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A Modified Method for Determining Energy of Fresh Faeces and Urine of Pig

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Introduction

In metabolism experiments, energy and nitrogen of feeds, faeces and urine must be determined accurately. Usually these determinations are carried out on the samples dried by various method. For faeces, samples are dried in a conventional air oven[1,15] or in a vacuum oven under reduced pressure[3,4], or freeze dried[6]. On the contrary, energy determinations are also carried out on fresh samples with the aid of some primers to facilitate combustion[3,4,8,12]. These methods have yielded different results, though drying of samples generally give lower values of energy and nitrogen. With regard to urine, samples are usually acidified first, and then a primer is used to absorb or hold it for air oven[10,11], or vacuum oven drying[2,10], or freeze dried[6]. In this case, various problems concerning the efficiencies have been reported.

This experiment was designed to establish a more efficient method for the determination of the faecal and urinary energies, and of the faecal nitrogen. We examined on the efficiencies of two primers, polyethylene film and cellulose powder, the efficient weight ratio of faecal sample to primer, and the effect of drying faeces and urine with or without sulphuric or hydrochloric acid.

Materials and Methods

1. Materials

Materials used in these studies were: (1) Fresh faeces and urine of pig (from our farm), (2) Polyethylene film (commercial, 0.03 mm thickness), (3) Cellulose powder (powdered filter paper-D, 40 mesh, Toyo Kagakukogyo K.K.) and (4) Corn starch (Tanabe amino acid research foundation).

2. Preparation of samples

1) Faeces. Fresh samples for energy determination were taken from well mixed fresh faeces of pig. Each bit (ca. 80 g) of the same faeces was dried under various conditions; freeze drying for 16 hr (FD), conventional air oven drying at 60°C for 24 hr (AD), and vacuum oven drying at 60°C, 200 mm Hg for 4, 6 and 12 hr (VD–4, VD–6 and VD–12). The dried samples of VD–4 and VD–6 were exceptionally placed in a dessicator, however, all others were placed in the open air for 7 days to be equilibrated with the atmospheric moisture. These air-dried samples were then ground in a Wiley mill to pass a 1 mm screen, and then about 1 to 2 g of them were used for the energy determination.

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2) Urine. The pig urine was acidified to pH 6 with either concentrated sulphuric (H_2SO_4) or hydrochloric acid (HCl), and 25 ml portions were taken for drying.

3. Primers

The primers used in these studies were polyethylene film (PEF) and cellulose powder.

4. Gross energy determination

Gross energy was determined on the following samples by using a Satake Kagaku Kikai "Kairyo-gata Nenken-shiki B" adiabatic bomb calorimeter.

1) Dried faeces. The calorific value of the dried samples was determined in a usual manner.

2) Fresh faeces. A sheet of PEF, $8 \times 10\ cm^2$ weighing 0.3–0.4 g, was made into a bag by a modified method of Nijkamp[10,11]. The sheet was folded over and sealed on the two edges leaving the upper end open. An amount of about 2 g of fresh faecal sample was put into this bag, the bag was folded in. For the execution of successful combustion, it was found to be effective to make the faecal sample in the bag flattened and rolled prior to the starting of combustion as illustrated in Fig. 1.

With regard to the cellulose as the primer, about 1 g of cellulose was at first placed on the top of 2 g of faeces in the metal combustion capsule, and was combusted in the bomb. However, this procedure was proved to be unsuccessful. Therefore, the faecal sample was sandwitched between the two portions of 0.5 g each of cellulose (Fig. 1).

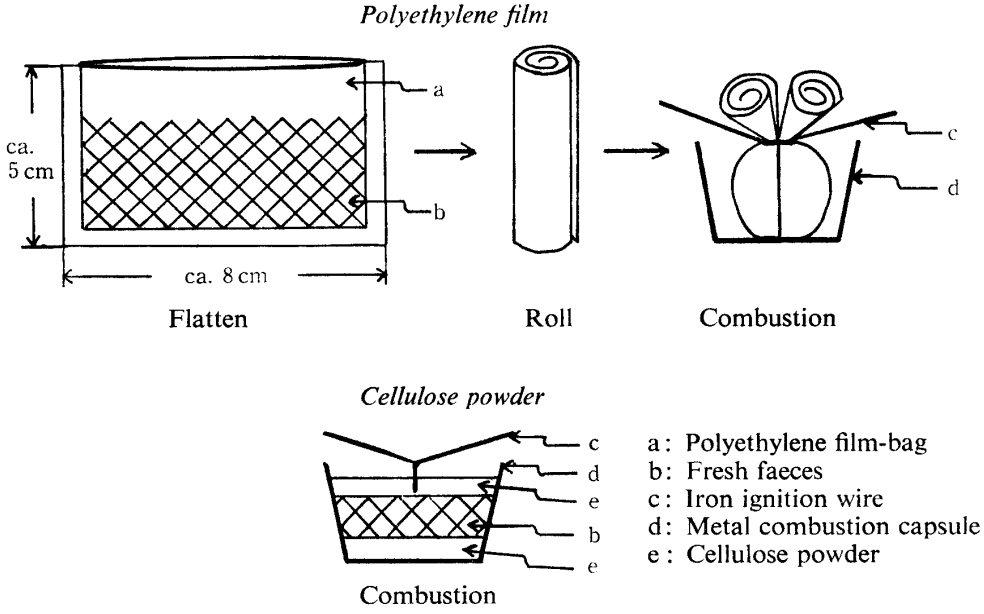

Fig. 1. Preparation of faecal sample for combustion.

3) Urine. A sheet of PEF was cut into a circle with a 10 cm diameter, weighing about 0.15 g. About 25 g of urine was pipetted onto the sheet in a tarred evaporating dish. The urine was dried in a conventional oven at 40°C, and then was applied for calorific value determination.

When cellulose was used, about 1 g of the cellulose powder was made into a cylindrical block and dried to a constant weight at 105°C. One ml of urine was added gently till the block became saturated, and was dried in a conventional oven at 40°C. This procedure was repeated so as to

absorb about 10 ml of the urine in the block. The cellulose block was then combusted in the bomb.

5. Nitrogen

All determinations were done in triplicate by the Kjeldahl method.

Results and Discussion

1. Selection of primer in the energy determination of fresh faeces

The results given in Table 1 show the PEF having a higher energy value than the cellulose. The PEF used here was found to have slightly higher value (ca. 3%) than those reported by Itoh and Tano[8] and Nijkamp[10], 11.068 and 11.071 kcal/g, respectively. The values of gross energy of faeces determined with PEF and cellulose showed a good agreement between the two primers, but the standard error in cellulose was higher than in PEF. PEF was, thus, concluded to be a more reliable primer for the determination of gross energy of fresh faeces.

Table 1. Gross energy value of fresh faeces obtained in aid of primer

Primer	kcal/g DM of faeces	Faeces Wt. g	Primer Wt. g
PEF	4.690±0.052	2.423±0.456	0.382±0.011
Cellulose	4.614±0.153	2.177±0.303	0.901±0.192

Mean±SE.

The calorific value of the PEF and cellulose powder was 11.395±0.031 and 4.169±0.040 kcal/g, respectively.

The following abbreviations are used in all the Tables of this paper: PEF, polyethylene film; DM, dry matter; FD, freeze drying; AD, conventional air oven drying; VD-4, VD-6 and VD-12, vacuum oven drying for 4, 6 and 12 hr.

2. Effects of the weight of sample and primer

A commercial corn starch as a reference and fresh faeces were used in this experiment. The results are shown in Table 2. The corn starch showed consistently lower value with higher sample weight. The significant factors were suspected to have been concealed in the sample weight. The energy value of fresh faeces was determined by varying the sample weight from 1 to 5 g, while PEF was kept at 0.2 to 0.3 g. No significant difference was noted between the obtained energy values, however, 5 g sample was incompletely combustible, occasionally, and 1 to 2 g sample was apt to give lower values. Consequently, the following experiments were conducted using 3 to 4 g fresh sample and about 0.3 g PEF. In these conditions the ratios of weight and calorific value of sample to primer were 3.5–4.2 and 1.5–1.8, respectively.

3. Effects of the drying methods on the faecal energy value

In the dried faeces the gross energy was determined without primer. The results are given in Table 3. The sample dried in a vacuum oven for a short time, VD-4, was not combustible. VD-6, the sample dried longer, showed incomplete combustion, giving low value with a relatively large standard error ($P<0.05$). The sample of VD-12 gave results similar to those of FD and AD.

Table 2. Effect of weight ratio of sample to primer on determination of gross energy value

Sample Wt. g	PEF Wt. g	Weight ratio of DM of sample to primer	kcal/g DM of sample	Calorific ratio of sample to primer
Corn starch (DM 87.01%)				
0.551	0.301	1.59	3.87±0.05[a]	0.54
1.511	0.322	4.08	3.81±0.05	1.80
0.550	0.508	0.94	3.86±0.05	0.32
1.550	0.491	2.75	3.80±0.05	0.92
1.033	—	—	3.82±0.00	—
2.064	—	—	3.79±0.05	—
Fresh faeces (DM 28.36%)				
1.053±0.022[a]	0.210±0.011[a]	1.42	4.81±0.11[a]	0.60
2.169±0.154	0.239±0.053	2.57	4.83±0.08	1.09
3.042±0.061	0.251±0.014	3.45	4.95±0.08	1.49
4.204±0.116	0.282±0.056	4.23	4.90±0.05	1.82
5.148±0.013	0.238±0.072	6.13	5.00±0.06[b]	2.69

a: Mean±SE.
b: Incomplete combustion was encountered, frequently.

The dry matter content of the VD-6 sample was 48.63%. Itoh and Tano[8] reported that corn starch samples containing 50% or more dry matter was combustible without any primer or drying, while Owens *et al.*[12] showed that silage samples with more than 55% dry matter required no additional primer for complete combustion. The energy value obtained on the fresh sample was, as shown in Table 3, somewhat higher, though not significant, than the value of dried ones. This result agrees with those reported by Bratzler and Swift[3], Colovos *et al.*[4] and Owens *et al.*[12].

Table 3. Effect of drying method on determination of faecal energy

Drying method	Sample Wt. g	DM in %	PEF Wt. g	kcal/g DM of sample	% of FD value
FD	1.68±0.20[a]	90.12	—	4.67±0.04[a]	100.0
AD	1.53±0.30	90.71	—	4.65±0.04	99.6
VD-4			—	not combustible	
VD-6	3.80±1.60	48.63	—	4.00±0.15*	85.7
VD-12	1.71±0.28	92.78	—	4.70±0.10	100.6
FD	1.46±0.29	93.53	—	4.64±0.02	100.0
AD	1.63±0.10	90.47	—	4.62±0.03	99.6
Fresh	3.98±0.14	24.13	0.32±0.06	4.73±0.05	101.9

*: Significantly different from FD value ($P<0.05$).
a: Mean±SE.

4. Effects of acidifying treatment on energy value and nitrogen content of faeces

The bulk faecal sample was mixed thoroughly and some part was taken for the analysis of fresh matter. The remainder was divided into two portions. One portion was mixed with 50%

H_2SO_4 at a rate of 1 ml per 100 g sample. It was then dried in two ways, AD and FD. The other unacidified portion was directly dried in similar ways. Energy and nitrogen were determined on these samples.

The effect of drying with or without acidification is shown in Table 4. Of these factors, drying effect on energy loss was greater than acidification. The losses encountered with acidified sample were less than those in the unacidified, especially in AD. The results are in agreement with those of Hartfiel[7] working on poultry faeces and of Uchida[14] on silage.

Both factors also affected the magnitude of the loss of faecal nitrogen. The losses reported here were up to 10 to 16% for AD and 4 to 5% for FD compared with the fresh value, respectively. Among these losses only the difference between the fresh sample and the AD without acidification was significant. Other workers[1,4,13,15] reported various losses of nitrogen on drying, ranging from 3.23 to 34.2%. The results obtained here are in agreement with the general observation.

Table 4. Effect of drying and acidification on determination of faecal energy and nitrogen content

Drying method	Acidification	DM in sample %	Energy			Nitrogen	
			Sample Wt. g	kcal/g DM of sample	% of fresh value	%/DM of sample	% of fresh value
Fresh	none	28.36	3.62±0.67[a]	4.92±0.06[a]	100.0	3.42±0.09[a]	100.0
FD	H_2SO_4	86.08	1.07±0.06	4.73±0.01	96.1	3.28±0.01	95.5
	none	84.80	1.04±0.05	4.75±0.03	96.5	3.25±0.04	95.0
AD	H_2SO_4	90.62	1.23±0.23	4.55±0.33	92.5	3.10±0.02	90.6
	none	91.04	1.16±0.08	4.44±0.02*	90.2	2.85±0.02*	83.3

*: Significantly different from fresh value ($P<0.05$).
a: Mean±SE.

During drying of the fresh samples volatile materials other than water may be evaporated, which is suspected to have resulted in some underestimation of the energy and nitrogen for the airdried samples. The magnitude of this loss must depend on the contents of these volatile materials[5,9,12,14].

5. Effects of primer, drying and acidification on urinary energy value

The results are shown in Table 5. The value obtained with cellulose as the primer was about 6% lower than that with PEF, but the difference was not significant. Although the number of

Table 5. Effect of primer, drying and acidification on determination of urinary energy value

Primer	Drying method	Acid	kcal/100 ml of sample	Calorific ratio of sample to primer
PEF	AD	H_2SO_4	9.17±0.08[a] (100.0)	0.5
Cellulose	AD	H_2SO_4	8.62±0.25 (94.0)	1.3
PEF	FD	H_2SO_4	5.83±0.05 (100.0)	
PEF	AD	H_2SO_4	5.93±0.05 (101.7)	
PEF	AD	HCl	5.94±0.09 (101.9)	

a: Mean±SE.

analysis was small, the use of cellulose was accompanied by a large standard error. Nijkamp[10,11] showed in comparison of these two primers that the results obtained with cellulose were usually 2–5%, even up to 10% occasionally, lower than those with PEF. The results of this experiment are in agreement with these observations.

The value for the FD sample was slightly lower, though not significant, than that of the oven dried. Urine may be dried either by FD or AD at 40°C, however, occasional occurrence of frothing in FD might result in some losses. With regard to the type of acidification, HCl produced higher value, 0–12%, than H_2SO_4, though the data are not listed in the Table. It was also shown by Nijkamp[10,11]. According to Nijkamp[10] when HCl was used in excess the difference became bigger because of the reaction of chlorine ion with the metallic wall of the bomb. Thus, although generally either acid may be used, the tendency of overestimating the energy value when even slightly in excess, would make H_2SO_4 the better choice.

Summary

A method for determining the gross energy of fresh faeces and urine of pig, and nitrogen contents of faeces was investigated under the comparison with various procedures.

Polyethylene film (PEF) was found to be a better primer than cellulose on the energy determination of both fresh faeces and urine.

While either sulphuric or hydrochloric acid might be used for storage of the samples, excess of hydrochloric acid tended to give gross overestimation of the energy, and hence, acidification of both faeces and urine by sulphuric acid seemed preferable.

The energy of fresh faeces could be directly determined without drying by combustion of fresh sample, 3 to 4 g, rolled into a PEF bag weighing about 0.3 g as shown in Fig. 1.

About 25 ml of urine sample could be combusted after acidification with sulphuric acid to pH 6, pipetted into a tarred PEF (ca. 0.15 g), and dried in a conventional air oven at 40°C.

Significant nitrogen losses were observed between the fresh and dried (air oven dried at 60°C, without acidification) faeces.

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