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Determination of Digestive Enzyme Activity in the Digesta from the Small Intestinal of Growing Pigs and Development of *in vitro* Evaluation System for Feed Bioavailability Using Artificial Small Intestinal Juice

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Abstact: The objective of this study was to determine enzyme activity in the digesta from the small intestine of growing pigs and develope an *in vitro* evaluation system for feed bioavailability using artificial small intestinal juice. Ten pigs (22.77±0.89 kg and fitted with a simple T-cannula at the jejunum) were used in a doubly 5×5 Latin square design. In each period, pigs were offered one of five diets differing in nutrient level for 14 days. The Standard diet (ST) contained 3400 kcal kg^{-1} of Digestible Energy value (DE) and 17% Crude Protein (CP). The DE for the other four diets was 3600, 3200, 3600 and 3200 kcal kg^{-1}, respectively while the CP level was 21, 13, 13 and 21%, respectively. The small intestinal digesta was collected for determining digestive enzyme activity. Meanwhile, feces were collected for determining *in vivo* feed digestibility. The results showed that the range of amylase, trypsin, chymotrypsin and lipase activities in the intestinal fluid of growing pigs was 15.52-251.43, 21.24-67.39, 3.45-19.17 and 0.02-3.59 U mL^{-1}, respectively. To establish an *in vitro* evaluation system for feed bioavailability, Artificial Small Intestinal Juice (ASIJ) was prepared with mixed enzyme reagents based on the mean activities of amylase, trypsin, chymotrypsin and lipase in the digesta used to evaluate the five diets by three-stage enzymatic incubation. By comparing the DE of the five feedstuffs from the *in vivo* method and the digestibility of the feedstuffs from the *in vitro* ASIJ analysis, mathematical models for predicting *in vivo* DE of *In Vitro* Dry Matter (IVDM), Organic Matter (IVOM) and DE (IVDE) was established (DE = 0.1076×IVDM+0.3741, R^2 = 0.34; DE = 0.1276×IVOM+1.6486, R^2 = 0.31; DE = 0.4625×IVDE+7.2065, R^2 = 0.71). There were no significant differences between the *in vitro* evaluation results and the developed *in vitro* method. Therefore, the system in this study based on ASIJ is a convinent and reasonably accurate method for *in vitro* evaluation of feed bioavailability.

Key words: Digestive enzyme activity, growing pig, *in vitro*, feed evaluation, intestinal fluid, China

INTRODUCTION

In vivo determination of the digestibility of feed is a costly and time-consuming process (Lowgren *et al*., 1989; Gasim-Boubaker *et al*., 2007; Iyeghe-Erakpotobor, 2007). A quick, economic and reliable *in vitro* method for determination nutrient digestibility is preferred. Given that animal digestion is a kinetic processes in which many factors (the temperature, pH, secretion of digestive enzymes, transit and absorption) are involved, *in vitro* simulation of the animal's digestive system remains challenging. Several *in vitro* procedures have been developed to predict *in vivo* feed digestibility of swine using pancreatinum to simulate the digestion of small intestine (Boisen and Fernandez, 1997; Huang *et al*., 2003; Noblet and Jaguelin-Peyraud, 2007; Regmi *et al*., 2008; Chumpawadee and Pimpa, 2009). However due to the affect of feed particle size, sample weight and the contribution of different enzymes during *in vitro* digestion, the repeatability and accuracy of *in vitro* evaluation methods are still not good enough for practical application. Enzyme composition and activity in animal digestive juice vary with feed types and developmental stage while the enzyme activity *in vitro* is fixed (Furuya *et al*., 1979; Boisen and Fernandez, 1997). Therefore, it is very important to choose the suitable enzyme type and activity to simulate *in vivo* digestion and evaluate the feed bioavailability by *in vitro* analysis. The objective of this study was to determine the enzyme activity in the small intestine of growing pigs with different feedstuffs and develop a simpler, more convinent and accurate method for *in vitro* evaluation of feed bioavailability based on artificially prepared small intestinal juice.

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MATERIALS AND METHODS

Diets, animals and experimental design: Five diets were prepared and used in this study (Table 1). The Standard diet (ST) consisted of corn, soybean and wheat bran and was formulated according to the requirements of growing pigs recommended by NRC (1998). The ST diet contained 3400 kcal kg^{-1} of Digestible Energy value (DE) and 17% Crude Protein (CP). The other four diets were High Energy and High Protein diet (HEHP), Low Energy and Low Protein diet (LELP), High Energy and Low Protein diet (HELP) as well as Low Energy and High Protein diet (LEHP), respectively. Energy content was adjusted by replacing some of the corn with soybean oil or wheat bran and the protein content was adjusted by replacing some of the soybean meal with zein or wheat bran. The DE for the HEHP, LELP, HELP and LEHP diets was 3600, 3200, 3600 and 3200 kcal kg^{-1}, respectively while the CP level was 21, 13, 13 and 21%, respectively.

Ten pigs (Pietrain x large white) weighing 22.77 kg were surgically fitted with simple T-cannula at the jejunum according to the procedures described by Chen *et al.* (1995). The silicone cannula was 1.1 cm in internal diameter. Before surgery, pigs were sedated with an intramuscular injection of ketamine and then subjected to halothane anesthesia. After surgery, penicilin was injected to eliminate inflammation. Pigs were individually housed in individual pens (1.4×0.45×0.6 m^3) in a temperature-controlled room (23°C±1). During the 10 days recovery period after surgery and before treatment, pigs were fed a commercial diet. A doubly 5×5 Latin square design was used in this study. In each period, pigs were offered one of the five diets for 14 days. The intestinal fluid was collected at 9:30-10:30, 13:30-14:30, 17:30-18:30 on days 10, 12 and 14 to determine digestive enzyme activity. Meanwhile, the feces were collected on days 7, 8 and 9 for determining *in vivo* feed digestibility. Digesta collection was conducted according to the procedures of Zhao *et al.* (2007). One and half hour after the first meal in the day, samples of jejunum digesta were collected 3 times (at 930, 1330 and 1730) in sterilized plastic bottle. Then, 5 mL jejunum digesta was transferred to a 10 mL centrifuge tube and centrifuged for 10 min at 1250× g at 4°C according to procedure of Furuya *et al.* (1979). Supernatant were divided into 2 mL aliquots and kept frozen (-20°C) until enzyme activity analysis. Fecal samples were weighed and frozen (-20°) immediately after collection. Before analysis, samples were freeze-dried and finely ground. Samples from each collection day were pooled. The procedures for animal treatment were approved by the Animal Care and Use Committee of China Agricultural University.

Table 1: Formulation and chemical composition of the experimental diets

	Diet[1]				
Items	HEHP	LELP	HELP	LEHP	ST
Component (%) (as fed basis)					
Corn	58.35	64.30	69.10	41.20	68.50
Wheat bran	0.00	19.85	6.80	22.75	3.10
Soybean meal	30.20	12.50	14.85	27.60	25.00
Zein	5.00	0.00	0.00	5.20	0.00
Soybean oil	3.20	0.00	6.00	0.00	0.00
Dicalcium phosphate	0.80	0.90	0.80	0.80	0.85
Calcium carbonate	1.00	1.00	1.00	1.00	1.10
Salt	0.45	0.45	0.45	0.45	0.45
Vitamin and mineral mixture[2]	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Nutrient content					
DE (kcal $kg)^{-1}$	3600.00	3200.00	3600.00	3200.00	3400.00
CP%	21.00	13.00	13.00	21.00	17.00

[1]HEHP, LELP, HELP and LEHP = Diets containing a Low or High Energy level and a low or high Protein level, ST = Standard diet; [2]The vitamin and mineral mixture provided the following (per kilogram of diet): 5,000 IU of Vitamin A, 1,000 IU of Vitamin D3 and 20 IU of Vitamin E; 2.0 mg of thiamin; 4.0 mg of riboflavin; 1.0 mg of pyridoxine; 20 μg of cobalamin; 15 mg of niacin; 9.9 mg of D-pantothenate; 200 μg of biotin; 1 mg of folic acid; 2.0 mg of menadione; 500 mg of choline chloride; 100.2 mg of Zn (ZnO); 10.0 mg of Cu ($CuSO_4 \cdot 5H_2O$); 37.0 mg of Mn (Mn_4O_8); 80.0 mg of Fe ($FeSO_4 \cdot 7H_2O$); 202 μg of I (Ca (IO_3) 2; 100 μg of Co ($CoCO_3$) and 150 μg of Se ($Na_2 \cdot SeO_3$) (Wilfart *et al.*, 2007)

Analysis of digestive enzyme activity from small intestinal juice: The 2 mL samples of jejunum digesta supernatant was thawed in 4°C water and used for determination of digestive enzyme activities. Trypsin was determined using Na-ρ-Toluolsulfonyl-l-arginine methlester hydrochloride (T426, Sigma Chemical Company, St. Louis, MO) as a substrate; Chymotrypsin was determined using N-benzoy1-l-tyrosine ethyl eser (B6125, Sigma Chemical Company, St. Louis, MO) as a substrate according to the method described by Wirnt (1974a, b). One unit of trypsin was defined as the activity of hydrolyzing 1 μ mol of substrate per min at 25°C, at pH 8.1. One unit of chymotrypsin was defined as the activity hydrolyzing 1 μmol of substrate per min at 25°C, at pH 7.8.

The α-amylase was determined by using soluble starch as a substrate according to procedures described by Dahlqvist (1962). One unit of amylase was defined as the activity liberating starch correspongding to 1 μmol of maltose per min at 25°C, at pH 6.9. Lipase was measured with Randox kit (L1 188, Randox. Laboratories, Antrim, UK). One unit of lipase was defined as the activity of hydrolyzing 0.1 μmol of triokein to diolein per min at 37°C, at pH 6.9. All digestive enzymes activities were expressed as units per mililiter (U mL^{-1}) of jejunal fluid (Zhao *et al.*, 2007).

Feed bioavailability analysis by the *in vivo* method: The five feeds and fecal samples were analyzed for Dry Matter (DM), Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE) and ash according to standard laboratory procedures (AOAC, 1990). The Gross Energy (GE) of feed, feces and undigested residues was calculated by an adiabatic bomb calorimeter (PARR 1281, Moline, IL, USA).

Table 2: Preparation of the artificial small intestinal juice

Commercial enzyme type	Commercial enzyme activity: Trypsin (U mg^{-1})	Chymotrypsin (U mg^{-1})	Lipase (U mg^{-1})	Amylase (U mL^{-1})	Additive in 50 mL flask	Enzyme activity in 54 mL[1] buffer of step 2
Trypsin (AMRESCO, 0458)	157.40030	0.2978	-	-	0.3751g	43.920[3]
Chymotrypsin (AMRESCO, 0164)	2.58142	93.7304	-	-	0.0980 g	6.890[3]
Lipase (SIGMA, L3126)	-	-	4.3680	-	0.1196 g	0.387[3]
Amylase (SIGMA, A3306)	-	-	-	22765. 3119	6.4150 mL	108.190[3]

[1]54 mL buffer = 25 mL phosphate buffer (0.1M, pH 6.0)+10 mL 0.2M HCl+1 mL pepsin solution+0.5 mL Chloramphemicol solution+10 mL of a phosphate buffer (0.2M, pH 6.8)+5mL NaOH (0.6M)+0.5 L 1M HCl or a 1M NaOH+2 mL mixed enzyme[2], 2 mL mixed enzyme activity = (Commercial enzyme activity×Additive in 50 mL flask)/((Enzyme activity in 54 mL Buffer of step 2)×50×54 mL); [3]Enzyme activity in 54 mL Buffer = Mean activity of small intestinal juice *in vivo* experiment

Feed bioavailability analysis by the *In Vitro* Pancreatin Method (IVP): Digestibility and bioavailability of the feed samples mentioned before *in vivo* experiment were then analyzed and estimated by the *in vitro* method described by Boisen and Fernandez (1997) which simulates gastric, small intestine and large intestine digestion. The samples were finely ground through a 1 mm mesh size screen and then 0.5±0.01 g of each diet was introduced into a 100 mL conical flask. About 25 mL of phosphate buffer (0.1 M, pH 6.0), 10 mL 0.2M HCl and 1 mL of a freshly prepared pepsin solution containing 25 mg pepsin (porcine, 2000 FIP-U g^{-1}, Sigma NO.7190) were added to the flask one by one. After the pH of the mixture was adjusted to 2.0 with a 1 N HCl or a 1 N NaOH solution, 0.5 mL of a chloramphernicol solution (0.5 g of chlorampphericol, Sigma No.C-0378, per 100 mL ethanol) was added to prevent bacterial growth. At last, the conical flasks were closed with a rubber stopper and kept at 39°C for 2 h in a horizontal shaking water bath.

About 2 h later, the pH of the mixture was adjusted to 6.8 by addition of a 1 N HCl or a 1 N NaOH solution after 10 mL of a phosphate buffer (0.2M. pH 6.8) and 5 mL of NaOH solution (0.6 M) were added to the conical flask. Thereafter, 0.1 mL of a freshly prepared pancreatin solution containing 100 mg pancreatin (porcine, Grade IV, Sigma No.P-1750) was added and the flasks were kept at 39°C for 4 h in a horizontal shaking water bath. After 10 mL of a 0.2M EDTA solution was added, pH of the mixture was then adjusted to 4.8 by addition of 30% acetic acid. At last, 0.5 mL mixed multi-enzyme (Viscozyme, 120FBG g^{-1}, Sigma No, V2010) was added and the flasks were incubated at 39°C for 18 h in a horizontal shaking water bath. After 18 h, 10 mL ethanol (96%) and 10 mL acetone (99.5%) were added to each of flasks while shaking to facilitate precipitation of OM in flasks. The undigested residues were collected in a filtration unit by using dried and preweighed filter paper (Whatman No. 541, Whatman Inc, Florham Park, MJ). The flasks were washed with deionized water until the flask was clean. The residues and the filter paper were dried for 4 h at 60°C and then continued to be dried at 103°C for 4 h until a constant weight was achieved and ashed at 500°C for 4 h.

Feed bioavailability analysis by the *In Vitro* Artificial Small Intestinal Juice Method (IVA): Bioavailability of the five diets was also analyzed by an adapted *in vitro* artificial small intestinal juice method based on the analyzed data of the digestive enzyme activity from the intestinal digesta. For Step 1 and 3, the new method was similar to the three-step multi-enzyme method developed by Boisen and Fernandex (1997) to mimic the digestion procedure in the stomach and large intestine.

However, Step 2 was revised by replacing pancreatin with a 2 mL mixture of trypsin, chymotrypsin, lipase and amylase to simulate the small intestinal digestion of the pigs. Four commercial enzyme were mixed in a 50 mL measuring flask based on the mean activity of amylase, trypsin, chymotrypsin and lipase found in the *in vivo* experiment. About 2 mL of artificial small intestinal juice were added to the buffer of Step 2 of the *in vitro* pancreatin method and the activity of each digestive enzyme in 54 mL buffer was equaled to the activity of the digestive enzyme in the small intestinal juice of swine (Table 2).

Calculation and statistical analyses: The *in vitro* DM or OM digestibility and DE were calculated using the following equation:

$$\textit{In vitro}\ \text{DM or OM digestibility} = \frac{\text{Sample DM or OM} - \text{Residue DM or OM}}{\text{Sample DM or OM}}$$

$$\textit{In vitro}\ \text{DE} = \text{Sample GE} - \text{Residue GE}$$

$$\textit{In vivo}\ \text{DE} = \text{Feed GE} - \text{Feces GE}$$

Data were analyzed according to the GLM and the REG procedure (SAS Inst. Inc., Cary, NC), the R^2 between the *in vitro* digestibility and the *in vivo* digestibility was determined. At the same time, regression equations were developed to predict the *in vivo* digestibility and bioavailability based on the *in vitro* digestibility.

RESULTS AND DISCUSSION

Digestive enzyme activity in small intestinal juice of growing pigs: The mean activity of amylase, trypsin, chymotrypsin and lipase within the experimental period were 108.19±9.01, 43.92±1.93, 6.89±0.48 and 0.416±0.12 U mL^{-1}, respectively. Although, the activity of amylase, trypsin, chymotrypsin and lipase increased with time throughout the experimental period, no statistically significant differences were observed (Table 3). A frequency distribution diagram based on the four digestive enzyme activities in the experiment indicated that the four enzymes' activities were present as a Gaussian distribution and the mean enzyme activity of the four enzymes appeared most frequency in the distribution (Fig. 1). The effect of diet energy and protein level on digestive enzymes activity in the small intestinal juice is show in Table 4 and no statistically significant differences were observed among the different dietary energy and protein levels ($p>0.05$).

Comparison of the digestibility values from *in vivo*, IVA and IVP analysis as well as development of a mathematical model: The results of DM, OM digestibility and DE obtained from the *in vivo* and IVA are shown in Table 5. A R^2 for positive relativity between *in vivo* and *in vitro* data was observed when data from LEHP group were excluded. The relationship between *in vitro* DE and IVA Digestibility of DM (IVDM), IVA digestibility of OM (IVOM), IVA Digestible Energy (IVDE) were established, the models were DE = 0.1076×IVDM+0.3741, R^2 = 0.34, DE = 0.1276×IVOM+1.6486, R^2 = 0.31, DE = 0.4625×IVDE+7.2065, R^2 = 0.71 (Table 5). The results of DM, OM digestibility and DE obtained from IVA and IVP are also shown in Table 5. Relationship between IVA DM, OM digestibility and IVP DM, OM digestibility of 5 feed samples in growing pigs are shown in Table 5. The R^2 between IVA DM, OM digestibility and IVP DM, OM digestibility was very high (R^2 = 0.97 and 0.92, Fig. 2 and 3).

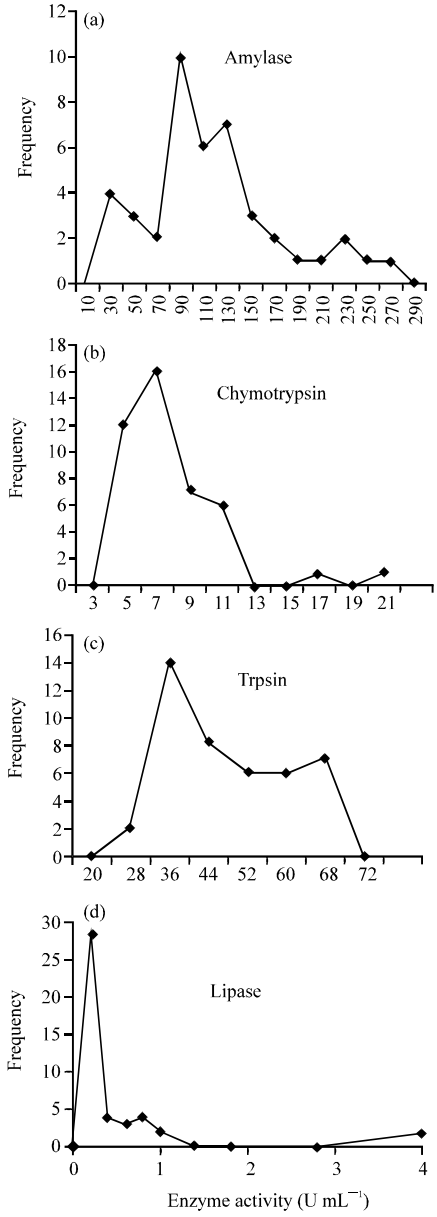

Fig. 1: Frequency distribution diagram of a) amylase; b) chymotrypsin; c) trypsin and d) lipase

Table 3: Change pattern of four digestive enzymes activities in each experimental period (Mean±SE)

	Amylase (U mL^{-1})		Trypsin (U mL^{-1})		Chymotrypsin (U mL^{-1})		Lipase (U mL^{-1})	
Items (days)	Activity[2]	RSD[1]	Activity[2]	RSD[1]	Activity[2]	RSD[1]	Activity[2]	RSD[1]
1-14	72.75±14.58	60.12	36.29±3.15	26.06	6.39±1.61	76.42	0.30±0.09	92.39
15-28	88.43±13.04	44.24	43.73±4.81	33.01	6.08±0.72	35.89	0.26±0.08	103.76
29-42	124.64±20.18	45.81	47.20±4.18	25.06	6.91±0.69	28.26	0.05±0.01	84.90
43-56	113.45±22.82	60.35	43.00±5.02	35.05	7.16±1.21	50.97	0.91±0.45	149.49
57-70	147.90±22.71	43.44	50.49±3.14	17.61	8.06±0.81	28.58	0.23±0.11	141.60
Source of variation (p)								
Time	0.05		>0.05		>0.05		>0.05	
Pig	0.01		0.01		0.12		0.52	

[1]The Residual Standard Deviation (RSD) is the root mean square of the residual error and applies to the whole model, not an individual estimate within the model; [2]The differences are not significant

Since, the *in vivo* determination of animal digestibility of feed is a costly and time-consuming process, a variety of *in vitro* prediction models to assess the nutritional value of feed has been established (Boisen and Fernandez, 1997; Huang *et al*., 2003; Noblet and Jaguelin-Peyraud, 2007; Regmi *et al*., 2008). Until now the most advanced method is the *In Vitro* Pancreatin (IVP) method described by Boisen and Fernandez (1997) which contains a three-step multi-enzyme system to simulate the stomach, small intestine and large intestine digestion. Pancreatin was used to stimulate the small intestine in this system. Although, the progress has been made, the accuracy, repeatability and relevance between *in vivo* and *in vitro* analysis is still a problem due to differences between the composition of the *in vitro* evaluation system and the actual biological system in the animal's gastrointestinal tract. To improve the *in vitro* evaluation system and to better compare the result of different *in vitro* experiments, *in vitro* procedures must be standardized, it is necessary to disclose the digestion kinetic process of animal.

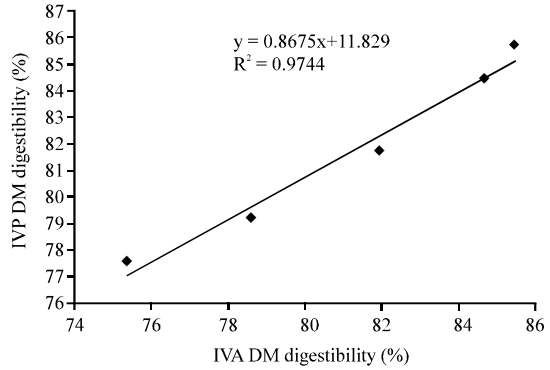

Fig. 2: Relationship between DM digestibility of the *in vitro* Artificial Small Intestinal Juice Method (IVA) and DM digestibility of the *in Vitro* Pancreatin Method (IVP) of 5 feeds in growing pigs

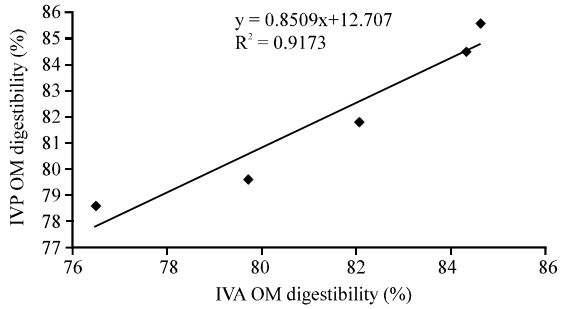

Fig. 3: Relationship between OM digestibility of the *in Vitro* Artificial Small Intestinal Juice Method (IVA) and OM digestibility of the *in Vitro* Pancreatin Method (IVP) of 5 samples in growing pigs

The small intestine containing a variety of digestive enzymes such as amylase, trypsin, chymotrypsin and lipase plays a very important role in the digestion of nutrients (REF). In this study, digestive enzyme activity from intestinal juice were systematically analyzed and an artificial small intestinal juice based on the analyzed data was developed to replace the pancreatin of the *in vitro* pancreatin method for better stimulation of the digestive process in the small intestine. In this study, researchers made a distribution graph of the digestive enzyme activity from the *in vivo* experiment. From Fig. 1, researchers could

Table 4: Effect of diet digestible energy/protein level on digestive enzymes activity in intestinal fluid (Mean±SE)

Items[1]		Digestive enzymes activity in small intestinal juice			
DE	CP	Amylase[2]	Trypsin[2]	Chymotrypsin[2]	Lipase[2]
LE	LP	104.17±21.13	42.99±5.67	6.42±1.30	0.53±0.34
HE	HP	101.81±21.60	43.52±4.20	7.86±1.63	0.37±0.13
ST	ST	118.25±17.09	45.10±3.63	6.47±0.68	0.17±0.09
LE	HP	97.04±22.37	44.83±4.94	6.10±0.75	0.54±0.38
HE	LP	113.7±21.70	43.15±3.87	7.18±0.81	0.22±0.06
Source of variation (p-value)					
DE		0.99	0.86	0.30	0.58
CP		0.78	0.67	0.63	0.78
DE x CP			0.58	0.77	0.88 0.94

[1]LELP = Low Energy and Low Protein diet, HEHP = High Energy and High Protein diet, ST = Standard Diet, LEHP = Low Energy and High Protein diet, HELP = High Energy and Low Protein diet; [2]The differences are not significant

Table 5: *In vivo* and *in vitro* DE, DM, OM digestibility of 5 diets and their R^2 with *in vivo* DE excluded LEHP diet (Mean±SE)

Items	LELP[1]	HEHP[1]	ST[1]	LEHP[1]	HELP[1]	R^2
DM digestibility (%)						
In vivo	85.53±1.51	82.61±2.10	85.62±5.33	86.25±1.09	84.24±5.41	0.57
IVA[2]	78.19±0.50	84.47±0.34	83.74±0.30	75.23±0.52	81.27±0.11	0.34
IVP[3]	79.23±0.94	85.69±0.87	84.43±0.72	77.60±0.28	81.70±0.36	0.29
OM digestibility (%)						
In vivo	87.82±1.97	85.29±2.40	87.66±3.58	88.42±0.97	86.39±3.12	0.69
IVA[2]	79.71±0.52	84.61±0.35	84.22±0.34	76.47±0.45	82.04±0.10	0.31
IVP3	79.51±0.55	85.59±0.90	84.45±0.76	78.61±0.36	81.81±0.38	0.28
DE (MJ kg^{-1})						
In vivo	11.65±0.01	12.51±0.05	12.04±0.03	12.08±0.04	12.64±0.05	1.00
IVA[2]	9.74±0.01	11.75±0.01	10.81±0.01	9.36±0.01	10.97±0.01	0.71

[1]LELP = Low Energy and Low Protein diet, HEHP = High Energy and High Protein diet, ST = Standard Diet, LEHP = Low Energy and High Protein diet, HELP = High Energy and Low Protein diet; [2]IVA= The *In Vitro* Artificial Small Intestinal Juice Method; [3]IVP = The *in Vitro* Pancreatin Method

find that the distribution of enzyme activity was a normal distribution and most enzyme activity was concentrated nearby the mean activity. The mean activity of the four digestive enzymes could represent digestive enzyme levels *in vivo* under real production conditions. Under the same conditions of the experimental animals and trial factors, digestive enzyme activities of chyme were affected by three factors. Kidder and Manners (1978) reported that except lactase, the other digestive enzymes activity in the proximal and distal of small intestine are relatively low while there are different feed nutrient digestibility in different small intestine parts such as digestibility of organic matter were 29.8% in the front of the small intestine while it reached 85.9% at the end of the small intestine.

Chen *et al.* (1995) showed that the digestive enzyme activity 170±15 cm away from the pylorus was higher than the activities 60-70 cm away from this location at the same time. Porcine small Intestinal Fluid (PIF) were taken in this position for the *in vitro* experiment, the result was closer to *in vivo*. In the trial, chime were collected in the pig jejunum 170±15 cm from the pylorus so that the digesta samples from the same location of the small intestine minimize the interference from collection site. The second is the time of collection. In order to obtain a representative small intestinal juice, the pigs were fed regularly and were fed after the samples were collected after 1.5 h of ending fed, the day 9:30-10:30, 13:30-14 :30, 17:30-18:30 for sample collection, digesta were collected for the next day to ensure the health of pigs. Thirdly, the nutrient levels of dietary, protein, fat and crude fiber are likely to affect the activities of digestive enzymes.

Five different energy and protein ratio diets were fed to pigs to determine digestive enzyme activities of small intestinal juice. Corring (1982) and Brannon (1990) reported levels of high-energy high-protein diet significantly increased the activity of amylase, trypsin, chymotrypsin and lipase in rats and pigs. Partridge *et al.* (1982) reported that activity of trypsin, chymotrypsin and lipase was not significantly affected by composition of different diet but amylase activity was significantly affected. The results were similar with Partridge *et al.* (1982) report as different energy levels on amylase, trypsin, chymotrypsin and lipase of porcine small intestinal juice were not significantly different probably due to fact that the starch content were not same in the five diets as by soybean oil was added to increase energy levels and not increase dietary starch content. Except the LPHE diet, the other diets were a relatively low crude fat content and this may also result in amylase and lipase being little difference. These results are simlar with Luo and Dove (1996) who reported that there were no difference of lipase, phosphatase, trypsin, chymotrypsin and amylase by adding 5% animal fat in weaning diets. Makkink reported that different protein levels in weaned piglets significantly increased trypsin and chymotrypsin activity. This experiment with different protein levels on enzyme activity of trypsin and chymotrypsin had no significant impact, on the one hand due to the impact of individual differences in swine, on the other hand due to growing pigs to digest system has been developed, the secretion of the enzyme were adequate to digest the nutrients in the diets.

In this trial, organic matter digestibility, dry matter digestibility and DE *in vivo* were generally higher than *in vitro*, especially for the high level fiber feeds, compareing *in vivo* and IVA OM digestibility (Table 5), IVA OM digestibility was lower than *in vivo* OM digestibility in a good agreement with the reports of others (Van der Meer and Perez, 1992; Noblet and Jaguelin-Peyraud, 2007). *In vivo* OM digestibility of LELP and LEHP is higher than other groups, CF level is 4.86 and 6.4% in LELP and LEHP, respectively. The results are supported by the findings of Axelsson and Eriksson (1953) who reported crude fiber content of dry matter of 6.57% was optimum as to gain in weight and a content of 7.26% as to feed efficiency. Table 5 also shows that *in vivo* digestibility is higher than *in vitro* digestibility, especially in LELP and LEHP diets. One reason was that crude fiber was difficult to digest *in vitro*, the other reason might be that the enzyme amount was limited in the *in vitro* method while *in vivo* it was not. The trial showed that IVA DM and OM digestibility is lower than the digestibility of IVP. The reason was that trypsin, chymotrypsin, lipase and amylase are main enzyme in pancreatinum but there were also some other enzymes besides four types of enzyme in pancreatinum, it causes the result of IVP OM digestibility higher than IVA.

After excluding the LEHP data, a significant R^2 between *in vivo* DE and IVA DM, OM digestibility was observed (R^2 = 0.36 and 0.32, respectively). The result was lower than early studies (Noblet and Jaguelin-Peyraud, 2007; Regmi *et al*., 2009). However, Chen reported that there was no strong correlation between *in vitro* dry matter digestibility and *in vivo* DE values of barley samples. In our trial, the relationship between *in vitro* dry matter digestibility or organic matter digestibility and *in vivo* DE was not strong, (R^2 = 0.36 and 0.32), the main reason may be the less calibration diet (only five) and early study used pancreatinum to simulate the small intestine digestion, pancreatinum are mixture including amylase, trypsin, chymotrypsin, lipase and other enzymes while ASIJ are only four enzymes. In this experiment, the regression equation between *in vitro* and *vivo* DE were

established where R^2 = 0.51. When the high-fiber diet group (LEHP) was removed, the regression equation was y = 0.4625x+7.2065, R^2 = 0.71. The results were similar with previous reports. Huang *et al.* (2003) found that there is a strong correlation between *in vitro* DE and *in vivo* DE of six barley (R^2 = 0.93). Regmi *et al.* (2008) reported similar results of barley (R^2 = 0.97, the regression equation y = 1.28x - 25.33). Noblet and Jaguelin-Peyraud (2007) also have similar results. There were some difference between *in vitro* DE and *in vivo* DE, *in vitro* DE need calibrate by regression equation to obtain *in vivo* DE, compared with *in vivo* value, there were better repeatability *in vitro* value, the difference between *in vivo* DE and *in vitro* DE mainly was that *in vivo* DE was the apparent DE, *in vitro* DE was true DE (Huang *et al.*, 2003); second, difference of feed particle size between *in vitro* and *in vivo* is another factor reported that increasing feed particle size will reduce the energy digestibility of pig feed. Therefore, *in vitro* DE value can not directly replace the *in vivo* DE value, *in vitro* DE value must be corrected to *in vivo* DE value. There was no significant difference between IVA digestibility and IVP digestibility ($p>0.05$), relationship between IVA DM, OM digestibility and IVP DM, OM digestibility was very high (R^2 = 0.97 and 0.92), to some extent, the ASIJ can replace Pancreatinum to simulate digestion of small intestine in growing pigs.

CONCLUSION

In this study because of kinds of feed, the approach of using 1 regression equation to predict DE content of different batches of feedstuff might not be suitable. Especially for young animals, *in vitro* method or prediction equation should be developed for individual feedstuffs or feedstuff categories based on the macronutrient profile, the most important thing was to find suitable enzyme activity to simulate digestion of pigs. Artificial small intestinal juice including trypsin, chymotrypsin, lipase and amylase could better simulate the small intestine of pigs. It provided a solution that *in vitro* enzyme activity was determined in different simulate stage for individual feedstuffs or feedstuff categories based on the macronutrient profile.

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