Estimation of in vivo digestibility with the laying hen by an in vitro method using the intestinal fluid of the pig

BY K. SAKAMOTO AND T. ASANO
Nippon Kayaku Co. Ltd, Takasaki-shi 370–12, *Japan*
AND S. FURUYA* AND S. TAKAHASHI
Department of Nutrition, National Institute of Animal Industry, Chiba-shi 280, *Japan*

(*Received* 9 *October* 1979 – *Accepted* 26 *November* 1979)

1. Dry matter and crude protein (nitrogen × 6·25) digestibility of four poultry diets determined by an in vitro method using the intestinal fluid of pigs was significantly correlated with corresponding in vivo digestibility values obtained with hens.
2. The intestinal fluid could be lyophilized and stored for at least 35 d without losing its activity on digestion.

Recently, a new in vitro method was proposed to predict the digestibility of diets for pigs (Furuya *et al.* 1979). The method is based on a simulation of gastric digestion followed by intestinal digestion. The test substance (food) is first incubated with acid pepsin (*EC* 3.4.4.1) followed by incubation with intestinal fluid obtained from a pig fitted with a simple cannula in the upper jejunum.

Since the over-all digestive efficiency of the chicken is considered similar to that of other non-ruminant animals (Hill, 1962), the in vitro method could be expected to give a good estimate of the in vivo digestibility of poultry diets. The objective of this study was to compare the in vivo procedure with the in vitro method in determining the digestibility of poultry diets. Also, we examined the activity change of the intestinal fluid when the fluid was lyophilized.

EXPERIMENTAL

Diets A, B, C and D shown in Table 1 were used in the present study. For the in vitro determinations each diet was ground in a laboratory mill with a 0·5 mm screen.

Expt 1. *The relationships between in vitro and in vivo digestibilities.* The in vitro dry matter (DM) and crude protein (nitrogen × 6·25; CP) digestibilities were determined by the method described previously (Furuya *et al.* 1979). The intestinal fluid was obtained from a pig (host animal) fitted with a simple cannula as described previously except that intestinal contents were centrifuged at 1500 ***g*** instead of 1250 ***g***.

Four White Leghorn hens, weighing 1300–1500 g, fitted with an artificial anus by the method of Ariyoshi & Morimoto (1956) were used for in vivo digestion determinations. They were housed in individual metabolism cages. The trial consisted of four periods of 7 d each. The four diets shown in Table 1 were fed in rotation to each hen. After the 4 d preliminary feeding, all faeces were collected for the following 3 d, dried in a forced-air oven at 60°, air-equilibrated, ground and sampled for analysis. The in vivo digestibilities of DM and CP were determined by the chromic oxide method.

* For reprints.

0007-1145/80/073S-2611 $01.00

Table 1. *Composition of the experimental diets (g/kg)*

Ingredient	Diet A	B	C	D
Ground maize	708·5	732·5	652·5	668·5
Soya-bean meal	150	120	100	120
Fish meal	100	50	—	50
Rice bran	—	50	200	50
Lucerne meal	20	20	20	20
Calcium carbonate	—	—	—	50
Dicalcium phosphate	—	—	—	30
Tricalcium phosphate	10	—	—	—
Sodium chloride	5	5	5	5
Vitamin and mineral mixture*	4·5	4·5	4·5	4·5
DL-methionine	1	1	1	1
L-lysine	—	1	1	—
Chromic oxide	1	1	1	1
Chemical composition				
Dry matter	864	856	851	863
Crude protein (nitrogen × 6·25)	186	160	134	150

* Providing (/kg diet): retinol 6 mg, cholecalciferol 100 μg, α-tocopherol 20 mg, thiamin 4 mg, riboflavin 20 mg, pyridoxine 4 mg, pantothenic acid 8·7 mg, nictotinic acid 4 mg, choline 276 mg, iron 3 mg, copper 0·3 mg, zinc 25 mg, manganese 40 mg, iodine 1 mg.

Expt 2. Effect of lyophilizing the intestinal fluid on in vitro digestibility. Effect of lyophilizing the intestinal fluid on in vitro DM and CP digestibilities were examined with diets A and C. The intestinal fluid was lyophilized immediately after preparation, stored in a desiccator at room temperature for 35 d, reconstituted in water and then used for in vitro digestibility determinations. The values for DM and CP were compared with those determined using intestinal fluid stored at −20° for 35 d without lyophilizing.

Chemical analysis. Cr_2O_3 was determined colorimetrically using the method of Brisson (1956). DM and CP in the food and faeces were determined by the method of the Association of Official Analytical Chemists (1970).

RESULTS AND DISCUSSION

Expt 1. The relationships between in vitro and in vivo digestibilities. The results shown in Table 2 indicate that the in vitro method gave a good estimate of in vivo digestibility. The following linear regression equations could be fitted to the values for the four tested diets: DM, $Y = 1{\cdot}06X - 0{\cdot}0321$ (r 0·98, $P < 0{\cdot}05$, residual standard deviation (RSD) ±0·008); CP, $Y = 1{\cdot}21X - 0{\cdot}1731$ (r 0·99, $P < 0{\cdot}05$, RSD ±0·008), where Y and X are in vivo and in vitro digestibilities respectively. The RSD values of the regression for DM and CP were comparable to the corresponding values obtained in our previous experiment with pigs (±0·011 and ±0·012 for DM and CP respectively; Furuya *et al.* 1979).

There was a slight dissimilarity between the two methods. For DM, the in vitro digestibilities tended to be lower than the in vivo values. To overcome biased estimates of digestibility the in vitro method should be standardized with samples of known in vivo digestibility in each in vitro experiment.

Expt 2. Effect of lyophilizing the intestinal fluid on in vitro digestibility. The results are given in Table 3. Lyophilizing the intestinal fluid and storing at room temperature for at least 35 d had no effect on the digestibility of DM and CP compared with values determined with intestinal fluid stored at −20° for 35 d. Previously we showed that the intestinal fluid

Table 2. *Expt* 1. *The relationships between in vitro and in vivo digestibilities of dry matter and crude protein (nitrogen* × 6·25*) in the experimental diets**

(Mean values with their standard errors for eight (dry matter) and four (crude protein) measurements (in vitro) and four hens (in vivo))

	Digestibility of:							
	Dry matter				Crude protein			
	In vitro		In vivo		In vitro		In vivo	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	0·784	0·003	0·799	0·004	0·870	0·004	0·870	0·005
B	0·763	0·002	0·778	0·010	0·836	0·001	0·844	0·007
C	0·717	0·004	0·721	0·010	0·775	0·004	0·758	0·014
D	0·721	0·004	0·741	0·011	0·830	0·004	0·824	0·009

* For details of diets, see Table 1.

Table 3. *Expt* 2. *Effect of lyophilizing the intestinal fluid on in vitro dry matter and crude protein (nitrogen* × 6·25*) digestibility in diets A and C**

(Mean values with their standard errors for six (dry matter) or three (crude protein) measurements)

	Digestibility of:							
	Dry matter				Crude protein			
	Frozen		Lyophilized		Frozen		Lyophilized	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	0·779	0·009	0·772	0·002	0·860	0·009	0·859	0·002
C	0·712	0·004	0·714	0·003	0·766	0·004	0·769	0·004

* Before use, portions of intestinal fluid were either frozen, −20°, for 35 d or lyophilized and stored in a desiccator at room temperature for 35 d. For details of diets, see Table 1.

can be preserved at −20° for at least 60 d without any obvious change in its activity for DM and CP digestion (Furuya *et al.* 1979).

These results indicate that this in vitro method is not species-specific to the host animal species, but yields reasonable digestibility estimates for species as dissimilar as pigs and chickens. Therefore, this method may be applicable to most non-ruminant animals. Also since lyophilization of the intestinal fluid does not alter its efficiency in the digestion determinations, the use of this in vitro method does not require the maintenance of a host animal in each laboratory.

The authors thank Dr K. Kameoka for supervision, and Dr D. Kubota for reading the manuscript. They also thank Dr Nancy R. Stevenson, C.M.D.N.J. – Rutgers Medical School, for her valuable comments on the manuscript and her help with the English language.

REFERENCES

Ariyoshi, S. & Morimoto, H. (1956). *Bull. natl Inst. agric. Sci.* G12, 37.
Association of Official Analytical Chemists (1970). *Official Methods of Analysis*, 11th ed. Washington, DC: Association of Official Analytical Chemists.
Brisson, G. J. (1956). *Can. J. agric. Sci.* 36, 210.
Furuya, S., Sakamoto, K. & Takahashi, S. (1979). *Br. J. Nutr.* 41, 511.
Hill, F. W. (1962). In *Nutrition of Pigs and Poultry*, pp. 3–17. [J. T. Morgan and D. Lewis, editors]. London: Butterworths.

Printed in Great Britain