The Apparent Digestibility of Corn By-products for Growing-finishing Pigs *In vivo* and *In vitro*

Liang Guo[1], Xiangshu Piao, Defa Li* and Songyu Li

China Agricultural University, No. 2. Yuanmingyuan West Road, Beijing, 100094, P. R. China

ABSTRACT : Two trials *in vivo* and *in vitro* were conducted, *in vivo* to determine the apparent digestibility of gross energy, crude protein, dry matter, acid detergent fiber, neutral detergent fiber and apparent digestible energy in 10 corn by-products. *In vivo* the diets included one basal corn diet, four corn gluten meal diets, four corn distillers dried grains with solubles diets and two corn distillers dried grains diets using the different methods, 12 crossbred barrows weigh 40±1.6 kg were allocated into individual metabolic crate, according to a 6×6 Latin square design. *In vitro* using flask technique, filter bag technique and dialysis tubing technique, the digestibilities of gross energy, crude protein and dry matter in corn gluten meal and corn distillers dried grains with solubles were investigated. Pepsin, pancreatin, intestinal fluid, rumen fluid and cellulase were used in incubation. The results showed that correlation coefficient was 0.73 in corn distillers dried grains with solubles between the digestibility of crude protein and acid detergent fiber *in vivo* ($p<0.01$), and correlation coefficient was 0.68 in corn distillers dried grains with solubles between the digestibility of gross energy and neutral detergent fiber *in vivo* ($p<0.01$). Apparent digestible energy (DE) of corn by-products in pig total tract was predicted by the percentage of crude protein (CP) and the content of gross energy (GE) in feedstuff. The equation: DE=5,601.09+26.69×CP %-0.5904×GE, (R^2=0.72). *In vitro*, filter bag technique was more convenient; furthermore, the digestibility for the treatments (pepsin+pancreatin+rumen fluid and pepsin+pancreatin+cellulase) was better. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 379-385)***

Key Words : Corn By-products, *In vivo*, *In vitro*, Growing-finishing Pigs

INTRODUCTION

Corn by-products are valuable protein sources for animals as unconventional feedstuffs. Corn gluten meal (CGM), corn distillers dried grains (DDG) and corn distillers dried grains with solubles (DDGS) attributed to the corn by-products, are produced by different processing techniques in many countries. Corn gluten meal comes from corns by the extraction of oil and starch for food. DDGS and DDG come from corns by the extraction of oil and alcohol using different processing techniques.

Corn by-products have a good effect on animal growing performance (Brenes et al., 1985; Ham et al., 1994; Coyle et al., 1996; Koelkebeck et al., 1999). The consume for determination of animal digestibility *in vivo* was greater. Therefore, there is a need to found a quick and reliable method. Some methods have been attempted for relationship between *in vitro* and *in vivo* (Furuga et al., 1979; Graham et al., 1989; Cone and Vander poel, 1993). The prediction of digestible nutrients of diets *in vitro* has been proposed (Boisen and Fernandez, 1995 and 1997). However, an *in vitro* method with a general validity for all kinds of feedstuffs should be established to measure the digestibility of nutrient.

Two trials were conducted to investigate correlations of the nutrient digestibility *in vivo* and *in vitro* in this study. Growing-finishing pigs were employed to estimate the apparent digestibility of crude protein (CP), gross energy (GE), dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and digestible energy (DE) of corn by-products for trial 1. Trial 2 was designed to apply multi-enzyme systems for investigating the *in vitro* digestibility of CP, DM and GE of corn by-products. Data from both trials were collected and used to find the correlations among digestibilities of CP, GE, ADF, NDF, DM and DE in corn by-products.

MATERIALS AND METHODS

Trial 1

10 corn by-products were obtained from different provinces in China. They consisted of four samples of corn gluten meal (CGM), with CP 63, 52, 47 and 32%, respectively, from Beijing, Ji Lin and Inner Mongolian; four samples of corn distillers dried grains with solubles (DDGS), with CP 30, 27, 26 and 25%, respectively, from Si Cuan, Ji Lin, Ha Er Bin and Hei Long Jiang; and two samples of corn distillers dried grains (DDG), with CP 31 and P 30%, respectively, from He Bei and Tang San. Twelve crossbred (Yorkshire×Landrace×Beijing Black) barrows, weighed 40±1.6 kg growing-finishing pigs used in this trial were from Beijing Liucun Animal Farm.

This trial involved 10 experimental diets of Chinese corn by-products and the diets were formulated by the different techniques (Sauer et al., 2000). The 10 experimental diets were divided into two experimental

* Corresponding Author: Defa Li. Tel: +86-10-62893588, Fax: +86-10-62893688, E-mail: defali@public2.bta.net.cn

[1] Tianjin Agricultural College, Tianjin, 300384, P. R. China.

Received June 25, 2003; Accepted December 2, 2003

Table 1. Ingredient and chemical composition of experimental diets (%)

Ingredient	Corn diet	Corn gluten meal (CP) diets				DDGS (CP) diets				DDG (CP) diets			
		63	52	47	32	26	30	25	27	31	22	24	30
Corn	96.35	77.08	77.08	72.33	67.44	67.44	67.44	67.44	67.44	67.44	67.44	67.44	67.44
Test feedstuff		20.0	20.0	25.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Dicalcium phosphate	1.44	1.15	1.15	1.08	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Limestone	0.91	0.73	0.73	0.62	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64
Salt	0.30	0.24	0.24	0.22	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
1% premix*	1.00	0.80	0.80	0.75	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Chemical composition													
CP	8.5	19.0	17.9	18.4	16.1	13.2	14.9	13.1	13.8	15.7	12.9	13.1	14.6
GE (kcal/kg)	3,761	4,057	3,934	3,983	4,124	4,079	4,160	4,079	4,089	4,251	4,050	4,019	4,169
Ca	0.58	0.63	0.56	0.55	0.62	0.52	0.63	0.59	0.53	0.57	0.81	0.50	0.66
Total P	0.89	0.51	0.48	0.76	0.72	0.98	0.91	0.83	0.41	0.87	0.72	0.93	0.54
NDF	15.5	18.9	19.1	21.5	14.1	30.1	27.8	29.0	24.2	23.4	19.8	21.1	28.9
ADF	3.4	3.0	5.6	3.8	3.7	7.5	6.9	7.5	6.87	6.5	5.9	5.2	6.0

* 1% premix provide the following per kg of complete diet: vitamin A, 5,512 IU; vitamin D_3, 2,200 IU; vitamin E, 6.1 IU; vitamin B_{12}, 0.028 mg; riboflavin, 5.5 mg; D-pantothenic acid, 13.8 mg; niacin, 30.3 mg; choline chloride, 551 mg; Mn, 100 mg; Fe, 100 mg; Zn, 100 mg; Cu, 150 mg; I, 1.4 mg; Se, 0.3 mg; Co, 1.0 mg.

Table 2. Multi-enzyme systems used *in vitro* incubation

Enzyme systems procedure	Time, pH and temperature in enzyme incubation
(1) Pepsin+(2) pancreatin	(1) 6 h, 39°C, pH 2.0, (2) 18 h, 39°C, pH 7.0
(1) Pepsin+(2) intestinal fluid	(1) 6 h, 39°C, pH 2.0, (2) 18 h, 39°C, pH 7.0
(1) Pepsin+(2) pancreatin+(3) cellulase	(1) 1 h, 39°C, pH 2.0, (2) 6 h, 39°C, pH 7.0, (3) 17 h, 39°C, pH 6.8
(1) Pepsin+(2) Pancreatin+(3) rumen fluid	(1) 1 h, 39°C, pH 2.0 (2) 6 h, 39°C, pH 7.0 (3) 17 h, 39°C, pH 6.8

groups. The trials were simultaneously conducted under the same condition. Each experimental group had six pigs and were fed diets according to a 6×6 Latin Square. The first group was fed diets containing one corn basal diet, four CGM diets and one DDG diet; while the second group was fed diets containing one corn basal diet, four DDGS diets and one DDG diet. Each experimental diet of Chinese corn by-products had six replicates. Each pig was fed diet for a 7 day period, which consisted of 4 days adaptation followed by 3 days collection of feces. The diets (Table 1) were based on nutrient requirements of pigs between 50 and 80 kg (NRC, 1998). Throughout the experiment, the barrows were individually housed in 0.5×1.5 m cast-iron metabolism crates equipped with a 0.25 m^3 round bottom feeder located at the front of the crate. The crates were located in an environmentally controlled barn with the temperature set at 18°C. The barrows were fed at 08:00 and 17:00 h each day. Feed intake was maintained at a constant level (3-4% body weight) for all pigs during each period of fecal collecting. The amount fed was the amount consumed by the pig eating the least during the trial adaptation phase. Water was added to the diets at feeding to form a moist mash. The barrows typically consumed their rations within 40 minutes of feeding. Collection of feces was started after the morning feeding, and the feces were collected for 9 h during each day of collection. Each collection was placed in freezer and stored at -20°C. At the completion of the third day collection, the two frozen feces samples were thawed and mixed with the third collection. About 500 g of the mixed feces were refrozen and stored at -20°C. Prior to analysis, the feces were thawed, then freeze-dried and analyzed.

Trial 2

The trials *in vitro* were conducted by flask technology, filter bag technology and dialysis tubing technology. Some digestive enzymes were applied to, which were pepsin, pancreatin, intestinal fluid, cellulase and rumen fluid. Corn by-products included one corn gluten meal (CP 47%) and one DDGS (CP 30%) and the feedstuffs were ground by a 1 mm screen. Four treatment groups in every technology trial, which were pepsin+pancreatin, pepsin+intestinal fluid, pepsin+pancreatin+cellulase and pepsin+pancreatin+ rumen fluid. Each treatment group had six replications. The samples were incubated by enzymes to investigate the digestibility of DE, DM and CP. The temperature, time and pH were presented in Table 2.

Trial materials were as follows : Pepsin (P 2000, Sigma, from porcine stomach mucosa, 1:10,000, 800-2,500 unit/mg); Pancreatin (P 1,500, U.S.P., Sigma, from porcine pancreas); Rumen fluid (Holstein rumen cannula cattle, China); Cellulase (C 2000, T. Reesei, China) and Intestinal fluid (freeze-dried pig intestinal fluid, Husbandry research institute, Beijing China). Dialysis tubing (D-9402, Sigma, retain protein of mol. wt. 12,000, width 76 cm, diameter 49 mm) was a kind of cellulose membrane only used in laboratory. Filter bag (F57, Sigma) was made from special

materials, with its pore diameter 30 microns. Parafilm (Chicago, IL, 60631, American) was used to seal; Whatman paper (3 MM CHR, Cat NO. 3030 861, English) was used to filtrate end-products; Filter bag sealing machine (ATWH200-220V, 220VAC, 1.5A, 50/60HZ, 330W); Stirrer (China, HZ-9202S, constant temperament).

HCl - NaCl buffer (0.1 M, pH 2.0; 8.5 ml HCl+3.2 g NaCl, then, adding distilled water to 1,000 ml);

Phosphate buffer (0.1 M, pH 7.0; A solution: 5.38 g $NaH_2PO_4 \cdot 2\ H_2O$+250 ml distilled water; B solution: 8.66 g $Na_2HPO_4 \cdot H_2O$+500 ml distilled water; then, A solution+B solution, adding distilled water to 1,000 ml);

Acetic acid - sodium acetate buffer (pH 5.0; A solution: 0.2 M, 3.53 ml acetic acid add distilled water to 300 ml; B solution: 0.2 M, 19.05 g sodium acetate solution add distilled water to 700 ml. Then, A solution and B solution are mixed to add distilled water to 1,000 ml);

- NaOH solution (1 M, 4 g NaOH, adding distilled water to 100 ml);
- HCl solution (1 M, 8.33 ml HCl, adding distilled water to 100 ml);
- Pancreatin solution (1.6 g pancreatin+2.0 ml phosphate buffer);
- Cellulase solution (2.5 g cellulase+10 ml acetic acid buffer);
- Pig intestinal fluid solution (1 g freeze-dried pig intestinal fluid+20 ml phosphate buffer).

The trials *in vitro* were based on previous studies (Boisen and Eggum, 1991; Boisen and Fernandez, 1995 and 1997; Deboever et al., 1988; Lowgren et al., 1989).

Flask technique

The trial procedures are as follow :

Pepsin−pancreatin : Step 1. The sample of about 1 g was added to a conical flask (100 ml volume); then 20 ml HCl - NaCl (pH 2.0) buffer and 0.1 g pepsin were added to the flask, which were sealed with a parafilm under anaerobic condition, and placed in the oscillator. The temperature of shaking bath was controlled to 39°C, shaking frequency was 60 times in a minute, and incubating time was 6 h.

Step 2. To the mixture, 20 ml phosphate buffer (pH 7.0) and 2.5 ml pancreatin solution were added, then, the flask with mixture was put into the shaking bath at 39°C for 18 h after sealed with a parafilm under anaerobic condition.

Step 3. Filtered with whatman filter paper, the undigested residues were separated, and dried at 105°C. DM, GE and CP in the residues were analyzed.

Pepsin−intestinal fluid : The methods were similar to flask technique (pepsin+pancreatin treatment), except pancreatin solution was not added to but 20 ml intestinal fluid solution was added to the flask.

Pepsin−pancreatin−rumen fluid : The methods of step one and step two were similar to flask technique (pepsin+pancreatin), except that the incubated time of pepsin and pancreatin was one hour and six hours respectively. The procedure of step three was that 10 ml rumen fluid was added to the flask with sample solution (step one and step two), while pH in flask solution was 6.8. Then, shaking at 39°C for 17 h.

Step 4. Step four was similar to the step three in flask technique (pepsin+pancreatin).

Pepsin−pancreatin−cellulose : The methods were similar to flask technique (pepsin+pancreatin+rumen fluid), rumen fluid was not added to the flask but 10ml cellulase solution was added to.

Filter bag technique

Pepsin − pancreatin : Step 1. The methods were similar to flask technique (pepsin+pancreatin) but the sample was added to a filter bag. The entrance of filter bag was sealed by sealing machine, and was put into the flask. The filter bag with undigested remnants was dried under 105°C for 4 h and analyzed.

Pepsin−intestinal fluid, pepsin+pancreatin+rumen fluid and pepsin+pancreatin−cellulose : The method was respectively similar to flask technique (pepsin+intestinal fluid, pepsin+pancreatin+rumen fluid and pepsin+pancreatin+cellulase) but the sample was added to filter bag.

Dialysis tubing technique

The dialysis tubing should be prepared beforehand. Firstly, the dialysis tubing was cut 20 cm long bag. Glycerin from surface of dialysis tubing was washed out by running water for 3-4 h. Then, Dialysis tubing was soaked by 0.3% (w/v) sodium sulfide solution at 80°C for one minute to remove off sulfur compounds, and washed with hot water (60°C) for two minutes, followed by acidification with 0.2% (v/v) sulfuric acid, rinsed with hot water to remove the acid. One end of dialysis tubing would be sealed by cord before adding sample and buffer, another end of dialysis tubing would be sealed by cord after sample and buffer was added to. The trial treatments are as follow:

Pepsin−pancreatin, Pepsin+intestinal fluid, pepsin+pancreatin+rumen fluid and pepsin−pancreatin+cellulose : The trial steps were respectively similar to that of the flask technique (pepsin+pancreatin treatment, pepsin+intestinal fluid treatment, pepsin+pancreatin+rumen fluid treatment and pepsin+pancreatin+cellulase treatment), except that the sample, enzyme and buffer were added into the dialysis tubing, then, the entrances of dialysis tubing with sample, enzyme and buffer were sealed by cord, the dialysis tubing was put into a bottle (500 ml volume), which contained 40 ml phosphate buffer. The sample solution in dialysis tubing was required to soak in the bottle of phosphate buffer. Finally, the bottle with dialysis tubing was sealed with a

Table 3. The chemical composition of feedstuffs

Feedstuff	DM %	CP %	GE %	NDF %	ADF %	ASH %
CGM						
63	89	63.0	5,152	11.0	3.7	1.7
52	88	52.0	4,819	18.9	3.2	3.5
47	91	47.5	4,752	9.8	3.3	1.8
32	91	32.0	4,405	27.4	2.4	3.4
DDGS						
26	90	26.4	4,791	53.4	38.7	2.3
30	92	30.1	5,103	45.9	22.4	1.5
25	91	24.8	4,491	47.6	25.5	3.4
27	90	27.0	4,753	40.1	11.5	4.1
DDG						
31	91	31.1	5,330	50.6	38.5	2.2
22	91	22.4	4,586	41.3	19.9	5.8
24	92	24.5	4,589	36.2	20.0	3.1
30	92	29.9	5,198	56.2	31.3	1.5

* CGM, Corn gluten meal; DDG, Corn Distillers Dried Grains; DDGS, Corn Distillers Dried Grains with Solubles.

parafilm and shaken in the oscillator bath. The end-products in dialysis tubing were filtrated, dried and analyzed.

Chemical analysis

Samples of corn by-products, trial diets and feces were analyzed for their crude protein (CP), gross energy (GE), dry matter (DM), ash, acid detergent fiber (ADF) and neutral detergent fiber (NDF) content using the methods of the AOAC (1990). Nitrogen (N) was analyzed using the Kjeldahl method (AOAC method 988.05). NDF and ADF were analyzed by Fiber Analyzer (ANKOM220); GE was analyzed by Bomb Calorimeter (PARR 1281). The apparent digestibility of CP, GE, DM, ADF and NDF in corn by-products was determined by the equation (Sauer et al., 2000):

$$D_A=[D_D-(D_B\times S_B)]/S_A$$

Where D_A was apparent digestibility of a nutrient in the assay feed ingredient (%); D_B was apparent digestibility of a nutrient in the basal feed ingredient (%); S_B was the contribution level (%) of a nutrient in the basal ingredient to the assay diet; S_A was the contribution level (%) of a nutrient in the assay ingredient to the assay diet.

Statistical analysis

Both the *in vivo* and *in vitro* trials had six replicates per treatment. Analyses of variance from filter bag method, dialysis tubing method, flask method *in vitro* and the results *in vivo* were carried out by comparing means according to the One-way ANOVA of SPSS. Four corn gluten meals, 4 DDGS and 2 DDG were separately compared their correlate coefficient with correlate of Kendall's test. Prediction equation was established using the General Linear Model Procedure.

RESULTS

The pigs remained healthy throughout experiment. The chemical composition of DDGS, DDG and corn gluten meal is presented in Table 3. The digestibility of diets and feedstuffs is presented in Table 4. The correlation

Table 4. Digestibility of the feedstuffs and the diets

	DE (kcal/kg)		Digestibility							
			CP %		NDF %		ADF %		GE %	
	Feedstuffs	Diets	Feedstuffs	Diets	Feedstuffs	Diets	Feedstuffs	Diets	Feedstuffs	Diets
Corn gluten meal										
63	4,230	3,910	93.0	84.0	-	66.5^{ab}	-	87.0^{a}	79.7	84.4
52	4,030	3,820	92.0	87.0	-	63.7^{a}	-	94.5^{b}	81.8	85.9
47	4,020	3,830	94.3	89.0	-	73.2^{b}	-	93.0^{b}	83.9	86.2
32	4,250	3,920	96.2	89.0	-	64.9^{a}	-	93.0^{b}	89.9	88.2
SEM	0.16	0.04	0.17	0.03		2.38		0.01	3.39	0.96
P value	0.58	0.26	0.52	0.07		0.06		0.00	0.18	0.08
DDGS (CP)										
26	3,470	3,650	66.0^{a}	71.0	76.6^{a}	68.5^{c}	91.0^{a}	91.0^{a}	67.7	80.1
30	3,570	3,650	75.8^{b}	77.0	60.6^{b}	60.0^{b}	94.0^{b}	93.0^{b}	61.0	77.4
25	3,580	3,610	69.2^{ab}	74.0	63.01^{b}	61.3^{b}	95.0^{b}	94.0^{b}	60.2	77.8
27	3,430	3,650	75.2^{ab}	76.0	61.5^{b}	51.2^{a}	94.7^{b}	94.0^{b}	62.4	78.7
SEM	0.14	0.05	2.03	0.02	4.16	2.07	0.01	0.001	2.71	0.97
P value	0.84	0.89	0.07	0.13	0.04	0.001	0.001	0.002	0.23	0.25
DDG (CP)										
31	3,250	3,420	80.0	76.8	68.6	50.7^{a}	92.9^{a}	91.8	66.4	76.7
22	3,560	3,540	84.0	77.0	64.8	49.7^{a}	93.7^{a}	91.8	71.4	78.9
24	3,180	3,520	77.7	74.2	52.4	56.4^{a}	82.5^{b}	92.3	72.1	81.8
30	3,430	3,590	74.5	75.0	67.6	63.9^{b}	93.0^{a}	92.8	66.4	79.4
SEM	0.16	0.06	0.03	0.02	5.53	2.62	0.006	0.005	3.49	1.28
P value	0.35	0.24	0.13	0.79	0.17	0.002	0.000	0.50	0.52	0.07

Table 5. Digestibility of CP, GE and DM from feedstuffs *in vivo* and *in vitro*

	Digestibility		
	CP %	GE %	DM %
Corn gluten meal (CP 47%)			
In vivo	94.3	83.9^{abc}	85.9^{c}
Filter bag method *in vitro*			
Pepsin-pancreatin	89.2^{b}	84.9^{bc}	84.2^{c}
Pepsin-intestinal fluid	86.4^{b}	79.1^{ab}	63.9^{a}
Pepsin-pancreatin-rumen fluid	86.0^{b}	85.2^{bc}	64.2^{a}
Pepsin-pancreatin-cellulase	88.8^{b}	87.8^{c}	86.2^{c}
Dialysis tubing method *in vitro*			
Pepsin-pancreatin	79.9^{a}	84.0^{bc}	84.5^{c}
Pepsin-intestinal fluid	86.9^{b}	81.7^{abc}	65.3^{a}
Pepsin-pancreatin-rumen fluid	85.2^{b}	79.6^{ab}	64.7^{a}
Pepsin-pancreatin-cellulase	85.7^{b}	86.2^{c}	87.4^{c}
Flask method *in vitro*			
Pepsin-pancreatin	84.1^{a}	78.3^{a}	74.5^{b}
Pepsin-intestinal fluid	86.2^{b}	59.6	51.4
Pepsin-pancreatin-rumen fluid	85.5^{b}	62.1	64.8^{a}
Pepsin-pancreatin-cellulase	88.9^{b}	62.4	61.3^{a}
SEM	1.68	2.03	1.25
P value	0.000	0.000	0.000
DDGS (CP 30%)			
In vivo	75.8^{ab}	61.0^{f}	74.9^{d}
Filter bag method *in vitro*			
Pepsin-pancreatin	77.2^{a}	50.6^{bc}	42.3
Pepsin-intestinal fluid	68.5^{b}	37.7	41.9
Pepsin-pancreatin-rumen fluid	77.5^{ab}	51.5^{bcd}	48.1^{bc}
Pepsin-pancreatin-cellulase	75.1^{a}	52.9^{bcde}	44.7^{a}
Dialysis tubing method *in vitro*			
Pepsin-pancreatin	83.2^{cd}	56.6^{def}	46.7^{ab}
Pepsin-intestinal fluid	78.2^{ab}	42.8^{a}	47.4^{abc}
Pepsin-pancreatin-rumen fluid	83.6^{d}	55.2^{cde}	49.9^{c}
Pepsin-pancreatin-cellulase	82.9^{cd}	56.8^{ef}	43.2
Flask method *in vitro*			
Pepsin-pancreatin	80.3^{bcd}	48.1^{b}	42.2
Pepsin-intestinal fluid	67.1	37.4	40.4
Pepsin-pancreatin-rumen fluid	78.9^{abc}	51.3^{bc}	48.3^{bc}
Pepsin-pancreatin-cellulase	83.8^{d}	41.9^{a}	42.8
SEM	1.44	1.67	1.02
P value	0.000	0.000	0.000

coefficient between the digestibility of GE and NDF of DDGS *in vivo* was 0.68 ($p<0.01$), while the correlation coefficient between the digestibility of CP and ADF of DDGS was 0.73 ($p<0.01$). DE was predicted by the percentage of CP and the content of GE in corn by-products, as follow:

$$DE=5{,}601.09+26.69\times CP\ \%-0.5904\times GE$$
$$(R^2=0.72)$$

(CP %: the percentage of crude protein in corn by-products; GE: the content of gross energy in corn by-products; DE: the apparent digestible energy of corn by-products in pig total tract).

The digestibility of GE, DM and CP *in vivo* and *in vitro* is presented in Table 5.

DISCUSSION

Digestibility of GE, CP, NDF and ADF *in vivo*

Corn by-products were used as protein sources for pigs. In the present study, corn gluten meal had the higher digestibility of CP and GE than that of DDGS and DDG. Thus, it is regarded that corn gluten meal can supply high quality protein and energy sources for animal. As observed in Table 4, there was significant difference ($p<0.01$) in the digestibility of ADF and NDF among four DDGS.

Two explanations are possible for this phenomenon. Firstly, it is likely that processing technique affected the chemical composition of corn by-products. Corn gluten meal came from corns after the extraction oil and starch; DDGS and DDG came from corns after extraction oil and alcohols, additionally, the process techniques were different between DDGS and DDG. Secondly, the chemical composition of corn by-products may have direct effect on the digestibility of nutrients.

It was shown in the present study that processing methods had some effect on the physical and chemical characteristics of feedstuffs. Similar results were also reported by Boisen and Eggum (1991) and Yang et al. (2001). Clearly, the digestibility was influenced by the characteristics of feedstuffs.

Although DDGS and DDG contained higher NDF and ADF, they had higher nutrients digestibility. This result showed that fiber did not affect the digestion of other nutrients in pig diets. Apparently, growing-finishing pigs had the potential ability of digesting fiber diet. This conclusion is in agreement with the previous study (Varel and Yen, 1997).

Furthermore, it was showed in this study that there was a significant correlation between digestibility of NDF and GE ($R^2=0.68$). In spite of the higher digestibility of ADF, significant correlation between ADF and GE was not found. This result demonstrated that NDF markedly influenced GE digestion. Meanwhile, it was also found that the digestibility of CP was not affected by the digestibility of NDF. However, it was significantly affected by the digestibility of ADF ($R^2=0.73$). Further studies should be carried out to establish correlation between CP and ADF in DDGS. Fortunately, for corn by-products in this study, the prediction equation of DE *in vivo* can be determined by its percentage of CP and GE.

Comparison of techniques *in vitro*

Filter bag technique was used to measure CF, NDF and ADF of food or feed with ANKTM fiber analyzer in many countries. However, there were very few reports applying this technique in determining the digestibility of nutrients *in*

vitro. Filter bag can make liquid or gas pass through freely; but the solid contents are retained in bag. In addition, filter bag do not contain N and can not be eroded below 72% sulfuric acid solution. It was indicated in this study that filter bag technique was superior to dialysis tubing technique and flask technique in evaluating the digestibility of CP and GE, as the filter bag technique was convenient. Some time should be saved without sample transferring and filtrating procedure. Dialysis tubing have dialysis performance, which can timely dialyze some reacting matters as gas, water and small chemical molecule. Clearly, particle size of sample and filtrating technique can influenc the digestibility of nutrients. With treatments (pepsin+pancreatin+rumen fluid and pepsin+pancreatin +cellulase), the sample digestibility was more stable. It was indicated that three-step enzyme methods tended to imitate the digestion *in vivo* efficiently.

Furthermore *in vitro*, many factors can affect digestibility such as pH, temperature, enzyme activity, substrate concentration, incubating time and feedstuff characteristics.

Comparison of digestibility *in vivo* and *in vitro*

It is shown in Table 5 that the CP digestibilities of corn gluten meal *in vivo* were markedly higher than that of *in vitro* ($p<0.01$); but the CP digestibilities of DDGS *in vivo* had lower tendency than that of *in vitro* ($p<0.01$); except that the CP digestibility in filter bag (pepsin+pancreatin+ rumen fluid) had not markedly difference from *in vivo*. For GE digestibility, corn gluten meal *in vivo* was significantly lower than that of filter bag and dialysis tubing (pepsin+ pancreatin+cellulase), but was significantly higher than GE digestibility of flask method ($p<0.01$); DDGS *in vivo* was significantly higher than that of *in vitro* ($p<0.01$). For DM digestibility, corn gluten meal *in vivo* was significantly higher than that of flask method ($p<0.01$); DDGS *in vivo* was significantly higher than that of *in vitro* ($p<0.01$).

There were four possible explanations for difference between *in vitro* and *in vivo*. Firstly, it was likely that the difference came from different trial techniques. Secondly, digestive environment was different between *in vivo* and *in vitro*, as pH, temperature, incubating time, enzyme activity, multi-enzyme compound systems, shaking frequency and sample size. Thirdly, the characteristics of corn by-products can affect the digestibility of nutrient. Fourty, it is explained that endogenous N might have a great influence on the digestibility *in vivo*, thus, it is showed in this study that the some nutrient digestibility *in vivo* was lower than *in vitro*. Boisen and Fernandez (1995) also reported that *in vivo* digestibility was influenced by endogenous loss.

CONCLUSION

It can be concluded that the digestibility *in vitro* with three-step enzyme was close to that *in vivo*. Filter bag method to evaluate the digestibility of nutrients in feedstuff was more convenient than that of flask method and dialysis tubing method. There was significant correlation between the digestibility of GE and NDF or between the digestibility of CP and ADF in pig diet. The prediction of DE *in vivo* from corn by-products can be determined by using the nutrient concentrations of the feedstuffs.

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemists, Arlington, Virginia.

Boisen, S. and B. O. Eggum. 1991. Critical evaluation of *in vitro* methods for estimating digestibility in simple stomach animals. Nutr. Res. 4:141-162.

Boisen, S. and J. A. Fernandez. 1995. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by *in vitro* analyses. Anim. Feed Sci. Technol. 51:29-43.

Boisen, S. and J. A. Fernandez. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by *in vitro* analyses. Anim. Feed Sci. Technol. 68:277-286.

Brenes, A., L. S. Jensen and H. M. Cervantes. 1985. Activation of hepatic microsomal mixed function oxidase system in broiler chicks by diet changes. Poult. Sci. 64:963-968.

Cone, J. W. and A. F. B. Vander Poel. 1993. Prediction of apparent ileal digestibility in pigs with a two-step *in vitro* method. J. Sci. Food Agric. 62:393-400.

Coyle, S., T. Najeeullah and J. Tidwell. 1996. A preliminary evaluation of naturally occurring organisms, distillery by-products, and prepared diets as food for juvenile freshwater prawn macrobrachium rosenbergii. J. Appl. Aquaculture. 6:57-66.

Deboever, J. U., B. G. Cottyn, J. I. Andries, F. X. Buysse and J. M. Vanacker. 1988. The use of a cellulose technique to predict digestibility, metabolism and net energy of forages. Anim. Feed Sci. Technol. 19:247-260.

Furuga, B. Y., S. K. Sakamoto and S. Takahashi. 1979. A new *in vitro* method the estimation of digestibility using the intestinal fluid of the pig. Br. J. Nutr. 4:511.

Graham, H., W. Lowgren and P. Aman. 1989. An *in vitro* method for studying digestion in the pig and comparison with *in vivo* ileal and faecal digestibility. Br. J. Nutr. 61:689-698.

Ham, G. A., R. A. Stock, T. J. Klopfenstein, E. M. Larson, D. H. Shain and R. P. Huffman. 1994. Wet corn distillers by-products compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. J. Anim. Sci. 72:3246-3257.

Koelkebeck, K. W., C. M. Parsons, R. W. Leeper, S. Jin and M. W. Douglas. 1999. Early postmolt performance of laying hens fed a low-protein corn molt diet supplemented with corn gluten meal, feather meal, methionine and lysine. Poult. Sci. 78:1132-1137.

Lowgren, B. Y., W. H. Graham and P. Aman. 1989. An *in vitro* method for studying digestion in the pig. Br. J. Nutr. 61:673-687.

National Research Council. 1998. Nutrient Requirements of Swine.

10th Ed. National Academy Press, Washington, DC.

Sauer, W. C., M. Z. Fan, R. Mosenthin and W. Drochner. 2000. Methods for measuring ileal amino acid digestibility in pigs. In: Farm Animal Metabolism and Nutrition (Ed. J. P. F. D. Mello). CAB International, Wallingford, UK. 279-305.

Varel, V. H. and J. T. Yen. 1997. Microbial perspective on fiber utilization by swine. J. Anim. Sci. 75:2715-2722.

Yang, J. S., H. J. Jung, Z. N. Xuan, J. H. Kim, D. S. Kim, B. J. Chae and In K. Han. 2001. Effects of feeding and processing methods of diets on performance, morphological changes in the small intestine and nutrient digestibility in growing-finishing pigs. Asian-Aust. J. Anim. Sci. 10:1450-1459.