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Development of a Dynamic System Simulating Pig Gastric Digestion

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ABSTRACT : The objective of this study was to develop a model for simulating gastric digestion in the pig. The model was constructed to include the chemical and physical changes associated with gastric digestion such as enzyme release, digestion product removal and gastric emptying. Digesta was collected from the stomach cannula of pigs to establish system parameters and to document the ability of the model to simulate gastric digestion. The results showed that the average pH of gastric digesta increased significantly from 2.47 to 4.97 after feed consumption and then decreased 140 min postprandial. The model described the decrease in pH within the pigs' stomach as $pH_t = 5.182e^{-0.0014t}$, where t represents the postprandial time in minutes. The cumulative distribution function of liquid digesta was $V_t = 64.509e^{0.0109t}$. The average pepsin activity in the liquid digesta was 317Anson units/mL. There was significant gastric emptying 220 min after feed consumption. The cybernetic dynamic system of gastric digestion was set according to the above data in order to compare with *in vivo* changes. The time course of crude protein digestion predicted by the model was highly correlated with observed *in vivo* digestion ($r = 0.97$; $p = 0.0001$), Model prediction for protein digestion was higher than that observed for a traditional static *in vitro* method ($r = 0.89$; $p = 0.0001$). (**Key Words :** Gastric Digestion Modelling, Pigs, Protein Digestion)

INTRODUCTION

Methodology for the evaluation of nutrient digestion is important in animal nutrition research because it allows not only estimation of the nutritive value of particular feedstuffs (Yang et al., 2007) but also the bioavailability of drugs (Hébrard et al., 2006) and feed supplements (Chiang et al., 2005; Fang et al., 2007). *In vitro* methods are economical and efficient because they do not require animals, they employ less manpower and decrease the variation associated with replicate measurements using traditional laboratory procedures. The *in vitro* digestibility of major dietary components in pig diets (Furuya et al., 1979; Babinszky et al., 1990; Boisen and Fernández, 1995) were similar to those values reported in previous *in vivo* feeding trails.

Several *in vitro* methods of estimating feed digestibility have been developed, and can be divided into single- (AOAC, 1980; Mertz et al., 1984), two- (Babinszky et al., 1990; Cone and van der Poel, 1993), or three-step (Vervaeke et al., 1979; Boisen and Fernández, 1991) models simulating the gastric digestion, the gastric/small intestinal digestion and the gastric/small intestinal/large intestinal digestion, respectively. In each, the first step simulates gastric digestion. In non-ruminants, such as pigs, digestion of crude protein begins in the stomach or gastric pouch (Keys and DeBarthe, 1974; Furuya et al., 1979). The current static models, which expose substrates to a digesta fluid or enzyme solution at a fixed pH and temperature for a fixed period of time, simulate digestion in the animal gut in a manner similar to a batch reactor. These conditions do not simulate the physiological environment of digestion in the pig gastrointestinal tract (GI). In the static *in vitro* gastric models, pepsin hydrolysis proceeds at pH 1.0 (Babinszky et al., 1990) to 2.0 (Boisen and Fernández, 1995), or in a 0.1 N HCl solution (Cone and van der Poel, 1993). *In vivo*, gastric pH decreases from 4.8 to 2.1 and 1.7, one and two hours, respectively, after ingestion of milk (Marteau et al., 1990; human). The amount of digesta within the GI tract and digesta transit time significantly affects nutrient digestive capacity. None of these conditions can be properly simulated in a batch, static model of *in vitro* digestibility.

Previous *in vivo* research described the gastric empty rate (Hunt and Stubbs, 1975; human; Weisbrodt et al., 1969; dog) using the equation: $V(t) = V_0 (1-e^{-Kt})$, where $V(t)$ represents the gastric digesta volume at time t postprandial,

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V_0 the initial volume and K a constant of digesta emptying rate. To date, the *in vivo* gastric residence time has only been realistically simulated in the pre-ruminant calf gastric model (Yvon et al., 1992) where liquid flow and the pH are controlled continually in real time.

The objective of the present study was to develop a dynamic *in vitro* model of digestion within the pig which would simulate, as closely as possible, the actual physiological processes which occur within the lumen of the pig stomach during nutrient digestion. This model was compared to digestibility parameters measured *in vivo* as well as a traditional static *in vitro* incubation technique.

MATERIALS AND METHODS

Experimental design and digestibility estimates

Animal preparation : The animal feeding protocol, surgery procedure and care were approved by the Animal Care and Use Committee of National Chung Hsing University. Materials required and procedure for cannulation were similar to those described by Low et al. (1985). Experiments were carried out on four Landrace castrated male pig. All surgery was performed with full aseptic precautions under halothane anaesthesia. The pigs were fitted, at a weight of 40-45 kg, with a cannula (polypropylene; barrel outer diameter 25 mm) in the fundic region of the stomach for a further study of the control of gastric emptying. The pigs were given water and antibiotic powder, for the first 24 hours after surgery. The normal diet was then gradually re-introduced and full intake was achieved 4-5 days after surgery. Collections of gastric digesta began 14 days after surgery.

In vivo gastric physiological parameters and digestibility : The gastric-cannulated pigs were used to examine the effects of feeding level on gastric physiological parameters and nutrient digestibility measurement. A Latin 4×4 experimental design was used. All pigs were housed in individual cages and fed one of four feeding level treatments during each treatment period. Dietary treatment intakes were determined by measuring *ad libitum* feed intake of the pigs during a three day pre-test. The average intake was 1,200 grams. Accordingly, experimental dietary intakes were limited to 17%, 33%, 66% and 100% of the average *ad libitum* pre-test maximum intake. Treatments were 200, 400, 800, or 1,200 g daily at 0800 and 1600 hours during the sample collection day. Each feeding level was given to each pig for 2 days, in a Latin-square design. Gastric digesta was collected once on the last day of each phase. Latin-square experiments were repeated three times. A commercial grower diet (18% CP; 13.40 MJ digestible energy/kg) was mixed with water at a ratio of 1:1 for wet feeding. PEG 4000 (Polyethylene glycol 4000; Merck, Darmstadt, Germany) was added to the wet feed at a level of 2% (w/v) as a liquid digesta marker. On trial days, liquid water intake was not allowed during the sampling period to prevent dilution of the liquid digesta marker. Polyvinyl chloride (PVC) tubing was used to connect the cannulas for sampling 20 g digesta every 20 minutes for five h. Postprandial pH, pepsin activity, gastric liquid phase dilution rate, and protein digestibility were measured on each digesta sample.

Static in vitro model for measuring digestibility : The protocol of the static *in vitro* digestibility trial was according to the method of Babinszky et al. (1990). Optimal pepsin concentration and incubation times for the static *in vitro* incubations were established in the following manner. The activity of pepsin (P-7000, Sigma Chemical Co., St. Louis, MO) concentration was studied using 500, 1,000, 2,000, 4,000, or 8,000 units/ml at 10, 30, 60, 120, or 240 minutes for each level of enzyme. Incubations without added enzyme were used as controls. One gram of sample was incubated at 39°C for each incubation period in a 50 ml

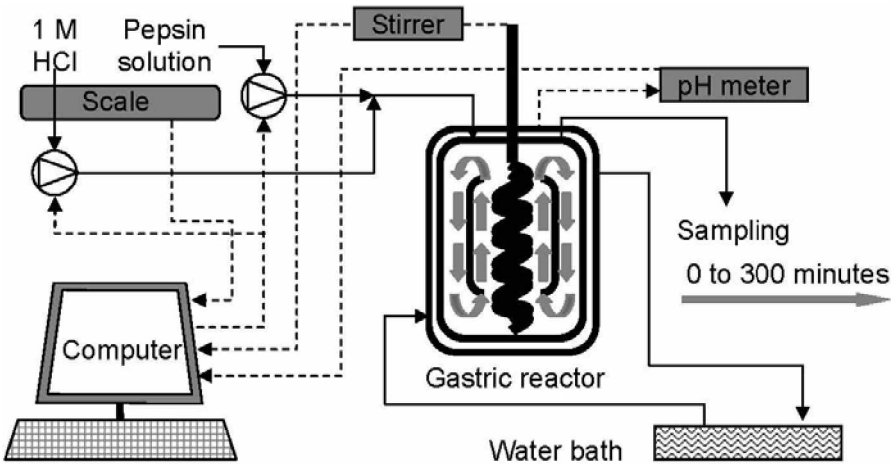

Figure 1. Schematic representation of the dynamic gastric digestion system.

beaker with 10 ml pepsin/0.1 M HCl at each concentration. The agitation of the magnetic stirring bars was controlled mechanically (direct-controlled magnetic stirrers, POLY15, VARIOMAG, Germany), which ensured equal stirring rates for each incubation. After incubation, 10 ml of 5% (w/v) TCA (tricloroacetic acid) was added and the sample was then poured through filter paper (Whatman No. 1). The amount of crude protein in the TCA-insoluble residue was determined.

Dynamic in vitro model for measuring in digestability : The dynamic model simulating gastric digestion in pigs was constructed using the LabView (Ver. 6.1, National Instruments Corp., USA) platform. This computer program has been designed to accept data obtained from *in vivo* studies in pigs, such as the pepsin activity, stomach digesta pH curves and secretion rates of gastric fluid into the system apparatus depicted in Figure 1. The reactor was a triple layer beaker. Water was pumped from a water bath around the secondary glass jacket to control the temperature inside the compartment. Sampled digesta was stirred using a helical polytetrafluoroethylene (PTFE) rod in the innermost glass tube such that the mixture of solid and liquid digesta was constantly mixed vertically to simulate peristaltic mixing *in vivo*. There were four holes at the top of the reactor, one for the stirring rod, two to allow the introduction of gastric digesta and 1 M HCl, the other for the removal of digesta. The secretion and removal of digesta occurred through perforated tubes by computer-controlled single action micro-tube pump (EYELA MP-3, Rikakikai Co. Tokyo, Japan) and peristaltic pump (PP-60, Biotop Process and Equipment, Taichung, Taiwan), respectively.

The test meals were prepared by mixing 60 g of the grower diet, which contained 0.5% Cr_2O_3 as solid digesta marker, with 60 ml 2% PEG 4000 and 90 ml of saliva/gastric electrolyte solution containing $CaCl_2$ (0.22 g/L), KCl (2.2 g/L), NaCl (5 g/L) and $2NaHCO_3$ (1.5 g/L). This solution was adjusted to a volume of 300 ml with water (Minekus, 1998).

Gastric fluid, consisting of saliva and gastric electrolyte solution with pepsin designed to mimic *in vivo* conditions in the stomach, was introduced into the dynamic incubation apparatus. The flow rate of gastric fluid in and out of the incubation vessel was set to mimic the conditions predicted by the model equation which was generated from direct observations within pigs for each level of intake during the *in vivo* study. The gastric volume was maintained at a constant level by controlling the rate flow of gastric secretion into the incubation vessel and controlling the removal of digesta to another compartment within the apparatus. The pH within the gastric compartment was controlled to simulate the *in vivo* conditions by the addition of 1 M HCl. The pH was recorded every minute during the incubation. Samples were collected once per 20 minutes for five hours. The sampled digesta were measured for pH values and protein digestibility, and digesta passage rates were estimated.

Chemical analyses

The pH values of digesta were measured by direct insertion of a pH electrode (TS-1, SUNTEX, Taiwan). The pepsin activity of digesta was analyzed according to the method of Anson (1938). Indigestible protein was defined as the fraction of TCA-insoluble residue. Crude protein (CP, Kjeldahl nitrogen) and dry matter (DM) were measured according to AOAC (1980). The marker/indicator concentrations of PEG 4000 and Cr_2O_3 in the digesta were both determined calorimetrically with a spectrophotometer (U-2001, HITACHI, Japan) according to the methods described by Ishikawa (1966) and Williams et al. (1962), respectively.

Calculations and statistical analyses

The digestibility of protein measured *in vivo* and within the dynamic system were expressed as the percentage of indigestible protein and PEG 4000/Cr_2O_3 relative to that in the diet. The static *in vitro* protein digestibility was calculated by the difference between the crude protein in the TCA-insoluble residue of the blank and incubated samples.

Gastric liquid phase dilution rates were estimated using the passage rate constant (k) of the water-soluble marker (PEG 4000) as calculated by the slope of the semilog plot of PEG 4000 concentration against time. The equation describing the curve was: $V(t) = V_0 e^{-Kt}$, where $V(t)$ is marker concentration at time t, V_0 is marker concentration at time 0, both expressed as a percentage of total intakes, and k is the dilution rate constant for PEG 4000 (modified from Colucci et al., 1990). The calculated curve fitting parameters were used to compare the variation between the dynamic system and the pre-set delivery curves, which were based on the *in vivo* digestibility data.

The effects of pepsin activity and incubation time on static *in vitro* protein digestibility were tested by one-way analysis of variance using the General Linear Model procedure of SAS (1999) and the means were compared by least significant difference. Differences between means at the probability level of $p<0.05$ were accepted as being statistically significant. Linear correlations between *in vitro* (static and dynamic) and *in vivo* data were calculated using the test of Pearson correlations.

RESULTS

pH value curves

The amount of feed significantly altered pH in the stomach of pigs during the *in vivo* digestibility study. The changing pH with time course model was described as pH_t

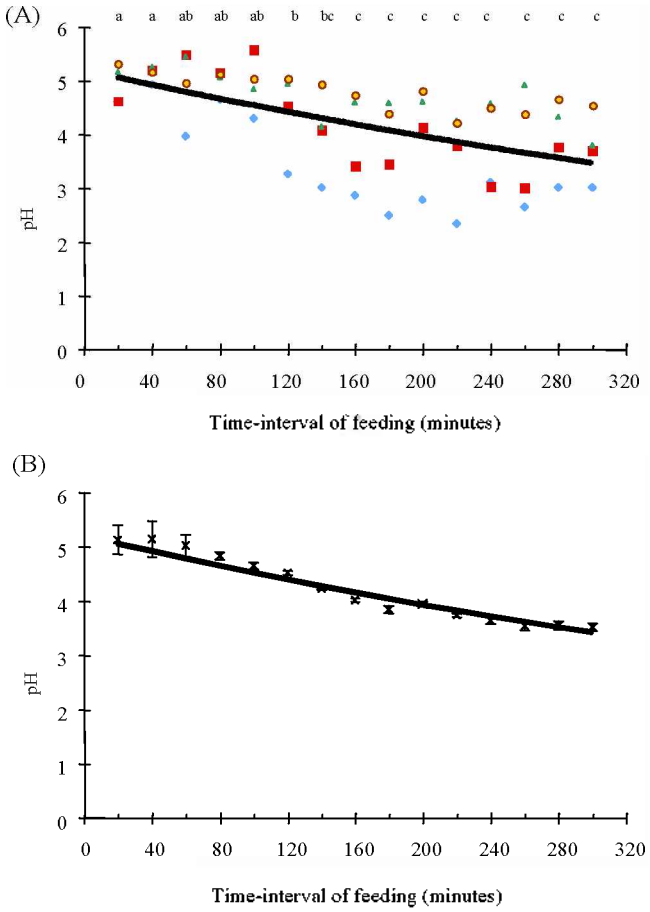

Figure 2. The pattern of pH values of gastric digesta (A) are means for four pigs with four feeding levels in a 4×4 Latin Square design. The meal sizes were 200 g (♦), 400 g (■), 800 g (▲), or 1200 g (●). The total *in vivo* data followed an exponential pattern of $pH_t = 5.182e^{-0.0014t}$, which compared with the results (×; mean±SE) from the dynamic system are shown as (B). Means of the combined data without same superscript are significantly different ($p<0.05$).

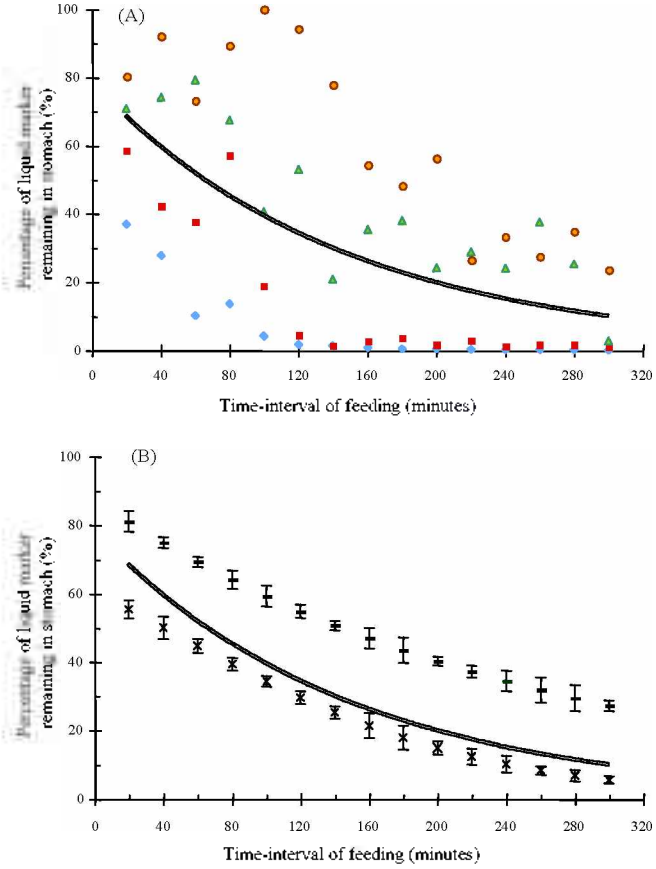

Figure 3. The amounts of liquid marker remaining in the stomach (A) are means for four pigs with four feeding levels in a 4×4 Latin Square design. The meal sizes were 200 g (♦), 400 g (■), 800 g (▲), or 1200 g (●). The total *in vivo* data followed an exponential pattern of $C_{t,\ in\ vivo} = 78.659e^{-0.0069t}$, which compared with the liquid (×; mean±SE) and solid (-; mean±SE) marker concentration remaining in the dynamic system are shown as (B).

$= 4.6417e^{-0.0021t}$ (200 g, $p = 0.002$), $5.4926e^{-0.0018t}$ (400 g, $p = 0.003$), $5.343e^{-0.0008t}$ (800 g, $p = 0.0128$), or $5.2936e^{-0.0006t}$ (1,200 g, $p = 0.096$) for each feeding level condition. The combined gastric pH curve for all *in vivo* data, $pH_{t,\ in\ vivo} = 5.182e^{-0.0014t}$ ($p = 0.0825$, Figure 2A), was used as the pre-set pH curve to establish incubation conditions in the dynamic *in vitro* model of digestibility.

The pre-set pH curve in the dynamic *in vitro* system closely simulated (Figure 2B) changes in pH noted during the *in vivo* digestibility study. The changing pH curve model, from dynamic system experiments, was described as $pH_{t,\ dynamic} = 5.360e^{-0.0016t}$ ($p<0.0001$).

Pepsin activity

The *in vivo* pepsin activity in gastric juice ranged from 240 to 865.4 Anson units/g digesta in all measurements taken until 5 h because of changes in digesta volume that were associated with the amount of feed consumed. There was no difference in pepsin activity amongst the five-hour postprandial measurements. Hence, 1,000 units pepsin/ml HCl dynamic were used in the *in vitro* system studies.

Gastric liquid phase dilution rate

The decreasing rates of liquid marker concentration in the stomach of pigs and in the dynamic *in vitro* system are shown in Figure 3. The gastric liquid marker passage rates measured *in vivo* (Figure 3A) and in the dynamic *in vitro* model (Figure 3B) were described as their time related changes in concentration. $C_{t,\ dynamic} = 74.998e^{-0.0083t}$ ($p< 0.0001$) and $C_{t,\ in\ vivo} = 78.659e^{-0.0069t}$ ($p = 0.0008$), respectively. The half time of liquid digesta marker was 48.8 min *in vivo* and 65.7 min *in vitro*.

Effects of experimental conditions of the static *in vitro* method on digestibility estimates

In the static *in vitro* trial, the effects of pepsin activities

Table 1. The effects of pepsin activities and incubation period on the *in vitro* protein digestibility (%)

	Pepsin activity (Anson units/ml)					p-value
	500 U/mL	1,000 U/ml	2,000 U/ml	4,000 U/ml	8,000 U/ml	
Incubation period (minutes)						
10	7.69	9.63	10.83	11.68	12.71	<0.0001
30	10.73	19.55	18.71	20.46	23.24	<0.0001
60	20.18	23.79	27.39	28.44	33.55	<0.0001
120	34.36	40.09	43.27	39.67	42.54	<0.0001
240	38.17	42.70	50.81	51.28	56.52	<0.0001
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

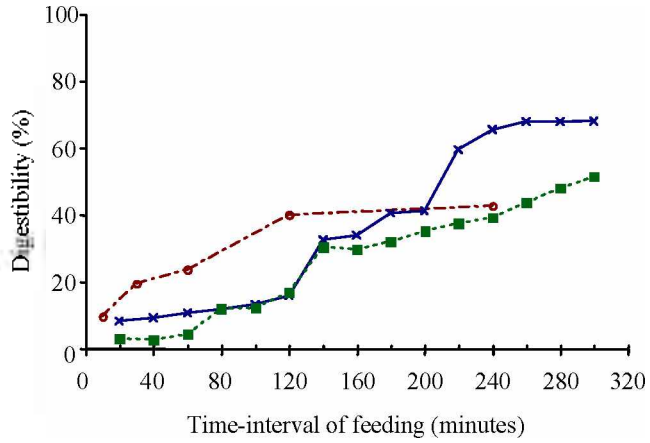

Figure 4. The gastric protein digestibility calculated from *in vivo* data —■—, static *in vitro* method - -○- -, and dynamic system —×—.

(p<0.0001) and incubation time (p<0.0001) on protein digestibility were considerable (Table 1). The 1,000 units/ml concentration of pepsin demonstrated the greatest protein digestibility and was used as the standard pepsin activity in the static *in vitro* model. A significant increase in protein digestibility occurred over the incubation period until 120 min.

Comparison of protein digestibility between methods

Figure 4 depicts protein digestibility measured by the *in vivo* study, the dynamic simulation model, and the static *in vitro* model. The correlation coefficients between the dynamic *in vitro* model and *in vivo* crude protein digestibility estimates (r = 0.97) were higher than that for the traditional static *in vitro* model (r = 0.89).

DISCUSSION

Gastric digestion is a key process in whole gastrointestinal tract digestion and absorption because it is alters the chemical and mechanical properties of the diet which affects the digestion and absorption in all other GI compartments. There have been few studies describing the physical and chemical changes in dietary matter exiting the pig's stomach *in vivo* (Low et al., 1985; Johansen et al., 1996). Moreover, both gastric digesta pH values and rate of digesta flow, the physiological dynamic conditions that have the greatest effects on gastric digestion, are rarely included within *in vitro* digestive parameters (Savalle et al., 1989).

In the present study, we determined these parameters in a controlled environment of diet and water intake to establish a dynamic model for simulating the pigs' gastric digestion and compared protein digestibility to that observed *in vivo* and to those protein digestibility estimates derived from a commonly used static *in vitro* model.

The pH values of gastric digesta were 2.47 before feeding (time 0), and increased to 4.97 at the first sampling point (20 min postprandial) during the initial *in vivo* study. The pH values significantly decreased 140 min after feeding (Figure 2). The gastric pH profile of all data was then entered into the dynamic *in vitro* model. In this dynamic system, the pH value increases from 2.42 to 5.12 during the ingestion of food and subsequently decreases due to acid secretion. These observations in the pigs receiving a standard experimental feed, as in the present study, do not agree with the human data from Marteau et al. (1990). Minekus et al. (1995) used the data of Marteau et al. (1990) to construct an *in vitro* digestion model for non-ruminants.

In the present study, *in vivo* gastric juice secretion by the pig reduces the liquid marker concentration in the digesta. The rate of change of gastric digesta water content, as determined by the dilution of the liquid digesta marker, can be described as an exponential equation. Excluding the effects of increasing feed consumption, the volume of gastric juice secretion as indirectly estimated by the increase in gastric digesta liquid over time can be described as $V_{t,\ in\ vivo} = 64.509e^{0.0109t}$ (p = 0.0004). This equation can be used to calculate the secretion rate of gastric juice which equals 0.7% per hour. This value agrees with the gastric secretion rate estimated by the *in vitro* model described by Minekus et al. (1995) of 0.5 ml/min for a 60 g sample.

The *in vitro* solid digesta marker (Cr_2O_3) concentration, described as $Cr_{t,\ dynamic} = -0.19t+79.95$, decreased linearly over time (p = 0.0081; Figure 3). This observation is in harmony with the hypothesis that liquids and semi-liquids conform to an exponential gastric flow pattern, while solids have a more linear pattern of gastric flow (Notivol et al., 1984).

In this study, the static *in vitro* protein digestibility estimates at 120 to 240 min of incubation agreed with the *in vivo* data when the concentration of pespin (1,000 units/ml 0.1 M HCl) was identical to that observed *in vivo* in the stomach of pigs. It is interesting that with a shorter incubation time, the *in vitro* protein digestibility was much higher than that observed *in vivo*. This is likely because, during *in vitro* incubation, substrate and enzyme are thoroughly mixed during the initial stage of the incubation procedure.

In contrast to the static model, the secretion of pepsin and electrolytes in the dynamic *in vitro* model was regulated according to the data based on gastric conditions in the pigs during the *in vivo* study. With this model, the environmental pH decreased slowly. At 300 min after the start of the incubation, the pH was 2.5 whilst 77% of the solid marker had passed through the stomach. The activities of enzyme and the physical condition in the stomach strongly influence digestive capacity. The digestibility of feed crude protein in the dynamic *in vitro* model increases with time in a manner similar to that observed in the *in vivo* pig trial (Figure 4). These data illustrate that the dynamic *in vitro* model, simulating *in vivo* gastric digestion, gives similar total protein digestibility estimates to the static *in vitro* model in which gastric environment has a fixed pH during a fixed period of time during incubation (Babinszky et al., 1990; Boisen and Fernández, 1995). Despite this fact, the dynamic *in vitro* model of digestion in the stomach of a pig could prove to be a more useful tool for understanding the kinetics of *in vivo* gastric digestion over time as well as total digestibility. The dynamic model represents less of an *in vitro* artifact than the static model. Unlike protein digestion *in vivo*, protein digestibility estimates generated by the static *in vitro* model do not change after 120 min of incubation, even though the *in vitro* model in this study used an enzyme (1,000 units/ml 0.1 M HCl):substrate ratio that was greater than that observed in the *in vivo* study. Previous studies of *in vitro* protein digestibility with static models have also used very high pepsin concentrations in the incubations. The enzyme:substrate ratio in earlier studies ranged from 5,000 (Boisen and Fernández, 1995) to 10,000 units/g diet sample (Babinszky et al., 1990; Cone and van der Poel, 1993). Although the previous cited sources used a different porcine pepsin preparation (art. 7190, Merck, Darmstadt, Germany), the activity of the pepsin product (501 Anson units/mg) was similar to the preparation (P-7000, Sigma Chemical Co., St. Louis, MO) used in the present study (525 Anson units/mg).

It is possible that enzymatic protein hydrolysis in the static *in vitro* models may be inhibited by the accumulation of digestion products or the self-hydrolysis of pepsin (Akeson and Stahmann, 1964; Robins, 1978). Increasing the enzyme concentration in the incubation solution of the static models may resolve the decreased protein hydrolysis at the later incubation periods (Table 1).

We conclude that problems inherent to the use of static *in vitro* models of gastric protein digestion can be avoided with the use of a dynamic *in vitro* model similar to that described in the present study. Therefore, a dynamic *in vitro* gastric digestion model may more effectively mimic *in vivo* physiological conditions.

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