Journal of Animal and Veterinary Advances 11 (12): 2043-2047, 2012
ISSN: 1680-5593
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The Additivity of Digestible Energy Values and Digestibility of Dry Matter, Organic Matter Determined by *in vitro* Method for Growing Pigs

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Abstact: The objective of this study was to determine if the supply of digestible energy, dry matter and organic matter in a mixture feedstuffs is equal to the total of the supply based on the digestible supply determined in the single feed ingredients. Nine feedstuffs were provided to formulate the three diets which consisted of corn, soybean and wheat bran from the same region, the diets were formulated according to the requirements of growing pigs recommended by NRC. The dry matter and organic matter digestibility and digestible energy of nine feedstuffs and three diets were determined by *in vitro* method which simulates gastric, small intestine and large intestine digestion, the estimated digestible energy value of diets were calculated with predicted equation described by the previous experiment, the calculated digestible energy value of diets were calculated based on digestible energy value in single ingredients. Results showed that there were no differences among direct determined digestible energy, estimated digestible energy and calculated digestible energy. The directly determined dry matter, organic matter digestibility in diets were compared with those calculated digestibility based on determination in single ingredients, result showed that there were no differences between directly determined and calculated dry matter and organic matter digestibility in diet mixtures. In conclusion, the supply of digestible energy, dry matter and organic matter in a mixture of feedstuffs is equal to the total of the supply based on the digestible supply determined in the corn, soybean and wheat bran by *in vitro* method.

Key words: Additivity, *in vitro* method, digestible energy, digestibility ingredients, dry matter

INTRODUCTION

Additivity of nutrients especial Digestible Energy (DE) is a crucial consideration in the formulation of diets for pig. It is assumed that the DE in individual feedstuffs can be added together to match the required DE supplied by the diet. However, few studies have supported this assumption. Information regarding additivity from pig (Imbeah *et al*., 1988; Furuya and Kaji, 1991) experiments showed that nutrients may not always be additive under certain circumstances but Fan *et al.* (1993) reported that digestible energy supply can be predicted from energy digestibilities estimated in single ingredients. These trial are based on *in vivo* experiment for determination of DE, it need a large number of experiment to determine DE since, many factors (livestock, feedstuff, the composition of diet, crude fiber content, ambient temperature) could lead to the difference of the DE value.

While *in vivo* determination of the DE of feed is a time-consuming and costly process (Lowgren *et al*., 1989), therefore it is necessary to find a fast and reliable method to assess the additiity of DE. *In vitro* method through simulating the animal's digestive system could quickly determinate the DE of different diets, it can determine the DE of feedstuff in a short time and then exclude the impact of experimental animals (Noblet and Jaguelin-Peyraud, 2007). The objective of this study was to determine the DE of a complete diet and its composition to verify whether the DE of feed can be additive or not to make preliminary research of additivity of DE for *in vivo* experiment.

MATERIALS AND METHODS

Diets and experimental design: Nine feedstuffs were prepared to formulate the three diets (Table 1), the three diets consisted of corn, soybean and wheat bran from the same region and the diets were formulated according to the requirements of growing pigs recommended by NRC (1998). Premix was provided by the Ministry of Agriculture Feed Industry Center.

The *in vitro* DE value of nine feedstuffs and three diets were determined by *in vitro* method and then the DE of the three diets was calculated based on the DE determined for the single ingredients.

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Table 1: Composition of three diets and nutrition levels of nine feedstuff

	Composition of diets and nutrition levels (%)										
Items (%)	Hebei corn	Hebei soybean meal	Hebei wheat bran	Shandon corn	Shandon soybean mean	Shandon wheat bran	Jiling corn	Jiling soybean meal	Jiling wheat bran	Premix	Total
Hebei diet	68.00	25.00	3.00	-	-	-	-	-	-	4	100
Shandong diet	-	-	-	68.00	25.00	3.00	-	-	-	4	100
JiLing diet	-	-	-	-	-	-	68.00	25.00	3.00	4	100
DM	87.61	87.52	88.62	86.42	87.97	88.87	86.29	88.19	88.69	-	-
CP	8.49	44.99	18.21	7.75	48.09	18.17	6.69	44.54	17.72	-	-
Ash	4.49	6.82	7.54	2.81	6.64	5.72	3.02	6.33	7.86	-	-
EE	3.57	0.77	2.60	4.24	0.84	3.75	3.64	1.12	2.74	-	-
NDF	9.73	13.96	40.88	12.46	14.52	35.90	9.51	17.09	31.53	-	-
ADF	1.65	6.59	11.12	1.87	5.39	9.22	1.76	6.26	10.27	-	-
Lys	0.26	2.98	0.78	0.24	3.05	0.77	0.21	2.85	0.72	-	-

[1]Premix provided the following per kg of complete diet for grower pigs: Vitamin A, 5,512 IU; Vitamin D_3, 2,200 IU; Vitamin E, 64 IU; Vitamin K_3, 2.2 mg; Vitamin B_{12}, 27.6 μg; riboflavin, 5.5 mg; pantothenic acid, 13.8 mg; niacin, 30.3 mg; choline chloride, 551 mg; Mn, 40 mg; Fe, 100 mg; Zn, 100 mg; Cu, 100 mg; I, 0.3 mg; Se, 0.3 mg

The estimated digestible energy value of diets were calculated with predicted equation based on the previous experiment using the regression equation:

$$y = 0.4625x + 7.2065, \quad R^2 = 0.71$$

Where:

X = The *in vitro* DE value

Y = The predicted DE value

Chemical analysis and bioavailability analysis by the *in vitro* method: Nine single feedstuffs and three diets were analyzed for 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 and undigested residues was calculated by an adiabatic bomb calorimeter (PARR 1281, Moline, IL, USA).

Digestibility and bioavailability of nine feedstuffs and three diets 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 samples was introduced into a 100 mL conical flask. About 25 mL of phosphate buffer (0.1 M, pH 6.0), 10 mL 0.2 M HCl and 1 mL of a freshly prepared pepsin solution containing 25 mg pepsin (porcine, 2000 FIP-U/g, 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 (0.2 M. pH 6.8) and 5 mL of NaOH solution (0.6 M) were added to the conical flask and then 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 0.2 M 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, 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.

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

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

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

Data were analyzed according to the GLM and t-test procedure (SAS Inst. Inc., Cary, NC). The observed DE and those calculated DE based on determinations in the single ingredients and predicted DE based on the equation were compared by means of GLM. The observed digestibility of OM and DM and those calculated digestibility based on determinations in the single ingredient were compared by means of a t-test.

RESULTS AND DISCUSSION

In vitro DE of nine feedstuffs and three diets were determined by *in vitro* method, the results shown in Table 2. Total DE value was calculated according to DE of single feedstuff and their percentage in the mixture diet, the results shown in Table 3. The predicted DE value was also shown in Table 3. There were no significant difference among *in vitro* measured values, calculated DE values and the predicted DE values ($p>0.05$). Similarly, total digestibility of DM and OM were calculated according to digestibility of single feedstuff and their percentage in the mixture (Table 4), results shown that there were no significant difference between the *in vitro* DM digestibility or OM digestibility and calculated digestibility of *in vitro* DM or OM values for the three diets ($p>0.05$).

The DE of a single feedstuff has been usually determined by *in vivo* method, it is not only time consuming but also affected by many factors, especially for some feedstuff that can not fed alone. However, the DE of single feedstuff is indispensable parameters in the formulation of the complete diets, additivity of DE is the basis and premise of formulation for the complete diets but the additivity of DE is uncertain. There are three opinion of additivity of DE, the first opinion is that the DE value of feedstuff could be added, its DE value remain stable no matter what kind of animal fed, the supply of DE in a mixture of feedstuffs is equal to the total of the supply based on the digestible supply determined in the single feed ingredients. Second opinion is that DE value of feedstuffs could not be added, the same kind of feed fed different animal, its DE value is different. It will be affected by animal species, diet levels, crude fiber content, ambient temperature and so on. The third opinion is weather the DE value of the feedstuffs could be added or not, it mainly decided by the types of feedstuffs in the formulation of diets if the characteristics of feedstuffs ingredient exits little difference, the DE of feedstuffs could be added if the characteristics of feedstuffs ingredient exits much difference, the DE of feedstuffs could not be added (Luo, 1983). There is a scarcity of information on the additivity of the DE value, the main reason may be that too much feedstuffs and the DE value of feedstuff were affected by

Table 2: DE and digestibility of DM and OM for feed stuff and diets with *in vitro* methods

	Digestibility of DM *in vitro* (%)							Digestibility of OM *in vitro* (%)							DE *in vitro* (MJ kg^{-1})		
Items	Determined values					Mean	CV%	Determined values					Mean	CV%	Determined values		Mean
Hebei corn	83.50	84.90	86.74	84.28	83.82	84.65	1.52	84.05	84.94	86.55	84.05	83.58	84.63	1.39	15.98	15.41	15.70
Hebei soybean meal	89.67	86.11	87.64	87.40	86.55	87.47	1.57	90.42	87.31	86.69	86.42	85.52	87.27	2.15	17.15	17.29	17.22
Hebei wheat bran	52.78	52.64	53.90	53.17	52.40	52.98	1.11	50.17	50.05	50.52	49.74	48.91	49.88	1.22	9.94	9.75	9.85
Hebei diet	86.29	85.90	84.69	85.71	85.17	85.55	0.73	86.53	86.17	83.62	84.72	84.14	85.04	1.49	15.69	15.37	15.53
Shandong corn	81.52	81.85	82.36	81.67	83.90	82.26	1.18	81.53	82.22	82.12	81.42	83.69	82.20	1.10	14.64	13.35	14.00
Shandong soybean meal	86.37	89.69	87.13	86.80	87.70	87.54	1.48	87.49	89.60	86.16	85.80	86.79	87.17	1.73	17.17	17.28	17.23
Shandong wheat bran	61.86	61.07	60.93	59.17	60.77	60.76	1.62	59.94	59.43	58.71	56.86	58.55	58.70	1.99	11.00	10.73	10.87
Shandong diet	82.48	84.22	84.39	83.53	83.05	83.53	0.96	82.99	84.63	83.22	83.30	81.78	83.18	1.22	15.00	15.04	15.02
Jiling corn	83.86	83.21	83.24	84.83	82.38	83.50	1.09	83.96	83.52	83.04	84.65	82.17	83.47	1.12	12.10	14.98	13.54
Jiling soybean meal	87.22	86.36	86.66	86.51	85.74	86.50	0.62	87.70	87.71	85.66	85.49	84.66	86.24	1.61	16.70	15.56	16.13
Jiling wheat bran	64.33	63.80	63.40	62.53	64.34	63.68	1.18	63.41	62.87	61.01	60.08	62.01	61.89	2.19	11.15	11.13	11.14
Jiling diet	84.08	81.46	83.71	85.90	85.03	84.04	1.99	84.68	81.80	82.62	84.96	84.03	83.62	1.63	15.24	14.50	14.87

Table 3: Comparision of DE among observed values, calculated values and predicted values

		DE value			
Items	Composition (%)	Observed values	Calculated values	Predicted values	p-values
Hebei corn	68.00	15.70	-	14.47	
Hebei soybean meal	25.00	17.22	-	15.17	
Hebei wheat bran	3.00	9.85	-	11.76	
Premix	4.00	-	-	-	
Hebei diet	100.00	15.53	15.28	14.39	>0.05
Shandong corn	68.00	14.00	-	13.68	
Shandong soybean meal	25.00	17.23	-	15.18	
Shandong wheat bran	3.00	10.87	-	12.23	
Premix	4.00	-	-	-	
Shandong diet	100.00	15.02	14.15	14.15	>0.05
Jiling corn	68.00	13.54	-	13.47	
Jiling soybean meal	25.00	16.13	-	14.67	
Jiling wheat bran	3.00	11.14	-	12.36	
Premix	4.00	-	-	-	
Jiling diet	100.00	14.87	13.57	14.08	>0.05

[1]Premix provided the following per kg of complete diet for grower pigs: Vitamin A, 5,512 IU; Vitamin D_3, 2,200 IU; Vitamin E, 64 IU; Vitamin K_3, 2.2 mg; Vitamin B_{12}, 27.6 μg; riboflavin, 5.5 mg; pantothenic acid, 13.8 mg; niacin, 30.3 mg; choline chloride, 551 mg; Mn, 40 mg; Fe, 100 mg; Zn, 100 mg; Cu, 100 mg; I, 0.3 mg; Se, 0.3 mg

Table 4: Comparision of digestibility of DM and OM between observed values and calculated values

		Digestibility of DM (%)			Digestibility of OM (%)		
Items	Composition (%)	Observed values	Calculated values	Difference	Observed values	Calculated values	Difference
Hebei corn	68.00	84.65	-	-	84.63	-	
Hebei soybean meal	25.00	87.47	-	-	87.27	-	
Hebei wheat bran	3.00	52.98	-	-	49.88	-	
Premix	4.00	100.00	-	-	100.00	-	
Hebei diet	100.00	85.55	85.02	0.53	85.04	84.86	0.18
Shandong corn	68.00	82.26	-	-	82.20	-	
Shandong soybean meal	25.00	87.54	-	-	87.17	-	
Shandong wheat bran	3.00	60.76	-	-	58.70	-	
Premix	4.00	100.00	-	-	100.00	-	
shandon diet	100.00	83.53	83.65	-0.12	83.18	83.45	-0.27
Jiling corn	68.00	83.50	-	-	83.47	-	
Jiling soybean meal	25.00	86.50	-	-	86.24	-	
Jiling wheat bran	3.00	63.68	-	-	61.89	-	
Premix	4.00	100.00	-	-	100.00	-	
Jiling diet	100.00	84.04	84.32	-0.28	83.62	84.18	-0.56

[1]Premix provided the following per kg of complete diet for grower pigs: Vitamin A, 5,512 IU; Vitamin D_3, 2,200 IU; Vitamin E, 64 IU; Vitamin K_3, 2.2 mg; Vitamin B_{12}, 27.6 μg; riboflavin, 5.5 mg; pantothenic acid, 13.8 mg; niacin, 30.3 mg; choline chloride, 551 mg; Mn, 40 mg; Fe, 100 mg; Zn, 100 mg; Cu, 100 mg; I, 0.3 mg; Se, 0.3 mg

many factors *in vivo* experiment, the error of DE value *in vivo* experiments could be conceal the truth. Therefore, it is precise way to determine the DE value of feedstuff by *in vitro* method (Regmi *et al*., 2009). Li *et al*. (1995) and Zhang *et al*. (1995) reported that there is no significant difference between the observed DE and calculated DE values by two step *in vitro* method and the diet level did not affect the additivity of DE. Shi and Nie (1985) reported the same result in this study, the DE of nine feed ingredients were determined by three step *in vitro* method and three diets were formulated based on nine feedstuffs ingredients, according to NRC (1998) nutritional requirements for swine, their DE were also determined by *in vitro* method. The study found there was no statistically significant difference ($p>0.05$) between that mixed DE value and the total of DE determined in the single feed ingredient because the DE of the nine feedstuffs and three complete diets do not determine with *in vivo* method therefore, it can not decide that DE of these feedstuffs could be added but Zhang *et al*. (1995) and Fan *et al*. (1993) reported that DE in a diet can be predicted from the DE in the ingredients by *in vivo* experiments.

There is few information about additivity of *in vitro* DM digestibility and OM digestibility in diets. Shi and Nie (1985) determined the DM digestibility and DE of sixteen feed ingredients and complete diets through *in vitro* and *in vivo* method, they found that *in vitro* dry digestibility and energy digestibility of the feedstuffs had good additivity but there was a significant difference between the *in vitro* digestibility of CP and *in vivo* CP digestibility ($p<0.01$). In this experiment *in vitro* dry matter and organic matter digestibility of nine feedstuffs and three complete diets were determined through three step enzymatic method which simulate the stomach intestine and large intestine digestion and found that *in vitro* DM and OM digestibility have good additive for corn, soybean and wheat bran.

CONCLUSION

In this study, the DE value were affected by many factors *in vivo* experiment, the noise of *in vivo* experiment may be conceal the true, it will reduce noise in the determination of DE by *in vitro* method in this study, the supply of DE, DM and OM in a mixture of feedstuffs is equal to the total of the supply based on the digestible supply determined in the corn, soybean and wheat bran.

ACKNOWLEDGEMENTS

This research was supported by the Natural Science Foundation of Beijing (No.: 6092006) and State Key Laboratory of Animal Nutrition (2004DA125184 (Group) 0815).

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