STUDIES ON THE FAECES

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Received September 23, 1968

In medical science the term coprology is frequently used. It means the science of faeces and is generally applied only to diagnostic study of faeces. However, the examination of the faeces reveals not only diagnostic criteria, it also throws light on the physiology and ecology of the animals. In this paper are presented some contributions to physiological coprology.

According to its origin the faeces material is generally divided in two fractions: food residues and metabolic material. This seems, however, to be a too simplified division. In fact there are in the faeces three different fractions (Paloheimo 1962 p. 1; 1966, p. 86): 1) An exclusively exogenous fraction (= food residues), containing substances which during the passage through the alimentary tract have undergone at most slight transformations. 2) An exclusively endogenous fraction, containing e.g. mucus, bile constituents, and calcium phosphates. 3) A bacteria fraction, containing living bacteria and bacteria debris, the origins of which are food substances as well as endogenous material.

Using the ultra-sound one can produce microscopic preparations in which the food residues of plant origin appear clean washed (Figs. 1 and 2). The picture in Fig. 3 is taken from an unwashed preparation. Fig. 4 shows a typical picture with bacteria debris. This picture is very different from Fig. 5 which is obtained from rumen fluid. Aggregations of living bacteria are found in the faeces only on the surfaces of undigested food particles (Fig. 6). Fig. 7 shows cells detached from the intestinal epithelium and Fig. 8 uric acid crystal globules from chicken excrements. All these pictures are taken in our department.

Sieve analysis of faeces

The sieve analysis can be employed for different purposes. If the quality of the diet of a wild animal is to be studied, a distribution of the faeces material into different sieves makes the task easier. Regarding farm animals, the sieving of the faeces reveals whether the concentrate food has been ground finely enough. Intestinal parasites and their segments

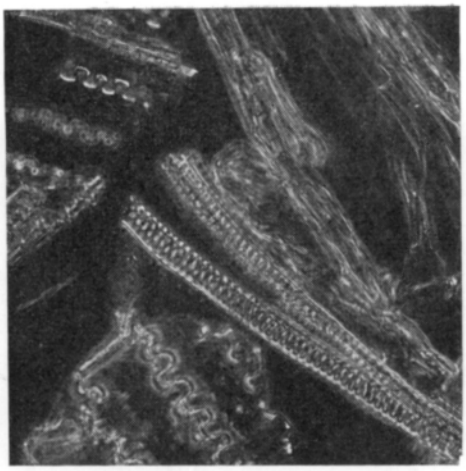

Fig. 1. Washed plant tissue fragments from from cow faeces. × 300.

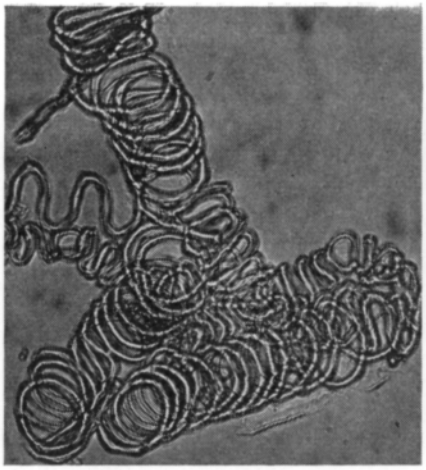

Fig. 2. Entangled spirals from plant tracheids. From cow faeces. × 400.

Fig. 3. Unwashed plant tissue fragments from cow faeces. × 400.

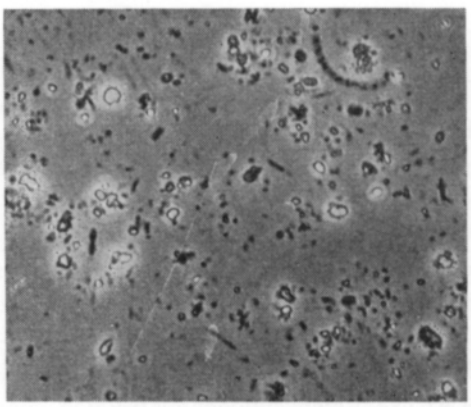

Fig. 4. Bacteria and bacteria debris from cow faeces. × 800.

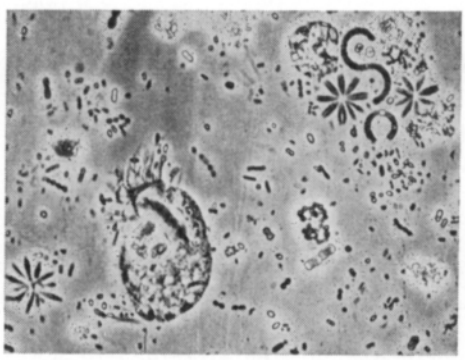

Fig. 5. Microorganisms in the rumen fluid of a cow. × 500.

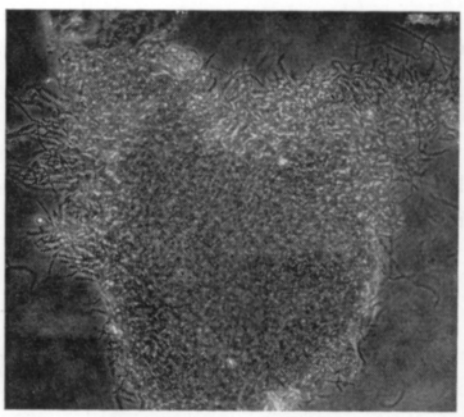

Fig. 6. Living bacteria on the surface of a food particle rich in nutrients. From cow faeces. × 300.

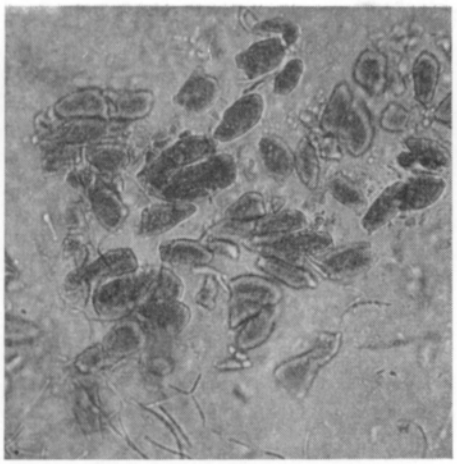

Fig. 7. Cells detached from the intestinal epithelium and attached on the surface slime of cow faeces. × 500.

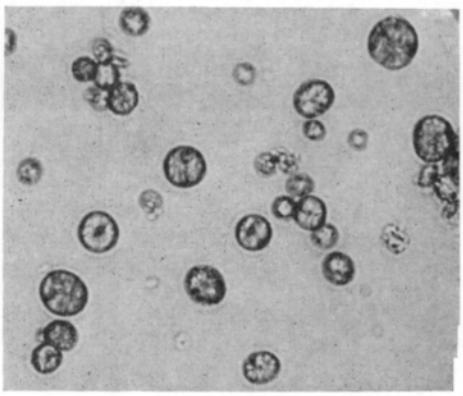

Fig. 8. Uric acid crystal globules from chicken excrements. × 800.

can be detected. The percentages of the fractions reveal the efficiency of the mastication. In this department the last mentioned purpose has been the most essential.

Sieving is a conventional procedure. Ideally, a sieve should effect a sharp separation between undersize and oversize particles, and the largest undersize particle should be only just smaller than the smallest oversize particle. In practice, appreciable portions of both fractions have the same size range. Especially in the faeces of herbivorous animals the food particles are mostly pin-formed and are retained on a sieve plate if they fall on it flatways but fall through if they fall longitudinally. By shaking the sieve or mixing the material upon it one gives each particle repeated possibilities to fall longitudinally. But even if the sieving is performed under running water, much time and water is needed before the sharp separation of oversize and undersize particles is completed. The undersize fluid is now sieved through the next sieve whereat the amount of water to be poured through the third sieve still increases. There are still two features which tend to restrict the fall of small particles through the sieve: a blinding of the sieve by the wedging of particles into the openings, and the sticking to each other of individual particles. In the sieving of faeces a preliminary shaking of the material with water is not sufficient for detaching the slime and bacteria from the vegetable particles, but an ultrasonic treatment is of considerable help in this respect. HELLSTRÖM (1958, p. 49) was probably the first to use ultrasonic treatment in studies on faeces. See also HELLSTRÖM and AAMISEPP (1965, p. 27). In the following paragraphs our method and some of its applications are presented.

Circular sieves 19 cm in diameter were used. The depth of the sieves was 5 cm. 4 sieves were used for each analysis. The sides of the square openings were 2.0 mm, 1.0 mm, 0.5 mm, and 0.1 mm.

The weight of the samples must depend on the dry matter content of the faeces. The dry matter of the sample should be about 3 g. If the faeces contain e.g. 15 % dry matter, a 20 g sample is convenient. The sample is mixed with water in a 200 ml beaker and decanted in portions into a larger beaker the total amount of water being about 200 ml.

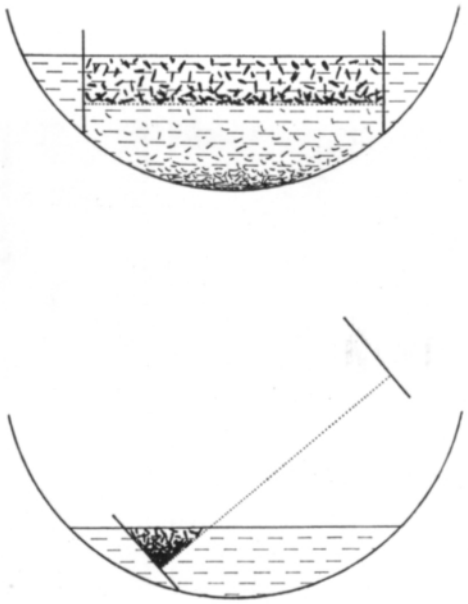

Fig. 9. Above: the sieve in the dish (primary position). Below: the sieve placed obliquely in the dish.

In the second beaker the mixture is treated with an ultrasonic probe (Blackstone) about 15 minutes in room temperature.

The mixture is poured from the beaker on the 2 mm sieve, and the undersize is allowed to fall into a pail. Then the sieve is placed in a large porcelain dish in which water is poured on the sieve until the water surface lies 2—3 cm above the sieve plate (Fig. 9). The suspension is then kept in motion with a spoon and at a moment when the particles are scattered as evenly as possible the sieve is abruptly raised. The undersize is poured into the pail and the treatment is repeated with the oversize. This time, as the undersize suspension is no longer very dense, one may half a minute after the raising of the sieve when the main part of the particles has sunk, lay the sieve slowly down whereat the water will rise again upon the sieve plate. The suspension is agitated and the sieve abruptly raised. This procedure can be repeated once or twice before the undersize is poured into the pail. When the sieve is again in the dish, water is poured on it and the treatment is continued until the undersize is practically free from particles. The sieve is now placed obliquely in the dish which must still contain water (Fig. 9), and from the lower part of the sieve the particles are ladled with a spoon into a weighed smaller dish. The drying of the sieve with the particles is not recommended. The further details in the removal of the final oversize from the sieve may be left to the analyser's own judgment. The water in the smaller dish is evaporated and the dry matter content determined and calculated as percentage of the faeces dry matter.

The undersize fluid poured into the pail after the sievings through the 2 mm sieve, is poured into the 1 mm sieve. The treatment is continued in the same way as with the 2 mm sieve. Then follow the sievings through the 0.5 mm sieve and, finally, through the 0.1 mm sieve. The amount of the undersize fluid increases gradually so that two or three pails are needed. The fluid from the last sieving (with the 0.1 mm sieve) can be discarded if one does not continue the fractionation by sedimentation. In the following paragraphs the fractions

are named: 2 mm, 1 mm, 0.5 mm, 0.1 mm, and < 0.1 mm. The value of the last fraction is obtained by subtraction. The 2 mm fraction may contain particles whose critical diagonal is considerably over 2 mm.

Table 1 shows the results of sieve analyses in which parallel determinations (a and b) have been made. The table shows that the a and b values agree rather well. The discrepancies are not likely to be caused so much by the analyser's work as by the differences in

Table 1. Parallel sieve analyses of some faeces samples, % on dry matter basis. (All figures for the < 0.1 fraction, including the average figures, are calculated by subtraction).

Fraction	Animal and feeding	a	b	Average	Animal and feeding	a	b	Average
2 mm	Cow I	3.0	4.6	3.8	Sheep	0.6	0.7	0.7
1	(Very rich	9.0	8.7	8.9	(Hay)	1.5	1.6	1.6
0.5	feeding)	18.9	19.3	19.1		9.3	9.7	9.5
0.1		19.1	21.1	20.1		37.6	38.0	37.8
< 0.1		50.0	46.3	48.1		51.0	50.0	50.4
2 mm	Cow II	3.4	3.7	3.6	Horse	9.4	8.7	9.1
1	(Medium feeding)	7.0	8.1	7.6	(Hay, oats)	15.4	17.0	16.2
0.5		18.4	17.5	18.0		29.3	26.8	28.1
0.1		22.1	20.9	21.5		18.7	20.1	19.4
< 0.1		49.1	49.8	49.3		27.2	27.4	27.2
2 mm	Heifer	1.7	2.0	1.9	Swine	3.9	3.3	3.6
1	(Mainly hay)	3.2	3.2	3.2	(Skim milk,	12.4	11.4	11.9
0.5		14.0	13.9	14.0	cereals, silage)	27.2	27.1	27.2
0.1		33.0	33.3	33.2		11.6	12.3	12.0
< 0.1		48.1	47.6	47.7		44.9	45.9	45.3
2 mm	Cow III	2.4	2.5	2.5	Cow IV	1.6	1.2	1.4
1	(Hay 6 kg)	3.3	3.6	3.5	(Hay 6 kg)	2.5	2.1	2.3
0.5		11.4	11.6	11.5		11.4	10.0	10.7
0.1		31.4	32.4	31.9		32.0	33.1	32.6
< 0.1		51.5	49.9	50.6		52.5	53.6	53.0
2 mm	Cow III	2.1	2.7	2.4	Cow IV	1.6	2.3	2.0
1	(Hay 6 kg,	3.7	4.1	3.9	(Hay 6 kg,	2.3	3.9	3.1
0.5	Sugar 1 kg)	12.6	11.4	12.0	Sugar 1 kg)	12.4	14.7	13.6
0.1		33.8	34.3	34.1		31.8	32.0	31.9
< 0.1		47.8	47.5	47.6		51.9	47.1	49.4
2 mm	Cow III	2.7	2.5	2.6	Cow IV	2.0	1.8	1.9
1	(Hay 6 kg,	4.6	3.5	4.2	(Hay 6 kg,	3.6	3.9	3.8
0.5	Sugar 2 kg)	17.0	18.3	17.7	Sugar 2 kg)	17.0	19.1	18.1
0.1		40.4	39.1	39.8		39.9	35.5	37.7
< 0.1		35.3	36.6	35.7		37.5	39.7	38.5

the samples. As faeces are not quite homogenous material, two fresh 20 g samples of the mixed primary sample may have a somewhat differing composition. Evidently the average values give a relatively correct picture of the character of the primary sample. The results obtained from the faeces of cows I and II, which were on the usual stall feeding of the University farm, are surprisingly similar in spite of the difference in the feeding intensity. The conformity appears in all fractions. In the sheep faeces, the three first fractions are considerably smaller perhaps owing to the sheep's habit of selection when eating hay, and/or to the more thorough mastication. For the horse, the sum of the three first percentage figures is 53.4, the corresponding sums for the cows I and II being 31.8 and 29.1. The percentage of the < 0.1 fraction is for horse lower than for any other animal presented in the table. The results pertaining to the cows III and IV are especially noteworthy because they demonstrate the usefulness of the sieve analyses. With the said cows a digestion trial was made in which the influence of sugar upon the digestibility of hay was studied. During all periods the cows received timothy hay 6 kg daily, during the second period 1 kg sugar

Table 2. Some average figures from the digestion trial with cows III and IV. (See also Table 1).

Food	Fraction < 0.1 mm	Digestibility of org. matter %
Hay 6 kg	51.8	64.1
Hay 6 kg, sugar 1 kg	48.5	62.7
Hay 6 kg, sugar 2 kg	37.1	50.4

was added, and during the third period the animals were given 2 kg sugar. The main results are collected into Table 2. From the sieve analyses we give here only the percentages of the < 0.1 mm fractions. It appears that 1 kg sugar per day had hardly any detrimental effect on digestibility. However, the sieve analysis revealed a distinct, although slight, decrease in the mastication effect. When the sugar ration was raised to 2 kg, the digestibility decreased considerably, while at the same time the coarsness of the faeces rose abruptly (the < 0.1 fraction decreased).

Table 3 shows that in the cases presented in the table, 54—76 % of the ash in the faeces went through all the sieves, the corresponding figures for the crude protein being 75—92. In many cases the ash percentage in the combined 2 mm + 1 mm fraction is rather high owing to the stones remaining on the coarser sieves. For the protein, the percentage is also often in the 2 mm + 1 mm fractions higher than in the 0.5 mm fraction. This may depend on the fact that the larger particles contain in their cells more indigested plant protein than the smaller ones. In cases where the 0.1 mm fraction contains more protein than the coarser fractions, slime and bacteria aggregations may have fastened on the sieve. In these cases one must remember that all nitrogen of the faeces does not belong to substances with a N-content of about 16 %, corresponding to the usual coefficient 6.25. The proteins of slime and bacteria, and especially bacteria debris, have a considerably lower N-content. The derivatives of chlorophyll and bile pigments are also relatively poor in nitrogen. Thus, if

Table 3. Ash and crude protein of the fractions.

Animal and feeding	Fraction	Ash % of		Crude protein % of	
		fraction	ash in faeces	fraction	crude protein in faeces
Cow II	2 mm + 1 mm	11.5	7.7	3.8	3.1
(Medium feeding)	0.5	7.2	8.5	3.4	4.2
	0.1	8.0	11.4	4.9	7.1
	< 0.1	22.9	72.4	24.5	85.6
Cow III	2 mm + 1 mm	17.9	7.6	2.8	1.5
(Hay 6 kg)	0.5	5.2	4.4	2.1	2.1
	0.1	5.1	11.7	2.0	5.5
	< 0.1	20.1	76.3	21.1	90.9
Cow III	2 mm + 1 mm	11.4	9.7	2.2	1.2
(Hay 6 kg, sugar 2 kg)	0.5	4.7	9.4	1.9	3.2
	0.1	4.1	19.4	0.9	3.3
	< 0.1	14.9	61.5	27.5	92.4
Sheep	2 mm + 1 mm	28.6	4.9	0.6	0.1
(Hay)	0.5	4.7	3.7	2.8	2.3
	0.1	5.0	15.7	2.6	8.0
	< 0.1	17.8	75.8	21.8	89.6
Horse	2 mm + 1 mm	4.7	14.4	3.9	9.7
(Hay, oats)	0.5	4.0	14.3	3.0	7.8
	0.1	7.4	17.0	3.8	7.3
	< 0.5	16.3	54.3	28.8	75.2
Swine	2 mm + 1 mm	10.3	9.8	5.6	4.4
(Skim milk, cereals, silage)	0.5	7.2	11.5	1.9	2.7
	0.1	13.6	9.2	4.0	2.6
	< 0.5	26.4	69.5	37.3	90.3

the crude protein percentage in the < 0.1 mm fraction is 24.5 (cow II), the real content of nitrogenous substance in this fraction is probably much higher.

Evidently the ultrasonic treatment used in the analysis has no appreciable breaking down effect on the vegetable particles of the faeces. On potato starch corns this treatment does not seem to have any effect whatever.

Extractions with 80 % and absolute ethanol

In developing the method for determination of the complex of cell wall substances in plant products PALOHEIMO and VAINIO (1965, p. 305) observed that two successive extractions with boiling 80 % ethanol and an after-extraction with absolute ethanol dissolved

from plant materials not only lipids and sugar but also significant amounts of ash ingredients and nitrogenous substances. E.g. from the lucerne grass dry matter in the first extraction with 80 % ethanol dissolved 35.3 %, in the second 2.8 %, and in the extraction with absolute ethanol 0.3 %. All extracts together contained 18.8 % ash and 25.5 % crude protein. The amount of dissolved ash was 62.7 % of the total ash, and the dissolved crude protein 36.3 % of the total crude protein. After such experiences it was interesting to see how the faeces material would react in similar extractions.

For the extractions 10 g fresh faeces were weighed, placed in a mortar, and ground, mixed with 80 % ethanol. The suspension was decanted into an Erlenmeyer flask, the residue was again ground with ethanol, and so on. Finally also the coarse remainder was transferred into the flask. The volume of 80 % ethanol should be 500 ml and the boiling time 4 hours. The mixture is filtered through a Whatman No. 4 paper using a funnel heater because some of the extracted substance is soluble only in a boiling solvent. The main part of the insoluble matter is left in the Erlenmeyer flask, and finally the substance restrained on the filter is rinsed into the flask with 80 % boiling ethanol. This is followed by another boiling with 80 % ethanol, this time for 2 hours, and 2 h. boiling in absolute ethanol. The three filtrates are united and the total amount of the extracts determined. The filtrates are quite clear when hot but after cooling dark sediment is observed which dissolves again if the mixture is heated.

In the following, as also in Tables 4 and 5, the term »Ethanol extract» is used for the sum of extracts obtained by the above described treatments. The term is not fully correct because 80 % ethanol was used as solvent. The extraction of faeces with boiling water is a difficult operation, and especially the obtaining of clear filtrates or, if centrifugation is used, clear supernatants is very difficult. On the other hand, the filtration after boiling with 80 % ethanol is quite easy. In preparing the ethanol extracts it is advisable to extract first with 80 % ethanol because in the boiling with absolute ethanol esterifications may occur. After the extractions with 80 % ethanol only a small amount of extractable substance is left for the after-extraction with absolute ethanol. It is advisable to begin the extractions with 80 % ethanol also because filtrations after these are easier than after extractions with absolute ethanol. Also more of the total extract is obtained if one begins with 80 % ethanol.

It is seen from the figures in Table 4 that the percentage of ethanol extract in faeces of herbivorous animals, swine, rat, and hen varies from about 12 to 23. The highest are the figures for mink 1 (56.5), calf 1 (45.1), and mink 2 (40.2). In the case of calf 1, the faeces were = meconium, and evidently the main part of the ethanol extract consisted of bile pigments. Also in some other cases these substances and their derivatives evidently have a significant share in the extract. As for the meconium, the extractions have not been exhaustive: with longer extraction times percentages above 60 can be reached. The figures in the columns 2, 3, and 4 are not additive because the ethanol extract contains nitrogenous substances and also ash constituents. The ash in faeces contains also stones, sand, and clay. Crude protein in Table 4 is a very conventional item (cf. p. 242). Especially regarding hen faeces it should be kept in mind that the excrements of birds contain uric acid (33 % N, 208 % crude protein) a considerable part of which is dissolved by 80 % ethanol. However, it appears from the table that the ethanol extract of the faeces contains significant amounts of nitrogenous substances, in the case of mink about half of the crude protein of the faeces.

Table 4. Ethanol extract of faeces, and some other items.

Feeding etc.: Cow 1, rich feeding in stall. — Cow 2, 7 kg hay. — Cow 3, pasture. — Calf 1, 7 days, milk. — Calf 2, newborn, faeces = meconium. — Sheep, hay. — Horse 1, hay and oats. — Horse 2, pasture and hay. — Horse 3, pasture. — Swine 1, cereal meal, kitchen offal, silage. — Swine 2, oat meal and skim milk. — Swine 3, molassed pulp and skim milk. — Human, wholemeal bread, milk, eggs, meat. — Rat, wholemeal bread, milk. — Mink 1, slaughter offal. — Mink 2, slaughter offal and pretreated cereal flour. — Hens 1 and 2, oats and protein concentrates.

Animal	1 Faeces dry matter %	2 % of faeces dry matter: Ash	3 % of faeces dry matter: Crude protein	4 % of faeces dry matter: Ethanol extract	5 Crude protein % of ethanol extract	6 Crude protein in ethanol extract % of crude protein in faeces
Cow 1	14.0	15.1	16.1	13.1	14.1	11.5
» 2	20.9	12.2	10.0	18.5	11.6	21.6
» 3	12.5	16.2	17.1	23.0	16.3	21.7
Calf 1	39.2	3.1	42.6	45.1	13.2	13.9
» 2	23.3	8.6	64.5	27.5	10.7	4.5
Sheep	36.9	11.0	10.3	15.3	12.4	19.0
Horse 1	23.3	7.2	8.8	16.5	11.2	20.6
» 2	17.9	16.3	10.2	17.6	10.3	18.1
» 3	18.1	11.2	9.1	16.0	10.7	18.8
Swine 1	25.7	19.9	17.1	17.2	7.6	7.6
» 2	34.4	12.1	8.3	11.9	9.1	12.8
» 3	26.0	24.0	31.6	21.7	21.0	14.4
Human	24.8	22.8	26.7	26.0	14.1	13.8
Rat	21.7	8.0	28.6	18.0	20.8	13.1
Mink 1	24.2	18.0	34.2	56.5	30.6	49.0
» 2	27.8	28.1	31.8	40.2	41.0	51.8
Hen 1*)	28.9	19.0	24.4	16.8	80.6	—
» 2*)	30.7	19.4	32.4	19.7	87.3	—

*) The stones were removed.

Table 5. Ash in the ethanol extract of faeces.

Animal[1])	Ash % of ethanol extract	Ash in ethanol extract % of ash in faeces
Cow 1	20.0	17.4
» 2	9.5	14.6
Calf 1	3.7	53.2
» 2	11.6	37.1
Sheep	11.2	15.7
Horse 1	14.2	31.7
Swine 1	14.8	12.8
Rat	5.8	12.5
Mink 1	13.4	42.2
» 2	9.3	13.2

[1]) The same animals are listed correspondingly in Table 4.

Table 5 shows that the ethanol extract of faeces can contain considerable amounts of ash. In some cases about 1/3 or 1/2 of the total ash is found in the ethanol extract. It should be pointed out that at least the ethanol extract of cow faeces may contain HCl-insoluble ash. E.g. in the case of Cow 2, nearly half of the extracted ash was not dissolved by HCl, and this was the case also with another cow on the medium stall feeding. Systematic studies on this point were not carried out.

As the above studies have shown that by extractions with 80 % ethanol followed by after-extraction with absolute ethanol considerable amounts of both nitrogen-free and nitrogenous substances can be extracted from faeces, it would seem advisable to use ethanol extractions as a preliminary step to a detailed examination of the occurrence of certain substances in faeces. Such an examination may prove helpful in finding several physiologically and even pathologically significant indications.

Summary

The purpose of this paper is to present some contributions to physiological coprology. A sieve analysis system for the fractionation of faeces is proposed and results obtained by this system presented. Ash and crude protein were determined from the fractions. The other part of the paper deals with the extraction of faeces first with boiling 80 % ethanol and subsequently with boiling absolute ethanol. Crude protein and, in some cases, also ash were determined from the extracts. The use of such extractions as a preliminary step towards a more detailed examination of certain substances in faeces is proposed.

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SELOSTUS

FAECESTUTKIMUKSIA

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Kirjoituksessa esitetään eräitä tutkimuksia fysiologisen koprologian alalta. Aluksi selostetaan seulonta-analyysijärjestelmä sonnan fraktioimiseksi. Tuhka ja raakaproteini on määritetty fraktioista. Järjestelmällä saatuja tuloksia esitellään. Kirjoituksen toisessa osassa käsitellään sonnan uuttamista ensin kiehuvalla 80-prosenttisella etanolilla ja sen jälkeen kiehuvalla absoluuttisella etanolilla. Raakavalkuainen ja eräissä tapauksissa myös tuhka on määritetty uutteista. Kysymyksessä olevan kaltaista uuttamismenetelmää ehdotetaan edeltäväksi vaiheeksi yksityiskohtaisempiin tutkimuksiin, joissa selvitetään tiettyjen aineiden esiintymistä sonnassa.