
magnesium	0.5 mg/100 ml	3

* 1 = Alexander, 1965; 2 = Davenport, 1961; 3 = Altman and Dittmer, 1968

† The dogs were killed 4-6 h after a meal of canned food and concentrations were measured

‡ The values were calculated from analyses of samples of juice being secreted at different rates in response to graded doses of histamine

§ Sham feeding stimulation

Dog gastric lipase is a 49 kDa glycoprotein containing 13% carbohydrate which is formed by a single polypeptide chain of 377-379 amino acid residues, acting on both long- and short-chain triglycerides. At pH 4 this lipase is 13 times more active on long-chain than on short-chain triacylglycerols (Carrière *et al.*, 1991). The lipase is irreversibly inactivated below pH 1.5, its activity also decreases significantly above pH 6.0 and is completely inactivated at pH 7.0. At pH values below 6.0, which normally prevail in the duodenum after ingestion of a liquid meal, a gastric lipase activity of ca. 90% is recovered (Carrière *et al.*, 1993).
The basal secretion rate of lipase is 606 ± 40 units/h (1 unit equals 1 µmol of butyric acid released from tributyrin per minute). Lipase is secreted in both the proximal and antral area of the canine stomach (Carrière *et al.*, 1992). The activity of lipase in the gastric juice varies from 0.9 to 3900 units/ml (Engel, 1946).
For pepsin the amount present after sham feeding ranged between 41-164 units/ml (mean 81). The factors responsible for the variability between the individual dogs are unknown (Villareal *et al.*, 1955). The amount of pepsin secreted can be influenced by hormones, such as adrenocorticotropic hormone (ACTH) which causes an increase in activity (Villareal *et al.*, 1955). Pepsin has an optimum activity at a pH of 2.0 maintained by gastric secretion of hydrochloric acid; its proteolytic activity decreases when chyme leaves the stomach, since it is irreversibly inactivated at neutral pH.

Acid secretion and pH

Gastric secretion is influenced by the amount of protein in a meal and by the volume of the meal (Carpentier *et al.*, 1988). Some hormones will indirectly effect the acidity of the stomach contents. ACTH increases hydrochloric acid production (Villareal *et al.*, 1955), and secretin decreases production through suppression of the release of gastrin (Jin *et al.*, 1994). The nervous system also plays a role in the secretion of hydrochloric acid as shown by Curtis Lawson *et al.* (1994): gastric acid secretion is doubled when a calcitonin gene-related peptide antagonist is infused.

Gastric pH can be measured by different methods: directly in samples taken from the stomach (Carrière *et al.*,1993; Banta *et al.*, 1979) or by radiotelemetry (Youngberg *et al.*, 1985). When measured in time a gastric pH curve can be mimicked (Figure 2). In the fasting state gastric pH fluctuates little with time, whereas postprandially there are wave-like patterns (Youngberg *et al.*, 1985). The gastric pH response varies with the type of meal ingested. After ingestion of a complete liquid test meal a pH drop below 4.0 was noticed within 10 to 20 min (Youngberg *et al.*,1985). With a meat based diet a drop in pH to below 6.0 was found after 60 min (Banta *et al.*, 1979; Carrière *et al.*,1993).

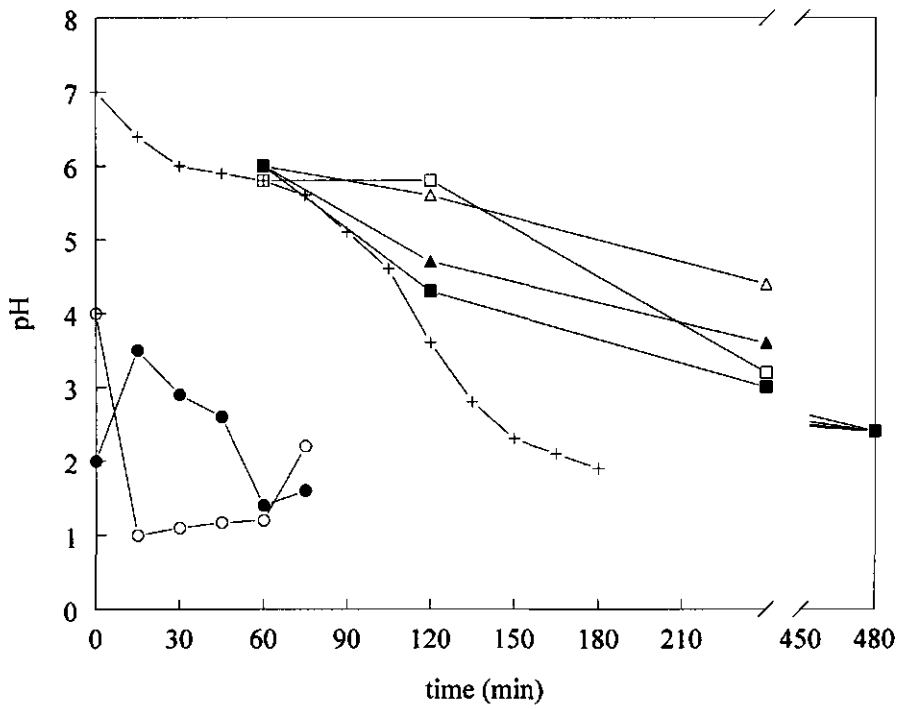

Figure 2 Changes in pH with time of gastric contents; ○ = fasted state, ● = fed state (Youngberg *et al.*, 1985), △ = cereal proximal stomach, ▲ = cereal distal stomach, □ = meat proximal stomach, ■ = meat distal stomach (Banta et al.;, 1979), + = fed state (Carrière *et al.*, 1993)

Gastric emptying

Gastric emptying plays an important role in the time enzymes are in contact with food in the stomach. Rates of emptying are influenced by many factors such as volume, energy content, viscosity, density and particle size of gastric contents (Cargill *et al.*, 1988; Ehrlein and Pröve, 1982; Horowitz *et al.*, 1994; Benini *et al.*, 1994; Dressman, 1986), temperature (Benini *et al.*, 1994; Teeter and Bass, 1982), body weight (Allan *et al.*, 1996) and amount of acid in the duodenum (Cooke, 1974; Cooke and Clarke, 1976). Also, a complex interaction exists between different meal components (Horowitz *et al.*, 1994).

The rate of gastric emptying of a liquid meal is determined by the pressure gradient between the stomach and the duodenum (Carrière *et al.*, 1993). In general, liquids empty in a monoexponential manner, whereas solids empty in a linear manner (Dozois *et al.*, 1971; Gupta and Robinson, 1988; Gué *et al.*, 1988; Hornof *et al.*, 1989) after a lag phase after ingestion (Horowitz *et al.*, 1994). This lag phase is probably dependent on the time taken for redistribution of food from the proximal into the distal part of the stomach and the time for grinding solid food into small particles (Horowitz *et al.*, 1994). After ingestion of a solid-liquid meal, the stomach retains solid food predominantly in the proximal part, until most (ca. 80%) of the liquid has disappeared (Horowitz *et al.*, 1994).

Carrière *et al.* (1993) determined gastric emptying by the concentration of a liquid marker (phenol red) in duodenal and gastric contents at 15 min intervals. They found an average gastric half-time of emptying of a liquid test meal of 75 ± 8 min. This was in agreement with the results obtained with the empirical equation described by Hunt and Stubbs (1975) :

$$t_{1/2} = V_0(0.18-0.17e^{-K})$$

where V_0 is the meal volume (ml) and K is its nutrient density (kcal/ml).

Both meal volume and density play a role in gastric emptying. Meyer *et al.* (1985) studied the effects of density of nondigestible solids in duodenal fistulated dogs. There was a trend for higher density particles to empty slower. The regulation of emptying by density depends upon the intraduodenal concentrations of products of digestion, either monosaccharides, acting through their osmotic pressure, or salts of fatty acids acting on duodenal receptors. Isocaloric concentrations of carbohydrates and triglycerides induce similar gastric emptying (Hunt and Stubbs, 1975). Although emptying of high-density meals is slower, more energy is transferred to the duodenum in a given time than with low-density meals (Hunt and Stubbs, 1975). A larger volume of fluid flows through the duodenum with meals of higher energy

density than with meals of lower energy density. The same effect occurs with foods differing in osmolarity (Hinder and Kelly, 1977). Apparently, a greater stimulation of endogenous secretion occurs with the meals of high energy density, and a greater diffusion of water occurs into the duodenal lumen because of the high osmolarity (Meyer *et al.*, 1989).

Gupta and Robinson (1988) studied the gastric emptying of liquids in the dog by collecting the effluent from a permanent Thomas cannula located in the duodenum about 15 cm from the gastroduodenal junction. The gastric emptying of volumes above 100 ml of water seems to follow an exponential curve with a half-time of discharge of ca. 10 min. In fasted dogs, gastric emptying of liquids starts immediately after administration of test meals and most of the volume (80-100%) is emptied within 40 min (Gupta and Robinson, 1988). Dozois *et al.* (1971) found a half-emptying time for liquid meals of 25 min. The discharge pattern of volumes of 100 ml or less was different from that of larger volumes. During the first 20-30 min after administration of water there was little or no emptying. Half of the administered volume was emptied between 35 and 45 min and the complete volume was emptied in 55-65 min (Gupta and Robinson, 1988). Lui *et al.* (1986) observed a longer gastric emptying time of 99.8 ± 27.2 (35-317) min after ingestion of 20 to 50 ml of water compared to the studies of Gupta and Robinson (1988) and Dozois (1971). The mean resting volume in the stomach in the fasted state was about 25 ml and administration of small volumes (≤ 100 ml) did not change the motility pattern. From these results it can be concluded that the volume transition to convert motility from a fasted to a fed state lies somewhere between 100 and 150 ml (Gupta and Robinson, 1988).

For liquid meal volumes smaller than 66 ml/kg of body weight, the volume emptied from the stomach in 30 min increased linearly with meal volume. For higher meal volumes, the gastric emptying did not increase linearly with increasing meal size, but seems to approach a maximum at 0.99 ± 0.052 ml/min/kg (Leib *et al.*, 1986). These results are in contrast with the results of Hunt and Stubb (1975) who conclude that nutrient density alone determines the volume of a meal emptied in 30 min, independent of the starting volume of a meal.

Meat leaves the stomach as particles smaller than 2 mm in diameter (Burrows *et al.*, 1985; Meyer *et al.*, 1985; Hinder and Kelly, 1977; Cullen and Kelly, 1996; Meyer *et al.*, 1979). It is therefore assumed that a food-containing stomach has a treshold or cut-off size, above which it retains material and below which it allows the food or particles to be moved to the duodenum (Meyer *et al.*, 1985). Breaking down digestible solids to a smaller size speeds up gastric emptying. In contrast, coarse, indigestible solids are retained in the stomach until digestion of other food components is completed. Such indigestible solids are not swept out

of the stomach until powerful, propulsive, gastric contractions in the fasting state take place (Hinder and Kelly, 1977).

Banta *et al.* (1979) studied the gastric emptying of both solids and liquids in dogs using two fluid markers; polyethylene glycol (PEG) and ^{51}Cr-labelled EDTA and radiopaque polyethylene tubing cut in particles of 2, 10 and 20 mm as a solid marker (Table 3). They concluded that the stomach appears to be the major site involved in regulation of particulate marker passage through the gastrointestinal tract; this is most obviously the case with the larger particles. Emptying of liquids (154 mM NaCl) and solids (solid plastic spheres, 1 cm in diameter) was also studied by Dozois *et al.* (1971) who concluded that the terminal antrum and pylorus are of minor importance in the regulation of gastric emptying of liquids but are of great importance in gastric emptying of solids. Allan *et al.* (1996), who used two different sizes solid particles, found no significant difference in the mean lag period between the large (5 mm) and small (1.5 mm) radiopaque markers.

Table 3 Gastric emptying time (min) for liquid and solids (different particle sizes) in dogs after feeding cereal- or meat-based diets (Banta *et al.*, 1979)

Diet	Liquid emptying time (min)*	Solid emptying time (min)*		
		2x2†	2x10†	2x20†
Cereal-based	102	186	564	600
Meat-based	108	606	642	666

* Time required for recovering 50% of the solid particles into the faeces

† particle size (mm x mm)

Miyabayashi *et al.* (1986) found a gastric emtpying time of 76 ± 16 min (range 30-120 min) in a barium sulfate contrast study.

By using external scintigraphy, Theodorakis (1980) found an average gastric half emptying time of 77 ± 23 min for 255 g of canned dog food in six beagles.

Scintigraphy was also used for dry kibble food to determine solid-phase gastric emptying in beagles and mongrel dogs (Hornof *et al.*, 1989). An average half-emptying time of 240 min was found in the beagles (ca. 12 kg ; n = 6) and 216 min in the mongrel dogs (5.4-35 kg; n = 5).

Arnbjerg (1992) studied the time of passage of various commercial food items through the stomach of dogs (25-30 kg) by radiography. The types of food used were (1) dried food with 10% moisture, (2) canned food with 70% moisture and (3) fresh food (fish) with 75% moisture. After food ingestion the animals had no access to water or to any other type of liquid. In group 1, the food remained unchanged in the stomach for 480 to 600 min (mean 534 min) after completion of the meal. After 900 ± 60 min the stomach appeared to be completely empty. In group 2, the food started to enter the duodenum after 270 ± 30 min. The stomach appeared to be empty 420-480 min after eating. In group 3, the food was observed in the duodenum 30 min after ingestion and emptying was complete 240-360 min after ingestion. However, this method is not very accurate due to problems with determining the very beginning of gastric emptying; besides, radiographs were taken at intervals of 60 min (Arnbjerg, 1992). The results of fresh meat emptying are in agreement with the results for labelled chicken liver reported by Cullen and Kelly (1996) who found a gastric emptying time of 214 ± 14 min, including a lag phase of 71 ± 9 min. However, Meyer *et al.* (1985) found a faster gastric emptying (180 min) for radiolabeled steak and liver in large breed dogs (20-25 kg body weight). Compared to Meyer *et al.* (1985) Burrows *et al.* (1985) observed comparable results in half-emptying time in large breeds (26-32 kg body weight) using different isocaloric commercial diets. Canned meat-based food (77% moist), dry cereal-based chow plus water (77% moist) and dry-cereal based food were emptied in 228 ± 36, 150 ± 36 and 144 ± 36 min respectively (differences not significant).

The large difference in half emptying time of dry food between the studies is probably due to the fact that sometimes, like in the study of Arnjberg (1992), the dogs had no access to water.

Cullen and Kelly (1996) used the equation of Elashoff *et al.* (1982) to calculate the emptying curves of liquids and solids in the dog (Table 4):

$$f=2^{-(t/t_{1/2})^{\beta}}$$

in which $t_{1/2}$ is the time (min) from the start of the meal until 50% of the meal has been emptied and β determines the shape of the curve. This equation can be used to summarize and compare data on gastric emptying between meals or between groups.

Table 4 Gastric emptying parameters of liquids and solids in dogs (Cullen and Kelly, 1996)

Parameter	Solids	Liquids
$t_{1/2}$ (min)	246 ± 14	148 ± 14
β	3.0 ± 0.4	1.4 ± 0.2
Lag (min)	71 ± 9	14 ± 8

An overview of the different studies on gastric emptying in dogs is shown in Table 5. Besides the effects of the food, motility (Azpiroz and Malagelada, 1984; Pröve and Ehrlein, 1982; Eagon and Kelly, 1993; Mandrek, 1991) and hormones (Curtis Lawson *et al.*, 1994; Jin *et al.* 1994; Landor and Wild, 1970; Patronella *et al.*, 1988; Jin *et al.*, 1994; van Kruiningen *et al.*, 1987) play an important role in the regulation of gastric emptying. Although these parameters are very important in gastric emptying, their effects are not discussed in this review because such parameters can not be used in *in vitro* models.

Pancreas

A very important aspect in the digestion of food is the secretion of pancreatic juice into the proximal small intestine mainly due to the action of electrolytes and digestive enzymes.

Composition of pancreatic juice

The electrolyte composition of the pancreatic juice released into the intestine varies among animal species and, in most animal species, with flow rate. Intermittent feeders (eating at intervals), such as the dog, mainly secrete the juice during the digestive phase after the ingestion of a meal (Stevens and Hume, 1995). The effects of different types and amounts of food are shown in Table 6.

Table 5 Overview of gastric emptying studies in dogs fed liquid meals, canned, fresh food or dry food

Method	Diet	Gastric emptying parameters	Reference
fluid marker : phenol red	liquid test meal 14 g protein 52 g carbohydrate 12.5 g lipid	$t_{1/2}$ = 75 ± 8 min	Carriere *et al.*, 1993
Thomas cannula	water >100 ml	$t_{1/2}$ = 10 min 80-100% has left the stomach after 40 min	Gupta and Robinson, 1988
fluid markers : PEG ^{51}Cr-EDTA	 cereal-based diet meat-based diet	mean gastric emptying* 102 min 108 min	Banta *et al.*, 1979
aspiration from the stomach	154 mM NaCl	$t_{1/2}$ = 25 min	Dozois *et al.*, 1971
Heidelberg capsule	20-50 ml of water	gastric emptying time 99.8 ± 27.2 min (range 35-317 min)	Lui *et al.*, 1986
barium contrast	barium sulfate suspension	gastric emtying time 6 ± 16.7 min (range 30-120 min)	Miyabayashi *et al.*, 1986
external scintigraphy	canned dog food	$t_{1/2}$ = 77 ± 23.3 min	Theodorakis*et al.*, 1980
radiography	canned food (70% moist)	first appearance duodenum: 270±30 min emptying complete: 420-480 min	Arnbjerg, 1992
radiography	fresh food (75% moist)	first appearance duodenum: 30 min emptying complete: 240-360 min	Arnbjerg, 1992
γ-activity measurement	radiolabelled steak radiolabelled liver	$t_{1/2}$ = 180 min $t_{1/2}$ = 180 min	Meyer *et al.*, 1985
scintigraphy	radiolabelled chicken ^{111}In-labelled beef broth	$t_{1/2}$ = 214 ± 14 min lag phase : 71 ± 14 min	Cullen and Kelly, 1996
radiopaque polyethylene tubing	 cereal-based diet meat-based diet	particle size (mm) 2x2 2x10 2x20 186 564 600 min 606 642 666 min	Banta *et al.*, 1979
scintigraphy	dry kibble food	beagles $t_{1/2}$ = 240 min mongrel $t_{1/2}$ = 216 min	Hornof *et al.*, 1989
radiography	dried food (10% moist), no access to water	radiographic appearance remained unchanged for 480 to 600 min (mean 534 min) emptying complete: 900 ± 60 min	Arnbjerg, 1992

* time required for 50% of the marker to leave the forgut or to be recovered in faecal material

Table 6 Amount, secretion time and composition of the pancreatic juice with different types and amounts of food given to dogs (Stevens and Hume, 1995)

Amount and type of food	Amount of juice (ml)	Time of secretion (min)	Dry matter (%)	Na_2CO_3 (%)
600 cm^3 milk	457	270	527	35
250 g bread	1624	465	322	56
100 g meat	1316	252	247	59

Because the amount and type of food play a role in the composition and secretion rate of the pancreatic juice secreted, the range of the values is very wide (Table 7).

Dog pancreatic lipase and its catalytic properties are very similar to those of humans and pigs. The lipase activity is stable above pH 4.0 and the amount delivered into the duodenum is shown in Figure 3 (Carrière *et al.*, 1993). The concentration slowly decreases in the duodenum and jejunum between meal ingestion and at the end of digestion (Figure 4) (Carrière *et al.*, 1993).

Besides lipase, chymotrypsin is also an important pancreatic enzyme with significant activity (Table 8). A very high induction of chymotrypsin activity is caused by feeding protein rich meals (especially animal protein) whereas lactose meals produce a very low chymotrypsin activity (1.45 ± 0.66 U/kg wet weight) (Kienzle, 1988).

Although the dog has no amylase activity in saliva, there is amylase activity in pancreas secretions. The amylase activity in pancreatic tissue of an adult dog has been reported to be 2316 ± 2017 (383 to 6625 U/g wet weight) (n = 16). As for chymotrypsin, maximum levels of amylase activity were found in the jejunum as well as in the ileum. In contrast to chymotrypsin activity, the activity of amylase is relatively high in carnivores, (Kienzle, 1988). The amylase output is increased by wheat bran supplementation in the diet, as well as bicarbonate output and pancreatic juice flow (Stock-Damgé *et al.*, 1983).

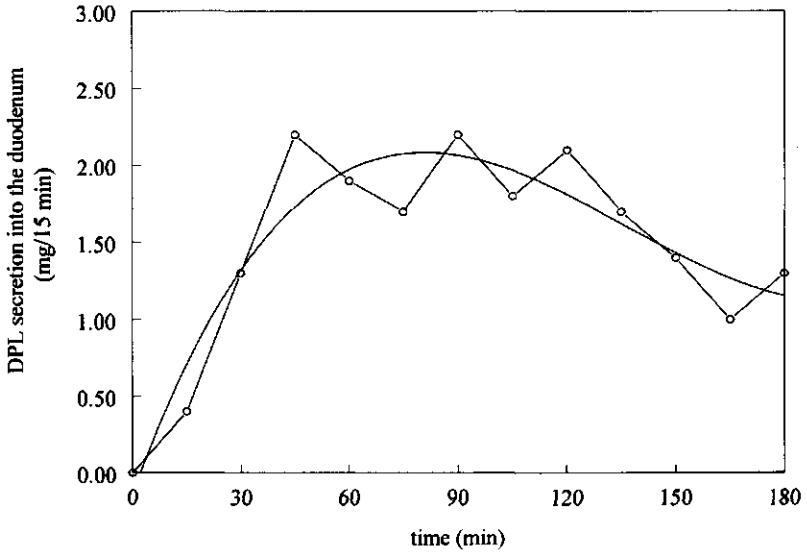

Figure 3 Rates of secretion of dog pancreatic lipase (DPL) secretion levels into the duodenum during digestion of a liquid test meal (14 g protein, 52 g carbohydrate and 12.5 g lipid) (Carrière *et al.*, 1993)

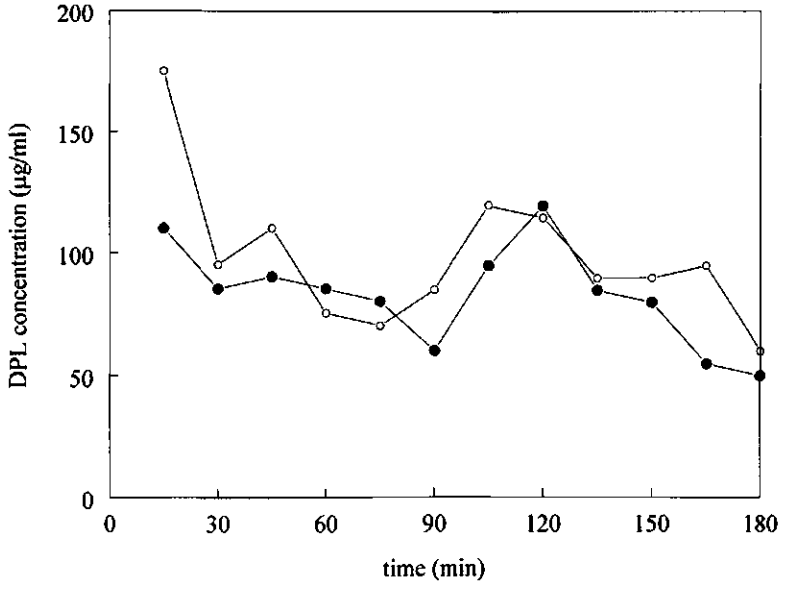

Figure 4 Dog pancreatic lipase (DPL) concentration in duodenal (○) and jejunal (●) contents, obtained from enzymatic activity measurement (Carrière *et al.*, 1993)

Table 7 Composition of dog pancreatic juice as reviewed by Altman and Dittmer (1968)

Property or constituent			Value	
pH			7.1-8.2	
secretion rate			0.2-1.1	ml/min
ash			8400-9700	mg/l
solids:	- total		14000-63900	mg/l
	- organic		4800-22000	mg/l
water			98.04	%
calcium			0.9-1.0	mmol/l
chloride			71-106	mmol/l
magnesium			0.1-1.7	mmol/l
potassium			2.5-7.0	mmol/l
sodium			142-162	mmol/l
bicarbonate			93-143	mmol/l
phosphate			0.4-1.8	mmol/l
glucose			250	mg/l
protein			5000-48000	mg/l
urea			240-585	mg/l
nitrogen	total		1000-9360	mg/l
		protein	748-843	mg/l
		nonprotein	180-840	mg/l
lactate			0.1-0.7	mmol/l
amylase*			23900-47500	mg/l†
lipase*			9.75-33.25	ml 0.05 N NaOH/ml‡
trypsin*			407.5-2440.0	mg tyrosin/ml§

* secretin-stimulated
† starch substrate
‡ olive oil emulsion substrate
§ casein substrate

Table 8 Activity (U/g wet weight) of chymotrypsin in the chyme of adult dogs in relation to the amount of protein in the diet (Kienzle, 1988)

Part of the intestine	Protein content in food dry matter		
	< 25% * n = 10	35-45%† n = 4	> 50% ‡ n = 4
duodenum	3.9 ± 6.2	31.0 ± 26.8	13.8 ± 8.1
jejunum	13.9 ± 15.3	53.1 ± 8.0	84.9 ± 100.1
ileum	20.4 ± 22.1	25.0 ± 8.4§	148.1 ± 99.8

* diets : meat meal with sucrose, meat meal with lactose, dry type of dog food, soya bean meal with tapioca starch
† diets : soya bean meal, soya bean meal with fat
‡ diets : raw meat, raw lungs
§ n = 11

Pancreatic enzymes levels can adapt to the type of food available. This adaptation is a physiological advantage that allows animals to digest food components and energy for metabolism as efficiently as possible (Ballesta *et al.*, 1990).
Besides food, hormones, such as pentagastrin, can also stimulate the exocrine secretion of the pancreas: directly, resulting in a protein-rich secretion and indirectly by virtue of their effect on gastric acid secretion and consequently the release of secretin and cholecystokinin when acid gains access to the duodenum (Gupta *et al.*, 1973).

Bile

Composition of dog bile

Bile is continuously produced in the liver and is partly stored in the gall bladder between meals or between periods of ingestion. In fasting dogs 29 to 53% (median 42%) of newly produced bile is stored in the gall bladder. The remainder is directly released into the duodenum (Rothuizen and de Vries-Chalmers, 1990). Bile is stored in a concentrated form and is actively evacuated into the duodenum in response to the ingestion of a meal (Camello *et al.*, 1994). Therefore bile from the gall bladder differs in concentration from bile directly secreted from the liver (Table 9).

Table 9 Composition of bile secreted from the gall bladder and from the liver of dogs (Nakayama, 1969; Altman and Dittmer, 1968); mean ± SD or range

		Gall bladder	Gall bladder	Liver
secretion rate	mg/kg body wt/24h			12.0 (5.2-52.5)
pH			5.18-6.97	7.1-8.5
dry matter	g/l		114-246	23-45
solid, total		196.6 ± 122.9		
salts	g/l		79-150	36669
calcium	mmol/l		131	1.9-3.6
chloride	mmol/l			70 (59-105)
iodine	g/l		130×10^{-6}-1130×10^{-6} *	
iron	g/ ml		0.9×10^{-3}-1.8×10^{-3}	18×10^{-3}-160×10^{-3}
magnesium	mmol/l			1.8 (1.1-2.5)
total phosphorus	g/ l		0.87- 2.8	0.1- 0.15
potassium	mmol/l			5.1-6.0
sodium	mmol/l			168 (150-203)
bicarbonate	mmol/l			7-34
choline, total	g/l		3.4-11.1	0.39-0.58
cholesterol	g/l	1.37 ± 0.75	0.8-1.0	0.004-0.15
total fatty acids	g/l	0.25 ±0.23	16.0-50.0	1.75-2.70
monoglycerides diglycerides triglycerides		2 ± 3 2 ± 4 0		
total protein	g/l		1.9-5.2	1.3-2.1
total lipid*	g/l	193.5 ± 49.8		
bilirubin	g/l	0.021 ± 0.014	0.92-1.70	0.42-0.55
total bile acids†		37.9 ± 20.6		
phospholipids		20.3 ± 15.4		

* Source of bile not specified

† Sum of cholic, chenodeoxycholic, deoxycholic, lithocholic, hyocholic, and hyodeoxycholic acids

Madrid *et al.* (1983) found, in a study with adult dogs (20-25 kg), comparable results as Altmann and Dittmer (1968) for basal secretion of bile (29 ml/kg/24 h), concentration of bilirubin (195 ± 14 mg/100 ml) and concentration of chloride (70 ± 4.5 mmol/l). Nakayama (1969), Washizu *et al.* (1989) and Wildgrube *et al.* (1986) studied the bile acid composition of gall bladder bile of dogs (Table 10). In the dog the bile acids are almost completely conjugated with taurine. More than 99% of the bile acids are conjugated with taurocholic acid, taurodeoxycholic acid and taurochenodeoxycholic acid.

Gall bladder emptying

In response to food ingestion the gall bladder contracts (Figure 5) and the pressure and the rate of emptying of the gall bladder increase. Emptying peaks are found at 30 min after a meal and the emptying decreases 2 h after food ingestion (Madrid *et al.*, 1983; Traynor *et al.*, 1984). Food is an inducer of circadian rhythms in the gall bladder. These contraction-relaxation cycles are synchronized with periodic dilution-concentration processes (Camello *et al.*, 1991).

The gall bladder empties only partially (5-65%; median 31%) after a meal (Rothuizen and de Vries-Chalmers, 1990). The time course of (partial) postprandial gall bladder emptying is reflected by the parameters of the power-exponential function. Values for the gall bladder half-emptying time $t_{1/2}$ of 47.3 ± 4.7 min and for the curve shape parameter S of 0.866 ± 0.036 were reported by Jonderko *et al.* (1994). During feeding the gall bladder bile concentration shows circadian rhythms peaking immediately before meal time in all biliary compounds (Camello *et al.*, 1991). This finding suggests that periodic food ingestion plays a role in the circadian rhythms of gall bladder bile composition (Camello *et al.*, 1991). The duodenal activity has also an influence on the output of bile components (Table 11). Drugs (Jonderko *et al.*, 1994) and hormones (Rothuizen and Vries de-Chalmers, 1990; Keane *et al.*, 1980; Stevens and Hume, 1995) also can influence the motility.

Table 10 Bile acid composition of gall bladder bile of the dog (% of total bile acid concentration)

Bile acid*	(Washizu *et al.*, 1990)	(Wildgrube *et al.*, 1986)	(Nakayama, 1969)
TUDC	1	-	n.d.
TC	73.2	74.3	n.d.
TCDC	6	5.3	n.d.
TDC	20.1	14.9	n.d.
TLC	0.1	n.d.†	n.d.
C	-	n.d.	77.4
CD	n.d.	n.d.	4.9
DC	n.d.	n.d.	17.7
LC	n.d.	n.d.	-
GUDC	-	-	n.d.
GC	0.4	-	n.d.
GCDC	-	-	n.d.
GDC	n.d.	-	n.d.
GLC	0.1	n.d.	n.d.

* TUDC, tauroursodeoxycholic acid; TC, taurocholic acid; TCDC, taurochenodeoxycholic acid; TDC, taurodeoxycholic acid; TLC, taurolithocholic acid; C, cholic acid; CD, chenodeoxycholic acid; DC, deoxycholic acid; LC, lithocholic acid; GUDC, glycoursodeoxycholic acid; GCDC, glycochenodeoxycholic acid; GDC, glycodeoxycholic acid; GLC, glycolithocholic acid

† n.d. = not determined

Table 11 Bile acid and bicarbonate outputs related to different phases of duodenal activity (Keane *et al.*, 1980)

Mean output (μmol/min)	Phase I*	Phase II*	Phase III*	Phase IV*
Bile acids	0.17 ± 14	23.6 ± 4.6	7.1 ± 1.6	2.7 ± 1.5
Bicarbonate	0	10.6 ± 2.5	21.3 ± 5.2	6.03 ± 5.3

* Phase I = period of complete motor quiescence (no spikes)
Phase II = gradual increase in spiking activity
Phase III = series of strong contractions with spikes on every slow wave
Phase IV = rapid return to quiescence and hence to phase I of a new cycle
(Eeckhout *et al.*, 1984)

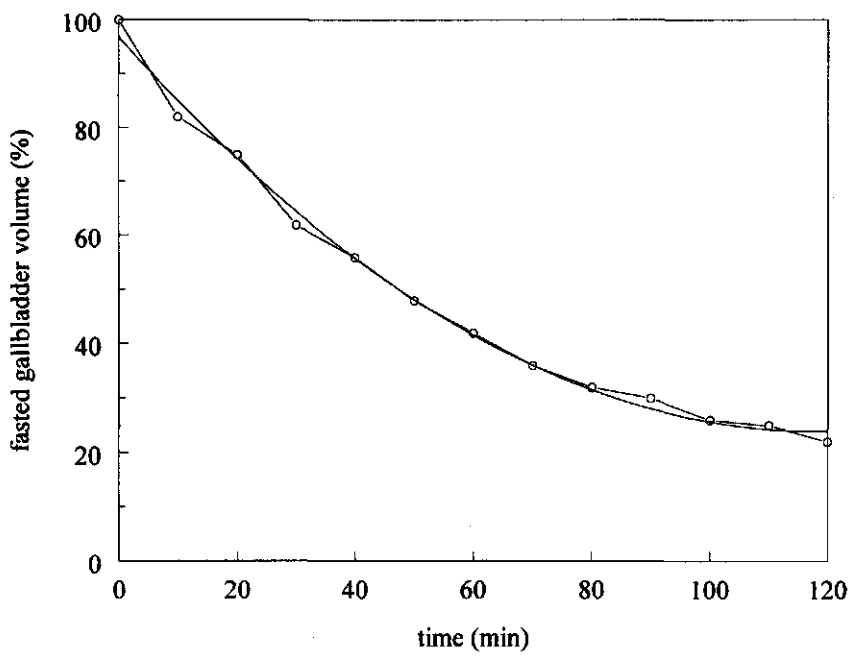

Figure 5 Time course of the meal-induced gall bladder emptying in dogs (Jonderko *et al.*, 1994)

Small intestine

Electrolytes and enzymes

The pH value, concentrations of constituents and activity of some enzymes in different parts of the small intestine are listed in Table 12. The results of the reviews of Altman and Dittmer (1968) and Alexander (1965) both show that there is a large variation between results of different studies.

pH

Digesta are rapidly neutralized as they pass from the stomach into the duodenum. The average pH in the proximal duodenum is 6.2 (6.0-7.2) both for a cereal- and a meat-based diet (Banta *et al.*, 1979). This is much lower than the pH of 8.4 reported by Florey and Harding (1934). The difference probably can be explained by the method used to measure the pH.

Gupta and Robinson (1988) studied the effect of administration of different volumes of water to a fasted dog on pH of the duodenal effluent collected from a permanent Thomas cannula (15 cm distal from the gastroduodenal junction). The mean pH of the duodenal discharge during one activity period without the administration of a test meal was 7.7. The pH was maintained between 7 and 8 after the administration of volumes up to 100 ml. This compares well with the results of Lui *et al.* (1986) who found a maximum pH of 7.7 at 20 min after gastric empyting. The pH gradually declined thereafter (pH 7.2 at 180 min after empyting). The overall mean intestinal pH was 7.3 ± 0.09. However, a reduction in pH of the effluent was seen as the volumes were increased to 100 ml or more. The range of pH for volumes up to 100 ml was 4.3 to 8.3 whereas it was 1.5-8.3 for 150 ml and more. From these results it can be concluded that the pH of the duodenal effluent in dogs indirectly depends on the volume of water ingested. Large volumes apparently induce acid secretion in the stomach (by stimulation of gastrin release) and thus lower the pH to values as low as 1.5 in the lumen of the proximal duodenum. The movement of acid chyme from the stomach into the small intestine stimulates the secretion of pancreatic juice into the duodenum. The large amount of bicarbonate in pancreatic juice and bile accounts for the increase in pH of digesta passing from the stomach to the duodenum (Banta *et al.*, 1979). Figure 6 shows the pH of the intestinal contents of beagles fitted with chronic duodenal and jejunal fistulae, inserted opposite to the biliary canal and 30 cm below the angle of Treitz, respectively (Carrière *et al.*, 1993).

Table 12 Composition of gastrointestinal content presented as mean and/or range (in parenthesis) in the duodenum, jejunum and ileum

		Duodenum*	Jejunum†	Ileum‡	Reference§
pH		84	6.8 (6.3-7.2)	(7.6-8.7)	1
inorganic matter	mg/g	926	-	-	1
organic matter	mg/g	615	-	-	1
solids, total	g/l	15.41 mg/g	17 (12-23)	(13-18)	1
calcium	mmol/l	-	1.4 (0.8-2.7)	(2.5-2.8)	1
magnesium	mmol/l	-	0.6 (0.1-1.0)	-	1
chloride	mmol/l	136 (130-140)	147 (141-153) 61 ± 3.4	78 (68-88) 101 (98-104) 54 ± 5.0	112
sodium	mmol/l	145 (136-150)	141 (126-152) 75 ± 2	151 (146-156) 109 ± 8	12
potassium	mmol/l	6.3 (4.5-8.0)	6.3 (4.2-10.2) 59 ± 3.5	4.7-6.8 44 ± 4.3	12
bicarbonate	mmol/l	17 (14-22)	22 (5-30) 20 ± 3.8	92 (70-114) 28 ± 4	12
phosphate	mmol/l	-	1.6 (0.6-4.0) 44.5 ± 5.5	- 41.5 ± 6.0	12
pepsin	units/ml	15	-	-	1
lipase (as fatty acid)	g/l	-	61 (49-74)	22 (18-29)	1
amylase (as reducing sugar)	g/l	-	600 (500-680)	240 (200-300)	1

* Brunner's glands and and duodenal mucosa fistula

† isolated loop or fistula ; during fasting

‡ loop or transplant; during fasting

§ 1 = Altman and Dittmer (1968); 2 = Alexander (1965)

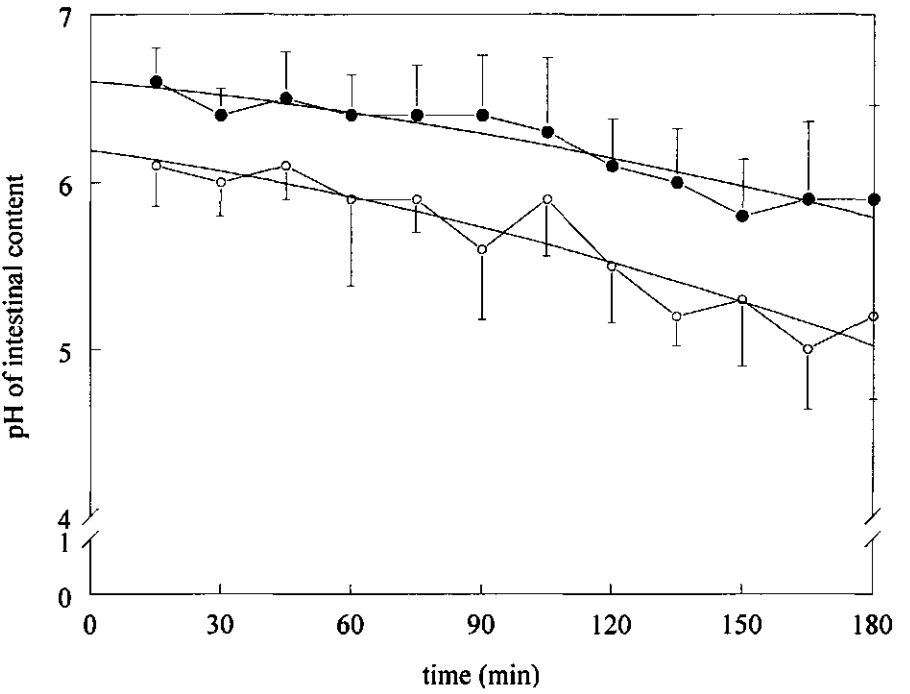

Figure 6 Curvefitted pH curves of the duodenal (○) and jejunal (●) contents measured in fistulated beagles (Carrière *et al.*, 1993)

Absorption

One of the main functions of the small intestine is the absorption of digested products. The absorption of water and electrolytes from the jejunal lumen has been studied in dogs using a 25 cm proximal jejunal Thiry-Vella loop and 400 g of a standard mixed canine meal (52% protein, 36% fat, 12% carbohydrates). In the basal period the F_{H2O} (flux of water) averaged 171 ± 44 µl/min, the net F_{Na+} averaged 20 ± 6 µmol/min and the F_{Cl-} averaged 10 ± 5 µmol/min (Yeo *et al.*, 1990). After ingestion of a standard mixed meal there was a significant increase ($P < 0.0001$) in the net absorption of water and electrolytes at all postprandial observations, peaking at 75 min after a meal. The maximum ΔF_{H2O} at 75 min after a meal was 206 ± 33 µl/min, while the maximum ΔF_{Na+} and ΔF_{Cl-} were 25 ± 4 and 17 ± 3 µmol/min, respectively (Yeo *et al.*, 1990).

This is in contrast with the results of Hakim *et al.* (1992) who studied the net absorption of water, glucose, electrolytes and folate in a 80 cm modified Thiry -Vella loop of the proximal

jejunum. The enteric neural continuity with the duodenum and proximal jejunum was maintained. All effluent was collected from the jejunostomy at 15 min intervals to determine net absorption during a fasting state and a fed state (meal of 500 g porcine liver) (Table 13).

Table 13 Recovery of test substances in effluent* of the proximal jejunal loop determined in 15 min interval samples after infusion of an electrolyte solution† (Hakim *et al.*, 1992)

Parameter	Amount infused	Median	Range
Fasting state			
H_2O (ml)	42	29.1	24.5-29.6
sodium (mmol)	5880	3590	3460-4200
potassium (mmol)	210	162	144-179
chloride (mmol)	5250	3710	3460-4150
glucose (µg)	42	2.3	1.31-3.80
folate (%)	-	66	35-77
$t_{1/2}$ (min)	-	5.6	4.0-8.0
Fed state			
H_2O (ml)	42	24.2	22.8-24.8
sodium (mmol)	5880	3590	3460-4200
potassium (mmol)	210	151	130-157
chloride (mmol)	5250	3710	3455-4150
glucose (µg)	42	1.21	0-2.47
folate (%)	-	54	31-66
$t_{1/2}$ (min)	-	7.3	6.1 -8.0

* six dogs, three experiments per dog at each time point

† isosmolar solution containing NaCl (120 mmol/l), $NaHCO_3$ (20 mmol/l), KCl (5 mmol/l), glucose (5.6 mmol/l), ^{3}H-folic acid (10 µCi/l), unlabelled folate, 1.5 µg/ml, and 5 g/l PEG labelled with ^{14}C-PEG (5 µCi/l)

When net absorption of water during fasting was compared to the absorption after feeding at different times, no statistically significant differences were detected ($P > 0.05$). However a tendency towards absorption of more infusate after feeding was noticed as indicated by median values. Similar results were found with sodium, potassium, chloride, glucose and folate.

Meyer *et al.* (1989) studied the absorption of water, sodium and potassium in the small intestine in ileal fistulated dogs during 25 feeding trials. Water was always absorbed in the small intestine at a rate of between 15 and 65 ml/kg body weight or 30 to 90% of the water intake. Sodium absorption in the small intestine generally corresponded with sodium intake. The absorption of potassium is strongly correlated to potassium intake. Sodium and potassium had an absorption of ca. 90% of the intake or more. Only with some specific foods (e.g. tapioca starch) absorption was less. The sodium concentration in the ileum varied from 0.3-3.0 g/kg chyme. The potassium concentration varied from 0.3-0.5 g/kg chyme. The sum of both ions reached a value of 90-130 mmol in the ileal chyme.

Intestinal transit

The movement of digesta along the gastrointestinal tract is regulated via structural and physiological properties of the digestive tract. It is also influenced by physical as well as nutritional characteristics of the diet (Clemens and Stevens, 1980).

Chyme is usually propelled through the small intestine in a distal direction, mainly by the direction of propagation of the small intestinal pacesetter potentials (Soper *et al.*, 1990). The motility of the intestine can be distinguished in different phases (Sarr and Kelly, 1980) and can be influenced by several factors, such as food components, hormones and the nervous system (Bueno *et al.*, 1981; Berhns and Sarr, 1994; Dreznik *et al.*, 1994; Eeckhout *et al.*, 1984; Neri *et al.*, 1991; Bueno *et al.*, 1987).

Miyabayashi *et al.* (1986) studied the small intestinal transit and emptying time in dogs given orally 60% w/v barium sulphate solution. In this study small intestinal transit time was determined by identifying the head of contrast medium column within the cecum or ascending colon. The small intestinal emptying time was defined as the passage of the contrast medium into the cecum and colon. Sequential radiographs were made every 30 min. A transit time of 73.0 ± 16.4 (range 30-120) min was found and a small intestinal emptying time of 214.0 ± 25.1 (180-300) min ($n = 5$, three samples/dog). More frequent measurements would produce shorter times, since the experimental design allowed sampling errors of 29

min. The results suggest that both small intestinal emptying time and transit time correlate positively with gastric emptying time.

Banta *et al.* (1979) used PEG and ^{51}Cr-labelled EDTA as a fluid and a solid marker, respectively, to determine the passage of liquids and particles in a meat diet through the gastrointestinal tract (Figure 7). The fluid marker passed rapidly through the stomach and small intestine regardless of the diet. In dogs fed a cereal diet the distribution of the marker after 4 h was ca. 25% in the stomach, 45% in the small intestine and 30% in the colon. Species with a simple stomach and a short, non-sacculated, non-voluminous colon, such as the dog, display no selective retention relating to the size of the particles. This means that no differences exist in the rate of transit between the different particles sizes of the marker (Clemens and Stevens, 1980).

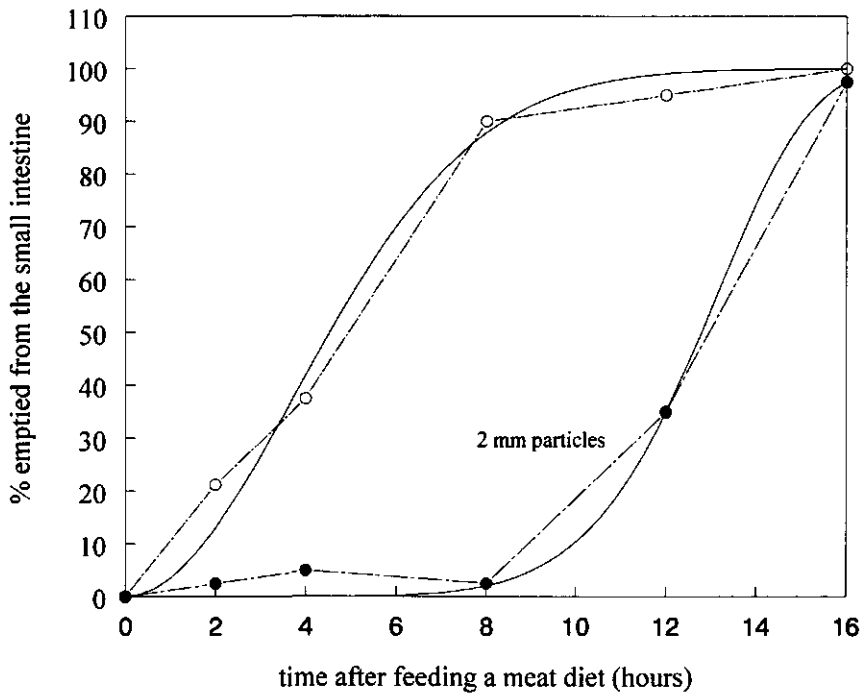

Figure 7 Percentage of liquids (○) and solids (●; 2 mm particles) emptied from the small intestine in a given time after feeding a meat diet (Banta *et al.*, 1979)

To determine the transit time of canned dog food the concentrations of labelled markers can be measured in ileal or jejunal samples using liquid scintillation spectroscopy (Tables 14 and 15) (Bueno *et al.*, 1981; Neri *et al.*, 1991).

Besides radiography, scintigraphy and radiopaque plastic markers, the oro-caecal transit time can be assessed by ingestion of an unabsorbable but fermentable carbohydrate in a test meal and determining the time needed for a sustained increase in exhaled hydrogen (Papasouliotis *et al.*, 1993). In a study by Papasouliotis *et al.* (1993), the median oro-caecal transit times were 105 min (range 45-135 min) for a standard meal of canned food, 113 min (range 53-203 min) for a standard meal with the addition of wheat bran and 105 min (range 75-195 min) after addition of guar gum. A possible explanation for the difference between the study of Bueno *et al.* (1981) and that of Papasouliotis *et al.* (1993) maybe the amount of fibre added to the diets: Bueno *et al.* (1981) added 30 g of fibre and Papasouliotis *et al.* (1993) added 7.7 g of fibre. The amount of fibre can influence the viscosity of the meal, which in turn influences the transit time.

The results of transit experiments can be misleading for two reasons, the single value ($t_{1/2}$) (1) in no way indicates how the bulk of material moved through the gastrointestinal tract and does not (2) consider the structure, volume or length of the digestive tract (Clemens and Stevens, 1980).

Table 14 Transit time and flow rate of digesta through a 200 cm jejunal segment from 2 to 4 h after feeding 500 g of a standard diet, or mixed with 30 g of bran, cellulose or gum (mean ± SD, n=6) (Bueno *et al.*, 1981)

	Control	Bran	Cellulose	Gum
Transit time* (min)	9.5 ± 2.7	12.2 ± 4.3	82.3 ± 10.3†	14.3 ± 3.6†
Flow rate of digesta (ml/h)	172 ± 41	161 ± 34	55 ± 16†	285 ± 54†

* retention volume calculated from the flow rate (ml/min) of digesta and the mean transit time (min)

† significantly different ($P < 0.05$) from control values

Table 15 Half-emptying time of the small intestine (min) in the fasting state, early postprandial state and late postprandial state (Neri *et al.*, 1991)

$t_{1/2}$	Fasting	EPP*	LPP*
ileum-ileum‡	16.3 ± 4.6	8.7 ± 2.7	5.1 ± 0.7†
ileum-colon‡	44.8 ± 6.7	25.2 ± 8.8	9.6 ± 3.4†

* EPP = early postprandial period, which extend from ingestion of the meal to its arrival in the terminal ileum
LPP = late postprandial period, which extend from the arrival of the meal in the terminal ileum to the end of the study (4 h after ingestion of the meal)

† $P < 0.05$ vs fasting

‡ ileum -ileum = from the infusion catheter 32 cm proximal to the ileocolonic sphincter (ICS) to the aspiration catheter 1 cm proximal to the ICS
ileum-colon = from the infusion catheter 32 cm proximal to the ICS to the cannula 5 cm distal to the ICS in the proximal colon

CONCLUSIONS

In order to develop *in vitro* systems for simulation of digestion it is necessary that the digestive processes take place in an environment which simulates these processes as measured *in vivo*. Therefor we gathered literature on various aspects of the physiology of the stomach and small intestine of the dog. In this respect it is important to note that literature on physical conditions and rate of passage is not unanimous. Between studies variation was noted in relation to diets fed, to the type of dog and to the experimental methods.

Parameters needed for simulation of digestion are transit times, pH, composition of the secretions and the rate of secretion in the different parts of the gastrointestinal tract. Based on the data in literature it can be concluded that the concentration of electrolytes in saliva and gastric juice are in the same range and that dogs saliva does not contain amylase. This means that for the secretion in the model the secretion of saliva and gastric juice can be combined. So by mimicking this by the computer program physiological conditions can be derived. Gastric juice should consist NaCl, KCl , $CaCl_2.2H_2O$, $NaHCO_3$ and the enzymes lipase and pepsin.

The mean secretion rate of gastric juice from literature is at a rate about 0.25 ml/min. To mimic the pH in the stomach the pH-curve found by Carrière *et al.* (1993) can be used.

Transit of the chyme through the stomach can be derived using the equation described by Elashoff *et al.* (1982). Physiological values for $t_{1/2}$ and ß should be 90 and 1, respectively.
For mimicking the secretion of pancreatic juice and bile porcine pancreas and bile extracts can be used. Besides pancreatic juice and bile also an electrolyte solution has to be added into the duodenum, consisting of NaCl, KCl and $CaCl_2.2H_2O$. Based on the literature the rate of secretion of pancreatic juice and the electrolyte solution should be in the same range as the secretion rate of the stomach juice. However the secretion of bile has to be higher than that of pancreatic juice (0.5 ml/min).
Based on the literature the pH in the small intestine can be set at 6.2 in the duodenum, 6.5 in the jejunum and 7.0 in the ileum. If these values can be mimicked in the model the condition may mimic the *in vivo* situation. Transit of chyme through the small intestine can be derived with a similar equation as used for transit through the stomach. Correct parameters to be used for the small intestine will be $t_{1/2}$ about 270 min and ß about 2.
In conclusion the literature gives sufficient data for the specific development of a dynamic model of the canine gastrointestinal tract on the basis of the general concept of the gastrointestinal tract model developed at TNO Nutrition and Food Research. A rather reliable composition of artificial saliva, gastric juice, bile and pancreatic juice can be made for secretion in the laboratory model of the canine gastrointestinal tract. Also the values derived for gastric and intestinal transit and for pH of the dog can be used to simulate the physiological situation when using the model as described. Although most parameters are influenced by factors such as meal components, hormones and the nervous system and although a large variation exists between individuals, a selection of these data can be made, within the physiological range, to mimic the parameters which are needed for the development of an *in vitro* dog model.
Based on these literature data and pilot experiments a protocol was set up and the computer programme was adapted to simulate the physiology of the dog. Preliminary results showed that successive physiological parameters, such as gastric and intestinal pH (Figure 8) and gastric emptying (Figure 9), could be mimicked.

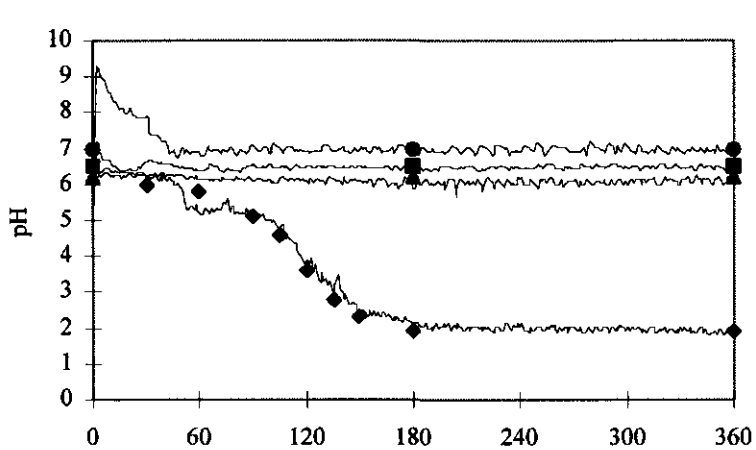

Figure 8 Comparison of the pH setpoints (markers) in the gastric and small intestinal compartments and the actual pH values in the canine model during an experiment with dry dog food

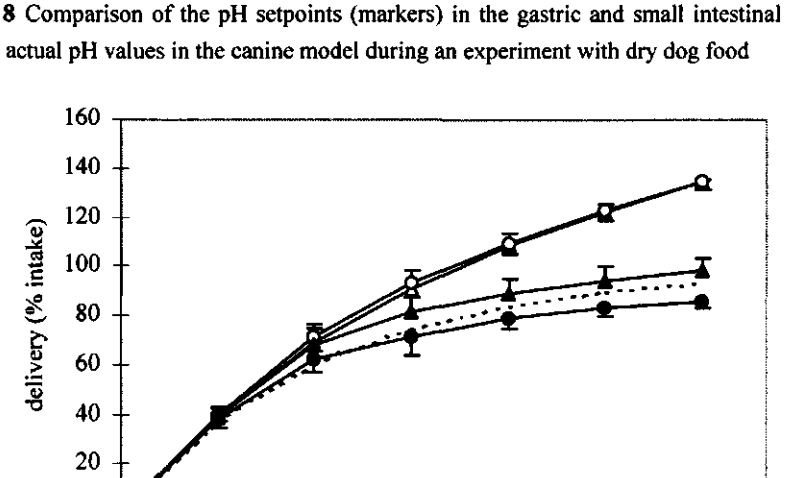

Figure 9 Comparison of the theoretical curve based on the equation for *in vivo* gastric emptying ($t_{1/2}$ = 90 and ß = 1; dashed line) and the actual emptying rate of fresh matter (= food + secretions, open markers) and dry matter (closed markers) of the gastric compartment during an experiment with dry dog food ground at particle sizes smaller than 3 mm (●) and smaller than 1 mm (▲)

3 DESCRIPTION OF A DYNAMIC *IN VITRO* MODEL OF THE DOG GASTROINTESTINAL TRACT AND AN EVALUATION OF VARIOUS TRANSIT TIMES FOR PROTEIN AND CALCIUM

Marianne Smeets-Peeters[1,2], Mans Minekus[1], Robert Havenaar[1], Gertjan Schaafsma[3], Martin Verstegen[2]

1 TNO Nutrition and Research, PO Box 360, 3700 AJ Zeist, The Netherlands

2 Wageningen University, Department of Animal Nutrition, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

3 Wageningen University, Department of Human Nutrition and Epidemiology, PO Box 8129, 6700 EV Wageningen, The Netherlands

Alternatives to Laboratory Animals (ATLA) **27**, 935-949, 1999

ABSTRACT

In order to manufacture complete and balanced dog diets it is important to know the nutrient requirements of dogs and the availability of nutrients. Because petfood manufacturers are restricted their options for (invasive) animal studies, due to ethical constraints, it is important to have alternatives for research on dog diets.

To simulate the gastrointestinal tract of the dog, the dynamic gastrointestinal tract model as described by Minekus *et al.* (1995) was further developed and modified. The model consists of four compartments simulating the stomach and small intestine (duodenum, jejunum and ileum). Each compartment is made of glass with inside a flexible wall which is squeezed by increasing the pressure of the water surrounding this flexible wall. In this way peristaltic movements and mixing are mimicked. The model is computer-controlled to simulate physiological parameters, such as pH, transit time and secretion of digestive juices, as derived from the literature.

To evaluate the model, gastric meal delivery as well as the effect of intestinal transit time on protein digestibility and availability for absorption of calcium in dog food were studied. The gastric meal delivery of dry dog food was identical to a preset curve, which was based on *in vivo* data of healthy dogs. Emptying time for canned dog food was somewhat slower then the preset values, probably due to the viscosity of the meal. The differences between the preset values and the measured delivery were not significantly different. The digestibility of protein and the availability for absorption of calcium increased with increasing the transit time in the model. A significant difference was found between medium and slow transit time for the nitrogen content in the ileal delivery effluent and the jejunal dialysates ($P < 0.05$). The same trend was seen for calcium (not significant). The overall conclusion is that the model is a useful tool for mimicking the gastrointestinal tract of dogs. Parameters such as pH, transit time and enzyme activities can be mimicked and kept within a physiological range.

INTRODUCTION

In dog food manufacturing the nutrient requirements of the dog and the availability of nutrients from the food must be taken into account. Information about the digestibility of the food and the availability of nutrients is essential to estimate the nutritional quality of food (Boisen and Eggum, 1991).

Digestion of food is a complex process which is affected by the chemical structure of nutrients in combination with the biochemistry and physiology of the digestive tract (Savoie, 1994). Besides the digestibility, the availability for absorption of nutrients is also determined by interactions between food components, which may have positive or negative effects on the intestinal absorption of nutrients. Thus, research on the digestibility of food and the availability for absorption of nutrients is a basic prerequisite for being able to manufacture complete and balanced diets for animals. For such research it is important to have predictive *in vitro* systems as an alternative to animal studies.

In vitro models can have advantages over *in vivo* experiments. In general, *in vitro* experiments are relatively easy to perform and less expensive. Besides, these models do not have ethical limitations which is an important factor for petfood manufacturers who are restricted in their options for (invasive) animal studies.

However, for a high extrapolation value, several aspects have to be taken into account in *in vitro* models. An *in vitro* model should simulate (a) a sequential use of digestive enzymes in physiological amounts, (b) an appropriate pH for the enzyme activities and addition of relevant co-factors such as bile salts and co-enzymes, (c) appropriate mixing at each step of digestion, (d) physiological transit times for each digestion step and (e) removal of digestion products (Longland, 1991). Hardly any of the models published so far meets all of these requirements (Boisen and Eggum, 1991). Especially the transit time of the food is often not taken into account.

Gastrointestinal models for man and pigs that do fulfil these prerequisites have been described by Minekus *et al.* (1995), Minekus (1998) and Minekus and Havenaar (1996, 1998). These models offer perspectives for the development of a gastrointestinal model for the dog. The aims of the present study were to develop such a model, to evaluate its functioning and to validate it from a technical point of view. Experiments were performed to determine gastric meal delivery with dry and canned dog foods. Also the effect of transit time of canned dog food through the small intestine on protein digestibility and the availability for absorption of calcium was studied. Several physiological aspects were taken into account in the development and validation of the dog model, such as pH, gastric and intestinal transit time and composition of the digestive juices.

MATERIALS AND METHODS

The gastrointestinal model

The dynamic *in vitro* gastrointestinal model (Figure 1 and 2; see Figures 1a and 1b Chapter 2) consists of four consecutive compartments, simulating the stomach, duodenum, jejunum and ileum. Each compartment is formed by two glass jackets with a flexible silicone wall inside. The flexible wall is surrounded by water which keeps the temperature at 37 °C. By increasing the water pressure the flexible walls are squeezed, thus simulating the peristaltic movements of the gastrointestinal tract. Transport of the food is regulated by a computer which calculates, on the basis of a preset gastric and ileal delivery curves, the amount of food to be transported in time by means of peristaltic valve systems between the different compartments. This peristaltic valve system has been described by Minekus *et al.* (1995). Besides the transit time of food and chyme, also the pH and secretion of electrolytes and enzymes are controlled by a computer. The pH is measured every minute and, when necessary, HCl is secreted into the stomach to decrease the pH, and $NaHCO_3$ is secreted in the compartments of the small intestine to hold the pH on the set-point levels. By means of semi-permeable hollow-fibre units (Cobe HG-400, Secon, Germany) connected to the jejunal and ileal compartments, absorption of the small-molecular-weight digestive products is mimicked.

MATERIALS AND METHODS

Transit time and pH profiles

To control the delivery of the meal from the stomach and ileal compartments, the equation described by Elashoff *et al.* (1982) was used:

$$f=100*(1-2^{-(t/t_{1/2})^{\beta}})$$

where f is the percentage of food delivered, $t_{1/2}$ is the time (min) after ingestion of the meal until 50% of the meal has been delivered and β determines the shape of the curve (e.g. lag phase). The parameters used to control gastric delivery are based on *in vivo* gastric delivery data on canned and dry dog foods (Banta *et al.*, 1979; Cullen and Kelly, 1996; Gué *et al.*, 1988), $t_{1/2}$ = 90 min and β = 1. The rate of gastric delivery was the same in all experiments.

In the small intestine three different transit times were mimicked representing a fast ($t_{1/2}$ = 210, β = 2.5), a medium ($t_{1/2}$ = 255, β = 2.0) and a slow ($t_{1/2}$ = 300, β = 2.5) transit of the food (Figure 3). The slow transit is comparable to the average physiological situation in healthy dogs after the ingestion of canned and dry dog foods (Banta *et al.*, 1979).

Based on literature data reviewed by Smeets *et al.* (1998), a pH profile was programmed to mimic the average physiological decrease in pH in the stomach after a meal (Carrière *et al.*, 1993). The set-points of the pH profile in the gastric compartment are shown in Figure 4. The pH in the different compartments of the small intestine was controlled at an average physiological level of 6.2 ± 0.2, 6.5 ± 0.2 and 7.0 ± 0.2 for the duodenal, the jejunal and the ileal compartment, respectively.

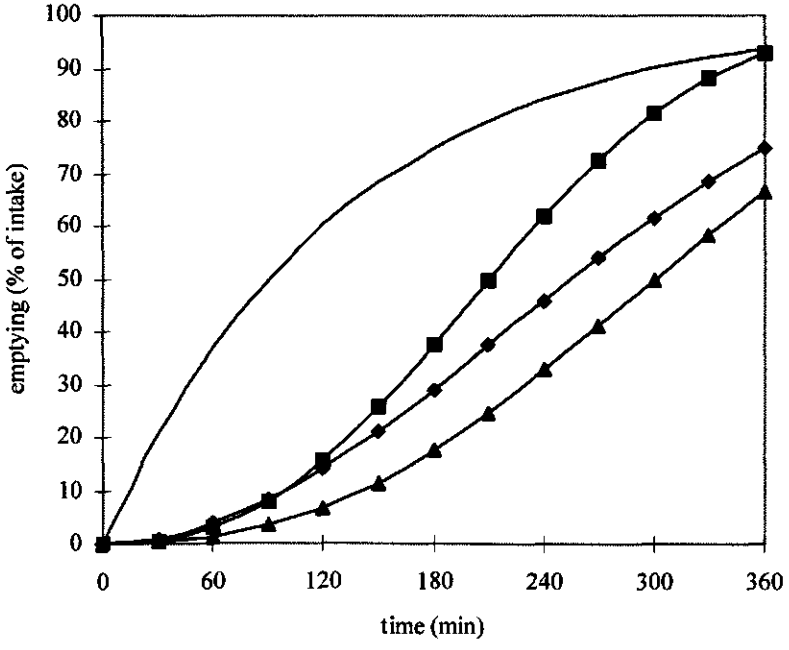

Figure 3 Preset values for gastric delivery (without marker) and ileal delivery curves (▲: slow, $t_{1/2}$ = 300 min, β = 2.5; ◆: medium, $t_{1/2}$ = 255 min, β = 2.0; ■: fast, $t_{1/2}$ = 210 min, β = 2.5). Markers do not represent measured values

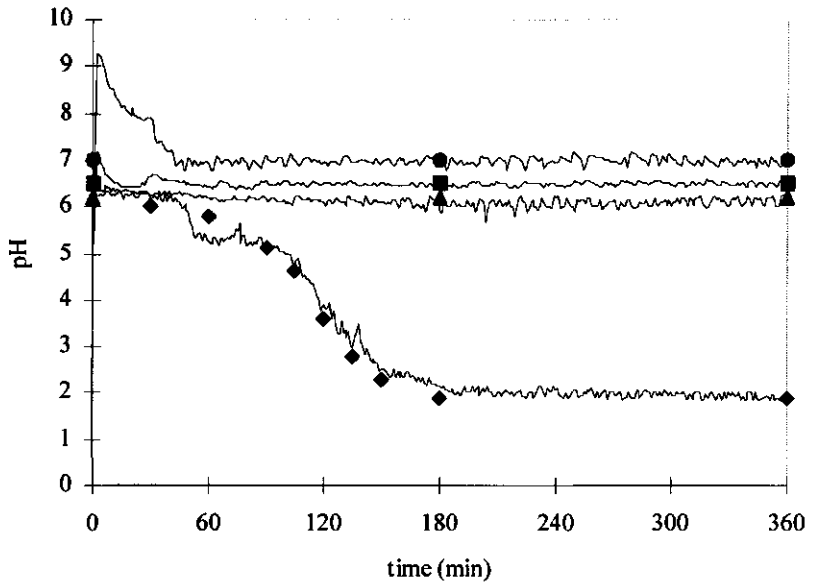

Figure 4 pH values in the different compartments of the gastrointestinal model. Set-points of the gastric compartment (◆), the duodenal compartment (▲), the jejunal compartment (■), and the ileal compartment (●). The lines through these markers represent the measured pH in the corresponding compartments during an experiment

Secretions

The composition of the different digestive juices are listed in Table 1. The solutions were prepared with in demineralized water. Based on literature (Altman and Dittmer, 1968) it was concluded that porcine and bovine products were good alternatives to canine digestive juices. The secretion rate for the juices was 0.25 ml/min, except for bile which had a secretion rate of 0.50 ml/min (Gürtler, 1967).

To mimic a physiological situation of the residues left from previous meals, the stomach and small intestine were filled with residues at the beginning of the experiments (Table 2).

Table 1 Composition and secretion rate of the digestive juices used in the *in vitro* gastrointestinal dog model

Juice	Composition* (g/l)	
Saliva	combined with gastric juice	
Gastric juice I	NaCl	3.5
	KCl	1.3
	$CaCl_2.2H_2O$	0.2
	$NaHCO_3$	0.25
	Lipase	0.5
	NaCl	3.5
Gastric juice II	KCl	1.3
	$CaCl_2.2H_2O$	0.2
	$NaHCO_3$	0.25
	Pepsin	0.15
Small intestinal electrolyte solution	NaCl	7.0
	KCl	0.5
	$MgCl_2.2H_2O$	0.53
Bile solution	Bile powder	60
Pancreatic juice †	Pancrex powder	10

* Nace, $CaCl_2.2H_2O$, $MgCl_2.6H_2O$, $NaHCO_3$ (Merck), and KCl (BDH), pepsin (from porcine stomach mucosa 2100 U per mg solid, Sigma), lipase (150,000 U/g, Rhizopus lipase F-AP 15, Amano Pharmaceuticals), trypsin (type III bovine pancreas, 12,700 U per mg solid, Sigma), porcine bile (Sigma) and pancreatin (Pancrex V Powder, Paines and Byrne, Greenford, UK)

† The pancreatic powder was dissolved in demineralized water, stirred for 20 min and centrifuged for 20 min at 4 °C at 9000 r.p.m. The supernatant was used as pancreatic juice

Table 2 Composition of the starting residues in the different parts of the gastrointestinal model at the beginning of the experiment

Compartment	Composition and amount of the residue
stomach	5 ml of gastric juice I and 5 ml of gastric juice II (pH 1.9)*
duodenum	15 ml bile solution*
	7 ml electrolyte solution*
	7 ml pancreatin solution*
	1 ml trypsin solution (200 mg/l)
jejunum	115 ml small intestinal electrolyte solution*
ileum	115 ml small intestinal electrolyte solution*

* See Table 1 for the composition of the solutions

Test meals

The amount of food and drinking water used in the model was 300 g with a dry matter content of 15%. To obtain this level the food was diluted with demineralized water.

To study the gastric delivery in the *in vitro* model, dry dog food (moisture 80, protein 260, fat 160, fibre 25, and ash 70 g/kg) and canned dog food (moisture 625, protein 65, fat 108, fibre 3 and ash 22 g/kg) were used. The dry dog food was ground in two different particle sizes: ≤1 mm and ≤3 mm. These particle sizes were based on the fact that food particles smaller than 2 mm will pass the pyloric sphincter *in vivo* (Cullen and Kelly, 1996; Meyer *et al.*, 1958; Burrows *et al.*, 1985). Another canned dog food (moisture 771, protein 65, fat 15, fibre 4, and ash 20 g/kg) was used to study the effect of intestinal transit time on the digestibility of protein and the availability for absorption of nitrogen and calcium.

Sampling and analyses

To study the gastric delivery, the total delivery was collected every hour after the peristaltic valves of the stomach (pylorus). These samples were weighed to determine fresh matter (food + salivary and gastric secretion) delivery. To determine dry matter delivery the samples were dried in an oven at 80 °C until no further loss of weight was detected.

To study the effect of transit time on the delivery and digestibility of protein and the availability for absorption of nitrogen and calcium, the total ileal delivery was collected every hour. The dialysis fluids from the jejunal and ileal compartments were collected every

two hours. At the end of the experiment the residual content of the pooled gastric and duodenal compartments and of the pooled jejunal and ileal compartments were collected. To determine the total amount of introduced nitrogen and calcium, samples were taken from the food, bile and pancreatin solution. The amount of nitrogen and calcium in the other solutions was negligible and therefore not analysed. After the experiment the model was rinsed with 0.2 M HCl to collect all the residual calcium. Based on the analyses of these samples a mass balance and the recovery of nitrogen and calcium were calculated.

All samples were frozen at -20°C until analysis. The amount of nitrogen was determined according to the Kjeldahl method. Calcium was analysed by atomic absorption spectroscopy after pooling the colonic, jejunal and ileal dialysate samples.

Calculations and statistics

Recovery of the nitrogen and calcium was calculated by the formula:

$$\text{recovery } (\%) = (N_{digesta}/(N_{food} + N_{secretions})) \times 100$$

where $N_{digesta}$ is the total amount of nitrogen or calcium in the samples collected, N_{food} is amount of nitrogen or calcium in the food and $N_{secretions}$ is the amount of nitrogen or calcium in the bile and pancreatin solution.

The data were evaluated using the F-test and Student's *t*-test. The level of significance was set at $P < 0.05$.

RESULTS

pH control

The measured pH values followed the preset profile for the stomach and the different parts of the small intestine (Figure 4). The results of only one experiment are shown because the pH curves of the other experiments were similar.

Gastric delivery

The average cumulative delivery of fresh and dry matter of dry dog food is shown in Figure 5. Because the delivery is expressed as a percentage of food ingestion, fresh matter (food + secretion) delivery is above 100%.

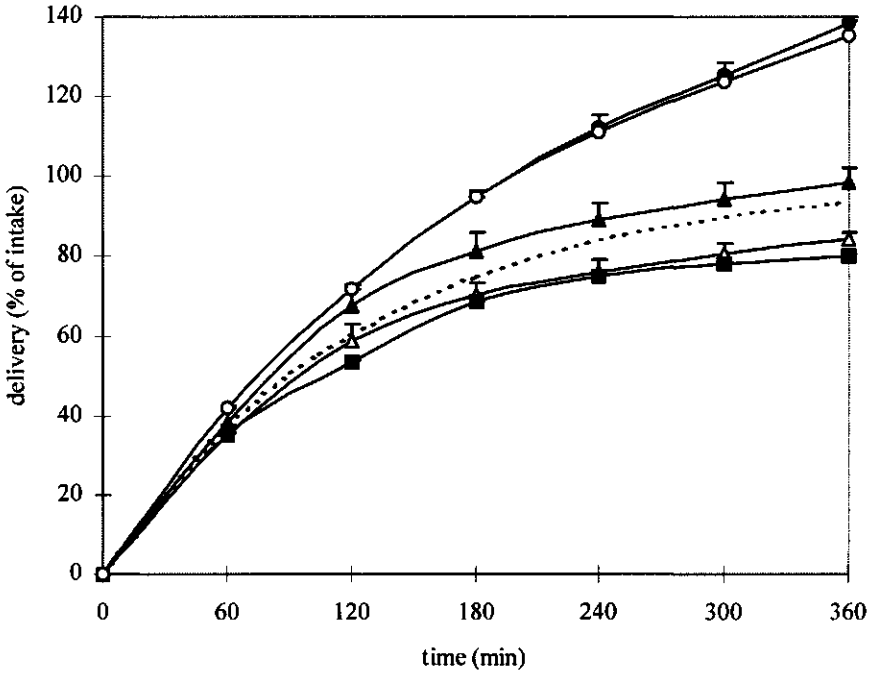

Figure 5 Gastric meal delivery (mean ± range, $n = 2$) for fresh matter (food + gastric secretions) and dry matter of dry dog food ground at particles ≤1 mm or ≤3 mm and of canned food in comparison with the average *in vivo* physiological gastric emptying of the dog (dashed line = preset gastric delivery curve; ● = fresh matter emptying of canned dog food; ○ = fresh matter emptying of dry dog food; ■ = dry matter emptying of canned dog food; ▲ = dry matter empyting of dry dog food, particles ≤ 1 mm; △ = dry matter emptying of dry dog food, particles ≤ 3 mm)

The results show that the dry food emptied according to the preset delivery curve. For food ground at particles ≤1 mm, the delivery seemed somewhat faster than the preset curve, whereas food ground at particles ≤3 mm emptied at a somewhat slower rate. The canned dog food emptied at a slower rate than the preset values. The results were very reproducible, but the meal delivery was not significantly different from the preset gastric delivery curve.

Protein delivery and digestibility

Nitrogen recovery in the different experiments was 96.1 ± 5.2%. Figures 6 and 7 show the results of the ileal delivery of nitrogen per hour and cumulative values, respectively. Most food was transported to the end of the ileum with the fast transit time (Figure 7). An equal amount of nitrogen was delivered from the 'ileo-caecal valve' (Figure 1; see Figure 1a Chapter 2) after 120 min when simulating a fast and a medium transit time (Figure 7). The overall cumulative ileal delivery of nitrogen was highest with the fast ileal delivery curve and lowest with the slow ileal delivery curve.

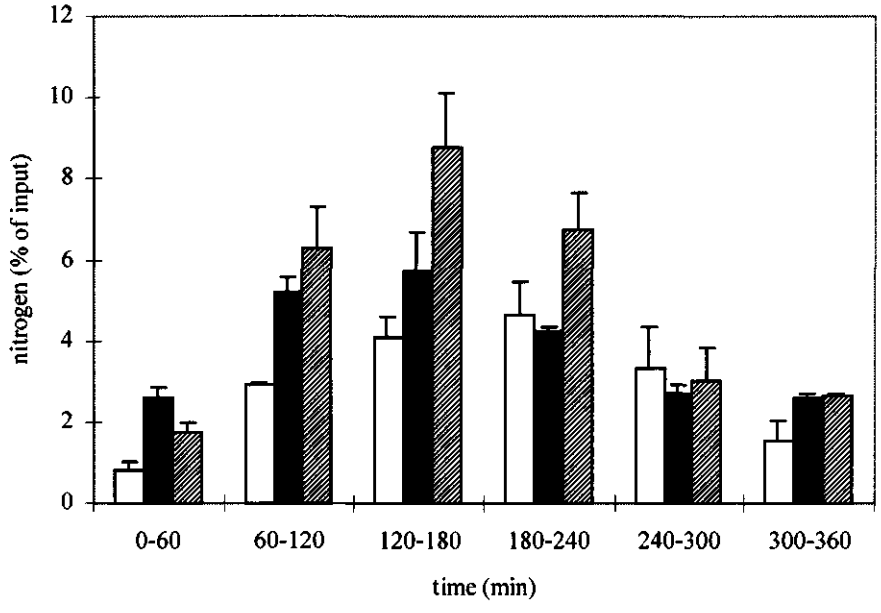

Figure 6 Average amount (± range, $n = 2$) of nitrogen in the samples collected per hour from the 'ileo-caecal valve' in experiments with different small intestinal transit times (white column, slow transit; black column, medium transit; hatched column, fast transit)

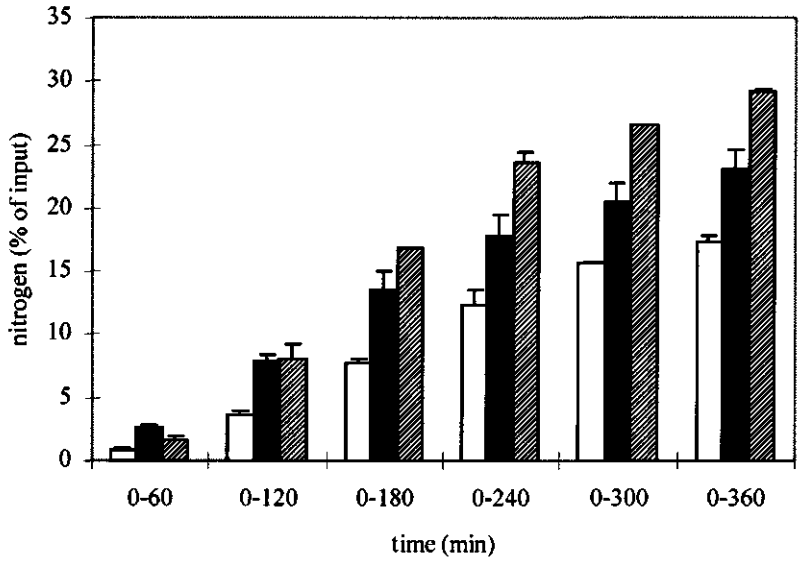

Figure 7 Average cumulative amount (± range, $n = 2$) of nitrogen in the samples collected per hour from the 'ileo-caecal valve' in experiments with different small intestinal transit times (white column, slow transit; black column medium transit; hatched column, fast transit)

Most of the nitrogen was absorbed from the jejunal compartment (Figure 8). There is a trend towards more absorption of nitrogen in the dialysate in the experiments with the slow delivery curves than with the medium and fast delivery curves.

The overall results of these experiments (Figure 9) show that the faster the ileal delivery the more nitrogen was delivered from the 'ileo-caecal' valve and the less is absorbed in the dialysis fluid. This finding is consistent with the preset delivery curves. The amount of nitrogen in the residues after 6 h was similar in all experiments, irrespective of the intestinal transit time.

The digestibility of protein was defined as the amount of nitrogen absorbed in the dialysis fluid from both jejunum and ileum. Based on this definition the protein digestibility was 72.5 ± 1.9%, 68.3 ± 3.9% and 62.3 ± 0.6% in the experiments with the slow, medium and fast delivery curve, respectively. The difference in protein digestibility between the slow and fast transit time was significant ($P < 0.05$).

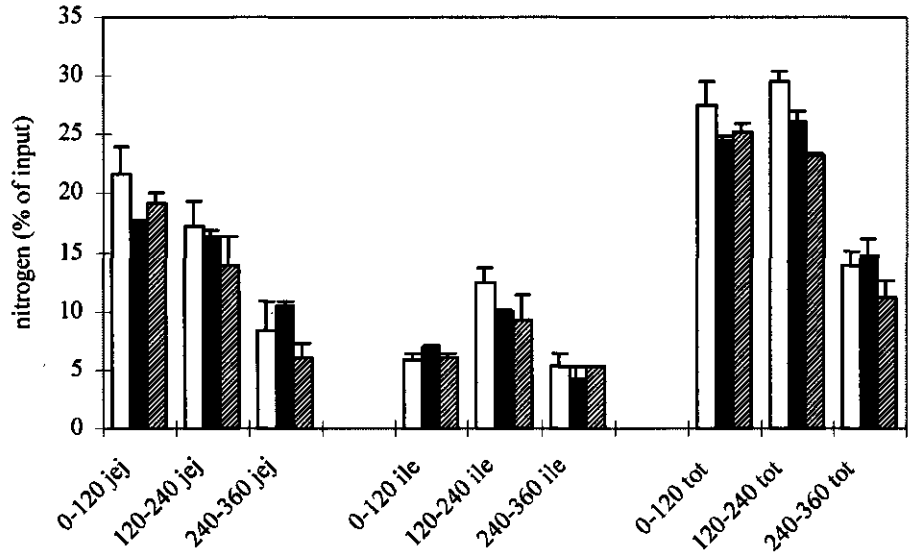

Figure 8 Average amount (± range, $n = 2$) of nitrogen in the jejunal (jej), ileal (ile) and total dialysates (tot) from 0-120 min, 120-240 min and from 240-360 min (white column, slow transit; black column, medium transit; hatched column, fast transit)

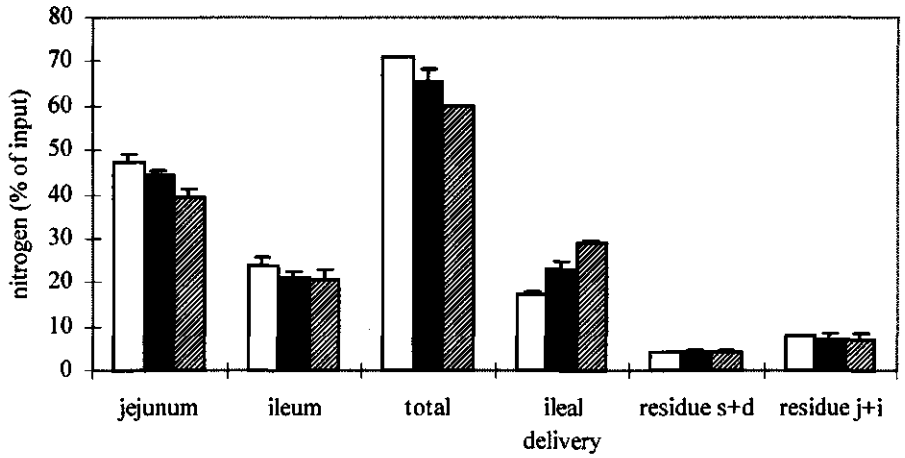

Figure 9 Average amount (± range, $n = 2$) of nitrogen in the jejunal, ileal and total dialysate, ileal delivery, residues in the stomach + duodenum (s+d) and residues in the jejunum + ileum (j+i) (white column, slow transit; black column, medium transit; hatched column, fast transit)

Calcium delivery and availability for absorption

The average (± SD) calcium recovery in all experiments was 94.9 ± 8.4%. The results are expressed as a percentage of recovery.

With respect to the ileal delivery of calcium the same trend was seen as with nitrogen: the faster the small intestinal transit time, the more calcium was delivered from the ileal compartment (Figure 10). In the dialysis fluid the opposite effect was found: the faster the transit time, the less calcium was absorbed into the dialysis fluid.

With respect to the availability for absorption of calcium, it was found that no more than 21.2% was absorbed in the dialysis fluid and 50-60% was delivered from the ileal compartment (Figure 10). No significant differences in availability for absorption of calcium were seen between the experiments with different transit times.

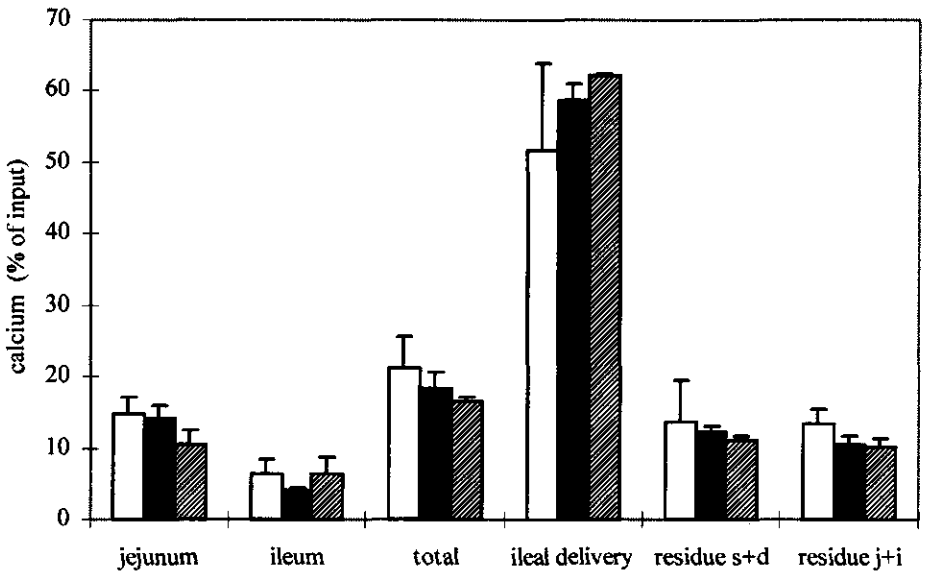

Figure 10 Average amount (± range, $n = 2$) of calcium in the jejunal, ileal and total dialysate, ileal delivery, residues in the stomach + duodenum (s+d) and residues in the jejunum + ileum (j+i) (white column, slow transit; black column, medium transit; hatched column, fast transit)

DISCUSSION

The gastrointestinal model as described by Minekus (1998) and Minekus and Havenaar (1996) is a useful basis for developing and validating a model that simulates the kinetics in the gastrointestinal tract of dogs. Parameters such as pH and transit time, based on the mean physiological condition in the gastrointestinal tract of dogs (Smeets-Peeters *et al.*, 1998), can be incorporated in the model in a reproducible manner.

Because particle size has an effect on the transit time of the food through the stomach *in vivo* (Meyer *et al.*, 1958; Cargill *et al.*, 1988; Sirois *et al.*, 1990), it is important to investigate the effect of differences in particle size on gastric meal delivery in the model. This is important in order to have an optimal comparison with the *in vivo* situation. In the *in vivo* situation food passes the pyloric sphincter in particles smaller than 2 mm (Cullen and Kelly, 1996; Meyer *et al.*, 1958; Burrows *et al.*, 1985). In order to mimic a physiological situation with respect to gastric delivery a peristaltic valve system has been developed by Minekus *et al.* (1995) which simulates the pyloric sphincter. The results of experiments with dry and canned dog food in the *in vitro* model showed that the food was delivered from the stomach according to the preset curve. The dry matter of the diet ground at particles <1 mm was delivered somewhat faster from the stomach compared to the preset curve while the dry matter of the diet ground at particles <3 mm was delivered at a somewhat slower rate. The amount of dry matter of canned dog food delivered from the stomach was below (although not significantly different from) the preset curve. This was probably due to the viscosity of the meal.

For both canned and dry dog food it can be concluded that gastric delivery was very reproducible and not significantly different from the preset curve. This was also seen in the experiments with the different ileal delivery curves, without altering the rate of gastric delivery. The amount of nitrogen left in the stomach after 6 h (residue) was similar in all experiments (Figure 9).

Besides the transit time through the stomach, also the transit time through the small intestine is important in view of digestion. Effects of transit time on digestibility have also been seen *in vivo* (Williams *et al.*, 1984; Zhao *et al.*, 1996; 1997). Zhao *et al.* (1996; 1997) showed that transit and absorption of nitrogen were influenced by the protein load in the diet. Inhibition of intestinal transit time may increase nitrogen absorption by increasing the residence time of nutrients in the small intestine, thus increasing the time available for digestion in and absorption from the luminal contents. The effect of residence time on protein digestibility and absorption of nitrogen was also demonstrated in the *in vitro* model (Figure 9). Most food

was transported to the end of the ileum with the fast transit time (Figure 7). An equal amount of nitrogen was delivered from the 'ileo-caecal valve' (Figure 1; see Figure 1a Chapter 2) after 120 min when simulating a fast and medium transit time (Figure 7). This is according to the preset meal delivery curves (Figure 3) for the slow and medium transit times, in contrast to that of the fast transit time. After the first two hours the rate of delivery should be the same. This is reflected in the amount of nitrogen measured in the ileal delivery samples after two hours (Figure 7). Protein digestibility of the food was increased when the transit time through the small intestine was increased. Prolongation of the experiments (until the food has completely been emptied from the small intestine) could only lead to more absorption of nitrogen in the dialysis fluids in the experiments with the medium and slow transit time, because after 6 h only 74.9% and 66.5%, respectively, has been emptied from the 'ileo-caecal valve'. In the experiments with the fast transit time almost all the food already has been emptied from the ileum (93.1%) (Figure 3). However, based on the amount of nitrogen measured in the residues of the small intestine in the different experiments, more absorption of nitrogen when prolonging the experiments is not to be expected. After 6 h an equal amount of nitrogen was measured in the small intestinal residues in all experiments (Figure 9). This means that most of the protein already has been digested and absorbed within 6 h. Thus, running an experiment for a longer period than 6 h would not lead to more absorption of nitrogen.

The relation between the transit time and the ileal delivery of calcium was the same as with protein. The faster the transit time, the less calcium was absorbed into the dialysis fluid. An unexpected result was the low availability for absorption of calcium from this dog food. Even with the slow delivery curve only some 20% of the calcium ingested was absorbed into the dialysis fluid. In the experiments with the medium and fast delivery curves this proportion even tended to be less. This is in contradiction to the 50-80% utilization of calcium reported by the NRC (1985). This low availability for absorption of calcium for absorption could be caused by the gelling agents used in the canned food. Some gelling agents, such as alginate and carrageenan, complex with calcium (Imeson, 1997). Dietary fibre or phytate in the diet can also have a negative effect on calcium bioavailability (Rossander *et al.*, 1992; Torre *et al.*, 1991). Further research is necessary to elucidate this aspect.

In conclusion, the model is technically able to mimic the dynamics of the gastrointestinal tract of the dog. Parameters such as pH, transit time and enzyme activities can be controlled reproducibly, resulting in digestibility conditions with a high resemblance with the *in vivo* situation. Both canned and dry dog food can be used in the model. Therefore, this model can

be a useful tool in experiments to study the digestibility and availability for absorption of different components of canine diets, including macro- and micronutrients and even pharmaceuticals. In interpreting these results it should be realized that *in vitro* models also have their limitations. In *in vitro* models neuro-hormonal feedback mechanisms nor a local or humoral immune system can be mimicked. In the model no mucosal cells are present. Some of these problems can be solved by combining *in vitro* models with biological models, such as cultured mucosal cells and intestinal segments. However, based on validations studies in the models for humans, calves and pigs it can be concluded that the model has a predictive capacity for the *in vivo* situation (1998).

Besides nutritional studies the model can also be used for other purposes, such as efficacy studies on food additives (e.g. enzymes) and functional food ingredients (e.g. pre- and probiotics). In order to develop the model further for these purposes, additional validation studies, such as comparisons with results obtained *in vivo*, are required.

4 VALIDATION OF A DYNAMIC *IN VITRO* MODEL OF THE CANINE GASTROINTESTINAL TRACT

Marianne Smeets-Peeters[1,2], Esther Schreijen[2], George Fahey, Jr.[3], Robert Havenaar[1], Gertjan Schaafsma[4], and Martin Verstegen[2]

1 TNO Nutrition and Research, PO Box 360, 3700 AJ Zeist, The Netherlands
2 Wageningen University, Department of Animal Nutrition, Marijkeweg 40, 6709 PG Wageningen, The Netherlands
3 University of Illinois, Department of animal sciences, Urbana 61801, USA
4 Wageningen University, Department of Human Nutrition and Epidemiology, PO Box 8129, 6700 EV Wageningen, The Netherlands

Submitted to the Journal of Animal Science

ABSTRACT

The objective of this study was to compare protein digestibility responses, using a dynamic, computer-controlled *in vitro* model of the gastrointestinal tract of the dog, with *in vivo* data measured in ileally cannulated dogs. Eight different dry dog foods were tested with the *in vitro* model. Six diets containing different cereal grains with varying starch forms, types, and concentrations, and two diets with different poultry-products were used to asses the protein digestibility responses of these diets. The results were compared with the ileal protein digestibility values of these diets measured in ileally cannulated dogs. It was not appropriate to calculate a correlation coefficient between results of both methods, because the differences in protein digestibility between diets were too small. One of the advantages of using the *in vitro* model is the capability of studying protein digestibility over time at different parts of the small intestine, which provide more insight about rate of digestion. The results obtained were very reproducible and the standard deviations obtained in the *in vitro* study were much lower than those for the *in vivo* study. It can be concluded that the model effectively ranks different diets with respect to their protein digestibility. This conclusion is based on the results found in this study combined with results found in other experiments using the *in vitro* model.

INTRODUCTION

Finding alternatives methods that simulate *in vivo* responses is an important area of study. With respect to alternatives for nutrition and drug research, a dynamic *in vitro* model of the gastrointestinal tract was developed by Minekus *et al.* (1995). To use this model for canine nutrition research, a close simulation of the dynamic conditions in the gastrointestinal tract of dogs is essential. Based on data derived from the literature about the physiology of the dog (Smeets-Peeters *et al.*, 1998), a dog model was developed (FIDO: *f*unctional gastro*i*ntestinal *do*g model; Smeets-Peeters *et al.*, 1999). Several factors can cause incomplete nutrient digestion in the gastrointestinal tract, e.g. crystallinity, nature of starch and composition of animal byproducts (Murray *et al.*, 1997; 1999). Therefore, six diets containing different cereal grains with varying starch forms, types, and concentrations and two diets with different poultry products were used to compare the protein digestibility of these diets *in vitro* and *in vivo*.

The objective of the present study was to assess the predictive capacity of the gastrointestinal model for the *in vivo* situation as regards protein digestibility.

MATERIALS AND METHODS

Gastrointestinal model

The model has been described in detail by Minekus *et al.* (1995) and Minekus and Havenaar (1996; 1998). Briefly, the dynamic *in vitro* gastrointestinal model (Figures 1 and 2; see Figures 1a and 1b Chapter 2) consists of four consecutive compartments, simulating the stomach, duodenum, jejunum, and ileum. Each compartment is formed by two connected glass jackets, each with a flexible silicone wall inside. The flexible wall is surrounded by water kept at a temperature of 38 ± 1°C. By increasing the water pressure, the flexible walls are squeezed, simulating the peristaltic movements. Transport of the food along the model is regulated by peristaltic valves between the separate compartments, which are controlled according to pre-set delivery curves of the chyme. Transit time of the food, the pH values, and the addition of electrolytes and enzymes are based on the physiological data of the dog (Smeets-Peeters *et al.*, 1998) and controlled by a computer and specifically developed software. A previous study with FIDO showed that transit time has an effect on the digestibility of protein and on availability for absorption of calcium (Smeets-Peeters *et al.*, 1999). The half emptying time of the food from the stomach is 90 min and from the small intestine 300 min. After 6 h 66.5% of the meal has passed the 'ileo-caecal valve (see Figure 1a, Chapter 2). By means of semi-permeable hollow fiber devices (Cobe HG-400, Secon GmbH Germany) connected to the jejunal and ileal compartments, absorption of water and digestion products is mimicked.

Experimental design

Diets

The diets used in this study aso have been investigated in ileally cannulated dogs (Murray et al., 1999). The cereal diets included one out of the six flours: wheat, corn, rice, potato, barley, or sorghum (Table 1). The animal byproduct diets included poultry by-product meal or fresh poultry (Table 2). The chemical composition of the flours and the animal by-products is reported in Table 3. The chemical composition of the total diets is given is Table 4. Dry dog foods were ground and diluted with demineralized water to a standardized DM content of 15%. Of this wetted food, 300 g was used in the model. All diets were tested in duplicate.

Table 1 Ingredient composition of the six starch flour-containing diets

Ingredient% DM† basis	Diet*					
	WF	CF	RF	PF	BF	SF
Starch source	49.1	43.6	44.1	50.4	51.9	44.2
Poultry byproduct meal	27.9	34.3	32.5	27.6	26.4	33.6
Poultry fat	13.1	12.2	13.4	12	11.7	12.4
Beet pulp	4	4	4	4	4	4
Calcium carbonate	1.5	1.5	1.5	1.5	1.5	1.5
Monosodium phosphate	1.2	1.2	1.2	1.2	1.2	1.2
Brewer's dried yeast	1	1	1	1	1	1
Vitamin mixture‡	0.4	0.4	0.4	0.4	0.4	0.4
Potassium chloride	0.6	0.6	0.6	0.6	0.6	0.6
DL-methionine	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride	0.3	0.3	0.3	0.3	0.3	0.3
Mineral mixture‡	0.3	0.3	0.3	0.3	0.3	0.3
Sodium chloride	0.1	0.1	0.1	0.1	0.1	0.1

* WF = wheat flour diet, CF = corn flour diet, RF = rice flour diet, PF = potato flour diet, BF = barley flour diet, SF = sorghum flour diet

† DM = dry matter

‡ For composition of the vitamin and mineral mixture, see Murray *et al.* (1999)

Sampling and analysis

For each hour, the total ileal delivery behind the 'ileo-caecal valve' (Figure 1, see Figure 1a N Chapter 2) was collected on ice. The dialysis fluids of the jejunal and ileal compartments were sampled every two hours. At the end of the experiment, the residual contents of the gastric plus duodenal compartments and, separately, of the jejunal plus ileal compartments were collected. To determine the input of exogenous and endogenous nitrogen (N) into the model, samples were taken from the food, bile, and pancreatic solution. All samples were frozen at -20°C until analysis. The amount of N was determined according to the Kjeldahl method (International Standard, ISO 5983, 1979).

Table 2 Ingredient composition of the poultry byproduct meal diet (PBPM) and as fresh poultry diet (FP)

Ingredient (% DM basis)	Diet	
	PBPM	FP
Poultry byproduct meal	6.59	-
Fresh chicken viscera	-	5.75
Fresh chicken necks and backs	-	5.5
Brewers rice	52.49	52.84
Dehydrated egg	18.32	18.45
Poultry fat	7.86	3.21
Beet pulp	4.95	4.99
Calcium carbonate	4.29	3.75
Dicalcium phosphate	4.4	4.41
Sodium chloride	0.55	0.55
Vitamin mixture*	0.22	0.23
Mineral mixture*	0.16	0.16
Choline chloride	0.16	0.16
Ethoxyquin	0.01	0.01

* For composition of the vitamin and mineral mixture, see Murray *et al.* (1997)

Calculations and statistical analyses

Recovery of the N was calculated by the formula

$$\text{recovery } (\%) = (N_{samples}/(N_{food}+N_{secretions}))*100$$

where $N_{samples}$ is the total amount of N found in the samples collected behind the ileo-cecal valve, the dialysis fluids, and the residual samples, N_{food} is the amount of N in the food (exogenous nitrogen) and $N_{secretions}$ is the amount of nitrogen in the bile and pancreatic solution (endogenous nitrogen). The amount of nitrogen in the gastric secretions was negligible and, therefore, not included in the formula. The following formulas were used to calculate the ileal digestibility:

$$IDC\ (\%) = (N_{dialysate}/N_{recovered})* 100 \qquad \text{(Formula 1)}$$

where IDC is the ileal digestibility coefficient of the nutrient expressed as a percentage of recovery, $N_{recovered}$ is the total amount of N recovered after an experiment of 6 h, and $N_{dialysate}$ is the total amount of N recovered from the jejunal plus ileal dialysates.

$$IDC\ (\%) = (100- (N_{ileal\ delivery}/ N_{recovered}))*100 \qquad \text{(Formula 2)}$$

where $N_{ileal\ delivery}$ is the amount of N in the total ileal delivery during 6 hours.

For the statistical analyses two samples of the eight different diets were tested singular. Data analyses were performed using the statistical software package (SAS, 1990) and a P-value < 0.05 was considered as significant. Data were analyzed by ANOVA, after Bartlett's test for testing the homogenicity of the variances. ANOVA was performed to test the overall effect of foods. When an overall significance was found, pairwise tests on individual means were performed using the least squares means method of SAS (1990).

Table 3 Chemical composition of the starch flours and the poultry products incorporated into experimental diets

	Diet*								
	WF	CF	RF	PF	BF	SF	PBPM	FPV	FPNB
Dry matter (%)	90.5	90.1	90.8	93.5	91.9	90.9	95.3	27.2	38.6
	% DM basis								
Organic matter	99.3	99.4	99.4	95.5	97.2	99.5	86.1	93.4	92.2
Crude protein	13.1	5.6	8.2	9.8	11.9	9.2	67.6	45.6	30.4
Fat	2.6	3.2	2.8	1.6	4.4	2.6	11.6	41.1	50.7
Starch	78.1	88.3	87.4	77.9	61	84.8			
TDF†	3.4	3	1	6.4	18.9	2.7			

* WF= wheat flour diet, CF = corn flour diet, RF = rice flour diet, PF = potato flour diet, BF = barley flour diet, SF = sorghum flour diet, PBPM = poultry byproduct meal; FP = fresh poultry (this diet was prepared by blending fresh poultry viscera (FPV) and fresh poulty necks and backs (FPNB) 60:40).

† Total dietary fiber

Table 4 Chemical composition of experimental diets

	Diet*							
Item	WF	CF	RF	PF	BF	SF	PBPM	FP
Dry matter (%)	96	96	96.1	95.8	95.6	96.3	95.9	95.6
				% DM basis				
Organic matter	93.4	92.6	92.5	90.9	93.1	92.4	89.5	89.9
Crude protein	29.5	30.6	29.9	27.5	28	31.9	20.9	22.5
Fat	18.8	18.3	18.8	16.7	16.9	18.7	13.5	15.5
Starch	40.3	37.4	38.2	39.1	40.4	35.4	44.5	42.9
TDF†	7.5	7.1	5.5	8.6	9.9	9.4	5.9	7

* WF= wheat flour diet, CF = corn flour diet, RF = rice flour diet, PF = pototo flour diet, BF = barley flour diet, SF = sorghum flour diet, PBPM = poultry byproduct meal; FP = fresh poultry

† Total dietary fiber

RESULTS AND DISCUSSION

The average recovery of the N in the experiments was 92.6 ± 6.1% (n = 16). The *in vitro* results for the ileal digestibility of protein of the eight diets were compared to the *in vivo* protein digestibility results obtained using ileally cannulated dogs (Figures 3 and 4). Results show that the standard deviation (SD) for the protein digestibility values between replicates was much lower in the *in vitro* study as compared to the *in vivo* study. This confirms that the studies in the model can be performed under highly controlled and reproducible circumstances (Smeets-Peeters *et al.*, 1999), without effects of biological variation, such as found in the *in vivo* study. Similar results were found by Minekus (1998) in studies with pig feeds and calf milk replacers in the model, simulating the gastrointestinal conditions of pigs and calves, respectively. The ileal protein digestibility in the *in vitro* model was comparable to that in ileally cannulated dogs. However, the ileal digestibility *in vitro* was calculated in a different way (Formulas 1 and 2) than for the *in vivo* experiments (Boisen and Moughan, 1996). Data imply that the model may be used as a tool to discriminate between

digestibilities of different diets and as a method for ranking of diets rather than a tool for exact quantitative prediction of the digestibility of diets. Using this as a startingpoint for adopting the model for digestibility studies with dogs, the correlation coefficient between *in vitro* and *in vivo* protein digestibility should be determined. However, this was not possible for this criterion because the digestibilities of these diets were all in the same (relatively small) range. For calculating a correlation coefficient, it is necessary to have a relatively wide range of both highly digestible and poorly digestible protein sources. In this study, only very highly digestible dog diets were available. However, based on experiments with pig feed and calf milk replacers, it is known that the correlation coefficient for protein digestibility is very high between *in vitro* and *in vivo* systems (Minekus, 1998).

In general it can be concluded that the ranking of the starch diets *in vitro* (Formula 1) is similar to the ranking *in vivo*, with the exception of the diet with the potato starch included. From the literature it is known that potato starch has unique effects on digestive physiology *in vivo*. For example, it has been found in rats that uncooked potato starch prolongs the total transit time in comparison to other starches (including cooked potato starches; Calvert, 1989, Lajvardi, 1993; Mathers *et al.*, 1997). The effects of potato starch on transit time *in vivo* can, however, not explain the significant difference found between the *in vivo* and *in vitro* results because the potatoes used to produce the potato starch were steam-cooked and peeled. Besides, a lower digestibility of protein is found in the *in vivo* study compared to the *in vitro* study. This is only possible in case of a faster passage time of the meal through the small intestine (due to less contact time with enzymes and less time for absorption), as also found in the model for dog foods (Smeets-Peeters *et al.*, 1999).

Because in the *in vivo* study the apparent ileal digestibility was measured, the concentration of endogenous protein is important. In the *in vitro* study, the same amount of endogenous protein was added in all experiments and was not influenced by diet. The higher the amount of endogenous N the lower the apparent digestibility *in vivo*. Mason *et al.* (1976) found a higher amount of N at the end of the small intestine of pigs when feeding raw potato starch compared to corn starch. In the study of Everts *et al.* (1996), similar differences were found between native potato starch and corn starch. Wünsche *et al.* (1987) who studied the effect of thermic treatment of potato starch on nutrient degradation in pigs, on the other hand, could not confirm the effect of a higher amount of N at the end of the small intestine.

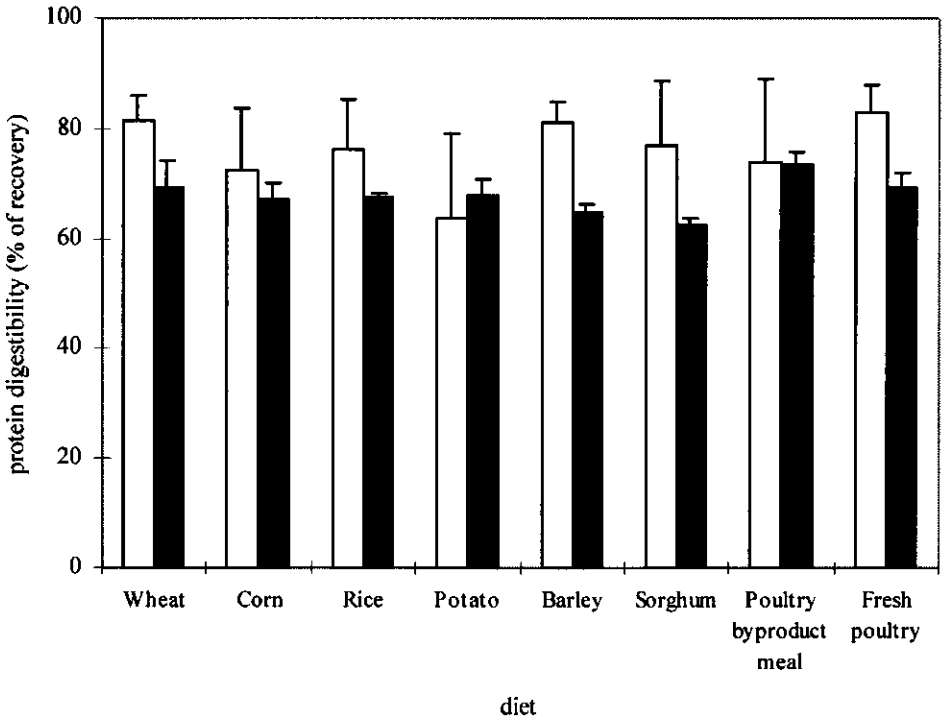

Figure 3 Comparison between the mean (± sd) protein digestibility values for ileally cannulated dogs (white bars; Murray *et al.*, 1997; 1999) and the mean (± range) protein digestibility values (% of input) in the *in vitro* gastrointestinal dog model (black bars) calculated according to Formula 1

It is not clear if (cooked) potato starch increases the endogenous nitrogen at the end of the small intestine *in vivo*. This could occur as a result of: 1) a higher secretion of N by digestive juices, 2) an increased microbial mass at the end of the small intestine by fermentation of (resistant) starches, or 3) movement of urea from the blood to the intestine. This movement of urea has been described by Beames and Eggum (1981) in a study using raw potato starch fed to rats.

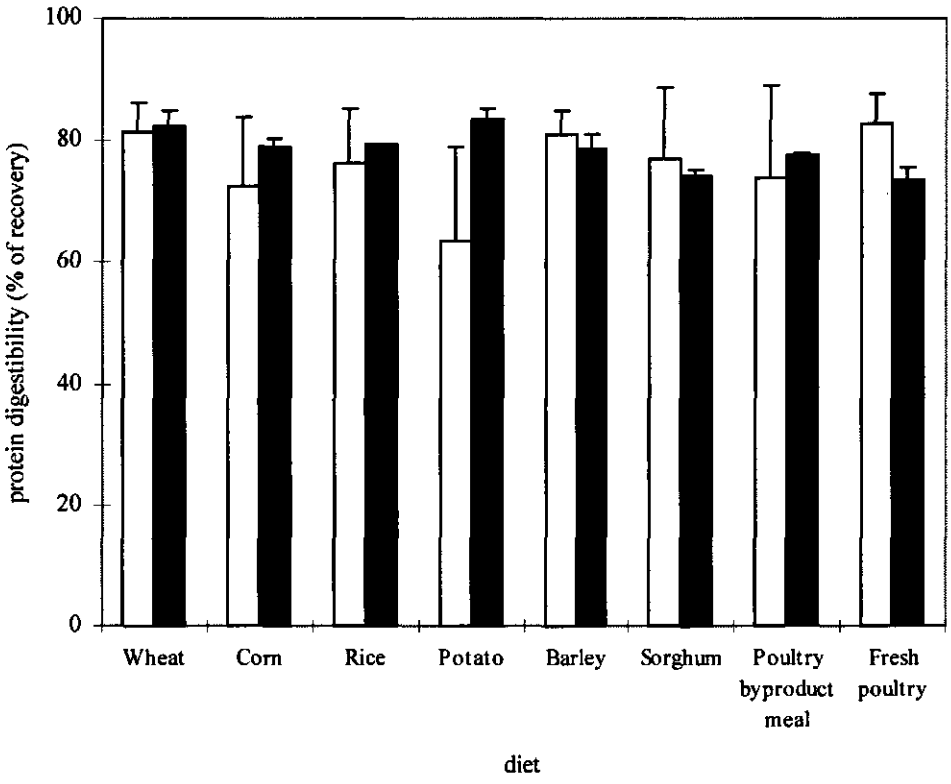

Figure 4 Comparison between the mean (± sd) protein digestibility values for ileally cannulated dogs (white bars; Murray *et al.*, 1997; 1999) and the mean (± range) protein digestibility values (% of input) in the *in vitro* gastrointestinal dog model (black bars). The *in vitro* data were calculated according to Formula 2 using different data, but from the same experiment as presented in Figure 3

Diets containing poultry products did not show a significant difference between treatments in the *in vitro* study (Figures 3 and 4). This is in contrast to the results of the *in vivo* study were the ileal protein digestibility of the diet with the poultry by-product meal was significantly lower ($P < 0.05$) than the diet with the fresh poultry (Murray *et al.*, 1997). Murray *et al.* (1997) ascribe differences in ileal protein digestibility to the quality of the chicken meat used in the diets, specifically the fact that the poultry byproduct meal contains parts of the bird having less nutritive value than the meat (e.g. becks, feet and feathers) This

is not an explanation for the results of our study because the diets used in both the *in vivo* and *in vitro* studies were the same.

One of the advantages of the *in vitro* model compared with *in vivo* studies is the ability to follow the digestibility of different subtrates over time (Figures 5, 6a and 6b). From Figure 5 it can be concluded that the difference in ileal delivery of N mainly occurs during the first 2 h of an experiment.After that the rate of delivery of N is comparable for all eight diets. Values which are statistical significant are presented in Table 5 ($P < 0.05$). The protein in these diets was readily digestible (Figures 6a and 6b). Most of the N (about 50%) is already absorbed from the jejunal compartment. When the data are expressed as a percentage of N in the jejunal and ileal dialysate, no significant differences were found between the diets ($P \geq 0.05$).

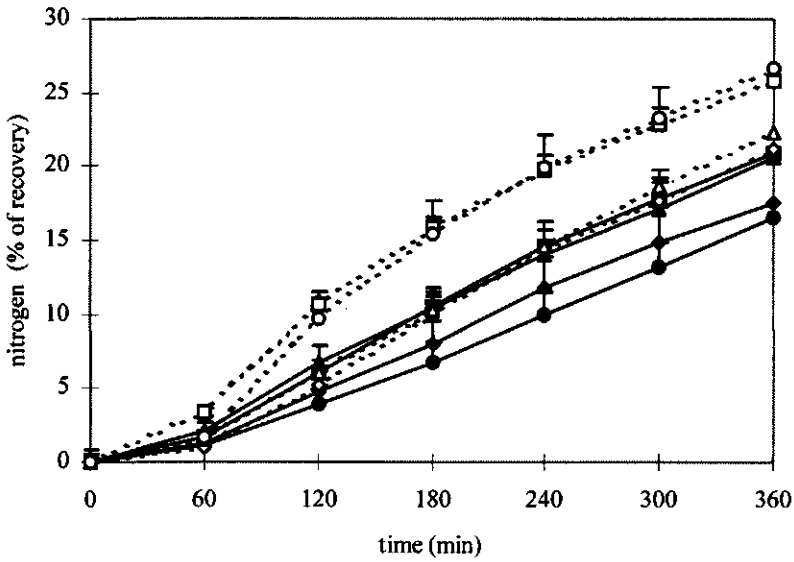

Figure 5 Mean cumulative ileal delivery of N (± range) of 8 different diets varying in starch source or in poultry product component in the *in vitro* canine gastrointestinal model. Solid lines: ◆ wheat, ■ corn, ▲ rice, ● potato and dashed lines:◇ barley, □ sorghum, ▵ poultry by-product meal, ○ fresh poultry

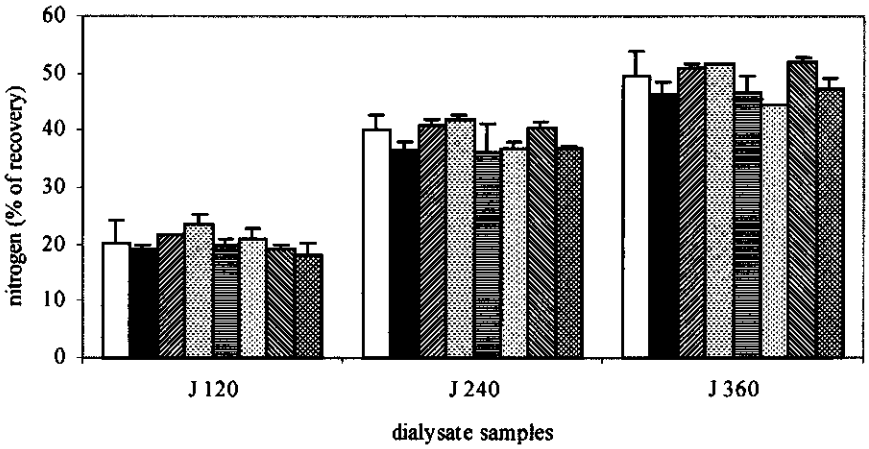

Figure 6a Mean cumulative absorption of N (± range) of 8 different diets varying in starch source or poultry product component in the jejunal (J) compartment measured at t = 120, t = 240 and t=360 min. White, wheat; black, corn; hatched (right), rice; broken horizontal lines, potato; horizontal lines, barley; brackets, sorghum; hatched (left), poultry byproduct meal; double hatched, fresh poultry. The data used were different from the data used in Figure 5, but from the same experiment

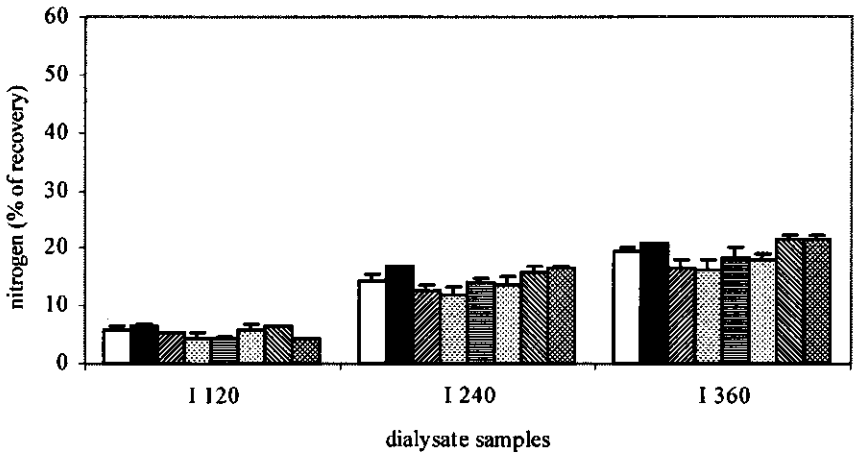

Figure 6b Mean cumulative absorption of N (± range) of 8 different diets varying in starch source or poultry product component in the ileal (I) compartment measured at t = 120, t = 240 and t = 360 min. Legend see figure 6a

Another advantage of the model is the possibility of discriminating between the N originating from endogenous and exogenous protein sources. The results presented in this paper were calculated using the total input of N (endogenous and exogenous). However, it is also possible to calculate digestibility as a percentage of N intake (exogenous). Although the ranking of the diets *in vitro* is still the same when using this type of calculation, it has effects on the absolute digestibility values. In general digestibility will be lower, based on ileal delivery, when using this type of calculation. Based on the N recovered from the dialysate, the digestibility will be higher. Also, blank experiments can be performed using this model. This gives the opportunity to attain information about the digestibility of the endogenous protein secreted into the GI tract. In this way, true digestibility can be calculated (Boisen and Moughan, 1996). Interactions between exogenous and endogenous protein during the digestion process are, however, not taken into account when using this method.

Table 5 Statistical evaluation of the cumulative ileal delivery data of N of 8 different diets varying in starch source or in poultry product component (see also Figure 5) in the *in vitro* canine gastrointestinal model

	Diet*							
Time (min)†	WF	CF	RF	PF	BF	SF	PBPM	FP
60	bc	bc	b	bc	c	a	bc	bc
120	bc	bc	b	c	bc	a	bc	a
180	bc	b	b	c	bc	a	bc	a
240	bc	b	bc	c	b	a	b	a
300	bc	bc	bc	c	bc	a	ab	a
360	cd	bcd	bcd	d	bcd	ab	abc	a

* WF= wheat flour diet, CF = corn flour diet, RF = rice flour diet, PF = potato flour diet, BF = barley flour diet, SF = sorghum flour diet, PBPM = poultry byproduct meal; FP = fresh poultry

† Diets not sharing common letters are significantly different ($P < 0.05$)

Although it possesses a lot of advantages the model has its limitations. Although physiological circumstances can be mimicked in the model, it is not possible to study the effects of different diets on physiological criteria (e.g. the effect of fibers on transit time). When it is known what, for example, the effect is of a fiber on transit time, then again the model can be used to simulate this circumstance.The consequences of the effect of changed transit time, due to the fiber, on the digestibility and availability of nutrients can be studied again.

Implications

The *in vitro* gastrointestinal tract model is a useful tool to predict protein digestibility of (dry) dog foods. The results are very reproducible and the deviations in the *in vitro* study are much lower than for *in vivo* studies. In addition, the model is a useful tool to perform kinetic studies which provide insight about rate of digestion of different substrates and diets. The limitations of the model for mimicking the special physiological reactions of the animal should be kept in mind in interpreting the results.

5 CALCIUM AND PHOSPHORUS AVAILABILITY OF CANNED DOG FOODS CAN BE MEASURED IN A DYNAMIC, COMPUTER-CONTROLLED GASTROINTESTINAL DOG MODEL

Marianne Smeets-Peeters[12], Lutien Groeneveld[2], Robert Havenaar[1], Martin Verstegen[1], Gertjan Schaafsma[3]

1 TNO Nutrition and Research, PO Box 360, 3700 AJ Zeist, The Netherlands

2 Wageningen University, Department of Animal Nutrition, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

3 Wageningen University, Department of Human Nutrition and Epidemiology, PO Box 8129, 6700 EV Wageningen, The Netherlands

Submitted to the Journal of Animal Physiology and Nutrition

ABSTRACT

The objectives of this study were 1) to measure availability of calcium and phosphorus in 3 commercially available diets with different concentrations of Ca and P, 2) to evaluate the effect of supplementing these diets with various components (e.g. Ca-gluconate, dicalciumphosphate, phytase) on availability, and 3) to evaluate the sensitivity of a dynamic, computer-controlled laboratory system of the gastrointestinal tract of the dog to measure availabilty.

Differences were found in the absolute amounts of Ca and P which were available for absorption, although the relative amounts were quite similar. This is probably related to the source of Ca and P as well as to the interaction with other dietary components. Addition of supplements did have a positive effect on the availability for absorption of Ca and P, as well as lowering the pH in the small intestinal compartments. Addition of phytase did have a positive effect on the availability for absorption of P and to a less extent of Ca. It is assumed that the model estimates the amount of Ca or P which will be available for absorption *in vivo*. The amount of Ca and P which will be absorbed *in vivo* is dependent on the physiological conditions of the animal.

INTRODUCTION

The bioavailability of minerals from the diet is an important issue. This is particularly relevant for dogs when it concerns calcium (Ca) and phosphorus (P). Both minerals are needed in correct amounts in the diets of dogs to allow adequate bone formation (Richardson and Toll, 1997). Ca and P are considered together because of their close metabolic association. The ratio of dietary Ca and P is important for good absorption but is of less significance than the absolute dietary contents of these minerals (NRC, 1985). A Ca:P ratio of 1.2:1 to 1.4:1 in dog diets is considered optimal for maximum utilization (NRC, 1985). However, the availability of the Ca and P should also be taken into account. A high chronic Ca availability and absorption in rapidly growing dogs of large breeds, for example Great Deanes, can give rise to several skeletal abnormalities (Hazewinkel *et al.*, 1985).

In the lumen of the small intestine, several dietary constituents may form complexes with minerals and trace elements, that may either be soluble or insoluble. The binding of minerals and trace elements to dietary fiber, fytic acid and some polyphenolic compounds may render these nutrients insoluble and decrease their absorption (Allen, 1982; Rossander *et al.*, 1992; Torre *et al.*, 1991) . On the other hand compounds like citric acid, ascorbic acid and some

amino acids and peptides may form soluble mineral complexes that are readily available for absorption (Rossander *et al.*, 1992). Another factor which influences the Ca absorption is the physiological status of the animal. It is well known that during periods of rapid growth as well as during pregnancy lactation Ca absorption from the diet is increased. Thus availability and absorption is affected by dietary and physiological factors. The dynamic model of the gastrointestinal tract of the dog (FIDO) has been developed to focus on dietary factors in relation to gastointestinal conditions.

The objectives of this study were :

1. to measure availability of Ca and P in 3 commercially available diets with different concentrations of Ca and P.
2. To evaluate the effect of supplementing these diets with various components (e.g. Ca-gluconate, dicalciumphosphate, phytase) on availability.
3. To evaluate the sensitivity of the model to measure availabilty.

MATERIALS AND METHODS

Gastrointestinal model

The basis of the system has been described in detail by Minekus *et al.* (1995) and patented for the USA and Europe (Minekus and Havenaar 1996; 1998). This dynamic *in vitro* gastrointestinal model (Figures 1 and 2; see Figures 1a and 1b Chapter 2) consists of four successive compartments, simulating stomach, duodenum, jejunum and ileum. Each compartment is formed by two glass jackets with a flexible silicone wall inside. The flexible wall is surrounded by water which keeps the temperature at body temperature. By increasing the water pressure the flexible walls are squeezed. In this way the peristaltic movements of the gastrointestinal tract are simulated. Transport of the food is regulated by peristaltic valves between the successive compartments according to preset emptying curves of the food. Transit time of the food, pH in the stomach and intestinal luminal contents and addition of the electrolytes and enzymes are based on the physiology of the dog (Smeets *et al.*, 1998; 1999) and controlled by a computer. By means of semi-permeable hollow fibre devices (Cobe HG-400, Secon GmbH Germany) connected to the jejunal and ileal compartments, absorption of the small molecular digested products is mimicked and the concentration of electrolytes in the model is controlled.

Diets

Three commercially available canned dog foods were used in this study (diet A, B, and C). The composition of these diets is reported in Table 1. A standard human breakfast (Vaquero *et al.*, 1992) was used as a control.

Table 1 Composition of the canned dog diets

	diet A (%)	diet B (%)	diet C (%)
Moisture	760	820	770
Crude protein	60	60	60
Crude fat	37	45	15
Crude fiber	2	5	4
Crude ash	11	25	15
Calcium	16	55	38
Phosphorus	14	45	28

Diet C was supplemented with Ca-gluconate mono-hydrate (Gluconal® CAM-P-OR, Glucona B.V., Veendam, the Netherlands), a low soluble Ca-mono-hydrogen phosphate (Windmill Dicalphos®, Tessenderlo Chemie, Vlaardingen, the Netherlands) and skim milk powder (Oxoid Ltd, Basingstoke, UK). The amounts of Ca and P added with the supplements to diets are reported in Table 2.

Table 2 Amount of calcium and phosphorus added to the diet with the supplements

Supplement	Calcium (mg)	Phosphorus (mg)
Calcium-gluconate	448	-
Dicalcium phosphate	448	346
Skimmed milk	224	-

Also the effects of the addition of phytase to diet C (4.8 mg; Natuphos 5500 FTU/kg, DSM Gist, Delft, the Netherlands) and of lowering of the pH (0.5 units in the duodenal compartment to pH 5.7 and 0.3 units in the jejunal compartment to pH = 6.2) were studied.

These pH changes were choosen to test whether small pH changes, which are still within the physiological range of the dog, influence the availability for absorption.
The amount of food used in the model was 300 g with a dry matter content of 15%. To attain this dry matter content the food was diluted with demineralized water.

Sampling and analyses

Every hour total ileal delivery aliquots were collected behind the 'ileo-caecal valve' (Figure 1, N; see Figure 1a Chapter 2). The aliquots were stored as separate samples (per hour) and as pooled sample (per 6 hours). The dialysis fluid of the jejunal and ileal compartments was sampled every two hours and stored as separate samples and as pooled samples. At the end of the experiment the residual contents of the gastric plus duodenal compartment and of the jejunal plus ileal compartment were collected. To determine the input of Ca and P into the model, samples of the food, bile and pancreatin solution were taken. All samples were stored at -20°C until analysis. Analyses of Ca and P were performed in samples of diet, pooled ileal delivery, pooled jejunal and ileal dialysis fluids, residues of the gastric plus duodenal compartment and of residues of the jejunal plus ileal compartment.The amount of Ca was determined, after ashing (500°C) and dissolving the ash in hydrochloric acid (0.6 M), according to the atomic absorption spectrometric method (NEN, 1984). The amount of total P was measured in the dissolved ash, by using a spectrophotometric method (International Standard, ISO 6491, 1979).

Calculations and statistics

Recovery of the Ca and P was calculated by the formula:

$$\text{recovery } (\%) = (M_{samples}/(M_{food}+M_{secretions}))*100$$

where $M_{samples}$ is the total amount of Ca or P found in the samples collected behind the ileo-caecal valve, the dialysis fluids and the residual samples, M_{food} is the amount of Ca or P in the food (exogenous Ca or P) and $M_{secretions}$ is the amount of Ca or P in the bile and pancreatin solution.The amount of Ca or P in the gastric secretion was negligible and therefor not included in the formula.The following formula was used to calculate the availability for absorption of Ca or P:

$$\text{Availability } (\%) = (M_{dialysate}/M_{recovered})* 100$$

where 'availability' is the total amount of Ca or P which is available for absorption expressed as a percentage of recovery, $M_{recovered}$ is the total amount of calium or P recovered after an experiment of 6 hours, and $M_{dialysate}$ is the total amount of Ca or P recovered from the jejunal plus ileal dialysates.

For statistical data analyses diet A, B, C and the standard breakfast were compared as well as the data from diet C and the diet C plus (supplement, pH or enzyme) data. Data analyses were performed using the statistical software package (SAS, 1990) and a P-value < 0.05 was considered as significant. Data were analysed by ANOVA, after Bartlett's test for testing the homogenicity of the variances (SAS/STAT, 1997). An ANOVA test was performed to test the overall effect of foods. When an overall significance was found, pairwise tests on individual means were performed using the least squares means method of SAS (1990).

RESULTS

The recovery of Ca in the experiments was 97.7 ± 13.9 % (n = 12). The absolute amounts of Ca absorbed in the dialysis fluids, corrected for recovery, was 59.0 ± 4.4 mg, 75.7 ± 3.0 mg, 70.7 ± 3.7 mg and for diets A, B, and C, respectively (Figure 3). This corresponds to relative amounts of 21.2 ± 0.0 % (diet A), 8.7 ± 0.7 % (diet B), and 11.0 ± 2.0 % (diet C) (Figure 4).

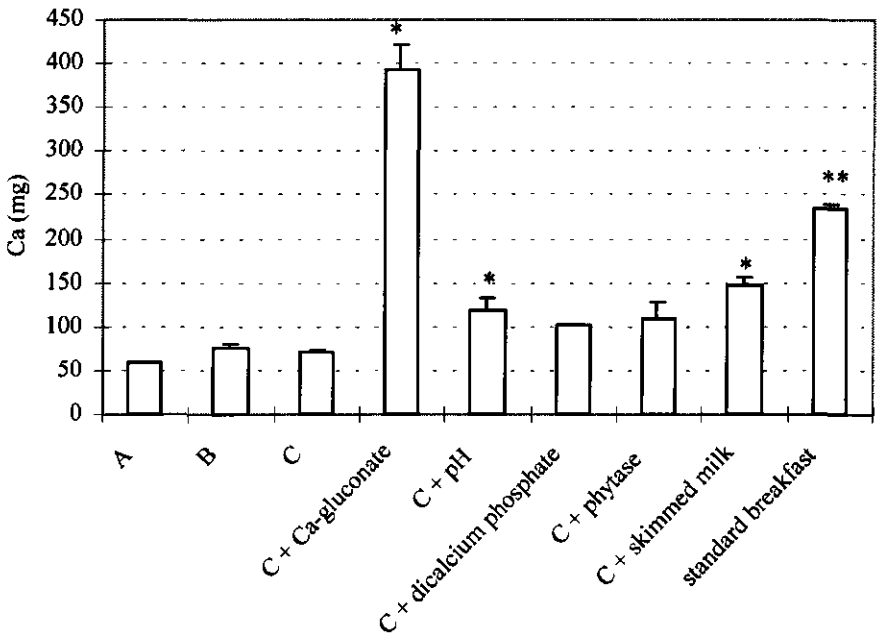

Figure 3 Mean (± range, n = 2) absolute amount of calcium (corrected for recovery) absorbed in the jejunal plus ileal dialysis fluids from diet A, B, and C (* significantly different from diet C (P < 0.05), ** significantly different from diet A, B and C (P < 0.05))

The recovery of P was 101.9 ± 20.0 % (n = 18). The availability of P was higher, both absolute and relative, than that of Ca (Figures 5 and 6). The absolute amounts absorbed were 157.6 ± 14.9 mg, 383.8 ± 16.6 mg, and 102.3 ± 12.5 mg for diet A, B, and C, respectively. The absorption expressed as percentage of input, corrected for recovery, was 43.8 ± 4.4% (diet A), 41.9 ± 0.9 % (diet B), and 24.3 ± 2.3% (diet C).

Addition of Ca-gluconate or skim milk powder increased significantly the (relative) amount of Ca ($P < 0.05$; Figures 3 and 4) absorbed. Phytase increased the relative absorption of Ca significantly ($P < 0.05$; Figure 4). Dicalciumphospate did not show any significant effects on the availability for absorption of Ca ($P > 0.05$; Figures 3 and 4).

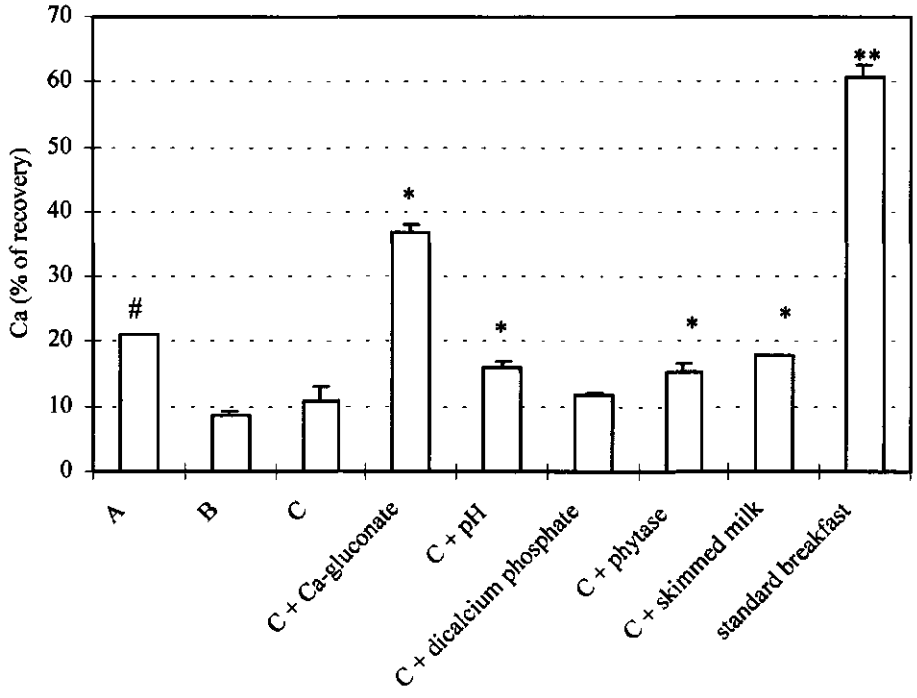

Figure 4 Mean (± range, n = 2) relative amount of calcium as a percentage of input (corrected for recovery), absorbed in the jejunal plus ileal dialysis fluids from diet A, B, and C (# significantly different from diet B and C ($P < 0.05$), * significantly different from diet C ($P < 0.05$), ** significantly different from diet A, B and C ($P < 0.05$))

Lowering the pH in the duodenal and jejunal compartment increased the absolute amount of Ca significantly ($P < 0.05$; Figure 3). Also the relative availability increased significantly ($P < 0.05$; Figure 4). Addition of supplements, lowering the pH or the addition of phytase had a significant increasing effect ($P < 0.05$) on the (relative) absorption of P (Figures 5 and 6).The standard breakfast, which was used as a control diet, had an absolute absorption of Ca of 234.4 ± 5.9 mg (Figure 3), and a relative absorption of 60.7 ± 2.2% (Figure 4) which was significantly higher ($P < 0.05$) than the respective values from canned dog foods. The amount of P absorbed from the standard breakfast was 326.1 ± 1.5 mg (83.2 ± 1.4 %).

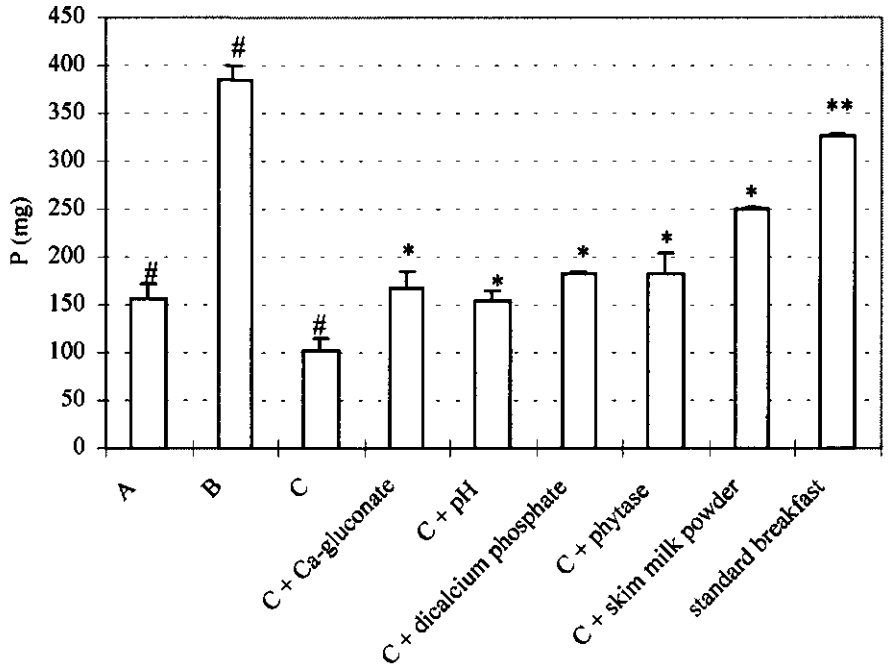

Figure 5 Mean (± range, n = 2) absolute amount of phosphorus (corrected for recovery) absorbed in the jejunal plus ileal dialysis fluids from diet A, B, and C (# significantly different from diet A, B and C ($P < 0.05$), * significantly different from diet C ($P < 0.05$), ** significantly different from diet A, B and C ($P < 0.05$))

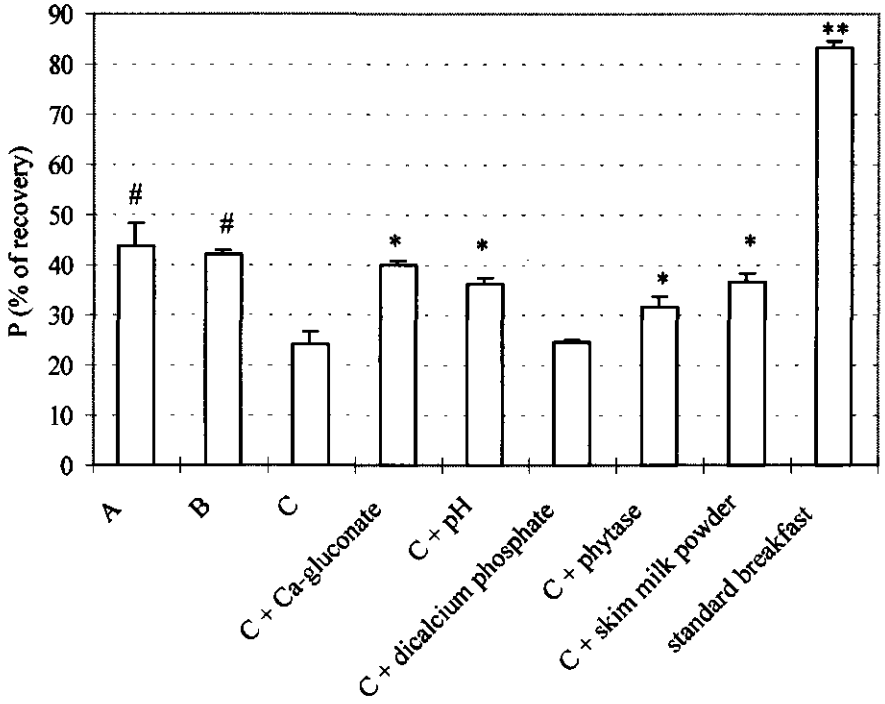

Figure 6 Mean (± range, n = 2) relative amount of phosphorus as percentage of input (corrected for recovery), absorbed in the jejunal plus ileal dialysis fluids from diet A, B, and C (# significantly different from diet C ($P < 0.05$), * significantly different from diet C ($P < 0.05$), ** significantly different from diet A, B and C ($P < 0.05$))

DISCUSSION

The amount of Ca and P absorbed *in vivo* is dependent on the physiological conditions of the animal but also on the chemical and physical properties of the diets. The model was developed to investigate these latter properties by simulating the digestive processes in the lumen of the gastrointestinal tract under standardized conditions. In this way nutritional factors which may influences the digestibility and availability of nutrients can be studied without having the physiological differences between animals.

In the present study the availability for absorption of Ca and P was studied in 3 commercially available canned dog diets. The availability for absorption of Ca found in our study was between 9 and 21 % and that of P between 24 and 43% without the addition of any supplement. From literature it is known that between 0 and 90% of the Ca from the food can be absorbed in adult dogs (Hazewinkel, 1989). Jenkins and Phillips (1960 a) found higher availabilities of P in dogs, when feeding the animals diets containing different concentrations P (29-76%). The driven force in the absorption in the *in vitro* model is the concentration gradient between the lumen and the dialysis fluid. Ca absorbed in the *in vitro* system is solubilized Ca, but also small soluble complexes can be absorbed by the dialysis system (5000 Dalton). Althoug it has been reported that *in vivo* also insoluble Ca complexes can be absorbed (Hanes *et al.*, 1999; Heaney *et al.*, 1990), it is generally accepted that also *in vivo* calcium solubility is the critical factor for absorption in the small intestine. This can be concluded from results of many studies showing negative effects of dietary factors that form insoluble complexes with calcium in the intestine, like phytate, oxalate, and long chain saturated fatty acids (Allen, 1982; Schaafsma, 1997).

The low availability for absorption of Ca from dog foods may be explained by the source of Ca used in the diets. Diet A contained less Ca per kg food than diet B and C, but the relative absorption of diet A was about twice as high, resulting in almost equal absolute amounts of Ca absorption. Besides the Ca sources, the differences in composition of the diets could also have influenced the availability for absorption.

From fat, especially long chain saturated fatty acids, it is known that it can have negatives effects on the Ca absorption, presumably due to the formation of Ca soaps (Cheng *et al.*, 1949; Freeman, 1969; Laval-Jeantet and Laval-Jeantet, 1976).

The standard breakfast, which was also tested in the model simulating the human GI conditions, was used as a control diet. In these studies the availability for absorption of Ca was approx. 80%, despite of the high fat content in this diet (Havenaar *et al.*, 1999). In the present study, using the dog GI conditions, similar results were found for the Ca availability of the standard breakfast. The fat in the standard breakfast is mainly polyunsaturated (63-65% linoleic acid), whereas in the canned dog diet it is probably mainly saturated. In *in vivo* studies with dogs the effects of fat on the Ca absorption were not found. In a study of Hallebeek and Hazewinkel (1998) no significant effects of fat on Ca absorption were found in a study with 6-month-old beagles (11.37 and 32.92 g fat/100 gr diet). Also Jenkins and Phillips (1960 b) did not found an effect on the Ca requirement when the fat content of a diet was increased from 3% (low fat diet) to 20 % (high fat diet). In the *in vitro* system, however, fat digestion takes place in the system but the fatty acids are not absorbed from the lumen.

To be able to absorb these fatty acids other membranes are needed which serve as dialyzing units. The use of suitable membranes for the absorption of fat and fat-soluble components is still under development. Without these membranes the model can be used to study the effect of different types of fatty acids (e.g. polyunsatureated fatty acids or saturated fatty acids) on the availability of minerals.

The results showed that the addition of supplements influences the amount of Ca or P absorbed from dog food. Addition of Ca-gluconate to the diet both increased the availability for absorption of Ca as well as for P ($P < 0.05$). Assuming that the extra amount of Ca absorbed, was due to the Ca-gluconate added to the diet, the availability for absorption of the Ca-gluconate was 71.9 %. This is comparable to the results found in the study using the human conditions in the *in vitro* model (Havenaar *et al.*, 2000). Also the amount of P measured in the dialysates was increased after supplementation with Ca-gluconate. This had not been expected because no extra P was added by the Ca-gluconate. Maybe the gluconate is preventing the formation of insoluble phosphate-mineral complexes. When this is the case, P stays solubilized and can be more easily absorbed in the jejunal and ileal dialysates.

The results implicate that the low availability for absorption is related to the foods. Both a control diet (standard breakfast) and the addition of a supplement showed the same results using the human protocol or the dog protocol. Besides the effect of the formation of Ca soaps or the source of Ca used in the diets, maybe processing of the canned dog diets cause the lower availability for absorption of Ca but also P (Hurell, 1989; Rossander *et al.*, 1992). Heat treatment, for example, results in inactivation of the enzyme phytase, which hydrolysates phytate. From phytate it is know that it can bind minerals and, thus, decreases the availability for absorption of minerals (Torre *et al.* 1991). Therefore, phytase was added to the diet in order to increase the digestibility of P and Ca. By improving the digestibility and availability for absorption of P, less P can be added to a diet without having problems to fulfil the animal's requirements. The lower amount of P in the diet, with a higher availability, decreases the amount of P which will be excreted by the animals. A lower excretion of minerals by the animals can help improve the environment. Addition of phytase increased the absorption of Ca with 4.3% (Figure 4) and of P with 7.4 % ($P < 0.05$). De Smet *et al.* (1999) found similar results in an *in vivo* study with adult beagle dogs. They added phytase to a dry dog food and found a significant effect on the availability of P (relative increase of 14 to 36%). They found however no effects of phytase on the availability of Ca. The explanation to the higher effect of phytase on the availability of P in this study compared to our study, is probably the use of a dry dog food by De Smet *et al.* (1999). It is well known that these diets contain more cereals, and therefore more phytate.

Lowering of the pH in the different compartments of the small intestine did also have a significant effect on the absorption of Ca and P ($P < 0.05$). The pH was lowered from 6.2 and 6.5 to 5.7 and 6.2 in the duodenal and jejunal compartment, respectively. Calcium tends to precipitate from solutions with a pH higher than 6.1 (Allen, 1982). After lowering the pH Ca solubility increases, thus having a positive effect on the availability for absorption.

From the experiments it can be concluded that the *in vitro* model of the gastrointestinal tract of the dog have can be used to determine dialyzable fraction of Ca and P from dog food. Small differences in the availability for absorption were measured in availability for absorption of Ca and P of canned dog diets whether or not with added supplements. When formulating a dog diet, not only the amount of Ca and P and the ratio of those minerals is important, but also the source of these minerals and the interaction with other dietary components in relation to their availability. Supplementation of the diets or lowering the pH the have positive effects on the amount of Ca and P which is available for absorption. The addition of phytase to canned dog food has a significantly positive effect on the availability for absorption of P and a slight effect on the Ca availability.

6 EFFECTS OF GELLING AGENTS ON THE DIGESTION AND ABSORPTION OF NUTRIENTS FROM CANNED DOG FOOD USING A DYNAMIC LABORATORY MODEL OF THE GASTROINTESTINAL TRACT OF DOGS

Marianne Smeets-Peeters[1,2], Robert Havenaar[1], George Fahey, Jr[3], Gertjan Schaafsma[4], Martin Verstegen[2]

1 TNO Nutrition and Research, PO Box 360, 3700 AJ Zeist, The Netherlands
2 Wageningen University, Department of Animal Nutrition, Marijkeweg 40, 6709 PG Wageningen, The Netherlands
3 University of Illinois, Department of animal sciences, Urbana 61801, USA
4 Wageningen University, Department of Human Nutrition and Epidemiology, PO Box 8129, 6700 EV Wageningen, The Netherlands

Submitted to the Journal of Animal Science

ABSTRACT

The digestibility and availability of nutrients from 7 canned dog diets were studied in a dynamic, computer-controlled *in vitro* model of the gastrointestinal tract of the dog. In this model physiological characteristics of the stomach and the small intestine of the dog (e.g., pH, passage time, activity of enzymes, and concentrations of electrolytes and bile) are mimicked. The diets used were chunks in gravy. The composition of the chunks was the same in all diets; the gravy differed in source and concentration of gelling agents used: carrageenan plus guar gum (1:1), carrageenan plus locust bean meal (1:1), and wheat starch. The concentrations used were 0.2% and 0.5%. As a control, the same diet without gelling agents added to the gravy was used. Neither the source nor the concentrations of the gelling agents had an effect on the digestibility of proteins or carbohydrates, the availability for absorption of calcium and phosphorus, or the viscosity and the buffering capacity of the intestinal contents. It can be concluded that the addition of gelling agents at low concentrations to the gravy does not have negative effects on the nutritional quality of the total diet.

INTRODUCTION

There are two sources of determinants of food digestibility: (1) the composition (the presence of and interaction between inhibiting and stimulating factors) and processing of the food, and (2) gastrointestinal factors, including transit time, pH, concentration of digestive enzymes and electrolytes, and bile acids.

The ability of dietary fibers (some of which can be used as gelling agents) to bind cations is one of the most important causes of reduced bioavailability of minerals, e.g. calcium, from the gastrointestinal tract (Kelly and Potter, 1990). Data on gelling agents used in canned dog food, e.g. carrageenan, indicates that they need calcium for gelation (Thomas, 1997). For other soluble viscous dietary fibers, it is known that they have marked effects on the absorption of glucose. Guar gum, for example, attenuates the postprandial glycemic response if added to an enteral formula fed to dogs. This effect is apparently due to the viscosity of the fiber source (Murray *et al.*, 1999).

The effect of viscous indigestible polysaccharides also could be due to an interference with the digestion and absorption of nutrients, resulting in decreased digestibility (Ikegami *et al.*, 1990).

The purpose of this study was to evaluate the effects of gelling agents on the digestibility of protein and carbohydrates, the availability for absorption of calcium and phosphorus, and on the viscosity and buffering capacity of digesta during the passage of canned dog foods using a dynamic *in vitro* model of the gastrointestinal tract of the dog.

MATERIALS AND METHODS

Gastrointestinal model

The dynamic, *in vitro* model of the gastrointestinal tract (GI tract) of the dog was described by Smeets-Peeters *et al.* (1999, 2000) (Figure 1; see Figure 1a Chapter 2). Briefly, the model consists of four compartments simulating the stomach, duodenum, jejunum and ileum. Each compartment is made of glass with a flexible silicone wall inside. This flexible wall is surrounded by water, which keeps the temperature in the model at body temperature. By increasing the water pressure, the flexible walls are squeezed, mimicking the peristaltic movements. Transit time of the food, pH, and the addition of digestive juices are mimicked according to physiological data (Smeets-Peeters *et al.*, 1998; 1999). A dialysis system, composed of semi-permeable hollow fiber membranes connected to the jejunal and ileal compartments mimics the absorption of digested products and water.

Experimental design

Diets

The ingredient composition and the chemical composition of the basic experimental diet (chunks in gravy; Rocofa B.V., Ittervoort, The Netherlands) used in these experiments are shown in Tables 1 and 2.

Three different gelling agents (Table 3) were used in two different concentrations:(1) 0.2% and 0.5% carrageenan + guar gum (1:1) (CG 0.2% or CG 0.5%), (2) 0.2% and 0.5% carrageenan + locust bean meal (1:1) (CL 0.2% or CL 0.5%), and (3) 0.2% and 0.5% wheat starch (S 0.2% or S 0.5%). The gelling agents were added to the gravy during the manufacturing process. The basic diet, without a gelling agent, was used as the control. The total amount of food used in the model was approximately 300 g with a dry matter content of 15%. To achieve this dry matter content, the food was diluted with demineralized water.

Table 1 Ingredient composition of experimental diet

Ingredient	Concentration (%)
Grounded chicken	254
Porcine organ meat	50
Porcine trachea	50
Fish	25
Wheat flour	80
Vitamins and minerals*	6
Caramel (sugar)	1
Water	534

* Provided contents per kg end product : calcium 0.35%, phosphorus 0.28%, sodium 2000 mg/kg, magnesium 100 mg/kg, potassium 2000 mg/kg, iron 17 mg/kg, copper 2.8 mg/kg, vit A 2000 IU/kg, vit D_3 200 IU/kg, vit E 20 mg/kg, vit B_1 1.1 mg/kg, vit B_2 1.0 mg/kg, vit B_6 1.2 mg/kg, vit B_{12} 21.3 μg/kg, panthotenic acid 6.4 mg/kg, biotin 20 μg/kg , and cholin 500 mg/kg

Table 2 Chemical composition of experimental diet

	Concentration (%)
Dry matter	20.44 %
	% DM basis
Crude protein	35.1
Crude fat	29.5
Crude ash	10.1
Crude fiber	0.4
Carbohydrates*	24.9
Calcium	0.35
Phosphorus	0.28

* Calculated amount (100% - crude protein - crude fat - crude ash - crude fiber)

Table 3 Chemical composition (g/kg) of the gelling agents

Item	CG*	CL	S
Moisture	100-120	100-120	130-150
Carbohydrates	800-820	800-820	810-830
Protein	40-50	40-50	36680
Fat	36803	36803	36585
Calcium	8	8	
Potassium	23	23	
Chloride	35	35	

* CG = carrageenan + guar gum (1:1), CL = carrageenan + locust bean meal (1:1), S = wheat starch

Sampling and analyses

To determine the input of exogenous nitrogen, calcium, and phosphorus, a sample of the food was taken before the 'feeding'. For simulation of the transit of food through the small intestine, the 'ileo-cecal valve' delivered intestinal contents according to a computer-controlled curve (Smeets-Peeters *et al.*, 1999). Over 6 h, approximately 65% of the (digested) food passed the ileo-cecal valve and this ileal delivery was collected on ice in 2 h aliquots. The samples were stored as pooled samples and as separate samples. Every 2 h, the dialyzed and absorbed liquids of the jejunal and ileal compartments were collected, mixed, and stored. At the end of each experiment (after 6 h) the residues in the gastric and duodenal compartments, and in the jejunal and ileal compartments, plus the dialyzing units were collected and stored. In order to study the carbohydrate absorption every 20 min a 1 ml (point)sample was taken from the dialysate stream of the jejunum and of the ileum for the first 3 h, and after that, samples were taken every hour. At the end of the experiment, the model was rinsed with 0.2 M HCl to collect all the residual calcium and phosphorus ('rinsed sample'). All samples were stored at -20 °C until analysis. The 'meal' sample, the ileal delivery samples, the jejunal and ileal dialysate samples, the residue samples, and the rinsed samples were analyzed for concentrations of nitrogen according to the Kjeldahl method (International Standard, ISO 5983, 1979), for calcium concentrations the samples were ashed at 500°C and dissolved in 0.6 M hydrochloric acid and measured using atomic

absorption spectroscopy (NEN, 1984). For total phosphorus concentrations the samples were also ashed at 500°C and dissolved in 0.6 M hydrochloric acid and measured using a spectrophotometric method (International Standard, ISO 6491, 1979).

Total glucose of samples taken from the dialysis stream was measured with a modified glucose test (Bergmeyer, 1974). Samples were initially mixed with 0.1 M sodium acetate-buffer (pH = 4.5) that contained at least 20 g/L amyloglucosidase (Amylo 300 ex. Quest Biocon, Ireland), of which the residual glucose was removed to reduce the background signal, to convert all the small saccharides into glucose equivalents. After 5 min of incubation (37°C), the glucose concentration was measured according to the hexokinase/glucose-6-phosphate dehydrogenase assay (Roche Diagnostics, Mannheim, Germany). Analysis was carried out according to the specifications of the manufacturer. This assay was automated using a Cobas Mira plus autoanalyzer (Roche Diagnostics, Mannheim, Germany). The viscosity of the ileal delivery and duodenal samples was measured using a Brookfield viscosity meter (LV-DVII+). The viscosity was measured at 30 rpm and 60 rpm at 37°C. These analyses resulted in a mass balance for nitrogen, calcium and phosphorus, data on the rate of digestion of the proteins and carbohydrates, and data on the availability for absorption of calcium, phosphorus, nitrogen, and glucose from the canned dog foods.

Calculations and statistical analyses

Nutrient recovery was calculated by the formula:

$$\text{recovery, \%} = (N_{samples}/(N_{food} + N_{secretions}))*100$$

where $N_{samples}$ is the total amount of a nutrient (nitrogen, calcium, or phosphorus) found in the samples collected behind the ileo-cecal valve, the dialysis fluids, and the residual samples, N_{food} is the amount of nutrient in the food, and $N_{secretions}$ is the amount of nutrient in the bile and pancreatin solution.

The following formula was used to calculate the ileal digestibility (or availability for absorption in case of calcium or phosphorus):

$$\text{IDC (\%)} = (N_{dialysate} / N_{recovered})*100$$

where IDC is the ileal digestibility coefficient of the nutrient expressed as a percentage of recovery, $N_{dialysate}$ is the total amount of the nutrient recovered from the jejunal plus ileal dialysates, and $N_{recovered}$ is the total amount of a nutrient recovered after an experiment of 6 h. The area under the curve (AUC) for glucose was calculated according to the linear trapezoidal rule (Garbrielsson and Weiner, 1997). For the statistical analyses, two samples of the seven different diets were tested singularly. Data analysis was performed using a statistical software package (SAS, 1990) and a P-value < 0.05 was considered as significant.

Data were analyzed by ANOVA, after Bartlett's test (SAS/STAT, 1997) for testing the homogeneity of the variances. The overall effect of foods was tested using ANOVA. When an overall significance was found, pairwise tests on individual means were performed using the least squares means method of SAS (1990).

RESULTS

Viscosity

The viscosity of the intestinal contents was very low and varied between 2.5 and 11.0 centipoise. No significant differences were found in viscosity measured at 30 or 60 rpm in the chyme collected behind the 'ileo-caecal' valve. The viscosity in the ileal delivery samples was measured at 120 min (t = 120), 240 min (t = 240) and 360 min (t = 360) after 'feeding'. The viscosity at t = 120 was lower for all diets (including the control diet) than that at t = 240 and t = 360 ($P < 0.05$). The viscosity was not significantly different between the diets except at t = 120 measured at 30 rpm (Table 4). These differences were not found at 60 rpm.

Glucose

Glucose absorption in the jejunal and ileal dialysis streams (Figures 2 and 3) did not show significant differences in time among diets, except for the glucose concentration in the jejunal dialysate at t = 100 (Table 5). The area under the curve (AUC) of the glucose concentrations in the jejunal and the ileal dialysates also did not show significant differences among diets.

Table 4 Mean viscosity (± range, n = 2) and P-values of the statistical analysis of the ileal delivery samples (30 *rpm*, *t* = 120 *min*) of canned dog diets with different gelling agents in different concentrations.

Viscosity (centipoise)	control*	CL 0.2%*	CL 0.5%	CG 0.2%*	CG 0.5%	S 0.2%*	S 0.5%
mean	4.5	4	2.25	4.75	5.25	5	3.25
range	0	0	0.25	0.75	0.25	0	0.75
P-value	control	CL 0.2%	CL 0.5%	CG 0.2%	CG 0.5%	S 0.2%	S 0.5%
control	x	n.s.†	n.s.	n.s.	P < 0.01	n.s.	n.s.
CL 0.2%		x	n.s.	n.s.	P < 0.01	n.s.	P < 0.05
CL 0.5%			x	n.s.	P < 0.01	n.s.	P < 0.05
CG 0.2%				x	P < 0.05	n.s.	n.s.
CG 0.5%					x	P < 0.01	n.s.
S 0.2%						x	P < 0.05
S 0.5%							x

* control = diet without gelling agent, CL = carrageenan + locust bean meal (1:1), CG = carrageenan + guar gum (1:1), S = wheat starch.

† n.s. = not statistically significant

Protein

The recovery of the nitrogen was 101 ± 9.3 % (n = 14). The ileal protein digestibility of the different diets varied from 70.2 to 75.8% (Table 6), but were not significantly different. Neither the amount of nitrogen measured in the different samples after six hours of experiments (Figure 4), nor the protein digestibility at t = 120, t = 240 or t = 360 between the diets (Figure 5) showed any significant differences.

Table 5 Mean glucose concentration (± range, n=2) and P-values of the statistical analysis of the jejunal dialysate samples (t=100 min) of canned dog diets with different gelling agents in different concentrations

Glucose (g/L)	control*	CL 0.2%*	CL 0.5%	CG 0.2%*	CG 0.5%	S 0.2%*	S 0.5%
mean	4.27	2.73	3.4	3.88	2.90‡	3.91	3.76
range	0.19	0.2	0.08	0.03		0	0.22
P-value	control	CL 0.2%	CL 0.5%	CG 0.2%	CG 0.5%	S 0.2%	S 0.5%
control	x	$P < 0.01$	$P < 0.01$	n.s.†	$P < 0.01$	$P < 0.01$	$P < 0.05$
CL 0.2%		x	$P < 0.05$	$P < 0.01$	n.s.	$P < 0.01$	$P < 0.01$
CL 0.5%			x	n.s.	n.s.	n.s.	n.s.
CG 0.2%				x	$P < 0.01$	n.s.	n.s.
CG 0.5%					x	$P < 0.01$	$P < 0.05$
S 0.2%						x	n.s.
S 0.5%							x

* control = diet without gelling agent, CL = carrageenan + locust bean meal (1:1), CG = carrageenan + guar gum (1:1), S = wheat starch.

† n.s. = not statistically significant

‡ n = 1

Calcium and phosphorus

The recovery of the calcium and phosphorus (n = 14) was 81.8 ± 11.3% and 86.6 ± 8.6%, respectively. The availability for absorption of calcium and phosphorus varied from 9.2 to 13.7% and 47.2 to 54.6%, respectively (Table 6). The seven different diets were not significantly different from each other with respect to the availability for absorption of calcium or phosphorus in the jejunal and ileal compartments (n = 2).

Neither the amount of calcium nor the amount of phosphorus measured in the various samples showed any significant differences among treatments (Figures 6 and 7).

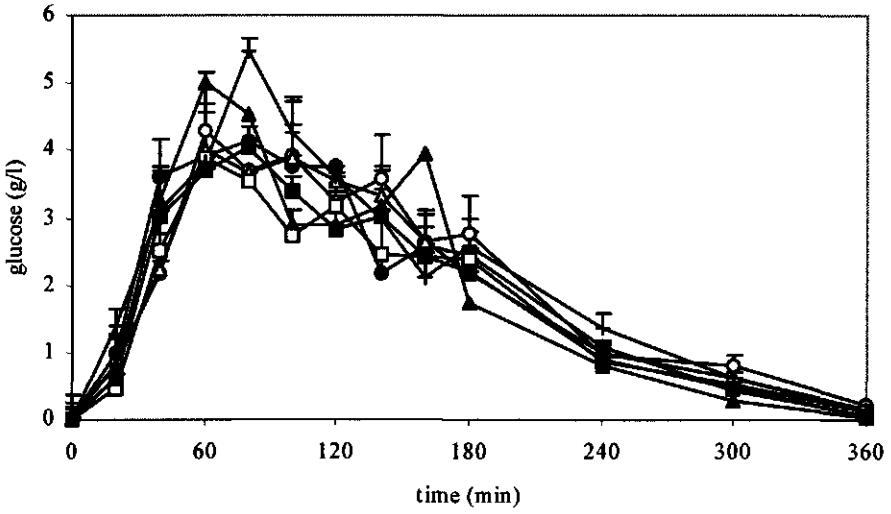

Figure 2 Mean (± range) glucose concentrations in the jejunal dialysis stream (n = 2) at different times after the intake of dog diets with 0.2% starch (○), 0.5% starch (•), 0.2% guar (△), 0.5% guar (▲), 0.2% locust bean meal (□), 0.5% locust bean meal (■) or without a gelling agent (+)

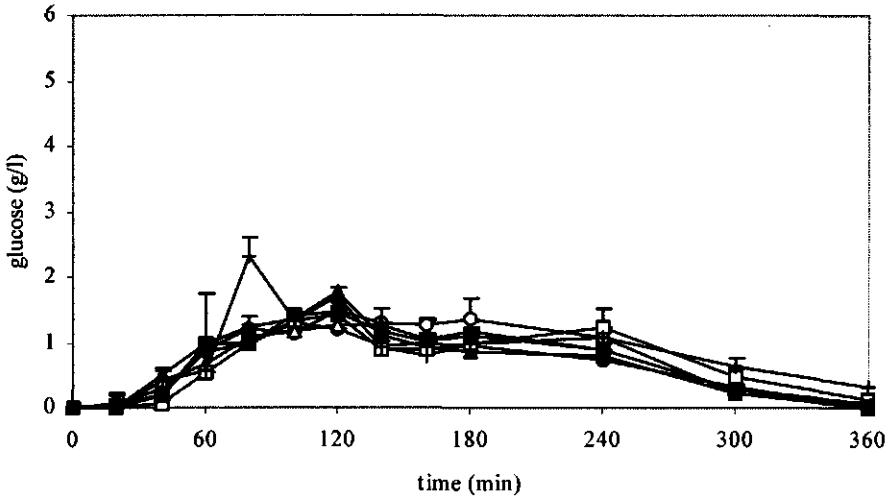

Figure 3 Mean (± range) glucose concentration in the ileal dialysis stream (n = 2) at different times after the intake of dog diets with 0.2% starch (○), 0.5% starch (•), 0.2% guar (△), 0.5% guar (▲), 0.2% locust bean meal (□), 0.5% locust bean meal (■) or without a gelling agent (+)

Table 6 Mean protein digestibility and mean availability for absorption of calcium and phosphorus (± range, n = 2) of canned dog diets with different gelling agents

Diet	Protein (%)*	Calcium (%)*	Phosphorus (%)*
Control	70.2 ± 0.4	13.7 ± 2.9	54.6 ± 5.3
Carrageenan + guar 0.2%	72.4 ± 1.0	10.0 ± 0.1	47.2 ± 1.6
Carrageenan + guar 0.5%	71.0 ± 3.8	9.6 ± 1.2	47.7 ± 0.4
Carrageenan + locust bean meal 0.2%	70.8 ± 1.7	13.8 ± 4.1	48.7 ± 0.1
Carrageenan + locust bean meal 0.5%	72.4 ± 2.4	9.8 ± 1.1	47.7 ± 3.6
Wheat starch 0.2%	73.4 ± 0.7	9.9 ± 0.4	51.1 ± 2.7
Wheat starch 0.5%	75.8 ± 1.2	9.2 ± 0.9	50.5 ± 2.2

* Digestibility and availability for absorption are expressed as a % of input corrected for the recovery

Buffering capacity

No differences in buffering capacity were found among diets. Both the amount of hydrochloric acid used to mimic the pH drop in the gastric compartment and the amount of sodium bicarbonate used in different compartments of the small intestine to increase the pH were similar in all experiments.

DISCUSSION

Polysaccharide food additives, such as carrageenan, serve advantageous functions in food products through their specific physical and colloidal properties without regard to digestibility (Jeanes, 1975). Because carrageenan alone results in a brittle gel, it is often combined with gallactomannans such as guar gum and locust bean meal (Fox, 1997). Just like carrageenan, galactomannans are not degraded by digestive enzymes. This is in contrast to non-resistant starches which are fully digested in the alimentary tract (Fox, 1997).

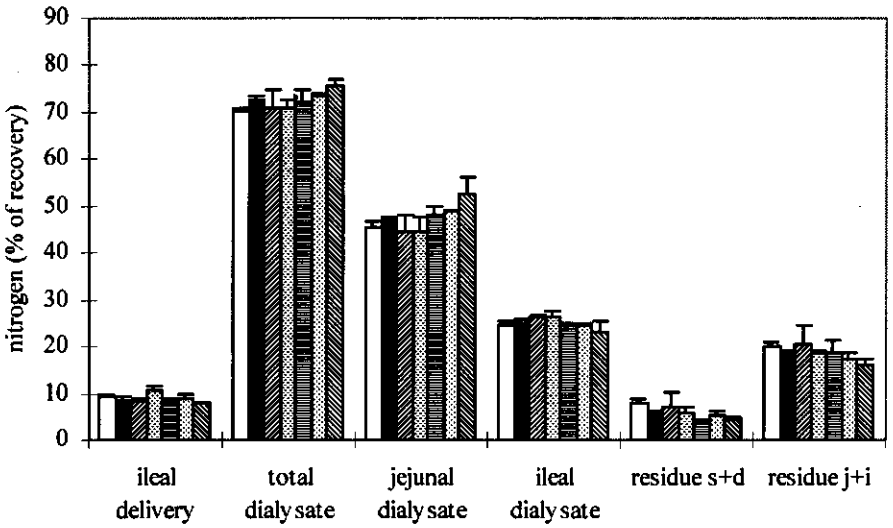

Figure 4 Mean (± range) amount of nitrogen (% of input corrected for recovery, n = 2) in the ileal delivery, total dialysate, jejunal dialysate, ileal dialysate, residue stomach + duodenum (residue s+d), and residue jejunum + ileum (residue j+i) taken from the dynamic model of the gastrointestinal tract of the dog 6 h after the intake of dog diets without a gelling agent (white) or with different gelling agents (CG 0.2% = black, CG 0.5% = hatched (right), CL 0.2% = broken horizontal lines, CL 0.5% = horizontal lines, S 0.2% = brackets, S 0.5% = hatched (left))

Levels of, for example, carrageenan plus guar gum used in petfoods, vary from 0.2 to 1.0 % for meat products and 0.1 to 0.2 % for chunks in gravy. Starches, on the other hand, are relatively inefficient thickeners, and must be used at much higher concentrations (4.0 to 6.0%) compared with 0.5 to 1.0% for galactomannans (Fox, 1997). However, to be able to compare the results regarding the effects on digestibility and availability for absorption of nutrients, the concentrations of the gelling agents used in this study were kept similar.

The viscosity at t = 120 min was significantly lower compared with the viscosity at t = 240 min and t = 360 min for all diets. This difference in time can be explained by the amount of food delivered from the stomach into the duodenal compartment and from the end of the small intestine into the 'large intestine' (sampling bottle). The amount of food delivered from the gastric compartment was 60, 84, and 94% at 120, 240 and 360 min, respectively. The amount delivered at the end of the small intestine was 7, 33 and 67% at 120, 240 and 360 min, respectively. However, part of the food had been digested and absorbed in the

jejunal and ileal dialysate in contrast to the gelling agent. This increasing amount of gelling agent in the compartments in time probably resulted in increasing viscosity. Another factor that plays a role in viscosity is water absorption. Via the dialysis system connected to the jejunal and ileal compartments, water was absorbed from the system, resulting in increasing concentrations of gelling agent.

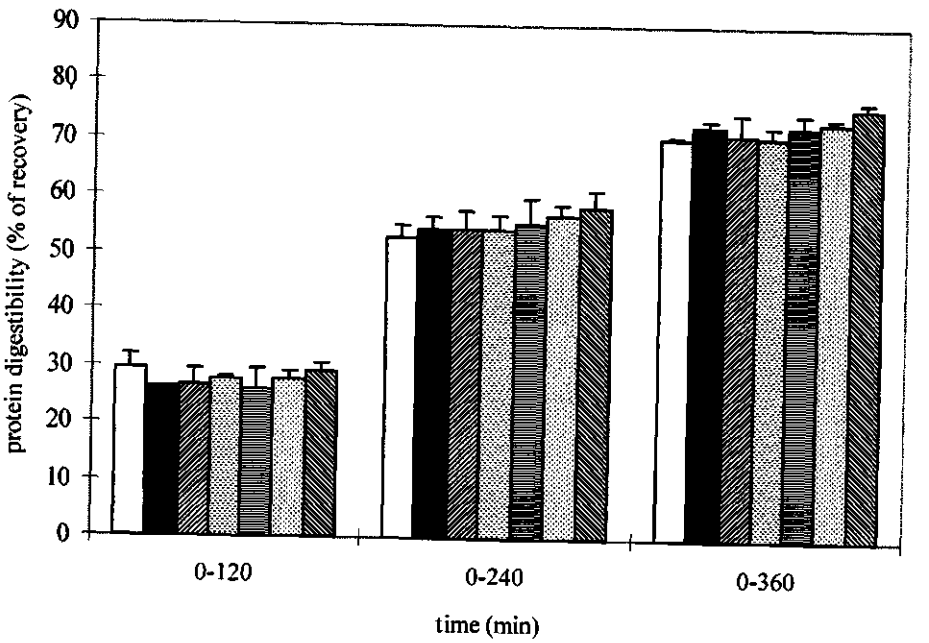

Figure 5 Average (± range, n=2) cumulative protein digestibility of dog diets with or without a gelling agent (legend see Figure 4)

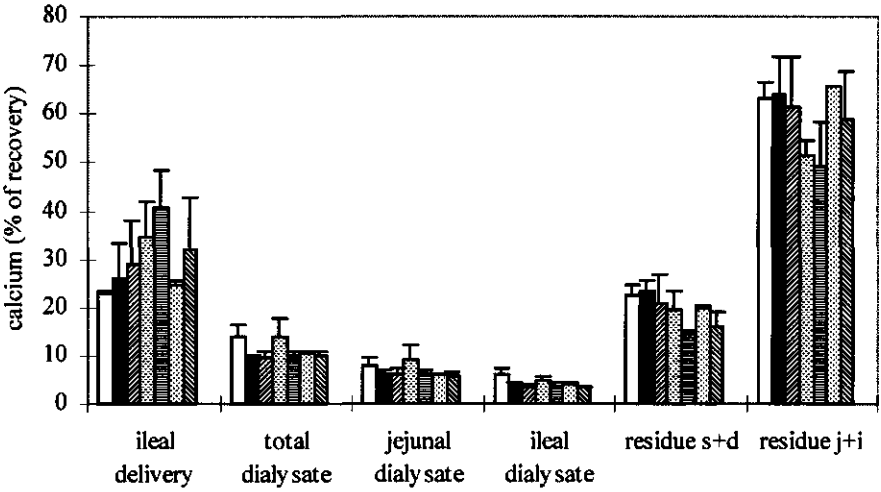

Figure 6 Mean (± range) amount of calcium (as a % of input corrected for recovery; n = 2) in the ileal delivery, total dialysate, jejunal dialysate, ileal dialysate, residue stomach + duodenum (residue s+d), and residue jejunum + ileum (residue j+i) taken from the dynamic model of the gastrointestinal tract of the dog 6 h after the intake of dog diets without or without a gelling agent (legend see Figure 4)

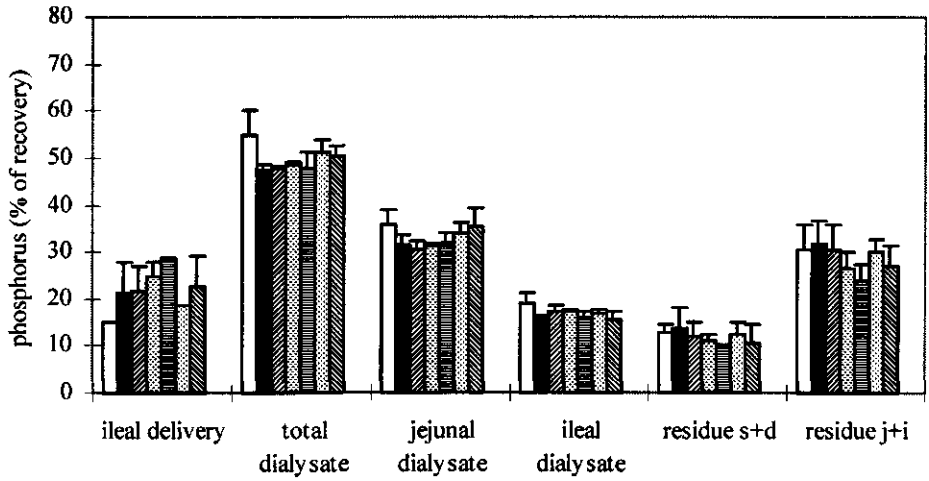

Figure 7 Mean (± range) amount of phosphorus (as a % of input corrected for recovery; n=2; legend see Figure 6)

Ikegam *et al.* (1990) found an increased viscosity of small intestinal contents for rats fed 5% guar gum (6 h after the latest meal and after homogenizing the intestinal fluid) compared to a control diet containing 5% sucrose. Homogenization, however, can have an effect on the viscosity, and the concentration used in this study was 10 times higher than in our study. This high concentration of guar gum is not realistic when used as gelling agent in petfood.
In the literature, different effects of dietary fibers on postprandial glycemic responses have been described. Murray *et al.* (1999) evaluated the glycemic response of dogs fed a liquid formula. They found that mean incremental area under the serum glucose response curves for dogs fed Glytrol® (soy fibre and gum arabic), Glucerna® (soy fibre), and IVF® (IVF = induced viscosity fiber; alginate) were lower ($P < 0.05$) than for a control (oat fibre and soy polysaccharide) treatment. Reppas and Dressman (1992) studied the relationship between postprandial blood glucose levels and meal viscosity by adding combinations of hydroxypropylmethylcellulose to glucose solutions (5 or 20%) in dogs. They concluded that hydroxypropylmethylcellulose retards the absorption of glucose and that this retardation was related to the viscosity of the glucose solution administered. They suggested further research to determine if this effect also occured when a complete meal was fed to the animals. Wolever *et al.*(1979) found that, in humans, the effect of fiber (guar gum) on the glycemic response was influenced by the way it was ingested. Higher levels of fiber had to be added to a solid meal than to a hydrated form to be effective. This effect might also play a role in dogs. For that reason, the present study was performed with complete, canned dog diets under standardized physiological conditions. Forster and Hoos (1977), who studied the influence of various gums (including carrageenan) on the intestinal absorption of glucose (5% solution) in rats, did not find effects on glucose absorption. They concluded that absorption of glucose was not influenced by the addition of 1 to 2% gum. This is in agreement with the results of the present study in which no differences were found in glucose absorption among diets with 0.2% and 0.5% gelling agent. The lowering effect of dietary fibers on the postprandial glycemic response as described by Murray *et al.* (1999) was not found for the fibers used as gelling agents in our study. If the effect on glucose absorption was caused by viscosity, then the lack of differences in glucose absorption can be explained by the absence of significant differences in viscosity measured in our study. It is also possible that the overall viscosity in our experiments was lower compared to the viscosity of the diets used by Murray *et al.* (1999). However, no data about viscosity were presented in their paper. Another possibility is that fibers slow nutrient absorption by increasing the viscosity of the gastric contents and reducing the rate of gastric emptying (Kelly and Potter, 1990). In our model, however, the transit time was standardized based on

physiological data of the dog after ingestion of solid food (Smeets-Peeters *et al.*, 1998; 1999). Also, the effects of viscous indigestible polysaccharides in the exocrine pancreatic-biliary function, which may depress the process of digestion and absorption (Ikegami *et al.*, 1990), are of no influence in the *in vitro* study due to standardization of the addition of digestive juices. Starch is a digestible gelling agent, in contrast to carrageenan, locust bean meal, and guar gum. However, it did not raise significantly glucose absorption. This can be explained by the low concentration of starch (0.2% and 0.5%) used in the gravy compared to the total amount of starch (± 25%) used in the diet.

There were no significant differences in viscosity and in glucose absorption in our study. Also no significant differences were found in the digestibility of protein among the different diets. This corresponds with the results of Rhee *et al.* (1981). In their study the concentration of carrageenan varied from 0 to 15% of the diet. The increase in carrageenan resulted in a decrease in nitrogen absorption in rats, but the decrease was only numerical (not significant). However, only carrageenan was included in the diet and not guar gum and locust bean meal like in our study.

According to Kelly and Potter (1990), availability for absorption of minerals can be influenced by dietary fiber. They found that carrageenan decreased the amount of calcium able to dialyze through a membrane. Only carrageenan was used in their study, in a mixture of κ-, λ-, and ι-carrageenan at a higher concentration (1%). They concluded that calcium was most likely bound to the sulfate group of the gum which keeps the calcium in an insoluble, non-dialyzable form. In our study, no significant effects of a combination of gelling agents up to 0.5% were found on the availability for absorption of calcium during digestion of a complete meal. The overall low availability for absorption of calcium was similar to other studies conducted using the *in vitro* gastrointestinal model (Smeets-Peeters *et al.*, 2000). Availability for absorption of phosphorus and the buffering capacity of the different diets were also not different.

In general it is known from the literature that dietary fibers or gelling agents can have effects on digestibility and availability for absorption of nutrients. However, hardly any experiments have been performed to study the effects of gelling agents in canned dog food (neither *in vivo* nor *in vitro*). The studies performed in dogs were mainly focused on the effect of fibers on glycemic response. Besides, the studies described in the literature are mainly focusing on just one gelling agent, and not, as in our study, the combination of gelling agents which are used in the manufacturing of petfood. The gelling agents used in our study (0.2 and 0.5% carrageenan plus locust bean meal (1:1), 0.2 and 0.5% carrageenan plus guar gum (1:1), and 0.2 and 0.5% starch) did not demonstrate any effect on the digestibility and

availability for absorption of different nutrients compared to a control diet without an added gelling agent. It can be concluded that the addition of gelling agents at low concentrations to the gravy does not have negative effects on the nutritional quality of the total diet. Further research is necessary to study the effects of higher concentrations of mainly starch (e.g. 1 and 5%) in gravy and of all gelling agents in all meat products at higher concentrations (e.g. 1%).

IMPLICATIONS

Carrageenan plus guar gum, carrageenan plus locust bean meal and starch (concentrations 0.2 and 0.5%) dit not have any effects on the digestibility or availability for absorption of the measured nutrients from a complete canned dog diet using an *in vitro* model of the gastrointestinal tract of the dog. Further research is necessary to study the effect of higher concentrations of the gelling agents or to study the effects in other types of diets (e.g. all meat products).

7 THE EFFECT OF VITAMIN D ON THE ABSORPTION OF CALCIUM IN DOGS. A STUDY USING THREE DIFFERENT METHODS

Marianne Smeets-Peeters[1,2], Marianna Tryfonidou[3], Robert Havenaar ,[1] Rob Onderwater , [1] Herman Hazewinkel[3], Martin Verstegen[2], Gertjan Schaafsma[4]

1 TNO Nutrition and Research, PO Box 360, 3700 AJ Zeist, The Netherlands
2 Wageningen University, Department of Animal Nutrition, Marijkeweg 40, 6709 PG Wageningen, The Netherlands
3 Utrecht University, Department of Clinical Sciences of Companion Animals, PO Box 80.154, 3508 TD Utrecht, The Netherlands
4 Wageningen University, Department of Human Nutrition and Epidemiology, PO Box 8129, 6700 EV Wageningen, The Netherlands

Submitted to Archives of Animal Nutrition

ABSTRACT

The studies presented in this paper describe the effect of vitamin D on the calcium (Ca) absorption with three different methods: *in vivo* in dogs with ^{45}Ca metabolic studies, in a dynamic *in vitro* system mimicking the gastrointestinal tract of the dog (FIDO), and in a biological model with intestinal segments of dogs. Two diets were tested containing 500 IU vitamin D (control; cVitD) and 4000 IU vitamin D (high vitamin D; hVitD), respectively.
In the *in vivo* study the overall absorption of Ca was studied at the start of complete weaning and during fast growth, i.e. 8 and 20 weeks of age. In the *in vitro* studies the availability for absorption of Ca from the diets was studied (FIDO) and active and passive absorption of Ca was measured in different segments of the small intestine of the dogs in these life stages.
Dogs, fed the hVitD diet revealed a significantly lower true absorption of Ca at 8 and 20 weeks of age than dogs fed the cVitD diet. The availability for absorption of Ca measured in FIDO was similar for both diets. With the intestinal segments no active transport was measured, neither in the biopsies of dogs fed the cVitD diet nor in those fed the hVitD diet. Both the study with dogs and the study with intestinal segments revealed a lower Ca absorption in the hVitD group than in the cVitD group at 20 weeks of age.
It can be concluded that vitamin D seems to downregulate the passive absorption of Ca and that the findings from the intestinal segments are in accordance with the findings of the *in vivo* study. The three different methods used in this study complement each other to get a complete picture of the influence of vitamin D on the regulation of the Ca absorption in young dogs.

INTRODUCTION

When formulating and producing pet foods, manufacturers must account for differences in calcium (Ca) availability in the various ingredients that are used and the interaction between ingredients. Absorption coefficients for Ca have been reported to vary between 0% and 90%, depending on the composition of the diet and the need of the animal. Within certain limits, Ca absorption efficiency increases when the calcium content of the diet decreases (Case *et al.*, 1995). Moreover calcium absorption is known to be increased under conditions of high physiological requirements, e.g. rapid growth, pregnancy and lactation.
The intestinal absorption of Ca occurs via two processes: an active transcellular saturable process and a paracellular diffusion process (Allen and Wood, 1994; Wasserman and Fullmer, 1995). The active process is dependent on calcitrol (1,25 dihydroxycholecalciferol),

the active metabolite of vitamin (Norman, 1990), whereas passive absorption is a function of concentration gradient. The first hydroxylation of vitamin D into 25-hydroxycholecalciferol takes place in the liver. The plasma level of 25-hydroxycalciferol has a rectilinear relationship with the amount of vitamin D consumed (Holick and Clark, 1978; Morris *et al.*, 1999). The second hydroxylation in the kidney leads to formation of the most active metabolite of vitamin D; 1,25-dihydroxycholecalciferol or calcitrol. Vitamin D and its metabolites are especially of importance for the Ca metabolism during rapid skeletal growth, i.e. in the prepubertal period. A chronic inappropriate high administration of vitamin D can lead to intoxication and disturbance of the Ca metabolism (Hazewinkel, 1996; Kallfelz and Dzanis, 1989).

The intestinal Ca transport system undergoes a process of maturation. At early development (during suckling period) the Ca transport through the intestinal wall is mainly passive in rats (Ghishan *et al.*, 1984). Active absorption during early post-natal development of pigs is neither regulated (Schröder *et al.*, 1993) nor stimulated by vitamin D (Schröder *et al.*, 1998). At a later stage passive absorption decreases, active absorption becomes more prominent and the whole mechanism is better hormonally regulated (Pansu *et al.*, 1983; Ghishan *et al.*, 1984). The vitamin D regulated Ca transport-system seems to be well developed at weaning age in rats (Halloran and DeLuca, 1981; Pansu *et al.*, 1983) and pigs (Fox *et al.*, 1985; Kaune *et al.*, 1992; Schröder *et al.*, 1990; 1993; 1998). Thus weaning age is suggested to be the cornerstone of this maturation process.

Dogs and cats, in contrast to omnivores and herbivores, do not produce adequate amounts of vitamin D under the influence of sunlight in the skin (Hazewinkel *et al.*, 1988; How *et al.*, 1994). The lack of the synthesis of pre-vitamin D in the skin has been attributed to a low concentration of 7-dehydroxycholesterol (the precursor of pre-vitamin D) (How *et al.*, 1994; Morris *et al.*, 1999). This makes these carnivores completely dependent on the content of vitamin D in the diet.

Studies in dogs were carried out on the development of the Ca transport system in the intestine beginning at weaning age (8 weeks) until the onset of puberty (20 weeks), with special emphasis on the effect of vitamin D on Ca absorption, concerning the active and passive component. Therefore, *in vivo* (^{45}Ca-balans) and *in vitro* Ca absorption tests with intestinal segments were carried out in a group of dogs fed a diet with vitamin D oversupplemented (hVitD) versus a group fed a diet with a vitamin D content (cVitD group) according to AAFCO (1998).

In the *in vivo* studies with dogs, true absorption of Ca in the intact animal was determined. To study the transport through the intestinal epithelium, intestinal segments (duodenum,

jejunum and ileum) of dogs were used in intestinal transport chambers. In these intestinal segments both active transport and passive diffusion were studied. The availability of Ca for intestinal absorption from the food matrix is largely dependent on the solubization of Ca during food digestion and is thus known to be inhibited by dietary factors that can form insoluble calcium complexes, such as phytate, oxalate and long chain fatty acids (Greger, 1999; Meyer and Mundt, 1983; Schoenmakers, 1998). Therefore, first the availability for absorption of Ca from the diets was investigated in an *in vitro* model of the gastrointestinal tract of the dog (FIDO = *f*unctional gastro*i*ntestinal *d*o*g* model) (Smeets-Peeters *et al.*, 1999; 2000).

The aim of the study was to evaluate if the three different methods, as mentioned before, could be complementary with respect to the availability and absorption of calcium.

MATERIALS AND METHODS

Diets

Two different isoenergetic dry dog foods (Table 1) were used in both the *in vitro* and the *in vivo* studies. One diet met the vitamin D requirements of AAFCO 1998 (cVitD; 500 IU/kg diet) and the other diet was eight times higher in vitamin D content (hVitD group; 4000 IU/kg diet).

Table 1 Composition of diets

Constituent		Diet cVitD	Diet hVitD
Ca	(g/100 gr dry matter)	8.65	8.74
P	(g/100 gr dry matter)	7.48	7.52
Vitamin D_3	(IU/kg diet)	500	4000
Dry matter	(gr/kg diet)	930	930

***In vivo* study in dogs**

Twenty-six Great Danes, originating from three different litters were divided at random into two groups at three weeks of age: a control (n = 19) and a high vitamin D group (n = 7). The pups were raised on a dry pellet food formulated to be comparable on the Ca and P content (Table 1). Partial weaning begun at three weeks of age and the pups received the dry food as gruel. Weaning was completed at six weeks of age with the pups removed from their dams and fed solely their diet (cVitD or hVitD, respectively) as dry food. The animals were weighed every 5 days and had a food intake restricted at two times their maintenance energy requirements during the whole study. The non-consumed amount of food was weighed and actual Ca intake (V_I) was calculated.

Ca true-absorption (V_a) was determined on the 8th and 20th week of age in 14 cVitD and 7 hVitD dogs by techniques as described previously (Hazewinkel *et al.*, 1987, 1991; Nap *et al.*, 1993; Schoenmakers, 1998). In short, dogs were kept individually in metabolic cages. The endogenous fecal excretion of ^{45}Ca (V_f) was determined by collecting faeces for three consecutive days after an intravenous injection of 0.1 and 0.4 MBq ^{45}Ca as $^{45}CaCl_2$ in water (NEN™ Life Science Products Inc., Boston, USA) at 8 and 20 weeks of age, respectively. On the fourth day of each period of Ca metabolic studies, equivalent amounts of ^{45}Ca activity to the intravenous doses was orally administrated and feces were collected for four consecutive days to determine the total fecal content of ^{45}Ca (V_F). On the assumption of a steady state during each investigation period, true absorption (V_a) could be calculated with the formula:

$$V_a = V_I - (V_F - V_f)$$

where V_I is the Ca intake, V_F is the total fecal content of ^{45}Ca and V_f is the endogenous fecal excretion of ^{45}Ca. The Utrecht University ethical committee for animal care and use approved all procedures.

Study in FIDO

The dynamic, *in vitro* model of the gastrointestinal tract (GI tract) of the dog was described by Smeets-Peeters *et al.* (1999, 2000) (Figure 1, see Figure 1b Chapter 2). This dynamic *in vitro* gastrointestinal model consists of four successive compartments, simulating the stomach, duodenum, jejunum and ileum. Mixing and transport of the food is regulated by peristaltic movements in and between the four compartments. Transit time of the chyme, its pH and the addition of the electrolytes and enzymes are adapted to the physiology of the dog

(Smeets-Peeters *et al.*, 1998; 1999) and controlled by a computer. By means of semi-permeable hollow fibre devices (Cobe HG-400, Secon GmbH Germany) connected to both the jejunal and ileal compartments, absorption of the small molecular digested products is mimicked and the concentration of electrolytes in the model is controlled.

The availability of Ca from both diets was tested in FIDO. The diets were ground to particles sizes ≤3 mm and diluted with demineralized water to a dry matter content of 15%. A total amount of 300 g (cVitD: n = 4 and hVit D: n = 2) was used.

Every hour total ileal delivery samples were collected behind the 'ileo-caecal valve' (Figure 1, N; see Figure 1a Chapter 2). The samples were stored as separate samples and as pooled samples (1-6 h). The dialysis fluid of the jejunal and ileal compartments was sampled every two hours and at the end of the experiment the residual contents of the gastric plus duodenal compartment and of the jejunal plus ileal compartment were collected. Also the jejunal and ileal dialysis samples were stored as separate and pooled samples. All samples were stored at -20°C until analysis.

Ca was determined by atomic absorption spectrometry (NEN, 1984). Recovery and availability of Ca was calculated as described previously (Smeets-Peeters *et al.*, 2000).

Study in intestinal segments from dogs

To investigate the *in vivo* transport of Ca across the intestinal mucosa, isolated small intestinal segments from dogs were placed in so called 'transport chambers'.

The experiments were carried out with tissue originating from five dogs at 8 weeks of age immediately at the first day of complete weaning (n = 3 cVitD; n = 2 hVitD), and from twelve dogs following the ^{45}Ca metabolic study at 20 weeks of age (n = 6 cVitD; n = 6 hVitD). The animals were euthanised with an overdose of pentobarbital, followed immediately by laparotomy and resection of the intestine. The following segments of the intestine were used: duodenum (a 5 cm segment was dissected 10 cm caudal to the ampulla of Vater), jejunum (a 10 cm segment at mid-jejunum), and ileum (a 5 cm segment at mid-ileum).

After rinsing the intestinal segment with ice-cold Dulbecco's modified eagle medium (DMEM; Gibco BRL, Life Technologies LTD, Paisley, Scotland), it was cut open in the longitudinal direction. The intestine was then placed with the mucosa on a flat underground and the mucosa layer was separated from the muscle layer with a blunt razor blade. Samples of the mucosa layer were taken with a 9 mm steel punch and were placed in transport chambers. The effective intestinal area in the transport chambers was 0.196 cm^2.

The experiment was started by adding 1.5 ml DMEM containing radiolabeled Ca (10 μM) (NEN™ Life Science Products, Inc. Boston, USA) and mannitol ([^{3}H]mannitol, 10 μM) (ICN Biomedicals, Irvine, USA) to the donor compartment (mucosal side) and 1.5 ml DMEM to the receptor compartment (serosal side). Table 2 gives an overview of which transport is measured in which compartment. By slowly passing carbogen (95% O_2 and 5% CO_2) through both the donor and receptor chamber, the medium was continuously mixed. The experiment was performed at 37°C. Samples of the receptor compartment were collected at 30, 60, 90, 120, and 150 min. Radioactivity was determined in the samples and the tissue (at the end of the experiment) by Liquid Scintillation Counting (LSC) using DOT-DPM™ (Digital Overlay Technique using the Spectrum Library and the External Standard Spectrum) for quench correction.
The permeability coefficient is calculated according to the formula:

$$Papp = \frac{dQ}{dt} * \frac{1}{A * C_0}$$

where Papp is the permeability coefficient, dQ/dt the initial rate of transport (dpm/s), A the area of the intestinal segment in cm^2 and C_0 the initial radioactivity at the donor side (dmp/ml).

Statistics

In vivo studies in dogs

The results of Ca-metabolic studies are expressed in mmol per kg body weight per day. Data were normally distributed. Therefore the results are expressed as means ± SEM. The significance of differences ($P < 0.05$), in all parameters was tested with the unpaired two tailed Student's t-test.

In vitro studies in FIDO and in intestinal segments from dogs

Analyses of the Ca availability and permeability data were performed using the statistical software package (SAS, 1990) and a P-value < 0.05 was considered as significant. Data were analyzed by ANOVA, after Bartlett's test for testing the homogenicity of the variances. An ANOVA test was performed to test the overall effect of foods. When an overall significance was found, pairwise tests on individual means were performed using the least squares means method of SAS (1990).

Table 2 Intestinal segments used for studying the transport of ^{45}Ca, ^{3}H-mannitol in dogs of 8 or 20 weeks old, which were fed a low or high vitamin D diet or a control diet

	CvitD†			HvitD†		
Intestinal segment	side‡	animals§	segment#	side‡	animals§	segment#
duodenum m=>s (8)*	-			-		
duodenum s=>m (8)	-			-		
jejunum m=>s (8)	+	3	9	+	2	6
jejunum s=>m (8)	+	3	8	+	2	3
ileum m=>s (8)	+	3	11	+	2	6
ileum s=>m (8)	-			-		
duodenum m=>s (20)	+	6	14	-		
duodenum s=>m (20)	+	6	14	-		
jejunum m=>s (20)	+	6	14	+	6	12
jejunum s=>m (20)	+	6	14	+	6	12
ileum m=>s (20)	+	6	14	+	6	12
ileum s=>m (20)	+	6	14	+	6	12

* Side of the segments used; m=mucosal side, s = serosal side. Number between brackets is the age of animals in weeks

† cVitD = control diet, hVitD = high vitamin D diet

‡ - not measured, + measured

§ number of animals used of which intestinal segments were taken

\# number of intestinal segment with which the experiments were performed

RESULTS

In vivo studies in dogs

Food intake was according to the calculated requirements, as dogs consumed the total amount of food given daily, which did not differ between groups. As a consequence, the V_i of Ca in the hVitD group did not differ significantly at 8 and 20 weeks of age (8.34 ± 0.17 mmol/kg BW and 6.23 ± 0.04 mmol/kg BW, respectively) from that in the cVitD group (9.09 ± 0.25 and 6.71 ± 0.2 mmol/kg BW, respectively) (Figure 1). V_f did not differ significantly between groups at 8 weeks of age (0.39 ± 0.03 mmol/kg BW). Whereas at 20 weeks of age it was significantly higher in the hVitD group (0.24 ± 0.01 mmol/kg BW, $P < 0.001$) compared to the cVitD group (0.17 ± 0.01 mmol/kg BW; Figure 2). The V_a was significantly lower in the hVitD group both after 8 weeks and 20 weeks: 6.91 ± 0.21 vs 5.69 ± 0.15 mmol/kg BW (cVitD vs hVitD at 8 weeks; $p = 0.001$) and 3.90 ± 0.13 vs 2.90 ± 0.12 mmol/kg BW, (cVitD vs hVitD at 20 weeks; $P < 0.001$) (Figure 2).

In vitro studies in FIDO

The mean recovery of the Ca in the GI model was 77 ± 14% (n = 6). The amount of Ca found in different samples of the GI model 6 h after 'ingestion' of the diet is shown in Figure 3. The mean availability for absorption of the Ca was 14 ± 7% (n = 4), and 8 ± 2% (n = 2) for the cVitD diet, and the hVitD diet, respectively. No significant differences were found between the different samples of the different diets tested ($p > 0.05$).

In vitro studies in intestinal segments from dogs

The results of the transport of Ca through the intestinal segments (jejunum and ileum) of dogs of 8 and 20 weeks old, are shown in Figures 4 and 5 and of the transport of mannitol in Figures 6 and 7.

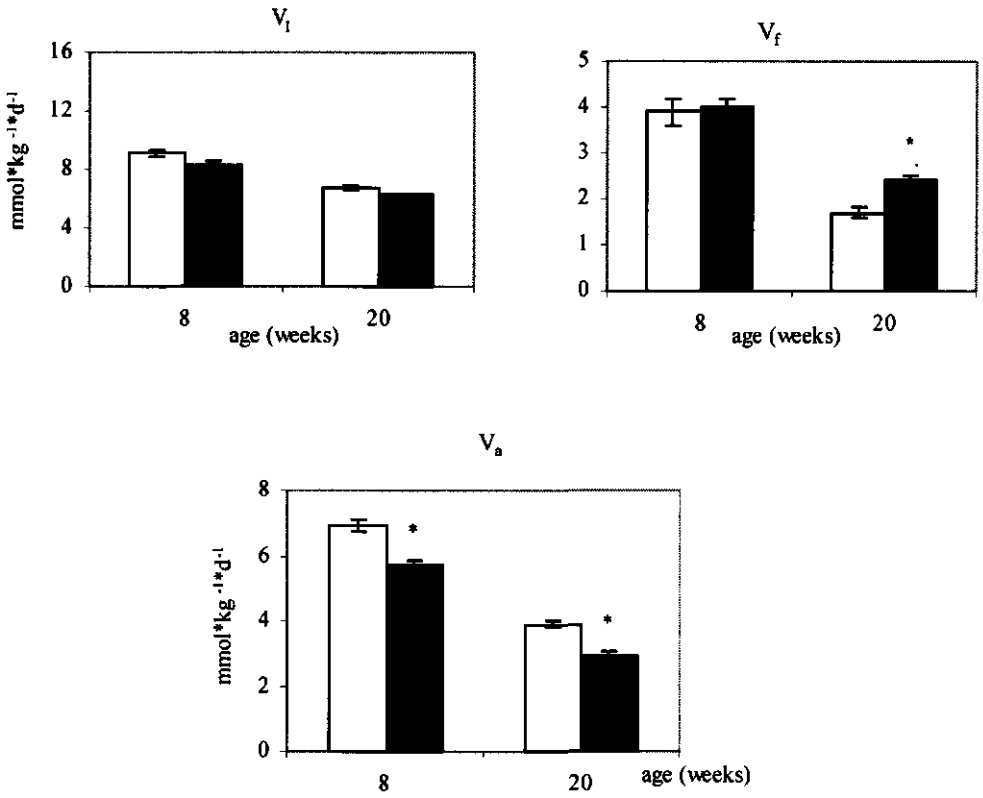

Figure 2 Ca intake (V_I), endogenous faecal excretion (V_f), and the true Ca absorption (V_a) in 2 groups of dogs fed with different vitamin D content (cVitD: 500 IU and hVitD: 4000 IU) as concluded by balance studies at 8 and 20 weeks of age respectively. Data are presented as mean ± SEM. Significant differences ($P < 0.05$) are indicated by * (cVitD = white bars and hVitD = black bars).

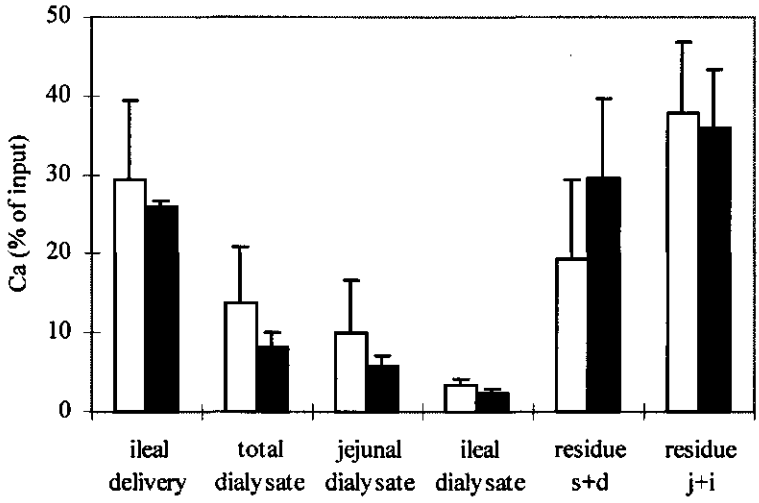

Figure 3 Mean (± sd) amount of calcium (as a % of input corrected for recovery) in different samples (s+d = stomach plus duodenum; j+i = jejunum plus ileum) taken from the dynamic model of the gastrointestinal tract of the dog, 6 hours after the intake of a cVitD diet (500 IU; white bars, n = 4) or a hVitD diet (4000 IU; black bars, n = 2)

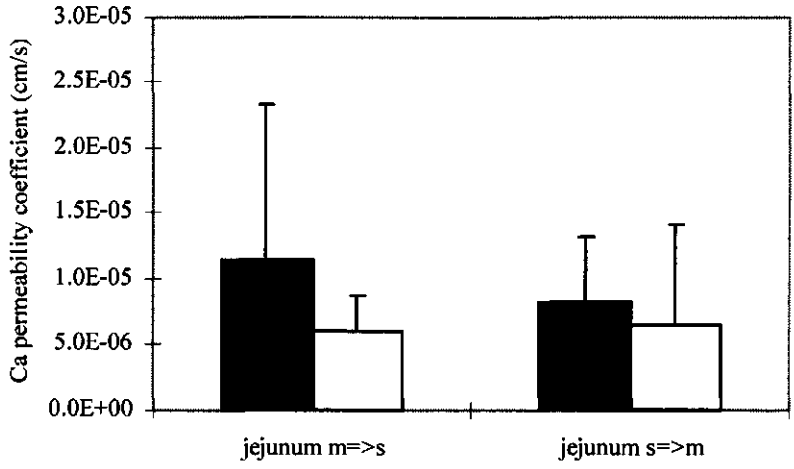

Figure 4 Mean calcium transport (± sd) in the jejunum of dogs of 8 weeks old eating a hVitD diet (500 IU; black bars) or a cVitD diet (4000 IU; white bars) (m = mucosal, s = serosal).

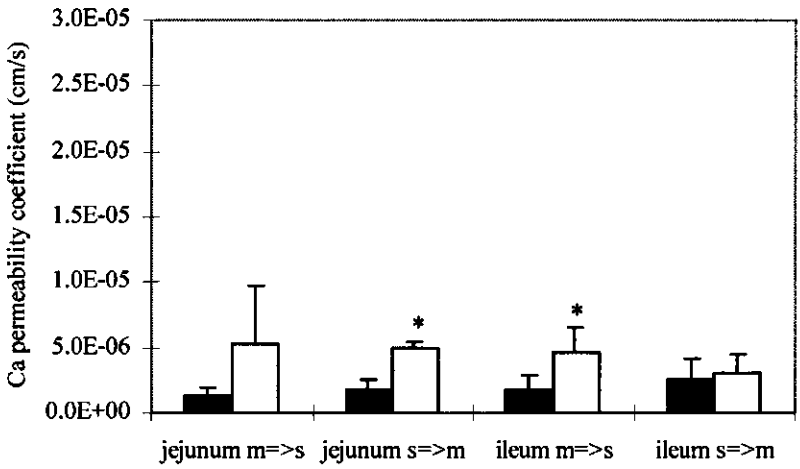

Figure 5 Mean calcium transport (± sd) in the jejunum and ileum of dogs of 20 weeks old eating a hVitD diet (500 IU; black bars) or a cVitD diet (4000 IU; white bars) (m = mucosal, s = serosal). Significant differences ($P < 0.05$) are indicated by *

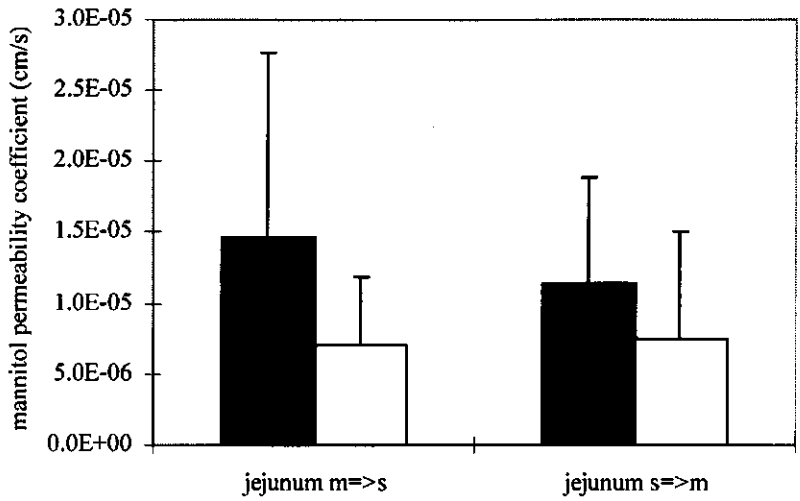

Figure 6 Mean ^{3}H mannitol transport (± sd) in the jejunum of dogs of 8 weeks old eating a hVitD diet (500 IU; black bars) or a cVitD diet (4000 IU; white bars) (m = mucosal, s = serosal)

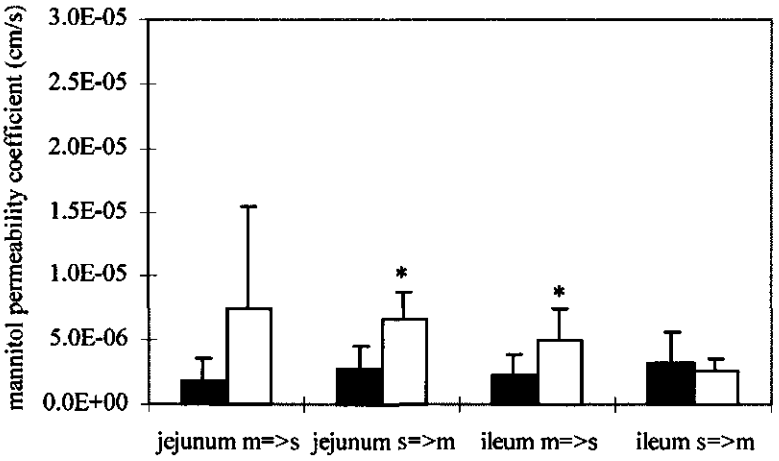

Figure 7 Mean ^{3}H mannitol (± sd) transport in the jejunum and ileum of dogs of 20 weeks old eating a hVitD diet (500 IU; black bars) or a cVitD diet (4000 IU; white bars) (m = mucosal, s = serosal). Significant differences ($P < 0.05$) are indicated by *

There were no differences in magnitude for Ca and mannitol absorption at the different ages, for the different diets ($P < 0.05$). However, in the hVitD group the transport of both Ca ($P < 0.05$) and mannitol through the segments of dogs of 8 weeks old is higher than through the segments of dogs of 20weeks of age. This difference was not seen in the cVitD group.
Transport of mannitol through the small intestine of dogs of 20 weeks of age was 2.9E-06, 7.5E-06, and 5.1E-06 cm/s for the duodenum, jejunum and ileum of the CVitD group, respectively. In the hVitD group the transport of mannitol was not measured in the duodenum and 1.8E-06 and 2.2E-06 cm/s in the jejunum and ileum, respectively. Transport of Ca through the small intestine of dogs of 20 weeks of age was 2.6E-06, 5.3E-06, and 4.6E-06 cm/s for the duodenum, jejunum and ileum of the cVitD group, respectively. In the hVitD group the transport of mannitol was not measured in the duodenum and 1.3E-06 and 1.8E-06 cm/s in the jejunum and ileum, respectively.

DISCUSSION

The aim of the study was to investigate if an *in vivo* study with Great Danes, a dynamic, *in vitro* model of the gastrointestinal tract of the dog, and a study with intestinal segments from dogs could be complementary to each other. To do so, experiments were performed to study the calcium availability and absorption under influence of vitamin D. The mechanism behind the effect of vitamin D is beyond the scope of this study, and will therefor not be discussed in detail.

The Ca balance study in Great Danes gives information on the overall absorption of Ca throughout the digestive tract without differentiating neither between active and passive Ca transport nor between the different compartments of the intestine.

The cVitD group has a true absorption of Ca (V_a) of 6.91 mmol/kg BW at 8 weeks of age. This is in accordance with the concept that young individuals have a high absorptional capacity of Ca. It is suggested that passive absorption is prominent at young age and especially post-natal until around weaning time (Pansu *et al.*, 1983). A fall in V_a is observed at 20 weeks of age, which can be explained by the preceding maturation of the Ca transport system, whereby passive absorption descends, and active absorption becomes more prominent, which is hormonally regulated by vitamin D (Ghishan *et al.*, 1984). This is supported by the fact that both the concentration of Ca binding protein (Pansu *et al.*, 1983) and the vitamin D receptor concentration (Halloran and DeLuca, 1981) in intestinal cells is positively correlated with active transport of Ca in studies with rats at weaning age.

Throughout the study Ca intake (V_I) was of comparable magnitude between both groups. Yet, in the hVitD group the V_a was significantly lower in comparison to the cVitD group at 20 weeks of age. This is in accordance with the findings in the transport chambers where Ca transport through the jejunum and ileum segments was significantly lower in the intestinal samples of the dogs of the hVitD group at 20 weeks of age when compared to those of the cVitD group. Ca is transported in the same trend as mannitol indicating passive transport of Ca and the component of active transport does not seem to change significantly in time. Besides that, the Ca transport in the jejunum and ileum in the hVitD group is even lower at 20 weeks of age than at 8 weeks of age and also lower than the cVitD group at the 20 weeks of age. This leads to the suggestion that vitamin D may have a down regulating effect on the passive absorption of Ca or even a more general and a-specific effect decreasing the paracellular transport in the intestinal mucosa.

The effect of vitamin D on Ca absorption in the intestine is a matter of great debate in the literature (Karbach, 1991; Wasserman and Fullmer, 1995; Norman, 1990). Enhancement of

the Ca ion transport across the paracellular way is reported (Karbach, 1991) but the studies were based on a relatively short term effect of vitamin D (specifically calcitriol). In addition to this, the effect of vitamin D on the paracellular transport system as a genomic effect of vitamin D on assembly and permeability of tide-junctional complexes (Chirayath *et al.*, 1998).

From the *in vitro* studies in FIDO it seemed that only 8-14% from the Ca of the diet will be available for absorption after digestion of the food. This is in agreement with results found in previous FIDO experiments (Smeets-Peeters *et al.*, 1999; 2000). Duflos *et al.* (1995) found that the amount of Ca solubilized throughout the intestine of a rat was 2.0-2.7%.This is even lower than the results found in FIDO in which the Ca should also be solubilized to be absorbed. No significant differences were found between the cVitD diet and the hVitD diet in FIDO. This was according to our expectations, because vitamin D was not expected to have influence on the availability of Ca from the diet.

One of the reasons for this low availability for absorption of Ca could be the source of the calcium used in the diet. Another possibility could be the fat source and content in the diet. Fat (especially long chain saturated fatty acids) can have negatives effects on the Ca absorption, presumably due to the formation of Ca soaps in the presence of fatty acids (Cheng *et al.*, 1949; Freeman, 1969; Laval-Jeantet and Laval-Jeantet, 1976). However, in an *in vivo* study in young Beagles this was not supported (Hallebeek and Hazewinkel, 1998) since an increase in dietary fat content from 10-30% by substitution of carbohydrates for beef tallow did not significantly affect Ca absorption. The percentage of Ca involved in Ca soaps was negligible when compared to Ca intake.

In this study the diet used the *in vivo* and in FIDO was the same. *In vivo* the true absorption varied between 47% in the hVitD group (20 weeks of age) and 76% in the cVitD group (8 weeks of age). A possible reason for this is that *in vivo* the fatty acids are absorbed from the intestine. The absorption of fatty acids is not (yet) possible in FIDO. This may cause a higher soap formation in FIDO, compared to *in vivo*. Other membranes should be incorporated in the model which also can absorb the products from the fat digestion. This part of the model is, however, still under development (FAIR, 1999).

In the experiments with intestinal segments the reproducibility was better in the experiments with segments of dogs of 20 weeks of age compared to those dogs of 8 weeks of age. The reason for this is that the tissues from the youngest dogs are much more fragile. Therefore it was much more difficult to separate the mucosal layer from the muscle layer, resulting in a lower quality of the segments used in the intestinal transport chambers.

From the results it can be concluded that at 20 weeks of age the mode of absorption of Ca is still mainly passive. The pattern of absorption of Ca, which can be absorbed in both a passive and an active way, is similar to the absorption of mannitol which is only absorbed in a passive way. Besides, no differences were seen in the transport of Ca from the mucosal side to serosal side and the other way around, also indicating passive transport of Ca. This could mean that in these Great Danes the maturation of the intestine is not yet completed after 20 weeks of age. In rats (Halloran et DeLuca, 1981; Pansu *et al.*, 1983) and pigs (Fox *et al.*, 1985; Kaune *et al.*, 1992; Schröder *et al.*, 1990; 1993; 1998) however it was found that the vitamin D regulated Ca transport-system seems to be well developed at weaning age.

No differences were found in the Papp-value between the duodenum, jejunum and the ileum of dogs of 20 weeks of age. It should be noted that the Papp value is only indicative for *in vivo* absorption. Aspects of residence time and concentration of Ca in the chyme should also be taken into account.

The absorption of both Ca and mannitol in the hVitD group showed a decrease at 20 weeks of age, compared to the age of 8 weeks. Mannitol shows also a lower absorption in the hVitD group, which suggests that vitamin D has an a-specific effect on paracellular transport. The vitamin D dependency of the nonsaturable component of Ca absorption is still subject of debate (Karbach, 1991; Wasserman and Fullmer, 1995; Norman, 1990).

Conclusively, the findings from the intestinal segments agree with those in the *in vivo* model and they are supplementing and thus lead to a complete picture of the regulation of Ca absorption. With the *in vitro* model of the GI tract of the dog the availability for absorption of Ca from the diet can be estimated. The three different methods can be complementary to get insight in the availability and absorption of Ca from a diet. Further studies are being done in order to elucidate the the regulatory effect of vitamin D in the Ca transport system in young dogs.

8 GENERAL DISCUSSION

IN VITRO MODELS

The petfood market follows trends of the human food industry. Health promotion, safety, indulgence and convenience are important topics and pet owners want the best for their pets. To fulfil the wishes of the pet owners and the needs of the pets, new and improved products are launched on the market. To be able to produce complete and balanced dog diets, it is necessary to know the needs of the dogs as well as the digestibility and availability of nutrients from a diet. Due to regulations, agreements and the public opinion, petfood manufacturers are restricted in performing (invasive) studies with dogs and cats. For that reason an alternative *in vitro* method has been developed (based on the model described by Minekus, 1995; 1998), simulating the dynamic conditions in the stomach and small intestine of dogs (FIDO, *f*unctional gastro*i*ntestinal *do*g model). Boisen and Eggum (1991) and Savoie (1994) have evaluated different *in vitro* methods for estimating the digestibility and absorption of food. The methods described include the dialysis cell method, the pH-drop and pH-stat method, and the digestion cell method. Both Boisen and Eggum (1991) and Savoie (1994) conclude that the choice of enzymes and incubation conditions and the need for equipment are dependent on the objectives of the study. Of course, it is always important to keep the objective of the study in mind, but on the other hand it is also important to realize what the physiological significance of the results will be. Therefore it is important to simulate the dynamic physiological conditions for digestion and absorption as closely as possible. With regard to FIDO this simulation is (partly) achieved by mimicking (1) physiological transit times and peristaltic movements in the stomach and small intestine, (2) concentrations of electrolytes and activities of enzymes related to the stomach and the different parts of the small intestine, (3) pH values in the different parts of the GI tract, and (4) absorption of digested products. Even more important is the combination of these factors in time in relation to the concentration of food in the different compartments of the system. Thus, the FIDO model is a combination of the methods evaluated by Boisen and Eggum (1991) and Savoi (1994). This, combined with the dynamic character of the model, gives a more realistic simulation of the physiological processes in the dog. Therefore, luminal processes can be studied in a more realistic fashion than in other *in vitro* models.

FIDO

The aim of the studies described in this thesis was the development, validation and application of a dynamic *in vitro* model of the gastrointestinal tract of the dog. To reach this aim, modifications were made to the model as described by Minekus (1995; 1998) to be able to test both dry and canned dog foods. The gastric compartment was modified which resulted in a better control of gastric emptying, mainly in the case of canned dog foods. After these modifications it was technically possible to test both types of dog food in the model (Chapter 3). Experiments were performed to validate the model in comparison to the *in vivo* situation. These experiments mainly focused on protein digestion (Chapter 4), but it is conceivable that the model can also be used for other components, such as carbohydrates, minerals or probiotics, to evaluate the digestibility of different diets or ingredients. This view is based on validation experiments mimicking the physiology of other species (pigs, pre-ruminant calves and human subjects (adults and baby's)) (Marteau *et al.*, 1997; Minekus, 1998; Larsson *et al.*, 1997).

In addition to providing knowledge of health aspects of petfood, research on the availability from minerals from petfood can be very useful to obtain insight into the environmental pollution caused by the excretion of minerals by dogs (and cats). In the Netherlands the dog population was 1.3 million in 1997 (Datamonitor, 1998). Assuming that the mean amount of faeces produced by a dog is about 150 g/day, this results in a total amount of 195,000 kg faeces each day. With this amount, also a substantial amount of minerals enters the environment. With respect to phosphorus (P), for example, it was measured that about 35% of the calcium of canned dog food is available for absorption (Chapter 5). The rest, 65%, will be excreted. Assumed that a dog (15 kg) eats 1 kg of canned food each day, with a P content of 0.3%, this would lead to a total daily excretion of (0.3/100) * (65/100) * 1.3 * 10^6 = 2535 kg P (0.925 million kg per year). Environmental pollution is a big issue in farm animal nutrition. In 1994, cattle excreted 60 million kg P, pigs 30.9 million kg and poultry 19 million kg (NRLO, 1996). In petfood, however, P excretion it is not considered as a source of pollution, but it obviously contributes to it. Research on the availability of minerals and the improvement of availability could well be performed in FIDO.

In general, this model should be seen as a tool to (1) investigate effects of food processing, (2) compare (rank) different diets, or (3) study luminal effects (e.g. pH) on the digestibility and availability of nutrients. Another interesting application of the model is the use of separate ingredients in the model. In this way separate ingredients or a combination of ingredients can be tested with or without the effect of processing. Testing separate dietary

ingredients is often not possible *in vivo*. Animals will probably not consume it and in longer-term experiments (which are necessary in studies *in vivo*) this would lead to insufficiencies in the animals.

Besides, the model can be useful as screening system in product development. The time to market for products to be launched should be as short as possible. This means that the time of product development should be short as well. The model is a tool that can be used to shorten the development time, because the experiments are less time consuming than *in vivo* studies.

Like other models, both *in vivo* and *in vitro*, FIDO also has its limitations. Some of the limitations, however, are the strength of the model. Hormonal or neural systems are not mimicked in the system. For that reason, feedback mechanisms that take place *in vivo* are not present. *In vivo* most physiological processes are regulated by both hormones and the nervous system. This causes biological variation among animals in the various physiological parameters. Experiments in the model are not influenced by these factors, which gives the opportunity to work under standard conditions. Due to these standard conditions the results of the experiments in FIDO are very accurate and reproducible. It is possible to develop a range of different standard tests that can be used to characterize a petfood in terms of digestibility of nutrients or availability of micronutrients for absorption. This makes the model a suitable tool for being incorporated in regulations for testing petfood.

REGULATIONS

The Association of American Feed Control Officials (AAFCO) has as a basic goal to provide a mechanism for developing and implementing uniform and equitable laws, regulations, standards and enforcement policies for regulating the manufacture, distribution and sale of animal feeds (including petfood). This should result in safe, effective and useful feeds. These regulations, which focus exclusively on the US market, deal with such topics as labelling, brand and product names, expression of guarantees, ingredients, drugs and petfood additives, and statements of energy content. To test diets the AAFCO has also developed feeding protocols for dog and cat foods to establish nutritional adequacy and to measure metabolizable energy (AAFCO, 2000).

A European version of the AAFCO does not exist. Within the EU, Fediaf (the European petfood industry federation) represents 16 national petfood industry associations towards EU institutions and other international bodies (http://www.fediaf.org). It promotes the views and interests of European petfood producers and aims at a legislative framework for the

production of safe, nutritious and palatable petfood. Fediaf cooperates closely with EU authorities but does not have any regulatory tasks itself, in contrast to the role of AAFCO.
EU legislation is under regular review to adapt to scientific and technological developments. Areas covered include:

- official requirements for the operation of pet food factories
- consumer information imposed by specific regulations on the labelling of petfood and on general advertising rules
- raw materials of animal and vegetable origin
- the use of additives in terms of utility, efficacy and innocuity
- health and sanitary measures and specific health certificates for trade and processing of meat products
- sampling and methods of analysis for checks on raw materials and finished products
- specific sanitary and safety inspections as well as checks on labelling and declarations
- the use of dietetic pet food for animals with a temporarily or irreversibly impaired metabolism.

The main difference between the AAFCO and EU regulations is that those of the EU do not regulate nutritional adequacy or the declaration or determination of metabolizable energy. Neither has the EU regulations with respect to packaging of pet foods. Although this lack of regulations gives the manufacturers much 'freedom', it can also be a threat to them. When a diet is produced according to regulations it can be accepted as save and nutritionally adequate. However, regulations with respect to testing of diets can result in costly operations and ethics can play a role in defining these regulations.
The necessity of proving that ingredients and diets meet all regulations combined with the limitation of the petfood manufacturers to perform (invasive) *in vivo* studies opens up new perspectives for *in vitro* models. FIDO can play a role in proving that a diet or ingredient meets all criteria laid down in regulations. In addition to the absence of ethical constraints, an advantage of the model is that experiments are less time consuming than *in vivo* studies. The minimum protocol for use in the determination of metabolizable energy of dog food, according to AAFCO, requires at least 6 animals which should be fed for at least 10 days (at least 5 days to acclimatize the test animals and at least 5 days as the collection period; AAFCO, 2000). Experience has shown that the model is very accurate and sensitive, which results in only two 6-hour experiments to determine the digestibility and availability of nutrients. These two experiments give comparable results in longer-term (e.g. 10-day)

experiments in dogs (Chapter 4). The advantages of FIDO makes the model a suitable tool for being incorporated in regulations on testing petfood.

FUTURE DEVELOPMENTS

Although fat is digested in the model, the fatty acids formed are not removed from the system. The semi-permeable hollow-fibre membranes used in the model for water-soluble components are not suitable for removing fatty acids and fat-soluble components. The fatty acids may interfere with other nutrients (e.g. calcium) which can have effects on the digestibility or availability for absorption of these nutrients. To enable absorption of fatty acids in the system other types of membranes should be used. Experiments were performed with membranes suitable for removing the fatty acids from the lumen, and the results look very promising. Validation experiments will be performed to compare fat digestion *in vivo* and *in vitro*. The fibres used for the absorption of fat-soluble components can be combined with the hollow-fibre membranes already used in the system, thus enabling the study of both fat- and water-soluble components in the *in vitro* model. This offers the opportunity to improve studying the availability of such compounds as fat-soluble vitamins (A, D, E and K). Vitamin D, for example, is not produced under the influence of sunlight in the skin of dogs and cats, in contrast to omnivores and herbivores (Hazewinkel *et al.*, 1988; How *et al.*, 1994). This makes these carnivores completely dependent on the vitamin D content of the food they consume. For that reason, it is important to study the availability of vitamin D from the diets, based on physiological properties.

In order to get as much information as possible from the products to be tested it can be useful to combine different techniques. Chapter 7 describes the use of a combination of different methods (*in vivo*, FIDO and *in vitro* studies with intestinal segments) to study the effect of vitamin D on the availability of calcium. Combining different methods yields more information on the amount that can be absorbed by the animal (which is dependent on the physiological conditions of the animal; *in vivo* method) and the availability for absorption of nutrients from the food (FIDO). Because no mucosal cells are present in this model, intestinal segment chambers were used to study the mode of transport (active or passive) and the absorption rate through the gut wall. To get more detailed information from the combined results, or to try to decrease the number of laboratory animals needed, combination of techniques needs further investigation.

The petfood market follows the trends in the human food industry. More and more new products are developed and introduced, such as functional foods (pro- and prebiotics) and

clinical foods. For the development of these products the model can be a useful tool. For example, for the development and testing of clinical foods the model can be used to simulate pathological circumstances (e.g. pancreatic insufficiency). In the development of probiotics for dogs, the model is a tool to test the sensitivity of different strains to differences in pH in the GI tract or the effect of bile salts. In that way the percentage of microorganisms entering the large intestine can be estimated because that is where these microorganisms are expected to work.

For that reason, it is also important to develop the large intestinal model for the dog (FIDO-2). Experience has been built up with human faeces to simulate the conditions in the large intestine (Minekus *et al.*, 1999). Recent experiments with microflora from the dog were performed successfully. Different inocula were used in this study: fresh faecal flora, cultivated faecal flora and cultivated caecal flora. The objective of this study was to determine if cultivated (standardized) faecal flora can be used to simulate the conditions in the caecum of the dog. It is of interest to simulate caecal circumstances because in that part of the large intestine most of the undigested nutrients will be fermented. The first results of the study with the large intestinal model showed that the different inocula were stable in the model for at least 8 days. The composition of the microflora of the cultivated faeces in the model was comparable to the composition in the caecum of dogs (Figure 1). With respect to the production of short-chain fatty acids (SCFAs), it can be concluded that the total amount and the ratio of SCFAs produced is within physiological ranges (Figure 2 and Table 1). Further experiments will validate this model for the dog and enable to mimic the whole GI tract. The combination of FIDO-1 and FIDO-2 is important to get a complete picture of the digestibility of nutrients in the ileum and the total tract and for the development of pro- and prebiotics, but also to study the effects of pharmaceuticals in the GI tract of dogs.

With respect to pharmaceuticals, the model can be used to simulate the luminal behaviour of pharmaceuticals. Luminal behaviour can be studied in different matrices (tablets, powder, controlled-release products) with or without the addition of different types of diets.

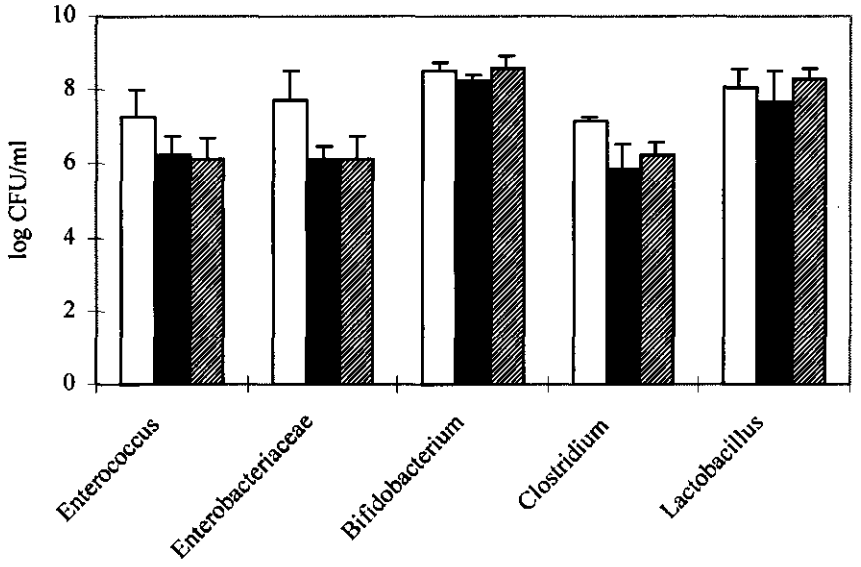

Figure 1 Mean (± sd) log number of bacteria per ml in the caecum of dogs (white bars, n=5), in FIDO-2 inoculated with fresh faeces from dogs (black bars, n = 10), and in FIDO-2 inoculated with cultivated faeces from dogs (hatched bars, n = 10)

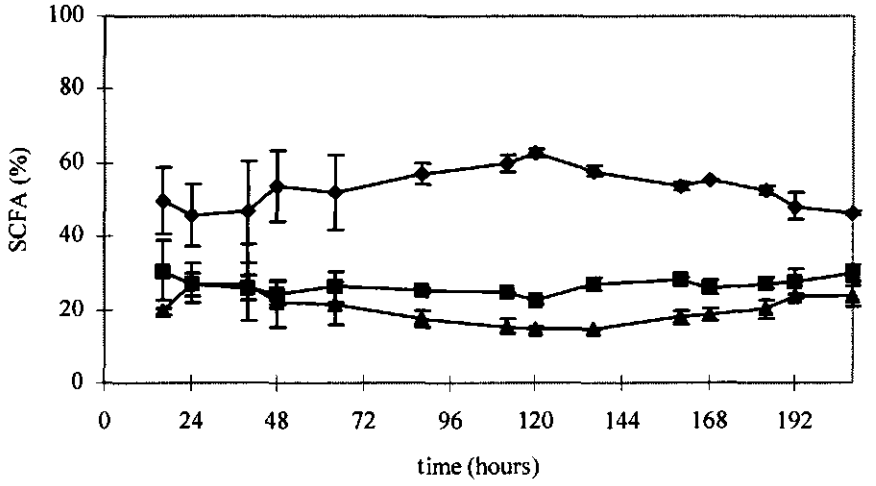

Figure 2 Mean ratio of short chain fatty acids (± range) produced in the lumen of FIDO-2 inoculated with fresh faeces from dogs (n=2; ◆ = acetate, ■ = propionate, ▲ = butyrate)

Table 1 Mean ratio of short chain fatty acids (SCFA) in a pooled caecal sample of dogs (n=5), and in the lumen of FIDO-2 inoculated with fresh faeces or cultivated faeces of dogs

Inoculum	Acetate (%)	Propionate (%)	Butyrate (%)	Total amount of SCFA (mmol/l)
caecum	58	33	99	175
fresh faeces	53	7	20	129
cultivated faeces	63	18	19	151

CONCLUDING REMARKS

The model described in this thesis is a good alternative to *in vivo* studies with dogs. It can help the scientists in petfood and ingredient research in getting more insight in the nutritional quality and safety of dog foods. In the phase of product development, studies in this model can reduce research time, which is important for decreasing the time to get the product onto market.

SUMMARY

INTRODUCTION

To produce a complete and balanced dog diet it is necessary to know the nutritional needs of the dog as well as the availability of nutrients from the diet. Petfood manufacturers are restricted in performing (invasive) studies with animals for ethical reasons. Therefore, it is necessary to search for *in vitro* alternatives to these studies as explained in Chapter 1. Based on a literature study on the physiology of the GI tract of the healthy, adult dog (Chapter 2), the dynamic *in vitro* model for human subjects, pigs and calves, as described by Minekus (1995), was modified to mimic the physiological conditions of the dog. The model is named FIDO (*f*unctional gastro*i*ntestinal *do*g model).

The aim of the study was to develop the *in vitro* model simulating the GI tract of the dog. The model should simulate the physiological conditions in the GI tract of the dog as closely as possible. In that way luminal processes as well as physical and chemical properties of diets could be investigated. This thesis describes the developmental experiments, the validation experiments in comparison to dog studies as well as application studies.

VALIDATION OF THE MODEL

Technical validation

The study started with an extended literature review on the physiology of the stomach and small intestine of the healthy, adult dog with a special emphasis on those parameters which are relevant for the development of the dog model (Chapter 2). With respect to digestion of food, such parameters as transit times, pH values, concentrations of electrolytes and activities of enzymes are important to mimic physiological conditions as closely as possible. Data found in the literature were translated to a computer program to simulate these parameters in FIDO. The features of the model are described in Chapters 2 to 7. Based on the simulation of the physiology of the dog dry and canned dog foods were used to test the technical possibilities of the model. After some technical modifications to the gastric compartment and the pre-filters connected to the jejunal and ileal compartments, a study was performed to test the effect of particle size of dry dog food on gastric emptying. Particles ≤ 3 mm emptied more slowly than particles ≤ 1 mm. The effect of transit time on the availability for absorption of nitrogen and calcium of canned dog food was also investigated. Like *in vivo*, in FIDO less nitrogen and calcium were available for absorption with faster transit times (Chapter 3).

Validation *in vitro* versus *in vivo*

Validation of the model in comparison to the *in vivo* situation was the next step in the development of the dog model (Chapter 4). Ileal protein digestibility and availability for absorption of nitrogen of eight different dry dog foods were tested in the model. The results were compared with data found *in vivo* with ileally cannulated dogs, performed at the University of Illinois. The experiments proved to be very reproducible and the results found in FIDO are similar to those found *in vivo* in the dogs.

Based on these findings and those of former experiments simulating pigs, calves and human beings, it can be concluded that the model is a suitable tool as an alternative to animal experiments in nutritional research.

APPLICATION OF THE MODEL

The validation study (Chapter 3) showed a low availability for absorption of calcium in the model. Based on these results it was decided to study calcium and phosphorus availability from three commercially available canned dog foods (Chapter 4). Effects of addition of calcium-phosphorus supplements or the enzyme phytase and the effect of a lower pH in the small intestine were also included in this study. A human standard breakfast was used as a control diet, because this diet had a high calcium availability under human conditions in the diet.

The results showed that the canned dog foods had a low availability for absorption for calcium (maximum 21%) and phosphorus (maximum 44%). Differences in relative availability of calcium and phosphorus were found among these diets, which can probably be explained by the source of these minerals. Also the low availability of these minerals can be attributable to the source. Another possible explanation of the low availability are the (saturated) fatty acids in the diet, which can form calcium soaps in the model.

The advantage of FIDO is that the availability for absorption of calcium and other nutrients can be studied without the influence of the physiological status of the animal. The real amount absorbed by the animal, however, cannot be studied. Absorption by the animal depends on its needs and the absorption is hence dependent on two different mechanisms: passive and active absorption. To get more insight into the absorption of calcium through the intestinal wall of the dog, experiments were performed with Great Danes (8 and 20 weeks of age; in cooperation with the Veterinary Faculty of Utrecht University), FIDO and intestinal segments (Chapter 7). The effect of vitamin D was taken into account in this study by studying two levels in the diet. The three different methods (*in vivo*, FIDO and intestinal

segments) are complementary and can be used to get a better understanding of the regulation of calcium absorption in the dog.

In Chapter 6 experiments are described to investigate the effect of gelling agents on the digestibility and availability for absorption of nutrients ('chunks in gravy' products). Three different (combinations of) gelling agents added to the gravy were used in this study in different concentrations (0.2% and 0.5%): carrageenan plus guar gum, carrageenan plus locust bean meal, and wheat starch. A diet without gelling agent was used as a control diet. Neither the gelling agent nor the concentration had any effect on digestibility of proteins and carbohydrates, availability for absorption of calcium and phosphorus, viscosity or buffering capacity in the intestinal content. From the results it can be concluded that addition of the gelling agents used does not affect the nutritional quality of the diets at the low concentrations tested.

CONCLUSIONS

The dynamic *in vitro* model of the GI tract of dogs simulates the physiological parameters very accurately and reproducibly. Transit time of food has an effect on digestibility and availability for absorption, just like in dogs. Also ileal protein digestibility in the model is similar to the data found *in vivo*. It is possible to investigate specific questions regarding dog food in this model (e.g. the effect of gelling agents on digestibility). Another important aspect is the fact that the experiments in FIDO can be performed under highly standardized condition, in contrast to *in vivo* studies in which biological variance among animals plays a role. This comes to expression in the reproducibility and sensitivity of the results from FIDO compared to results of *in vivo* studies.

The dynamic *in vitro* model of the gastrointestinal tract of the dog is a suitable alternative to *in vivo* studies with respect to digestibility and availability for absorption of nutrients from different types of dog food, such as canned and dry dog foods.

SAMENVATTING

INLEIDING

Om een complete en gebalanceerde voeding voor de hond te produceren is het noodzakelijk om de nutriënten behoefte van de hond te weten alsook de beschikbaarheid van de nutriënten uit de voeding. Omdat diervoerfabrikanten beperkt zijn in het uitvoeren van (invasieve) studies met honden is het belangrijk om alternatieven te hebben voor dit soort onderzoek.
Het doel van de studies, beschreven in dit proefschrift, was de ontwikkeling, validatie en toepassing van een alternatieve methode voor onderzoek. Gebaseerd op een intensieve literatuurstudie naar de fysiologie van het maag-(dunne)darmkanaal van de hond (hoofdstuk 2) is het dynamisch *in vitro* maagdarm model zoals beschreven door Minekus (1995) specifiek aangepast aan de condities in de maag en dunne darm van de gezonde, volwassen hond. Dit hondenmodel kreeg de naam FIDO (*f*unctional gastro*i*ntestinal *d*og m*o*del). Vervolgens is het model gevalideerd voor zowel de technisch aspecten alsook voor de *in vivo* situatie. Daarnaast is in de gevoeligheid van het model getest en geëvalueerd. Verder onderzoek naar specifieke vraagstellingen zoals de vertering en beschikbaarheid voor absorptie van nutriënten uit hondenvoeding werd uitgevoerd met dit model.

ONTWIKKELING VAN HET MODEL

Het model is opgebouwd uit vier compartimenten die achtereenvolgens de maag, het duodenum, het jejunum en het ileum nabootsen. Elk compartiment bestaat uit een glazen buitenwand met daarin een flexibele siliconen huls. Deze huls is omgeven door water dat ervoor zorgt dat de temperatuur in het model op lichaamstemperatuur blijft. Daarnaast kunnen deze hulzen samengeknepen worden door het verhogen van de waterdruk. Op deze manier worden de peristaltische bewegingen van het maag-darm kanaal nagebootst. Geleidelijke passage van de voeding door de opeenvolgende compartimenten vindt plaats door middel van peristaltische kleppen tussen elk van de compartimenten. De zuurgraad in elk van de compartimenten wordt nauwkeurig gecontroleerd met pH elektroden en aangepast cq gehandhaafd door middel van secretie van zuur en bicarbonaat. Dit betekent dat er in de maag een fysiologische daling van de pH na inneming van een maaltijd plaatsvindt (van pH 7.0 naar pH 1.9 binnen 3 uur). In het duodenum wordt de voeding vervolgens weer geneutraliseerd (pH=6.2), terwijl in het jejunum en het ileum de pH verder oploopt naar, respectievelijk pH 6.5 en pH 7.0. Daarnaast vindt er ook secretie plaats van verteringssappen in de maag en het duodenum. In de maag worden de enzymen lipase en pepsine en een elektrolyten-oplossing toegevoegd en in het duodenum vindt secretie plaats van pancreatine,

gal en een elektrolyten oplossing. Aan zowel het jejunum als het ileum compartiment zijn dialysesystemen gekoppeld die zorgen voor de absorptie van de verteringsproducten en water. Daarnaast wordt met behulp van dit dialysesysteem de concentratie elektrolyten in het model op fysiologische condities gehouden.
De genoemde parameters zijn vanuit de literatuur vertaald in een computerprogramma. Dit computerprogramma controleerd tijdens een experiment continue of de gemeten waarden overeen komen met de waarden in de computer. Indien er afwijkingen worden geconstateerd worden deze bijgestuurd door middel van de computer. Het hele model op deze manier computer gestuurd en er vindt continue dataregistratie plaats tijdens de experimenten.

VALIDATIE VAN HET MODEL

Technische validatie

Om de technische mogelijkheden van het model te bestuderen zijn een aantal proeven uitgevoerd met zowel droogvoer als blikvoer (hoofdstuk 3).
De zuurgraad in de verschillende compartimenten speelt een belangrijke rol bij de vertering van voeding en de beschikbaarheid van nutriënten. Het is daarom noodzakelijk om de pH in de verschillende compartimenten te reguleren. In het model is de pH daling in de maag na een maaltijd nauwkeurig en reproduceerbaar te simuleren, alsook de daaropvolgende pH stijging in de verschillende compartimenten van de dunne darm.
Naast de pH is ook de passagesnelheid van de voeding door het maag-darm kanaal een belangrijke factor bij de vertering en beschikbaarheid van nutriënten. De passagetijd van de voeding wordt door middel van de computer gereguleerd aan de hand van een vooraf ingestelde ledigingscurven. Deze ledigingscurven komen overeen met de fysiologische maaglediging en darmpassage van de voeding bij de hond. Om te controleren of de passage van de voeding tijdens een experiment overeenkomt met deze ingestelde curven is onderzoek uitgevoerd naar de 'fresh matter' lediging (= lediging van zowel voeding als verteringssappen) en de droge stof lediging van zowel een droogvoer als een blikvoer bestudeerd is. Om het effect van deeltjesgrootte op de maaglediging te bepalen werden de experimenten uitgevoerd met droogvoer vermalen tot deeltjes $\leq$ 3 mm en $\leq$ 1 mm. Uit de resultaten blijkt dat de maaglediging van droogvoer en blikvoer identiek is en zeer reproduceerbaar. Grotere deeltjes blijken iets trager te ledigen dan kleine deeltjes. Ook het effect van de passagetijd door de dunne darm is bestudeerd. In de experimenten is uitgegaan van drie verschillende passagesnelheden voor de dunne darm: een trage, een matig snelle en een snelle passage. De maagledigingssnelheid was bij deze experimenten identiek. Bij deze

experimenten is het effect van passagetijd van de voeding door de dunne darm op de eiwitvertering en de beschikbaarheid van calcium (Ca) voor absorptie bestudeerd. Uit de resultaten blijkt dat hoe sneller de passage van de voeding door de dunne darm hoe minder Ca en stikstof (= maat voor de eiwitvertering) beschikbaar kwam voor absorptie. Een opmerkelijke bevinding is de lage beschikbaarheid voor absorptie van Ca in deze experimenten (max 20 % bij de trage passage).

Validatie *in vitro* ten opzichte van *in vivo*

Naast een technische validatie van het model (*in vitro*) is de relatie met de situatie in de hond (*in vivo)* ook zeer belangrijk. Om dit te bestuderen zijn acht verschillende droogvoeren in dit model getest op de eiwitvertering. De eiwitvertering van deze voedingen werden tevens getest ileum-gefistuleerde honden (honden met een kunstmatige uitgang op het einde van de dunne darm). Op deze manier kon een goede vergelijking tussen de *in vivo* en *in vitro* eiwitvertering gemaakt worden. Uit de resultaten (hoofdstuk 4) blijkt dat de experimenten zeer reproduceerbaar zijn en dat er een duidelijke overeenkomst bestaat tussen de *in vivo* situatie en de *in vitro* situatie.

Naar aanleiding van deze bevindingen en van eerdere bevindingen met het model waarbij het varken, het kalf en de mens nagebootst werden, kan geconcludeerd worden dat het model een geschikt alternatief is voor dierproeven met betrekking tot voedingsonderzoek.

TOEPASSING VAN HET MODEL

Uit de resultaten van de validatie studie, bleek dat Ca uit de voeding een opmerkelijk lage beschikbaarheid voor opname had (hoofdstuk 3). Er werd daarom een studie uitgevoerd om de beschikbaarheid van Ca en fosfor (P) voor opname uit blikvoer te bestuderen (hoofdstuk 5). Hiervoor werden drie commercieel verkrijgbare blikvoedingen gebruikt. Tevens werd bestudeerd of de beschikbaarheid van het Ca en P verhoogd kon worden door middel van het toevoegen van Ca- en P-supplementen en het enzym fytase, en door middel van het verlagen van de pH in de dunne darm. Als controle voeding werd een standaard ontbijt meegenomen, waarvan de beschikbaarheid van Ca bekend was, zowel in het model alsook in de mens. Uit de resultaten bleek dat ook bij deze blikvoedingen de beschikbaarheid voor opname van Ca (max. 21%) en P (max. 44%) in het model laag was. Het standaardontbijt had een vergelijkbare beschikbaarheid van Ca in het hondenmodel als in de mens en het model van de mens. De lage beschikbaarheid van Ca uit het hondenvoer wordt mogelijk veroorzaakt door de (verzadigde) vetten in het voer. Verzadigde vetzuren kunnen zepen vormen met het Ca,

waardoor het Ca onoplosbaar wordt. Hierdoor is opname in het dialysesysteem niet meer mogelijk. In het hondenvoer werd een duidelijk verschil waargenomen in de relatieve mate van beschikbaarheid van Ca en P. Dit wordt mogelijk veroorzaakt door de Ca- of P-bron. Bot bijvoorbeeld, bevat veel Ca en P maar dit is slecht beschikbaar Ca en P.

Met het *in vitro* maag-darmmodel kan de beschikbaarheid van Ca en andere nutriënten uit het voer onderzocht worden. De precieze hoeveelheid die door het dier wordt opgenomen kan echter niet met dit model bepaald worden. De opname van Ca door het dier is afhankelijk van de behoefte. Bovendien wordt Ca in de hond op twee manieren opgenomen : actief en passief. Om inzicht te te krijgen in de opname van Ca door de darm van de hond zijn experimenten uitgevoerd met Duitse Doggen (6 en 21 weken oud) om de opname in honden te bepalen. Met FIDO werd de beschikbaarheid voor opname van Ca uit het voer bepaald en, als derde studie, werd met stukjes darm (darmsegmenten) van de Duitse Doggen de opname door de darmwand bepaald (hoofdstuk 7). Met deze laatste methode kan onderscheid gemaakt worden tussen actieve en passieve opname van Ca. De honden kregen een dieet met een normale hoeveelheid vitamine D (beïnvloed mogelijk de opname van Ca) en een verhoogde concentratie vitamine D. Er werden geen verschillen waargenomen in FIDO voor wat betreft de beschikbaarheid van het Ca uit het voer. De groep met het hoog vitamine D voer leek op de langere termijn een verminderde opname van Ca te vertonen. Dit werd zowel waargenomen in de studie met de honden, alsook in de studie met de darmsegmenten. De verschillende methoden vullen elkaar aan en op deze manier kan een compleet beeld verkregen worden van de regulatie van de Ca opname.

In hoofdstuk 6 staat een studie beschreven, waarin het effect van bindmiddelen op de vertering en beschikbaarheid van voedingsstoffen bestudeerd wordt. In deze studie werden 3 verschillende (combinaties van) bindmiddelen getest in twee concentraties (0.2% en 0.5%): carrageen plus guar gom, carrageen plus johannesbroodpitmeel en tarwezetmeel. Tevens werd een controle voeding meegenomen, waaraan geen bindmiddel aan de saus werd toegevoegd. De producten die gebruikt werden waren allemaal de zgn. 'balletjes in saus'. Noch het bindmiddel, noch de concentratie van het bindmiddel had een effect op de vertering van eiwitten en koolhydraten, de beschikbaarheid van Ca en P, de viscositeit of de bufferende capaciteit van de inhoud van de dunne darm. Uit de resultaten kan daarom geconcludeerd worden dat toevoeging van deze bindmiddelen in lage concentraties geen negatieve effecten heeft op de nutritionele kwaliteit van het complete voer.

CONCLUSIES

Het model blijkt een zeer geschikt alternatief te zijn voor *in vivo* studies met betrekking tot onderzoek naar de vertering en beschikbaarheid voor absorptie van nutriënten uit verschillende hondenvoedingen. Technisch gezien is het model in staat om er zowel droogvoer als blikvoer in te onderzoeken. Uit de resultaten blijkt bovendien dat de ingestelde condities in het model ook goed overeen komen met de gemiddelde *in vivo* gegevens: pH waarden worden nauwkeurig binnen fysiologische grenzen gehouden, de maaglediging volgt de ingestelde fysiologische lediging voor zowel droogvoer als blikvoer. Bovendien heeft passagesnelheid, net als *in vivo*, invloed op de resultaten van de eiwitvertering en de beschikbaarheid voor absorptie van calcium. Ook de verteringscoëfficiënten van verschillende soorten droogvoer komen goed overeen met de gegevens gevonden bij ileum-gefistuleerde honden.

Het blijkt mogelijk te zijn om specifieke vraagstellingen met betrekking tot hondenvoedingen te onderzoeken in het *in vitro* maag-darmmodel voor de hond.

Een belangrijk aspect is bovendien dat de proeven zeer gestandaardiseerd uitgevoerd kunnen worden, in tegenstelling tot in *vivo* studies waar de biologische variatie tussen individuele honden een rol speelt. Dit komt sterk tot uiting in de reproduceerbare resultaten die verkregen worden in het beschreven model.

LITERATURE CITED

A

AAFCO. Association of American Feed Control Officials. Official publication (1998). AAFCO Nutrient profiles for dog foods, pp 127-133.

AAFCO. American Feed Control Officials Incorporated (2000). Official Publication

Allan F.J., Guilford W.G., Robertson I.D., and Jones B.R. (1996). Gastric emptying of solid radiopaque markers in healthy dogs. *Vet. Radiol. Ultrasound* **37**, 336-344.

Allen L.H. (1982). Calcium bioavailability and absorption: a review. *Am. J. Clin. Nutr.* **35**, 783-808.

Allen L.H., and Wood, R.J. (1994). Calcium and Phosphorus (chapter 7). In: Modern nutrition in health and disease. Eds. M.E. Shils, J.A. Olsen, and M. Shike. Lea and Febiger, Philadelphia.

Altman P.L., and Dittmer D.S. (1968). Digestion and absorption. *Metabolism*, 237-306.

Altweb (2000). http://altweb.jhsph.edu/science/regs/reg.htm.

Alexander F. (1965). The concentration of electrolytes in the alimentary tract of the rabbit, guinea pig, dog and cat. *Res. Vet. Sci.* **6**, 238-244.

Arnbjerg J. (1992). Gastric emptying time in the dog and cat. *J. Am. Anim. Hosp. Assoc.* **28**, 77-81.

Azpiroz F., and Malagelada J.R. (1984). Pressure activity patterns in the canine proximal stomach: response to distension. *Am. J. Phys.* **247**, G265-G272

B

Ballesta M.C., Manas M., Mataix F.J., Martinez-Victoria E., and Seiquer I. (1990). Long-term adaptation of pancreatic response by dogs to dietary fats of different degrees of saturation : olive and sunflower oil. *Br. J. Nutr.* **64**, 487-496.

Banta C.A., Clemens E.T., Krinsky M.M., and Sheffy B.E. (1979). Sites of organic acid production and patterns of digesta movement in the gastrointestinal tract of dogs. *J. Nutr.* **109**, 1592-1600.

Beames R.M., and Eggum B.O. (1981). The effect of type and level of protein, fiber and starch on nitrogen excretion patterns in rats. *Br. J. Nutr.* **46**, 301-313.

Benini L., Brighenti F., Castellani G., Brentegani M.T., Casiraghi M.C., Ruzzenente O., Sembenini C., Pellegrine N., Caliari S., Porrini M., and Vantini I. (1994). Gastric emptying of solids is markedly delayed when meals are fried. *Dig. Dis. Sci.* **39**, 2288-2294.

Bergmeyer H.U. (ed.) (1974). Methods of Enzymatic Analysis (2nd ed., vol. 3) pp 1196-1201. Verlag Chemie, Weinheim. Academic Press, Inc., New York.

Berhns K.E., and Sarr M.G. (1994). Duodenal nutrients inhibit canine jejunal fasting motor patterns through a hormonal mechanism. *Dig. Dis. Sci.* **39**, 1665-1671.

Boisen S., and Eggum B.O. (1991). Critical evaluation of *in vitro* methods for estimating digestibility in simple-stomach animals. *Nutrition Research Reviews (NRR)* **4**, 141-162.

Boisen S., and Moughan P.J. (1996). Different expressions of dietary protein and amino acid digestibility in pig feeds and their application in protein evaluation: a theoretical approach. *Acta Agric. Scand. Sect. A, Animal. Sci.* **46**, 165-172.

Bueno L., Fioramonti J., and Ruckebusch Y. (1975). Rate of flow of digesta and electrical activity of the small intestine in dogs and sheep. *J. Phys.* **249**, 69-85.

Bueno L., Praddaude F., Fioramonti J., and Ruckebusch Y. (1981). Effect of dietary fiber on gastrointestinal motility and jejunal transit time in dogs. *Gastroenter.* **80**, 701-707.

Burrows C.F., Bright R.M., and Spencer C.P. (1985). Influence of dietary composition on gastric emptying and motility in dogs: potential involvement in acute gastric dilatation. *Am. J. Vet. Res.* **46**, 2609-2612.

C

Calvert R.J., Otsuka M., and Satchithanandam S. (1989). Consumption of raw potato starch alters intestinal function and colonic cell proliferation in the rat. *J. Nutr.* **119**, 1610-1616.

Camello P.J., Pozo M.J., and Madrid J.A. (1991). Ultradian rhythms in canine gallbladder bile composition. *J. Interdis. Cycle Res.* **22**, 281-291.

Cargill R., Caldwell L.J., Engle K., Fix J.A., Porter P.A., and Gardner C.R. (1988). Controlled gastric emptying. 1. effects of physical properties on gastric residence times of nondisintegrating geometric shapes in beagle dogs. *Pharm. Res.* **5**, 533-536.

Carpentier Y., Woussen-Colle M.C., and Graef de J. (1977). Gastric secretion from denervated pouches and serum gastrin levels after meals of different sizes and meat concentrations in dogs. *Gastroenter. Clin. Biol.* **1**, 29-37.

Carrière F., Laugier R., Barrowman J.A., Priymenko N., and Verger R. (1993). Gastric and pancreatic lipase levels during a test meal in dogs. *Scand. J. Gastroenterol.* **28**, 443-454.

Carrière F., Moreau H., Raphel V., Laugier R., Benicourt C., Junien J., and Verger R. (1991). Purification and biochemical characterization of dog gastric lipase. *Eur. J. Biochem.* **202**, 75-83.

Carrière F., Raphel V., Moreau H., Bernadac A., Devaux M., Grimaud R., Barrowman J.A., Benicourt C., Junien J., Laugier R., and Verger R. (1992). Dog gastric lipase: stimulation of its secretion *in vivo* and cytolocalization in mucous pit cells. *Gastroenterol.* **102**, 1535-1545.

Case L.P., Carey D.P., and Hirakawa D.A. (1995). Overnutrition and supplementation, pp 293-301. In: Canine and Feline Nutrition. A resource for companion animal professionals. Mosby-Year Book, Inc., St. Louis.

Cheng A.L.S., Morehouse M.G., and Deuel H.J. (1949). The effect of the level of dietary calcium and magnesium on the digestibility of fatty acids, simple triglycerides, and some natural and hydrogenated fats. *J. Nutr.* **37**, 237-256.

Chauncey H.H., Henriques B.L., and Tanzer J.M. (1963). Comparative enzyme activity of saliva from the sheep, hog, dog, rabbit, rat and human. *Arch. Oral Biol.* **8**, 615-627.

Chirayath M.V. Gajdzik L., Hulla W., Graf J., Cross H.S., and Peterlik M. (1998). Vitamin D increases tight-junction conductance and paracellular Ca^{2+} transport in Caco-2 cell cultures. *Am. J. Physiol.* **274**, G389-G396.

Clemens E.T., and Stevens C.E. (1980). A comparison of gastrointestinal transit time in ten species of mammal. *J. Agri. Sci.* **94**, 735-737.

Cooke A.R. (1974). Duodenal acidification: role of first part of duodenum in gastric emptying and secretion in dogs. *Gastroenterol.* **67**, 85-92.

Cooke A.R., and Clarke E.D. (1976). Effect of first part of duodenum on gastric emptying in dogs: response to acid, fat, glucose, and neural blockade. *Gastroenterol.* **70**, 550-555.

Cullen J.J., and Kelly K.A. (1996). Functional characteristics of canine pylorus in health, with pyloroplasty, and after pyloric reconstruction. *Dig. Dis. Sci.* **41**, 711-719.

Curtis Lawson D., Manthyn C.R., and Pappas T.N. (1994). Effect of CGRP antagonist, alpha-CGRP 8-37, on acid secretion in the dog. *Dig. Dis. Sci.* **39**, 1405-1408.

D

Datamonitor (1998). New opportunities in pet food 1999. London, UK.

Davenport H.W. (1961). Gastric secretion. In Physiology of the Digestive Tract (pp 85-112). Chicago: Year book medical publishers incorporated.

De Smet B., Hesta M., Seynaeve M., Janssens G., Vanrolleghem P., and De Wilde R.O. (1999). The influence of supplemental alpha-galactosidase and phytase in a vegetable ration for dogs on the digestibility of organic components and phytate phosphorus. *J. Anim. Phys. Anim. Nutr.* **81**, 1-8.

Dozois R.R., Kelly K.A., and Code C.F. (1971). Effect of distal antrectomy on gastric emptying of liquids and solids. *Gastroenterol.* **61**, 675-681.

Dressman J.B. (1986). Comparison of canine and human gastrointestinal physiology. *Pharm. Res.* **3**, 123-131.

Dreznik Z., Meininger T.A., Barteau J.A., Brocksmith D., and Soper N.J. (1994). Effect of ileal oleate on interdigestive intestinal motility of the dog. *Dig. Dis. Sci.* **39**, 1511-1513.

Duflos C., Bellaton C., Pansu D., and Bronner F. (1995). Calcium solubility, intestinal sojourn time and paracellular permeability codetermine passive calcium absorption in rats. *J. Nutr.* **125**, 2348-2355.

E

Eagon J.C., and Kelly K.A. (1993). Effects of gastric pacing on canine motility and emptying. *Am. J. Phys.* **265**, G767-G774.

Eeckhout C., Vantrappen G., Peeters T.L., Janssens J., and De Wever I. (1984). Different meals produce different digestive motility patterns. *Dig. Dis. Sci.* **29**, 219-224.

Ehrlein H., and Pröve J. (1982). Effect of viscosity of test meals on gastric emptying in dogs. *Quart. J. Exp. Phys.* **67**, 419-425.

Elashoff J.D., Reedy T.J., and Meyer J.H. (1982). Analysis of gastric emptying data. *Gastroenterol.* **83**, 1306-1312.

Engel C. (1946). Die Verdauungsenzyme der Vertebraten. In: Tabulae Biologicae (pp. 213-245). Ed.: H.J. Vonk, J.J Mansour-Bek., and E.J. Slijper. Amsterdam: Dr. W. Junk.

European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (1996). Part III: conduct of procedure; Article 6, paragraph 2. http://www.uku.fi/laitokset/vkek/Sopimus/convention.html.

Everts H., Dekker R.A., Smits B., and Cone J.W. (1996). Digestion of maize starch and native pea starch in the small intestine of pigs. *Proc. Nutr. Soc.* **55**, 59A.

F

FAIR CT97-3100 (1999). Model systems *in vitro* and *in vivo* for predicting the bioavailability of lipid soluble components of food. Progess report for the period from 1.11.98 to 31.10.99. Year 2.

Florey H.W., and Harding H.E. (1934). Further observations on the secretion of Brunner's glands. *Path. Bact.* **39**, 255-276.

Förster H., and Hoos I. (1977). Tierexperimentelle Untersuchungen zum Einfluβ von pflanzlichen Hydrokolloiden (Quellstoffen) auf die intestinale Resorption. *Z. Ernährungswiss.* **16**, 140-149.

Fox J.E. (1997). Seed gums. In: Thickening and Gelling Agents for Food (2nd ed.) pp 262-283. Ed.: A. Imeson. Blackie Academic Professional, London, UK.

Fox J., Maunder E.M., Randall V.A., and Care A.D. (1985). Vitamin D-dependent rickets type I in pigs. *Clin. Sci.* **69**, 541-548.

Freeman C.P. (1969). Properties of fatty acids in dispersions of emulsified lipid and bile salt and the significance of these properties in fat absorption in the pig and the sheep. *Br. J. Nutr.* **23**, 249-263.

G

Gabrielsson J., and Weiner D. (1997). Pharmacokinetic/Pharmacodynamic. Data analysis: Concepts and Applications (2nd ed.) pp 140-142. Swedish Pharmaceutical Press, Stockholm.

Ghishan F.K., Parker P., Nichols S., and Hoyumpa A. (1984). Kinetics of intestinal Ca transport during maturation in rats. *Ped. Res.* **18**, 235-239.

Greger J.L. (1999). Nondigestible carbohydrates and mineral bioavailability. *J. Nutr.* **129**, 1434S-1435S.

Gué M., Fioramonti J., and Bueno L. (1988). A simple double radiolabeled technique to evaluate gastric emptying of canned food meal in dogs. Application to pharmacological tests. *Gastroenterol. Clin. Biol.* **12**, 425-430.

Gupta P.K., and Robinson J.R. (1988). Gastric emptying of liquids in the fasted dog. *Int. J. Pharm.* **43**, 45-52.

Gupta S., Elder J.B., and Gillespie I.E. (1973). Exocrine secretory response of the pancreas to pentagastrin and to a meat meal in dogs. *Digest.* **9**, 482-489.

Gürtler H. (1967). Physiologie der Verdauung und Resorption. In Lehrbuch der Physiologie der Haustiere pp 192-331. Ed.: E. Kolb. Jena: VEB Gustav Fischer Verlag.

H

Hakim N.S., Walters A.M., Zinsmeister A.R., and Sarr M.G. (1992). Net absorption of water, electrolytes, glucose, and folate from the *in vivo*, neurally isolated canine jejunum. *Surgery* **111**, 394-401.

Hallebeek J.M., and Hazewinkel H.A.W. (1998). Effect of isoenergetic substitution of dietary fat (beef tallow) for carbohydrates (wheat starch) on the calcium absorption in the dog. *J. Anim. Phys.Anim. Nutr.* **78**, 60-66.

Halloran B.P., and DeLuca H.F. (1981). Appearance of the intestinal cytosolic receptor for 1,25-dihyroxyvitamin D3 during neonatal development in the rat. *J. Biol. Chem.* **256**, 7338-7342.

Hanes D.A., Weaver C.M., Heany R.P., and Wastney M. (1999). Absorption of calcium oxalate does not require dissociation in rats. *J. Nutr.* **129**, 170-173.

Havenaar R., Lelieveld J., Geuskens M., and Van Dokkum W. (2000). Availability for absorption of calcium form calcium supplements added to an European and Asian breakfast, in a dynamic gastrointestinal model. (Submitted)

Havenaar R., and Minekus M. (1996). Simulated assimilation. *Dairy Industries International* **61** (9), 17-23.

Hazewinkel H.A.W. (1989). Calcium metabolism and skeleletal development in dogs. Nutrition of the dog and cat. Waltham symposium 7. Cambridge University Press, Cambridge.

Hazewinkel H.A.W. (1996). Calciotropic hormones and bone metabolism (chapter 10). In: Clinical endocrinology of dogs and cats. Ed.: A. Rijnsbesk. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hazewinkel H.A.W., Goedegebuure G.A., Poulos P.W., and Wolvekamp W.T.C. (1985). Influences of chronic calcium excess on the skeletal development of growing Great Danes. *J.Am. Anim. Hosp. Assoc.* **21**, 377-391.

Hazewinkel H.A.W., Hagino H., Bouillon R., Goedegebuure S.A., Voorhout G., van den Brom W.E., and Bianda T. (1987). Influence of different calcium intakes on calciotropic hormones and skeletal development in young growing dogs. *Front. Horm. Res.* **17**, 221-232.

Hazewinkel H.A.W., How K.L., Bosch R., Goedegebuure S.A., and Voorhout G. (1988). Ungenügende Photosynthese von Vitamin D bei Hunden. In: Ernährung, Fehlernährung, und Diätetik bei Hund und Katze. Ed.: H. Meyer and E. Kienzle. Institut für Tierernährung. Tierärztliche Hochschule Hannover. Hannover, Germany.

Hazewinkel H.A.W., van den Brom W.E., Voorhout G. and Van W.A. (1991). Calcium metabolism in Great Dane dogs fed diets with various calcium and phosphorus levels. *J. Nutr.* **121**, S99-106.

Heaney R.P., Recker R.R., Weaver C.M. (1990). Absorbability of calcium sources: the limited role of solubility. *Calcif. Tissue Int.* **46**, 300-304.

Hinder R.A., and Kelly K.A. (1977). Canine gastric emptying of solids and liquids. *Am. J. Phys.* **233**, E335-E340.

Holick M.F., and Clarke M.B. (1978). The photobiogenesis and metabolism of vitamin D. *Fed. Proc.* **37**, 2567-2574.

Hornof W.J., Koblik P.D., Strombeck D.R., Morgan J.P., and Hansen G. (1989). Scintigraphic evaluation of solid-phase gastric emptying in the dog. *Vet. Radiol.* **30**, 242-248.

Horowitz M., Dent J., Fraser R., Sun W., and Hebbard G. (1994). Role and integration of mechanisms controlling gastric emptying. *Dig. Dis. Sci.* **39**, 7S-13S.

How K.L., Hazewinkel H.A., and Mol J.A. (1994). Dietary vitamin D dependence of cat and dog due to inadequate cutaneous synthesis of vitamin D. *Gen. Comp. Endocrinol.* **96**, 12-18.

Hunt J.N., and Stubbs D.F. (1975). The volume and energy content of meals as determinants of gastric emptying. *J. Phys.* **245**, 209-225.

Hurell R.F. (1989). Food manufacturing processes and their influence on the nutritional quality of foods. Nutritional impact of food processing. Ed.: J.C. Somogyi, and H.R. Müller. Karger, Basel. Biblioteca nutritio et dieta, no. 43.

I

Ikegami S., Tsuchihashi F., Harada H., Tsuchihashi N., Nishide E., and Innami S. (1990). Effect of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rats. *J. Nutr.* **120**, 353-369.

ISO (International Organization for Standardization). Animal feeding stuffs- Determination of nitrogen content and calculation of crude protein content, 1979 (ISO 5983), Geneve, Switzerland.

ISO (International Organization for Standardization). Animal feeding stuffs-Determination of total phosphorus content-Spectrophotometric method, 1979 (ISO 6491), Geneve, Switzerland.

J

Jeanes A. (1975). Digestibility of food polysaccharides by man: a review. In: Physiological Effects of Food Carbohydrates pp 336-347. Ed.: A. Jeanes, and J. Hodge. American Chemical Society, Washington DC.

Jenkins K.J., and Phillips P.H. (1960a). The mineral requirement of the dog. I. Phosphorus requirement and avalability. *J. Nutr.* **70**, 235-240.

Jenkins K.J., and Phillips P.H. (1960b). The mineral requirement of the dog. II. The relation of calcium, phosphorus and fat levels to mininal calcium and phosphorus requirement. *J. Nutr.* **70**, 241-246.

Jin H.O., Lee K.Y., Chang T.M., Chey W.Y., and Dubois A. (1994). Secretin: a physiological regulator of gastric emptying and acid output in dogs. *Am. J. Phys.* **267**, G702-G708.

Jin H.O., Lee K.Y., Chang T.M., Chey W.Y., and Dubois A. (1994). Physiological role of cholecystokinin on gastric emptying and acid output in dogs. *Dig. Dis. Sci.* **39**, 2306-2314.

Jonderko K., Ferre J., and Bueno L. (1994). Noninvasive evaluation of kinetics of gallbladder emptying and filling in the dog. *Dig. Dis. Sci.* **39**, 2624-2633.

K

Kallfelz F.A., and Dzanis D.A. (1989). Overnutrition: An epidemic problem in Pet Animal Practice? *Vet. Clin. North Am.* **19**, 433-446.

Kamath P.S., Hoepfner M.T., and Phillips S.F. (1987). Short chain fatty acids stimulate motility of the canine ileum. *Am. J. Phys.* **253**, G427-G433.

Karbach U. (1991). Segmental heterogeneity of cellular and paracellular calcium transport across the rat duodenum and jejunum. *Gastroenterol.* **100**, 47-58.

Kaune R., Kassianoff I., Schröder B., and Harmeyer J. (1992). The effects of 1,25-dihydroxyvitamin D-3 deficiency on Ca(2+)-transport and Ca(2+)-uptake into brush border membrane vesicles form pig small intestine. *Biochim. Biophys. Acta* **1109**, 187-194.

Keane F.B., DiMagno E.P., Dozois R.R., and Go V.L.W. (1980). Relationships among canine interdigestive exocrine pancreatic and biliary flow, duodenal motor activity, plasma pancreatic polypeptide, and motilin. *Gastroenterol.* **78**, 310-316.

Kelly B.J., and Potter N.N. (1990). Dialyzable calcium from milk processed with soluble fiber-containing gums, thickeners, and cocoa. *J. Food Sci.* **55**, 1004-1007.

Kienzle E. (1988). Enzymaktivität in Pancreas, Darmwand und Chymus des Hundes in Abhängigkeit von Alter und Futterart. *J. Anim. Phys. Anim. Nutr.* **60**, 276-288.

L

Landor J.H., and Wild R.A. (1970). Oestrus and gastric secretion in the dog. *Gut* **11**, 855-858.

Larmas M., and Scheinin A. (1971). Studies on dog saliva. I. Some physico-chemical characteristics. *Acta Odont. Scand.* **29**, 205-214.

Larsson M., Minekus M., and Havenaar R. (1997). Estimation of the bio-availability of iron and phosphorus in cereals using a dynamic *in vitro* gastrointestinal model. *J. Sci. Food Agri.* **73**, 99-106.

Lajvardi A., Mazarin G.I., Gillespie M.B., Satchithanandam S., and Calvert R.J. (1993). Starches of varied digestibilities differentially modify intestinal function in rats. *J. Nutr.* **123**, 2059-2066.

Laval-Jeantet A.-M., and Laval-Jeantet M. (1976). Interactions entre les lipides et le calcium en nutrtition expérimentale et humaine. *Path. Biol.* **24**, 213-225.

Leib M.S., Wingfield W.E., Twedt D.C., and Williams A.R. (1986). Gastric emptying of liquids in the dog: effect of volume. *Am. J. Vet. Res.* **47**, 1019-1021.

Lui C.Y., Amidon G.L., Berardi R.R., Fleisher D., Youngberg C.A., and Dressman J.B. (1986). Comparison of gastrointestinal pH in dogs and humans: implications on the use of the beagle dog as a model for oral absorption in humans. *J. Pharm. Sci.* **75**, 271-274.

Longland A.C. (1991). Digestive enzyme activities in pigs and poultry. In: *In Vitro* Digestion for Pigs and Poultry pp 3-18. Ed.: M.F. Fuller. CAB International, Wallingford, Oxon, UK.

M

Madrid J.A., Salido G.M., Mañas M., Martinez-Victoria E., and Mataix F.J. (1983). Use of a bidirectional cannula to study biliary secretion in conscious dogs. *Lab. Anim.* **17**, 307-310.

Mandrek K. (1991). Diameter and wall thickness recording of canine pylorus with implantable miniature ultrasonic transducers. *Dig. Dis.* **9**, 325-331.

Marteau P., Minekus M., Havenaar R., and Huis in 't Veld J.H.J. (1997). Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: Validation and the effects of bile. *J. Dairy Sci.* **80**, 1031-1037.

Mason V.C., Just A., and Bech-Andersen S. (1976). Bacterial activity in the hind-gut of pigs. 2. Its influence on the apparent digestibility of nitrogen and amino acids. *Z. Tierphysiol. Tierernähr. Futtermittelkde* **36**, 310-324.

Maskell I.E., and Johnson J.V. (1993). Digestion and absorption. In: The Waltham Book of Companion Animal Nutrition pp 25-44. Ed.: I.H. Burger. Oxford: Pergamon press.

Mathers J.C., Smith H., and Carter S. (1997). Dose-response effects of raw potato starch on small-intestinal escape, large-bowel fermentation and gut transit time in the rat. *Br. J. Nutr.* **78**, 1015-1029.

Meyer H., Behfeld T., Schünemann C., and Mühlum H. (1989). Intestinaler Wasser, Natrium und Kalium Stoffwechsel. *Adv. Anim. Phys. Nutr.* **19**, 109-119.

Meyer H., and Mundt H.C. (1983). Use of bone meal in feed rations in dogs. Arch. DTW. *Dtsch. Tierarztl. Wochenschr.* **90**, 81-86.

Meyer J.H., Dressman J., Fink A., and Amidon G. (1985). Effect of size and density on canine gastric emptying of nondigestible solids. *Gastroenterol.* **89**, 805-813.

Meyer J.H., Thomson J.B., Cohen M.B., Shadchehr A., and Mandiola S.A. (1979). Sieving of solid food by the canine stomach and sieving after gastric surgery. *Gastroenterol.* **76**, 804-813.

Minekus M. (1996). In-vitro modelling moves closer to the real thing. *Feed Mix* **3**, 36-38.

Minekus M. (1998). Development and validation of a dynamic model of the gastrointestinal tract. PhD Thesis. University of Utrecht, The Netherlands.

Minekus M., and Havenaar R. (1996). *In vitro* model of an *in vivo* digestive tract. US Patent. Patent number 5,525,305.

Minekus M., and Havenaar R. (1998). Reactor system. European patent no. 0642382. European Patent Bulletin 98/07, art 97 (4) and (5) EPC, dated 11.02.98

Minekus M., Marteau P., Havenaar R., and Huis in 't Veld J.H.J. (1995). A multi compartmental dynamic computer controlled model simulating the stomach and small intestine. *ATLA* **23**, 197-209.

Minekus M., Smeets-Peeters M., Bernalier A., Marol-Bonnin S., Havenaar R., Marteau P., Alric M., Fonty G., and Huis in 't Veld J.H.J. (1999). A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. *Appl. Microbiol. Biotechn.* **53**, 108-114

Miyabayashi T., Morgan J.P., Atitola M.A.O., and Muhumuza L. (1986). Small intestinal emptying time in normal beagle dogs. A contrast radiographic study. *Vet. Radiol.* **27**, 164-168.

Morris J.G., Earle K.E., and Anderson P. A. (1999). Plasma 25-hydroxyvitamin D in growing kittens is related to dietary intake of cholecalciferol. *J. Nutr.* **129**, 909-912.

Murray S.M., Fahey G.C. Jr., Merchen N.R., Sunvold G.D., and Reinhart G.A. (1999). Evaluation of selected high-starch flours as ingredients in canine diets. *J. Anim. Sci.* **77**, 2180-2186.

Murray S.M., Patil A.R., Fahey G.C. Jr., Merchen N.R., and Hughes D.M. (1997). Raw and rendered animal by-products as ingredients in dog diets. *J. Anim. Sci.* **75**, 2497-2505.

Murray S.M., Patil A.R., Fahey G.C. Jr., Merchen N.R., Wolf B.W., Lai C.-S., and Garleb K.A. (1999). Apparent digestibility and glycaemic responses to an experimental induced viscosity dietary fiber incorporated into an enteral formula fed to dogs cannulated in the ileum. *Food Chem. Toxicol.* **37**, 47-56.

N

National Research Council (NRC; 1985). Nutrient requirements of dogs. Washington D.C.: National Acadamy Press.

Nakayama F. (1969). Composition of gallstone and bile: species difference. *J. Lab. Clin. Med.* **April**, 623-630.

Nap R.C., Hazewinkel H.A.W, and Van den Brom W.E. (1993). ^{45}Ca kinetics in growing miniature poodles challenged by four different dietary levels of calcium. *J. Nutr.* **123**, 1826-1833.

NEN3349 (1984). Animal feeding stuffs-Determination of calcium, potassium, copper, magnesium, manganese, sodium, iron and zinc contents. AAS method. Standards of the Netherland Normalization Institute, Delft, The Netherlands.

Neri M., Phillips S.F., Fich A., and Haddad A.C. (1991). Canine ileocolonic sphincter: flow, transit, and motility before and after sphincterotomy. *Am. J. Phys.* **260**, G284-G289.

Norman A.W. (1990). Intestinal calcium absorption: a vitamin D-hormone-mediated adaptive response. *Am. J. Clin. Nutr.* **51**, 290-300.

NRLO, Nationale Raad voor Landbouwkundig Onderzoek (1996). Drastische verbetering van de nutriëntenbenutting in de dierlijke productie. NRLO-rapport nr. 94/3.

P

Pansu D., Bellaton C., and Bronner F. (1983). Developmental changes in the mechanisms of duodenal calcium transport in the rat. *Am. J. Phys.* **244**, G20.

Papasouliotis K., Muir P., Gruffydd-Jones T.J., Cripps P.J., and Blaxter A.C. (1993). The effect of short-term dietary fibre administration on oro-caecal transit time in dogs. *Diabetologia* **36**, 207-211.

Patronella C.K., Vanek I., and Bowen J.C. (1988). *In vivo* measurement of gastric mucus pH in canines: effect of high luminal acidity and prostaglandin E2. *Gastroenterol.* **95**, 612-618.

Pröve J., and Ehrlein H.J. (1982). Motor function of gastric antrum and pylorus for evacuation of low and high viscosity meals in dogs. *Gut* **23**, 150-156.

R

Reppas C., and Dressman J.B. (1992). Viscosity modulates blood glucose response to nutrient solutions in dogs. *Diabet. Res. Clin. Pract.* **17**: 81-88.

Rhee M., Pittz E.P., and Abraham R. (1981). Effect of combinations of *Irideae* carrageenan and cellulose on the absorption of some nutrients form the alimentary tract of rats. *Ecotox. Environ. Safety* **5**, 1-14.

Richardson D.C., and Toll P.W. (1997). Relationship of nutrition to developmental skeletal disease in young dogs. *Vet. Clin. Nutr.* **4**, 6-13.

Rossander L., Sandberg A.-S., and Sandström B. (1992). The influence of dietary fibre on mineral absorption and utilisation. In: Dietary fibre-a component of food. Nutritional function in health and disease. Ed.: T.F. Schweizer, and C.A. Edwards. Springer-Verlag, London.

Rothuizen J., and de Vries-Chalmers R. (1990). Post prandial and cholecystokinin-induced emptying of the gall bladder in dogs. *The Veterinary Record* **126**, 505-507.

S

Sarr M.G., and Kelly K.A. (1980). Patterns of movement of liquids and solids through canine jejunum. *Am. J. Phys.* **239**, G497-G503.

SAS (1990). SAS User's Guide (Version 6, 1st Ed) SAS Inst. Inc., Cary, NC.

SAS Language (1990). Reference version 6, First edition. SAS Institute Inc. Cary, NC.

SAS/STAT Software (1997). Changes and Enhancements through Release 6.12. pp 355. SAS Inst. Inc., Cary, NC.

Savoie L. (1994). Digestion and absorption of food : usefulness and limitations of *in vitro* models. *Can. J. Physiol. Pharmacol.* **72**, 407-414.

Schaafsma G. (1997). Bioavailability of calcium and magnesium. *Eur. J. Clin. Nutr.* **51**, S13-S14.

Schoenmakers I. (1998). Modulation of calcium regulation by excessive calcium intake in dogs. Studies during different stages of development. PhD Thesis, Utrecht of University. Universal Press, Veenendaal, The Netherlands.

Schröder B., Dahl M.R., and Breves G. (1998). Duodenal Ca^{2+} absorption is not stimulated by calcitrol during early postnatal development of pigs. *Am. J. Phys.* **275**, G305-G313.

Schröder B., Kaune R., and Harmeyer J. (1990). Studies of the porcine intestinal calcitriol receptor in pseudo-vitamin D deficiency rickets type I. *Clin. Sci. Colch.* **79**, 409-414.

Schröder B., Kaune R., Schlumblom C., Breves G., and Harmeyer J. (1993). Evidence for vitamin D-independent active calcium absorption in newborn piglets. *Calc. Tissue Intern.* **52**, 305-309.

Sirois P.J., Amidon G.L., Meyer J.H., Doty J., and Dressman J.B. (1990). Gastric emptying of nondigestible solids in dogs: a hydrodynamic correlation. *Am. J. Phys.* **258**, G65-G72.

Smeets-Peeters M.J.E., Groeneveld L.H., Havenaar R., Verstegen M.W.A. and Schaafsma G. (2000). Calcium and phosphorus availability of canned dog foods can be measured in a dynamic, computer-controlled gastrointestinal dog model. *J. Anim. Phys. Nutr.* (Submitted).

Smeets-Peeters M.J.E., Minekus M., Havenaar R., Schaafsma G., and Verstegen M.W.A. (1999). Description of a dynamic gastrointestinal *in vitro* model of the dog and evaluation of the different transit times for protein and calcium. *ATLA* **27**, 935-949.

Smeets-Peeters M.J.E., Schreijen E.M.W.C., Fahey G.C. Jr., Havenaar R., Schaafsma G., and Verstegen M.W.A. (2000). Validation of a dynamic *in vitro* model of the canine gastrointestinal tract. *J. Anim. Sci.* (submitted)

Smeets-Peeters M.J.E., Watson T., Minekus M., and Havenaar R. (1998). A review of the physiology of the canine digestive tract related to the development of *in vitro* systems. *Nutr. Res. Rev.* **11**, 45-69.

Soper N.J., Geisler K.L., Sarr M.G., Kelly K.A., and Zinsmeister A.R. (1990). Regulation of canine jejunal transit. *Am. J. Phys.* **259**, G928-G933.

Stevens C.E., and Hume I.D. (1995). Neuroendocrine control. In: Comparative Physiology of the Vertebrate Digestive System pp 268-287. Cambridge: Cambridge university press, UK.

Stevens C.E. and Hume I.D. (1995). Secretion and absorption of electrolytes and water. In: Comparative Physiology of the Vertebrate Digestive System pp 229-267. Cambridge: Cambridge university press, UK.

Stock-Damgé C., Bouchet P., Dentinger A., Aprahamian M., and Grenier J.F. (1983). Effect of dietary fiber supplementation on the secretory function of the exocrine pancreas in the dog. *Am. J. Clin. Nutr.* **38**, 843-848.

T

Teeter B.C., and Bass P. (1982). Gastric emptying of liquid test meals of various temperatures in the dog. *Proc. Soc. Exp. Biol. Med.* **169**, 527-531

Theodorakis M.C. (1980). External scintigraphy in measuring rate of gastric emptying in beagles. *Am. J. Phys.* **239**, G39-G43.

Thomas W.R. (1997). Carrageenan. In:Thickening and Gelling Agents for Food (2nd ed.) pp 45-59. Ed.: A. Imeson. Blackie Academic Professional, London, UK.

Torre M., Rodriguez A.R., and Saura-Calixto F. (1991). Effects of dietary fiber and phytic acid on mineral availability. *Crit. Rev. Food Sci. Nutr.* **1**, 1-22.

Traynor O.J., Dozois R.R., and DiMagno E.P. (1984). Canine interdigestive and postprandial gallbladder motility and emptying. *Am. J. Phys.* **246**, G426-G432.

V

Van Kruiningen H.J., Wojan L.D., Stake P.E., and Lord P.F. (1987). The influence of diet and feeding frequency on gastric function in the dog. *J. Am. Anim. Hosp. Assoc.* **23**, 145-153.

Vaquero M.P., Van Dokkum W., Bos K.D., Wolters M.G.E., Schaafsma G., and Luten J.B. (1992). *In vitro* availability of calcium, magnesium, iron, copper and zinc form white or brown bread separately or in combination with other foods. *Rev. Esp. Cienc. Tecnol. Aliment.* **32**, 47-58.

Villareal R., Ganong W.F., and Gray S.J. (1955). Effect of adrenocorticotrophic hormone upon the gastric secretion of hydrochloric acid, pepsin and electrolytes in dogs. *Am. J. Phys.* **183**, 485-494.

W

Washizu T., Ikenaga H., Washizu M., Ishida T., Tomoda I., and Kaneko J.J. (1990). Bile acid composition of dog and cat gallbladder bile. *Jap. J. Vet. Sci.* **52**, 423-425.

Wasserman R.H., and Fullmer C.S. (1995). Vitamin D and intestinal calcium transport: facts, speculations and hypotheses. *J. Nutr.* **125**, 1971S-1979S.

Wildgrube H.J., Stockhausen H., Petri J., Fussel U., and Lauer H. (1986). Naturally occurring conjugated bile acids, measured by high-performance liquid chromatography, in human, dog and rabbit bile. *J. Chromatogr.* **353**, 207-213.

Williams N.S., Meyer J.H., Jehn D., Miller J., and Fink A.S. (1984). Canine intestinal transit and digestion of radiolabeled liver particles. *Gastroenterol.* **86**, 1451-1459.

Wolever T.M.S., Jenkins D.J.A., Nineham R., and Alberti K.G.M.M. (1979). Guar gum and reduction of post-prandial glycaemia: Effect of incorporation into solid food, liquid food, and both. *Br. J. Nutr.* **41**, 505-510.

Wünsche J., Mein M., Hennig U., Borgmann E., Kreienbring F., and Bock H.-D. (1987). Influence of a thermic treatment of potato products on nutrient degradation in the digestive tract of pigs. 1. Passage and digestibility of the nutrients in various sections of the digestive tract. *Arch. Anim. Nutr.* **37**, 169-188.

Y

Yeo C.J., Bastidas J.A., Schmieg R.E., and Zinner M.J. (1990). Meal-stimulated absorption of water and electrolytes in canine jejunum. *Am. J. Phys.* **259**, G402-G409.

Youngberg C.A., Wlodyga J., Schmaltz S., and Dressman J.B. (1985). Radiotelemetric determination of gastrointestinal pH in four healthy beagles. *Am. J. Vet. Res.* **46**, 1516-1521.

Z

Zhao X., McCamish M.A., Miller R.H., and Wang L., Lin H.C. (1997). Intestinal transit and absorption of soy protein in dogs depend on load and degree of protein hydrolysis. *J. Nutr.* **127**, 2350-2356.

Zhao X., Miller R.H., McCamish M.A., and Wang L., Lin H.C. (1996). Protein absorption depends on load-dependent inhibition of intestinal transit in dogs. *Am. J. Clin. Nutr.* **64**, 319-323.

LIST OF PUBLICATIONS

PAPERS

Minekus M., Smeets-Peeters M.J.E., Bernalier A., Marol-Bonnin S., Havenaar R., Marteau P., Alric M., Fonty G., and Huis in 't Veld J.H.J. (1999). A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. *Appl. Microbiol. Biotechnol.* **53**, 108-114

Smeets-Peeters M.J.E, Groeneveld L.H., Havenaar R., Verstegen M.W.A., and Schaafsma G. (2000). Calcium and phosphorus availability of canned dog foods can be measured in a dynamic, computer-controlled gastrointestinal dog model. *J. Anim. Phys. Nutr.* (submitted).

Smeets-Peeters M.J.E. and Havenaar R. (1999). Dynamisch *in vitro* maag-darmmodel van de hond. *Dier-en-Arts* **14** (11), 317-321.

Smeets-Peeters M.J.E., Minekus M., Havenaar R., Schaafsma G., and Verstegen M.W.A. (1999). Description of a dynamic gastrointestinal *in vitro* model of the dog and evaluation of the different transit times for protein and Ca. *Alternatives to Laboratory Animals (ATLA)* **27**, 935-949

Smeets-Peeters M.J.E., Schreijen E.M.W.C., Fahey Jr., G.C., Havenaar R., Schaafsma G., and Verstegen M.W.A. (2000). Validation of a dynamic *in vitro* model of the canine gastrointestinal tract. *J. Anim. Sci.* (submitted)

Smeets-Peeters M.J.E., Tryfonidou M.A., Havenaar R., Onderwater R., Hazewinkel H.A.W., Verstegen M.W.A., and Schaafsma G. (2000). The effects of vitamin D on the absorption of calcium in dogs. A study using three different methods. *Arch. Anim. Nutr.* (submitted)

Smeets-Peeters M.J.E., WatsonT., Minekus M., and Havenaar R. (1998). A review of the physiology of the canine digestive tract related to the development of *in vitro* systems. *Nutrition Research Reviews (NRR)* **11**, 45-69

Venema K., van Nuenen M., Smeets-Peeters M., Minekus M., Havenaar R. (2000). TNO's *in vitro* large intestinal model: an excellent screening tool for functional food and pharmaceutical research. *Ernährung* (submitted)

CONFERENCE PAPERS

Havenaar R., Minekus M., Zeijdner E., Smeets-Peeters M., Van Nuenen M., and Venema K. (2000). A dynamic, computer-controlled model of the gastrointestinal tract to study the nutritional quality. Submitted for publication in: In: *Proceedings Agricontrol 2000. International Conference on Modelling and Control in Agriculture, Horticulture and Post-harvested Processing.* Wageningen, The Netherlands

Havenaar R., Zeijdner E., Minekus M., and Smeets-Peeters M. (2000). A dynamic *in vitro* model of the gastrointestinal tract: a tool for the selection of functional micro-organisms. INRA symposium, France. In: *Reproduction Nutrition Development 40 (2): 224.*

Smeets M. (2000). In-vitro model of the canine GI tract. In: *Proceedings Petfood Forum 2000, pp* , Chicago, USA

Smeets-Peeters M., and Havenaar R. (1999). A dynamic *in vitro* model of the stomach and small intestine of the dog. In: *Proceedings of Petfood Technologies '99.* Zentralfachschule der Deutschen Süßwarenwirtschaft, Solingen-Gräfrath, Germany

Smeets-Peeters M., Havenaar R., Schaafsma G., and Verstegen M. (1999). Validation of a dynamic computer-controlled *in vitro* model of the canine gastrointestinal tract. In: *Proceedings World Congress on Alternatives to Laboratory Animal Science, pp 432,* Bologna, Italy

DANKWOORD

DANKWOORD

FIDO, pootje ! Dan...de beloning.
De beloning ligt hier inmiddels voor me, in de vorm van dit proefschrift. Er is echter een aantal mensen aan wie ik nog een 'pootje' verschuldigd ben. Mede dankzij deze mensen is het proefschrift geworden tot datgene wat nu voor me ligt.
Allereerst natuurlijk mijn 'baasjes': Prof. Hautvast, Gertjan Schaafsma, Martin Verstegen en Rob Havenaar. Professor Hautvast, u bent in het begin als promotor nauw betrokken geweest bij dit project, een taak die u later heeft overgedragen aan Gertjan. U zorgde ervoor dat het overzicht goed behouden bleef, iets wat in het begin niet altijd even gemakkelijk was. Bedankt hiervoor. Gertjan, toen het duidelijk was dat jij de taak van prof. Hautvast ging overnemen vond je het tijd om TNO te verlaten om elders een nieuwe uitdaging aan te gaan. Nu mijn promotieonderzoek bijna afgerond is, ben je weer terug bij TNO. Ondanks je periode van afwezigheid bij TNO was je er altijd als het nodig was. Ik ben blij dat ik nu weer met je mag samenwerken. Martin, tot nôw tôw heb ich altiêt in het Hollands tegen uch gekaltj, mer nôw is de tiêt aangebroeëke det det neet mieër hooftj. Ze hoove os nôw neet mieër te kinne verstoan. Daorum in het plat : Martin, bedanktj dej-je er altiêt woortj. Gae hetj mich altiêt het geveul gegaeve det ich beej uch terecht kos als det noeëdig mocht zeen. Auch wil ich Mariet bedanke veur de hertelikheid en gastvreejheid woeë det ich altiêt met ontvange woort. Martin en Mariet, bedanktj ! Rob, zonder jouw goede raad, suggesties en tijd was FIDO nooit groot geworden. Het was nooit teveel moeite voor je om mijn vragen te beantwoorden of mijn teksten voor de zoveelste keer te bekijken. Bedankt voor alle tijd die je voor me vrijgemaakt hebt.
De samenwerking met mensen van andere instituten was erg nuttig en leerzaam. Prof. Fahey, from the University of Illinois, USA, thank you very much for your cooperation in my project. You gave me the opportunity to perform the validation studies, which were a very important part of my project. I hope we will continue this cooperation in the future. Prof. Hazewinkel en Marianna Tryfonidou van de Universiteit Utrecht, jullie wil ik bedanken voor de zeer prettige samenwerking in de vitamine D/calcium studie. De samenwerking is toevallig tot stand gekomen maar ik hoop dat deze zeker een vervolg gaat krijgen. De vitamine D/calcium studie is ook mede succesvol geworden door een aantal collega's bij TNO: Han van der Sandt, Carolien Versantvoort, Andries Gilde en Rob Onderwater bedankt voor de inwijding in de geheimen van die 'andere' *in vitro* methode.
De collega's van de Universiteit Wageningen wil ik bedanken. Ondanks het feit dat ik erg weinig in Wageningen geweest ben, hebben jullie mij toch altijd het gevoel gegeven dat ik

erbij hoorde. Een speciaal woord van dank aan Huug Boer en Jane-Martine Muijlaert die het mogelijk hebben gemaakt een aantal analyses uit te kunnen voeren op de universiteit.
En natuurlijk niet te vergeten: mijn collega's bij TNO. De mensen van de analyse afdeling en de afdeling statistiek wil ik bedanken voor al het werk wat ze voor mij verzet hebben. Dirk van der Heij bedankt voor de redigeren van de engelse teksten. En dan natuurlijk het TIM-team. Mans, mede dankzij jou heb ik een plekje kunnen verwerven binnen het TIM-team. En zoals je ervaart wordt dit plekje, letterlijk gezien, steeds groter (groter bureau, kasten etc.). Ik beloof je dat ik niet meer dan de helft van de kamer inneem. Evelijn, samen kwamen we hier binnen als onervaren studentjes. We hebben veel tijd doorgebracht in de 'stagiare' kamer en we hebben hier veel plezier gehad. Ik hoop dat we, ook in de toekomst nog veel plezier zullen hebben bij TNO. Ik vind het erg leuk dat je samen met Leanne mijn steun en toeverlaat bent tijdens de laatste loodjes Alle collega's van het TIM-team wil ik bedanken voor de prettige samenwerking: Jan, Jeffrey, Mark, Koen, Marleen, Cyrille en Eugène, bedankt ! In de loop van de afgelopen jaren is er ook een groot aantal stagiares geweest die het TIM-team versterkt hebben. Met name Elke, Miranda, Esther en Lutien wil ik bedanken voor de bijdrage die ze geleverd hebben aan FIDO.
Leanne, ik ben blij dat je ondanks alle drukte spontaan 'ja' hebt gezegd toen ik vroeg of je mijn paranimf wilde zijn. Bedankt voor je vriendschap en we gaan flink feesten de komende periode !
Pap en mam, bedankt voor de steun die jullie me altijd gegeven hebben in alles wat ik deed. Dankzij deze steun heb ik dit allemaal kunnen bereiken. Dankjewel, ik ben trots op jullie !!!
Lieve Erwin, zonder jou had ik mijn 'aio-dipjes' waarschijnlijk niet overwonnen. Je bent er altijd als het nodig is en ik hoop dat we er nog heel lang er voor elkaar kunnen zijn.

Nogmaals bedankt allemaal. Mede dankzij jullie kan ik dan nu zeggen : FIDO, AF !!

Marianne
=

CURRICULUM VITAE

CURRICULUM VITAE

Marianne (Maria Johanna Elisabeth) Smeets-Peeters werd geboren op 8 november 1970 te Weert. In 1990 haalde zij haar VWO diploma aan de Philips van Horne Scholengemeenschap te Weert. Datzelfde jaar begon zij met de studie Gezondheidswetenschappen, met als afstudeerrichting Biologische Gezondheidskunde, aan de toenmalige Rijksuniversiteit Limburg te Maastricht (nu Universiteit Maastricht). Haar afstudeerstages, met als onderwerpen "COPD. Onderzoek naar de vicieuze cirkel hypothese" en "Fermentation of dietay fibre in the TNO colon model. Development of a colon model and a method to analyse fermentation products", deed zij op resp. de afdeling Microbiologie van het Academisch Ziekenhuis te Maastricht (AZM) en de afdeling Dierlijke en Humane Voeding bij TNO Voeding te Zeist, om in augustus 1995 haar doctoraal te behalen. Van augustus tot en met december 1995 was zij als uitzendkracht werkzaam bij TNO Voeding (Zeist) bij de afdeling Dierlijke en Humane Voeding. Per 1 januari 1996 trad zij als assistent in opleiding (AIO) in dienst van de Landbouwuniversiteit Wageningen (nu Wageningen Universiteit) bij de leerstoelgroep veevoeding (Prof. dr. ir. M.W.A. Verstegen). Als AIO maakte ze daar onderdeel uit van de 'Graduate School WIAS' (Wageningen Institute of Animal Sciences). Het onderzoek dat zij gedurende vier jaar verrichtte is uitgevoerd bij TNO Voeding in Zeist (Prof. dr. ir. G. Schaafsma en Dr. R. Havenaar) en is beschreven in dit proefschrift. Sinds 1 januari 2000 is zij in dienst van TNO Voeding, afdeling Voedingsfysiologie, en werkzaam als productmanager petfood.

Marianne (Maria Johanna Elisabeth) Smeets-Peeters was born on 8th of November 1970 in Weert, the Netherlands. In 1990 she finished secondary school at the 'Philips van Horne Scholengemeenschap' in Weert. In the same year she started her study Health Sciences at the University of Maastricht, the Netherlands. Her practical research was performed at the Department of Microbiology of the Academical Hospital in Maastricht. She finished her study in August 1995. From August until December 1995 she worked at TNO Food and Nutrition Research in Zeist, the Netherlands, on the development of an *in vitro* model of the large intestine. In January 1996 she started as a PhD student at the Department of Animal Nutrition at the Wageningen Agricultural University (now called Wageningen University; Prof. dr. ir. M.W.A. Verstegen). She participated in the graduate school 'Wageningen Institute of Animal Sciences' (WIAS). Her working place was at TNO Nutrition and Food Research (Prof. dr. ir. G. Schaafsma and Dr. R. Havenaar), department of Nutritional Physiology, were she will continue her work as productmanager petfood.