A New *in vitro* Method for Estimating Digestibility of Animal Feeds

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Digestibility experiments have a considerable value in the estimation of nutritive values of animal diets. However, the determination of digestibility is not only tedious and time-consuming but also requires large quantities of diets.

Recently, the author proposed a new *in vitro* method to estimate the digestibility of diets for pigs. The method is based on a simulation of gastric followed by intestinal digestion. Test substances (feed) are first incubated with acid pepsin followed by incubation with intestinal fluid obtained from a pig fitted with a simple cannula in the upper jejunum. This method was also applied to estimate the digestibility of poultry diets, and activity changes of the intestinal fluid when the fluid was lyophilized were examined.

The present paper reports results of study on the *in vitro* method for the estimation of digestibility using the intestinal fluid of the pig.

Standard procedures in *in vitro* digestion

The method had two stages. In the first stage, 0.5 g each of duplicate samples of each diet was weighed into 100 ml Erlenmeyer flask, to which 20 mg pepsin in 10 ml 0.075 M-hydrochloric acid was added and incubated with shaking at 80 oscillations/min for 4 hrs at 37°C in a water-bath. At the end of the first incubation period, the content was neutralized with 0.2 M-sodium hydroxide. In the second stage, 10 ml of intestinal fluid was added and the digestion mixture was incubated for an additional 4 hrs at 37°C.

A female pig weighing approximately 25 kg was used as a host animal to obtain the intestinal fluid for *in vitro* digestion experiments. The pig was fitted with a simple ('T'-shaped) cannula in the upper jejunum 500 mm beyond the pylorus and distal to the common bile duct. Approximately 500 g of intestinal contents was removed daily between 10.00 and 11.00 a.m. through the cannula and centrifuged for 10 min at 1250 or 1500 g. The supernatant fraction (intestinal fluid) was used immediately or stored at −20°C for *in vitro* digestion experiments.

At the completion of the second incubation the content of the flask was transferred to 120 ml centrifuge-tube and centrifuged immediately for 10 min at 1250 g. The supernatant was resuspended in 50 ml water and recentrifuged for 10 min at 1250 g. The second supernatant fraction was discarded. The insoluble residue in the tube was mixed with a little water and filtered through a weighed filter paper. The paper containing the residue was dried for 5 hrs at 105°C and transferred to a Kjeldahl flask for determination of crude protein (CP). The digestibilities of dry matter (DM) and CP were calculated:

$$1-\frac{R}{S}$$

where R is the weight of the oven-dry sample residue and S is the weight of the sample for each constituent.

For the *in vitro* determinations each diet was ground in a laboratory mill with a 0.5 (poultry diets) or 1 (pig diets) mm screen.

Table 1. Chemical composition (%) of pig diets (diets A-H) and hen diets (diets I-L)

	Diets											
	A	B	C	D	E	F	G	H	I	J	K	L
Dry matter	87.8	87.0	87.0	86.3	87.3	85.9	86.1	86.2	86.4	85.6	85.1	86.3
Crude protein	24.7	20.3	15.1	13.2	19.5	18.8	16.3	15.9	18.6	16.0	13.4	15.0

Relationship between in *vitro* and *in vivo* digestibilities

The *in vitro* method was compared with the *in vivo* method for the determination of DM and CP digestibility with 8 pig diets and 4 poultry diets (Table 1). For *in vivo* digestion studies, 8 male pigs, weighing approximately 20 kg at the beginning of the experiment, and 4 hens, weighing 1300–1500 g, fitted with an artificial anus, were used. The *in vivo* method was described in the original papers[1,2].

Fig. 1 shows the relationships between *in vivo* (with pigs) and *in vitro* digestibilities of DM and CP. Linear regression equations $Y=1.04X+0.0806$ (r 0.98, $P<0.01$, residual standard deviation, $RSD \pm 0.011$) and $Y=1.70X-0.6092$ (r 0.98, $P<0.01$, $RSD \pm 0.012$) could be fitted to DM and CP respectively for 7 tested diets. In this calculation, the diet A was excluded because ingredients, chemical composition and physical form (particle size) of this diet were considerably different from those of the other diets and, as a result, the relationship between *in vivo* and *in vitro* digestibilities was different from those of the other diets, as shown in Fig. 1.

Fig. 2 shows the relationships between *in vivo* (with hens) and *in vitro* digestibilities for 4 poultry diets. The following linear regression equations could be fitted to the values of *DM* and *CP* : *DM*, $Y=1.06X-0.0321$ (r 0.98, $P<0.05$, $RSD \pm 0.008$; *CP*, $Y=1.21X-0.1731$ (r 0.99, $P<0.05$, $RSD \pm 0.008$).

Although the *in vitro* digestibilities obtained with the present method were highly correlated with the *in vivo* digestibilities, the former was different from the latter in ab-

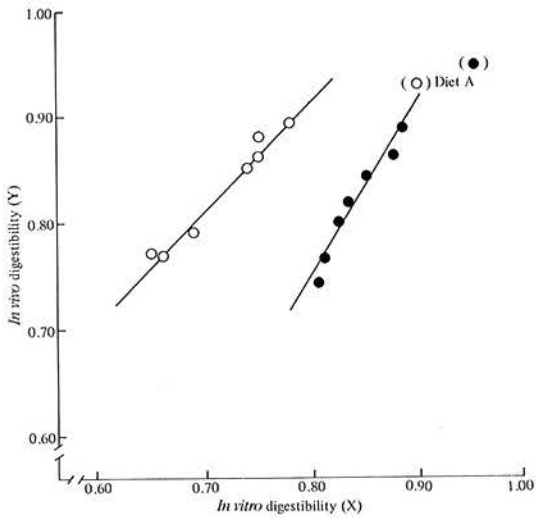

Fig. 1. Relationship between *in vivo* and *in vitro* digestibilities of dry matter (○) and crude protein (●) of pig diets.

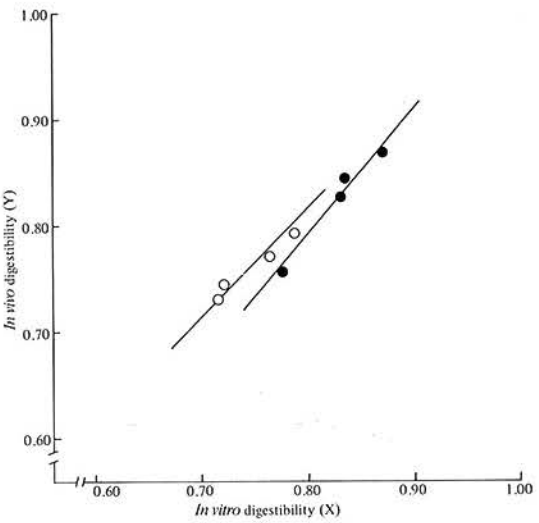

Fig. 2. Relationship between *in vivo* and *in vitro* digestibilities of dry matter (○) and crude protein (●) of hen diets.

Table 2. Effect of lyophilizing the intestinal fluid on *in vitro* dry matter and crude protein digestibility of diets I and K[1,2]

	Dry matter				Crude protein			
	Frozen		Lyophilized		Frozen		Lyophilized	
Diets	Mean	SE	Mean	SE	Mean	SE	Mean	SE
I	0.779	0.009	0.772	0.002	0.860	0.009	0.859	0.002
K	0.712	0.004	0.714	0.003	0.766	0.004	0.769	0.004

1) Prior to use, aliquots of intestinal fluid were either frozen at −20 °C for 35 days or lyophilized and stored in a desiccator at room temperature for 35 days.
2) Mean values with their standard errors for 6 (dry matter) or 3 (crude protein) measurements.

solute values, especially in the case of pigs. For DM, the *in vivo* digestibilities with pigs tended to be higher than the *in vitro* values, and for CP a reverse relationship. These observations indicated that to increase the accuracy of the *in vitro* method, some standard samples of known *in vivo* digestibilities should be included in each *in vitro* experiments and their values should be used for corrections.

Effect of lyophilizing the intestinal fluid on *in vitro* digestibility

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

The results are given in Table 2. Lyophilized intestinal fluid gave the same digestibility of DM and CP as determined with intestinal fluid stored at −20°C. Previously the author showed that the intestinal fluid can be preserved at −20°C for at least 60 days without any obvious change in its activity for DM and CP digestion. These results indicate that the use of this *in vitro* method does not require the maintenance of a host animal in each laboratory.

Discussion and conclusion

The present method for the estimation of *in vivo* digestibility has many advantages. This method requires only small amounts of sample and no special apparatus, and many samples can be handled in a single experiment; in our laboratory 8 to 16 samples of unknown digestibility, together with 4 standard diets, are used in duplicate in each trial. The period of time required is very short; determinations of DM and CP digestibility are usually completed within 2 days.

This *in vitro* method seems to be not specific to the host animal species, but it gives reasonable digestibility estimates with different species like pigs and chickens. Therefore, this method may be applicable to most non-ruminant animals.

However, there is a limitation to this method at present. Any digestion occurring in the large intestine, such as that of fibrous material, is excluded from the measurement by this method. Therefore, for feed samples with higher crude fibre contents such as roughage, the correlation of DM digestibility with *in vivo* digestibility would probably be lower than that obtained in the present study. For more exact estimation of *in vivo* DM digestibility of diets with high roughage contents it may be necessary to add a third digestion stage for crude fibre digestion to the present two-step procedure.

References

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2) Sakamoto, K. et al.: Estimation of *in vivo* digestibility with the laying hen by an *in vitro* method using the intestinal fluid of the pig. *Br. J. Nutr.* [In press].