Use of Chromic Oxide-paper as an Indicator in Digestibility Studies with Pigs

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Abstract Three experiments were conducted to evaluate the use of chromic oxide-paper which contained approximately 42% of chromic oxide, on dry matter basis, as an indicator for the determination of the digestibility with pigs. The first and the second experiments compared the fecal recovery of chromic oxide-paper with that of chromic oxide-powder and of 4N-HCl insoluble ash, respectively. The third experiment studied the effects of collection time of feces on the digestibilities of dry matter, crude protein and energy using the chromic oxide-paper method. The fecal recovery of chromic oxide administered as paper was in the range of 99.2-101.5% and the digestibilities determined by this method were practically identical to those obtained by the total collection method. The fecal recoveries of the chromic oxide administered in the form of powder and of the 4N-HCl insoluble ash were 89.8 and 90.9%, respectively, and digestibilities determined by those two indicator methods were significantly ($P < 0.05$) lower than those determined by the chromic oxide-paper and total collection methods. There was no significant difference ($P > 0.05$) in the digestibilities among the three collection periods (08:30-12:00, 12:00-15:30 and 15:30-08:30), indicating that the time for fecal collection is not critical.

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In determining the digestibility of animal diets the total collection method and indicator methods are commonly used. Indicator methods have the advantage that they dispense with the quantitative measurement of feed intake and feces output. Although chromic oxide is most widely used as the indicator in pig digestion trials, some workers have been hesitant about accepting the chromic oxide method because of their inability to quantitatively recover 100% of the ingested indicator^{1,2)}. Recovery rates of 75 to 87% of chromic oxide have been reported by MOORE³⁾ and ISHIKAWA⁴⁾.

Recently the use of 4N-HCl insoluble ash as an alternative to either chromic oxide or total collection have been evaluated in determining the digestibility in pigs^{1,2)}. However, the results obtained with the use of insoluble ash have not always been satisfactory. In some trials reported by MCCARTHY *et al.*^{1,2)} the digestibilities of energy or crude protein obtained by the insoluble ash method were lower than those obtained by total collection method, which suggested that some loss of insoluble ash might have occurred.

The objective of the present study was to evaluate the use of chromic oxide-paper developed for ruminants by CORBETT *et al.*⁵⁾, for the determination of the digestibility with pigs.

Materials and Methods

Preparation of chromic oxide-paper and purification of chromic oxide-powder Chromic oxide-paper which was purchased from Nippon Kayaku Co. Ltd., Tokyo was shredded into pieces of about 1×1 mm, weighing about 0.2 mg. It contained approximately 42% chromic oxide on the dry matter basis. Chromic oxide-powder which was purchased commercially was purified as described by WHITBY and LANG⁶⁾.

Feeding and preparation of feces samples Pigs were placed individually in the metabolism cages and were given 40 g/kg body weight once a day at 08:30 of one of the experimental diets shown in Table 1. In each experiment, pigs were weighed at the beginning of the preliminary period and kept on a constant ration throughout the experimental period. Water was allowed ad libitum.

Feces were collected and were dried in a 60°C forced-air oven. Then, dried feces were allowed to equilibrate with air moisture, weighed, ground through a 1 mm screen in a laboratory mill and sampled for subsequent analyses.

Experiment 1 The administration of chromic oxide-paper was compared with the administration of chromic oxide-powder by examining daily fecal recovery of chromic oxide. Also, the digestibilities of dry matter and crude protein obtained by the

Table 1. Compositions of the experimental diets*

Ingredient	Diet 1	Diet 2
Ground yellow corn	30	22
Milo	—	22
Barley meal	23	22
Wheat bran	29.9	12
Defatted rice bran	5	4
Soybean meal	4	9
White-fish meal	3	4
Alfalfa meal	2.5	2.5
Calcium carbonate	0.6	0.7
Dicalcium phosphate	0.8	0.8
NaCl	0.45	0.5
Vitamin and mineral mixtures**	0.4	0.4
L-lysine	0.1	—
DL-methionine	0.15	0.1
Composition (by analyses)		
Dry matter (%)	87.7	86.8
Crude protein (N×6.25, %)	14.4	15.1
Gross energy (kcal/g)	—	3.87

* 0.1% of chromic oxide-powder or chromic oxide-paper was added to each diet. **Providing (per kg diet): Vitamin A, 16,000 IU; Vitamin D, 3,200 IU; Vitamin E, 16 IU; thiamin, 3.2 mg; riboflavin, 16 mg; Vitamin B₆, 3.2 mg; pantothenic acid, 7 mg; niacin, 3.2 mg; choline, 221 mg; folic acid, 1.6 mg; Zn, 48 mg; Fe, 40 mg; Mn, 40 mg; Cu, 8 mg; I, 0.8 mg.

two chromic oxide methods were compared with those obtained by the total collection method.

Six castrated male pigs of an average initial weight of 50 kg were given diet 1 for a 3-day preliminary period and were allotted to two treatments of three pigs each, during the following 10-day collection period, in which 0.1% of either chromic oxide-paper or chromic oxide-powder had been added to diet 1. Feces were collected once daily.

Experiment 2 The fecal recovery of chromic oxide in the pig administered with the chromic oxide-paper was compared with that of the acid insoluble ash. Also, digestibilities of dry matter, crude protein and energy obtained by chromic oxide-paper, acid insoluble ash and total collection methods were compared.

Four castrated male pigs, weighing approximately 25 kg at the beginning of the experiment, were given diet 2 to which 0.1% chromic oxide-paper had been added. The experiment consisted of a 7-day preliminary period and a 10-day collection period. Feces were collected once a day.

Experiment 3 Three castrated male pigs, weighing approximately 25 kg at the beginning of the experiment were used to examine the effects of time of collection of the feces on digestibilities of dry matter, crude protein and energy by the determination with chromic oxide-paper method. They were given diet 2 to which 0.1% chromic oxide-paper had been added. After a 7-day preliminary feeding, feces were collected at 08:30, 12:00 and 15:30 daily throughout a 4-day collection period.

Chemical and statistical analyses Gross energy was measured for feed and feces by the use of a bomb calorimeter, and dry matter and nitrogen were determined by methods of AOAC⁽⁷⁾. Chromic oxide and acid insoluble ash was determined by the method described by BRISSON⁽⁸⁾ and by MCCARTHY *et al.*⁽¹⁾, respectively. The content of chromic oxide in diets containing chromic oxide-paper was obtained by calculation from the chromic oxide content of the paper. Data were statistically analyzed by a *t*-test.

Results and Discussion

Experiment 1 Fig. 1 is a graphic representation of the daily fecal recovery of

Fig. 1. Daily fecal recovery of chromic oxide in pig by days after initial feeding in Experiment 1 (O, chromic oxide-paper; ●, chromic oxide-powder).

Table 2. Fecal recoveries of chromic oxide in pig administered with chromic oxide-powder or chromic oxide-paper and digestibilities of dry matter and crude protein determined by the methods of markers and total collection (Experiment 1, Diet 1)*

Method	Recovery (%) of chromic oxide	Digestibility (%)	
		Dry matter	Crude protein
Chromic oxide-powder	89.8±1.3 ^a	74.4±0.2 ^a	72.9±0.8 ^a
Chromic oxide-paper	101.5±1.4 ^b	77.8±0.3 ^b	76.6±0.6 ^b
Total collection	—	77.2±0.2 ^b	75.9±0.5 ^b

* Values represent mean±standard error of the mean for three pigs. Recoveries of chromic oxide and digestibilities were calculated based on the analyses of an aliquot of the total feces voided in days 5-10. ^{a,b}Values in the same column with different superscripts are significantly different ($P<0.05$).

chromic oxide in the pig administered with chromic oxide-paper or chromic oxide-powder during a 10-day collection period. This indicates that recovery of chromic oxide in the administration with both paper and powder comes into equilibrium in less than 3 or 4 days after the initial feeding of the diet containing chromic oxide. In Table 2, the recovery of chromic oxide in the pig administered with chromic oxide-paper in the feces collected from 5 th to 10 th days was compared with that administered as chromic oxide-powder. The mean recovery of chromic oxide in the administration with chromic oxide-paper was achieved completely and therefore, the mean digestibilities of dry matter and crude protein agreed with the values obtained with the total collection method. While, in the case of the chromic oxide-powder, the mean digestibilities of dry matter and crude protein were significantly ($P<0.05$) lower than those obtained with total collection method because of a low fecal recovery of chromic oxide. These results are consistent with the findings of other workers^{1,3,4}.

Experiment 2 The mean recoveries of markers in the feces and the mean digestibilities of dry matter, crude protein and energy obtained by the three methods are presented in Table 3. The mean recovery of acid insoluble ash in the feces was

Table 3. Fecal recoveries of markers and digestibilities of dry matter, crude protein and energy in pig determined by the methods of chromic oxide-paper, acid insoluble ash and total collection (Experiment 2, Diet 2)*

Method	Recovery (%) of marker	Digestibility (%)		
		Dry matter	Crude protein	Energy
Chromic oxide-paper	99.2±0.9 ^a	79.6±0.2 ^a	79.3±0.7 ^a	78.8±0.3 ^a
Acid insoluble ash	90.0±1.3 ^b	77.5±0.3 ^b	77.2±0.6 ^b	76.6±0.3 ^b
Total collection	—	79.8±0.1 ^a	79.5±0.6 ^a	78.9±0.2 ^a

* Values represent mean±standard error of the mean for four pigs. Recoveries of marker and digestibilities were calculated based on the analyses of an aliquot of the total feces voided in 10 day collection period. ^{a,b}Values in the same column with different superscripts are significantly different ($P<0.05$).

significantly ($P < 0.05$) lower than that of chromic oxide in the pig administered with the chromic oxide-paper. The mean digestibilities of dry matter, crude protein and energy by acid insoluble ash method were significantly ($P < 0.05$) lower than those obtained by chromic oxide-paper method or total collection method. McCARTHY *et al.*^{1,2)}, in the experiments using pigs, have reported higher digestibilities of crude protein and energy when they compared the results of total collection method with those of the acid insoluble ash method.

The fecal recovery of chromic oxide administered as chromic oxide-paper was almost achieved completely and therefore, no significant differences ($P > 0.05$) between the mean digestibilities of dry matter, crude protein and energy obtained by chromic oxide-paper method and those by total collection method were observed. These results are consistent with those in Experiment 1 with diet 1.

Experiment 3 Effects of collection time of feces on the digestibilities as determined by the chromic oxide-paper method were examined in Experiment 3 and the results were shown in Table 4. There was no significant difference ($P > 0.05$) among the collection periods in the mean digestibilities of dry matter, crude protein and energy. These results indicate that the time of fecal collection is not critical for the components of the diet examined.

Table 4. Influence of the time of sampling the feces on the digestibilities of dry matter, crude protein and energy in pig determined by the chromic oxide-paper method (Experiment 3. Diet 2)*

Collection time	Digestibility (%)		
	Dry matter	Crude protein	Energy
08 : 30—12 : 00	78.9 ± 0.7	75.5 ± 1.6	78.2 ± 0.7
12 : 00—15 : 30	79.6 ± 0.6	76.9 ± 0.7	78.8 ± 0.7
15 : 30—08 : 30	78.7 ± 0.7	76.6 ± 1.1	77.5 ± 0.8
Total collection	79.7 ± 0.3	77.1 ± 0.9	78.8 ± 0.3

* Values represent mean ± standard error of the mean for three pigs. Digestibilities were calculated based on the analyses of an aliquot of the total feces voided in each collection time in four day collection period. No significant differences ($P > 0.05$) were noted within comparison groupings.

The indicator used in the studies of digestibility should satisfy the following requirements; a) its fecal recovery is almost complete, and b) there should be little variation with time in the ratio of the fecal concentrations of indicator and nutrients studied.

Chromic oxide conventionally administered as the form of powder was, in the past, considered to be an accurate indicator. However, considerable doubt was cast on this assumption when workers such as MOORE³⁾ found that the fecal recovery of chromic oxide was not complete and therefore, the digestibilities determined by the chromic oxide method were lower than those obtained by the total collection method.

Recently there has been the development of an alternative method of estimating the digestibilities in pig¹⁾. This method involves the use of acid insoluble ash as indicator. However, the fecal recovery has not always been complete^{1,2)}. Also, a low fecal recovery of acid insoluble ash was shown in the present study (Experiment 2).

The present experiments were conducted to evaluate the use of chromic oxide-paper as an indicator. The results indicated that the fecal recovery of chromic oxide in the administration with paper was almost achieved completely and that there was no significant difference among the digestibilities obtained with the three collection periods. Therefore, shredded chromic oxide-paper seems to satisfy the requirements mentioned above and to be an ideal indicator for the study of digestibility in pigs.

A disadvantage of chromic oxide-paper is that the amount added to diets must be weighed accurately for the calculation of chromic oxide content in diets. Direct measurement of chromic oxide content in diets would be unreliable because of variations occurring in sampling. Before weighing the paper it must be equilibrated with respect to moisture content in the environment.

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豚の消化試験における指標物質としての 酸化クロムペーパー

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豚における消化率測定のため指標物質として、酸化クロムペーパーについて、糞中への回収率および採糞時刻にともなう消化率の変動を子豚を用いて検討した。供試した酸化クロムペーパーは、酸化クロムを乾物換算で約42%含有する市販のもので、これを1mm四方に切断し飼料に0.1%配合して用いた。実験1および2では酸化クロムペーパーの糞中への回収率を、それぞれ、従来から指標物質として用いられている酸化クロム粉末および酸不溶性灰分の回収率と比較した。その結果、酸化クロムペーパーとして投与した酸化クロムの糞中への回収率は99.2-101.5%となり、それによって算出した消化率は全

糞採取法による値と大差なかった。しかしながら、粉末として投与した酸化クロムおよび酸不溶性灰分の回収率はそれぞれ、89.8および90.9%となり、これらを指標物質として算出した消化率は全糞採取法あるいは酸化クロムペーパー指標物質法による値よりも有意に($P<0.05$)低かった。実験3では、1日を3時刻、すなわち、08:30-12:00, 12:00-15:30および15:30-08:30に区分して採糞し、各時刻における消化率を比較したが、乾物、粗蛋白質およびエネルギーのいずれの消化率においても有意差は認められなかった。

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