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DIVISION OF CHEMISTRY

The Total Fatty Acids and Other Ether-Soluble Constituents of Feedstuffs

BY

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THE TOTAL FATTY ACIDS AND OTHER ETHER-SOLUBLE CONSTITUENTS OF FEEDSTUFFS.

BY J. B. RATHER, ASSISTANT CHEMIST.*

In previous publications of this Experiment Station (Fraps and Rather, Bulletins Nos. 150 and 162) it has been shown that the unsaponifiable matter in the ether extract of hays and fodders averages about 58 per cent. of the total extract, and is of much lower digestibility than the saponifiable matter. It has also been shown that chloroform extracts comparatively large percentages of material from hays and fodders previously extracted with ether, and that this extract contains fatty acids. A method was devised by means of which it was possible to separate the constituents of the extract into three fractions: unsaponified (largely wax alcohols), uncolored saponified (fatty acids) and colored saponified (chlorophyll and related compounds). The above method will be hereinafter designated the Digestion Method. The low digestibility of the ether extract of hays and fodders was shown to be due to the presence of wax alcohols, waxes, chlorophyll, and other substances not as easily digested as fats or as fatty acids. In the hays and fodders examined the unsaponified matter of the ether extract varied from 36 to 72 per cent. and averaged 58 per cent.

It had already been shown by Stellwaag (*Land. Versuch. Stat. 37*, 148) that the ether extract of seeds and of concentrated feeding stuffs contain unsaponifiable matter. This varied from 34.5 per cent. for malt germ to 0.5 per cent. for cocoanut cake. In most of the feeds examined, however, from 2 to 8 per cent. of the ether extract consisted of unsaponifiable matter.

In view of the above results, it appears that a rapid and reasonably accurate method is desirable for the systematic removal of unsaponified matter in the determination of fats.

HISTORICAL.

The writer has been unable to find any mention of an analytical method for the determination of fats in feeding stuffs, suitable for routine work, that makes any provision for the removal of unsaponifiable matter.

Attempts have been made to produce a purer ether extract by filtering through animal charcoal. Hayward states (*Ann. Rpt. Md. Exp. Sta.*, 1890, 90-103) that the use of animal charcoal gives lower results than the official method, varying from 0.1 per cent. in corn meal to 3.6 per cent. in dried tomatoes, and averaging 1.5 per cent. In the light of our present knowledge of the nature of the constituents of the ether extract of feeding stuffs, it is evident that the possibility of getting a pure fat by such means is slight.

*Under the general direction of G. S. Fraps, Chemist.

Poole (*J. Am. Chem. Soc. 19*, 877-881) recommends a method for the determination of fat in feces in which the ether extract is saponified with alcoholic potash, the chloresterol removed from the soap with ether, and fatty acids determined in the residue.

The method of Liebermann and Szekely (*Pfluger's Archiv. 72* (1898) 360-366), for the determination of fat, consists of saponifying the fat in the sample without extracting the sample with ether, by means of aqueous potassium hydroxide, dissolving the liberated fatty acids in petroleum ether, titrating an aliquot with 0.1/N alcoholic potash and phenolphthalein, and weighing the resulting soap. The amount of fat which corresponds to the soap is then calculated. This method is recommended by Haas and Hill (*Chemistry of Plant Products*, London, 1913, p. 26) as a reliable and convenient method applicable to the determination of fat in fodders, meat, feces, and physiological work in general. The method is, however, open to several serious objections. It makes no provision for the elimination of unsaponifiable matter, or for the elimination of chlorophyll products, both of which are present in large amounts in the petroleum-ether soluble matter of hays and animal excrements therefrom. The calculation of the fat depends on the assumption that the fatty acid is stearic or some other fatty acid, that the fats are present as triglycerides, and that there are no free fatty acids present. The mean molecular weight of the fats of plant products vary with the nature of the sample under examination, the fats are not necessarily present as triglycerides, and free fatty acids, always present to a considerable extent, may make up 87 per cent. (Stellwaag, *loc. cit.*) of the so-called fat.

Kumagawa and Suto (*Biochem. Zeit. 8*, 212) determine fat in plant products by saponifying the sample direct with 5/N aqueous sodium hydroxide, acidifying and shaking the fatty acids out with ether in a separatory funnel. The ethereal solution is filtered, evaporated, dried and taken up with light petroleum. The solution is filtered, evaporated, and dried to constant weight. The unsaponifiable constituents of the ether extract of plants are readily soluble in petroleum ether, and that solvent undoubtedly dissolves chlorophyll when that substance is present. The method, therefore, cannot be considered to give accurate results.

It has long been known that ether does not extract all of the ether-soluble constituents of plant and animal products, but the extraction is generally considered to be complete enough for all practical purposes. However, in the case of human feces, fatty acids may be present as calcium soaps, which are not soluble in ether. A number of methods have been devised for the extraction of such fatty acids in feces, most of them depending upon the solubility of the fatty acids in the ether after treatment of the soap with hydrochloric acid. Among methods of this type may be mentioned that of Folin and Wentworth (*J. Biol. Chem. 7*, 421-6), in which ether saturated with HCl gas is used to extract the feces.

Dormeyer (*Pfluger's Arch. Physiol. 61*, 341-343) states that only 75 to 85 per cent. of the fat of meat can be obtained by extracting 100 hours with ether. He proposes digestion of the meat with pepsin-hydrochloric acid and shaking the fat out with ether. Voit, on the

other hand (*Ztschr. Biol. 35,* 555-582) claims that when the ether extraction is properly carried out, with small amounts of substance as free from water as possible, 24 hours' extraction will remove 95 per cent. of the fat. Tangl and Weiser (*Pfluger's Arch. Physiol. 72,* 360-366) state that Liebermann and Szekley's method gives as good results as Dormeyer's method on meat and feces. Bogdanow (*Pfluger's Arch. Physiol. 68,* 431-433) states that meat extracted with alcohol after previous extraction with ether yields almost as much ether-soluble matter as was extracted by ether from the sample direct. He regards this excess over the original ether extract as fat intimately mixed with something insoluble in ether but soluble in alcohol.

Browne (*Proc. 20th Conv. A. O. A. C.*) states that pepsin digestion of residues from ether extraction renders part of some substances soluble in ether. He cites steer feces and mixed feeds containing molasses as examples.

Methods for the determination of unsaponifiable matter of fats and oils fall into two divisions: (1) extraction of the soap solution with solvents, either hot or cold, and (2) extraction of the dried soap with solvents. A number of the methods are described by Lewkowitsch (*Chem. Technology and Analysis of Oils, Fats and Waxes,* Vol. I, 5th Ed , pp. 456-460) and will not be described here.

The method proposed in this bulletin differs from both these general processes in that the fatty acids, after saponification, acidification and solution in ether, are precipitated from that solvent with alkali. This process, so far as the writer is aware, has never been used before.

We have been unable to find any mention of the use of *alcoholic* soda or potash, the basis of another method proposed in this bulletin, as a solvent for fats. Alcoholic soda probably dissolves less non-fats than aqueous soda; soap solutions in alcohol are more easily manipulated, and fats are soluble in alcohol alone. The advantages of alcohol over water thus appear to be considerable. The use of aqueous alkali would not prevent the contamination of the soap with unsaponified material, because the latter is both soluble in soap solutions, and emulsifiable with aqueous alkali.

AN IMPROVED METHOD FOR THE DETERMINATION OF TOTAL FATTY ACIDS AND OTHER CONSTITUENTS OF THE ETHER EXTRACT.

As mentioned above, a new process was devised for the separation of the unsaponified matter from the fatty acids. This method for the separation of the constituents of ether extracts into three fractions is essentially as follows: Saponify the ether extract, acidify and dissolve in ether, precipitate the fatty acids from ethereal solution with aqueous alkali and remove by washing with water. Acidify the soap with acetic acid and shake with petroleum ether to dissolve fatty acids and then with ethyl ether to dissolve the residue.

The novel feature of the above process consists in the method of separating the unsaponified from the saponified material. The method of fractionating the saponified matter into colored and uncolored portions

is essentially the same as that already reported from this Station (Fraps and Rather, Bulletin No. 162).

This method, designated the Precipitation Method, is given in detail below.

Extraction of Ether-Soluble Matter: Dry 10 grams of the sample, or 5 grams if it contains more than 4 per cent. ether extract, and extract with redistilled ether in a suitable continuous extractor for 16 hours. The extraction flask should have a capacity of 75 to 150 c.c. and should be of such a type that the extract may be readily and completely poured out. Knorr flasks should not be used. If it is desired to determine the total ether extract, the flask should be dried and weighed before the extraction. Evaporate off the ether after the completion of the extraction, and dry and weigh if the percentage of ether extract is desired.

Determination of Unsaponifiable Matter: Add 20 c.c. of approximately 2/N alcoholic sodium hydroxide, and boil under a reflux condenser for one hour. Evaporate nearly to dryness and add 3½ c.c. glacial acetic acid, or its equivalent in weaker acetic acid. Add 50 c.c. of redistilled ether and warm to dissolve the extract. Add 25 c.c. water and warm a minute more. Transfer to a 500 c.c. pear-shaped separatory funnel with a short neck and wash out the flask with 5 successive 20 c.c. portions of ether, pouring the washings in the funnel. Turn funnel on its ide and shake gently. The two layers should now be clear and the aqueous layer nearly colorless. Draw off the lower layer into a 500 c.c. Erlenmeyer flash. Add to the ethereal solution 10 c.c. of a warm 1:2 aqueous sodium hydroxide solution, turn the funnel on its side and shake gently. Allow the precipitate to settle, add 25 c.c. warm water, hold funnel in vertical position and give rotary motion. Allow the two layers to separate and draw off the clear aqueous solution into the Erlenmeyer flask, leaving any emulsion in the funnel. Repeat and then shake gently as above with 5 successive 30 c.c. portions of cold water, allowing a short time for the two solutions to separate, and add the washings to the soap solution in the Erlenmeyer flash. Transfer the ethereal solution to a tared 200 c.c. flask, evaporate or distil the ether and dry to constant weight in a steam oven at 100° C.

Correction for Fatty Acids in Unsaponified (for material high in fatty acids and low in unsaponified only): Add 20 c.c. of 0.2/N hydrochloric acid to the ethereal solution of the unsaponified matter in the separatory funnel before evaporation, stopper and shake vigorously. Draw off the aqueous layer and discard. Evaporate ethereal solution and weigh. Heat to boiling with 20 c.c. alcohol, titrate with N/10 sodium hydroxide and phenolphthalein, running a blank on the alcohol. Multiply the corrected reading by .28 (or .56 if 5 gm. is taken). The result is percentage fatty acids in the unsaponified matter. Subtract this from the percentage of unsaponified matter and add it to the fatty acids. This amount of fatty acids dissolved in the ethereal solution of unsaponified matter is on an average 16 milligrams for concentrated feeding stuffs, and this figure may be used instead of determining the correction if very accurate figures on the percentage of unsaponifiable matter are not desired.

Determination of the Fatty Acids: Heat the soap solution on a steam bath to remove dissolved ether, shaking gently from time to time. This must be done carefully to avoid frothing of the soap. Cool the soap solution nearly to room temperature under the tap, add 8 c.c. of glacial acetic acid or its equivalent in weaker acetic acid and extract in the separatory funnel with 40 c.c. of redistilled petroleum ether, distilling below 75° C., shaking violently. Draw off aqueous layer, hold funnel in a vertical position, giving it a rotary motion, and let stand a minute. This will cause the suspended matter to settle in a compact mass at the bottom of the funnel. Draw off this portion into the flask containing the aqueous mixture. Extract the aqueous mixture 3 times more in a similar manner. By using a pear-shaped funnel, and following the above procedure, filtration to remove suspended matter may generally be avoided. Shake well with two 50 c.c. portions of water to remove suspended matter and traces of inorganic substances, allowing any emulsion to go into aqueous mixture. Extract the latter a fifth time with petroleum ether, and after washing this extract twice with small portions of water, add to the other extracts. Evaporate and dry to constant weight. If the evaporation is carried to completeness on a steam bath, the flask being turned on its side to facilitate removal of gasoline fumes, the product may be dried to constant weight in 3 or 4 hours. This fraction is fatty acids.

Determination of Saponified Residue: Acidify further the aqueous residue from the extraction of the fatty acids, with hydrochloric acid, warm and extract 5 times as above with 40 c.c. portions of ethyl ether. Wash the combined extracts twice with 50 c.c. portions of water, evaporate and dry to constant weight. Include any suspended matter in this fraction.

Tests of the method were made by examining the groups of ether-soluble material obtained with it, by the Digestion Method already developed in this laboratory (*loc. cit.*) and by other means. The various factors affecting the accuracy of the process are discussed below. The results are expressed in percentage of the original sample.

Completeness of Saponification: Incomplete saponification will cause low results in the saponifiable material and high results in the unsaponifiable. That the saponification is complete at the end of 1 hour under the conditions of the method is shown by the following tests:

1. Samples of ether extract of wheat bran, corn chops, and milo maize chops were saponified for 1 hour with 20 c.c. 1/N alcoholic soda and with 20 c.c. 2/N alcoholic soda. The unsaponified was determined by the Precipitation Method. The results follow:

		Per cent Unsaponified	
		1N Alkali	2N Alkali
12996	Wheat bran	.21	.24
12999	Corn (chops)	.13	.12
13030	Milo Maize (chops)	.20	.18
	Average	.18	.18

Concentration of the alkali did not decrease the percentage of unsaponified.

2. The unsaponified matter was determined by the Precipitation Method in three hays and three excrements, the product was then resaponified and the unsaponified determined by the Digestion Method of Fraps and Rather. The results follow:

	Unsaponified Matter, Per cent.	
	A. By Precipitation Method.	B. In (A) by Digestion Method.
7980	.47	.43
7991	.43	.43
7999	1.02	1.02
8002	.68	.68
8011	1.17	1.13
8013	2.14	1.99
Average	0.98	0.95

The results are practically the same in all cases. It is to be expected that the results would be slightly lower by (B) because of the large amount of manipulation required.

Unsaponified Material in Fatty Acids: This would be due to incomplete separation of the two groups. Determinations by the Digestion Method showed it to be negligible in amount.

In percentage of the original sample the results were as follows:

	Maximum	Minimum	Average
12 Concentrated feeding stuffs	.04%	.00%	.02%
6 Hays and excrements, 20 determinations	.05%	.01%	.03%

Fatty Acids in Unsaponified Material: This could be due to incomplete washing of the ethereal solution with water to remove soap, or to hydrolysis of the soap to form free fatty acids. The acids were set free and determined by the process described in the Precipitation Method, it being found that no hydrochloric acid remained upon evaporation and drying after such treatment. The results are as follows:

	Maximum	Minimum	Average
12 Concentrates, 42 determinations	.50%	.04%	.16%
12 Hays and excrements, 27 determinations	.09%	.00%	.05%

The fatty acids in the unsaponified material of the concentrates varied greatly, even in duplicate determinations on the same samples. Increasing the number of washings of the ethereal solution of the unsaponified to eight did not decrease the amount of fatty acids in the unsaponified fraction. We believe the presence of the acids is due to hydrolysis of the soap and not to incomplete removal of the latter. We think that the correction given in the Precipitation Method should always be applied in the examination of plant and animal products rich in fat and low in unsaponifiable material.

The agreement of the determinations of fatty acids in the unsaponified matter is much more satisfactory in the case of products poor in fat and high in unsaponified matter. The unsaponified in the fatty acids, and the fatty acids in the unsaponified, very nearly balance each other in determinations on these products. The difference is only 0.02 per cent., well within the limit of error of determination.

Completeness of the Extraction of the Fatty Acids by Petroleum Ether: The fifth extraction of the fatty acids with petroleum ether was dried and weighed separately as a test of the completeness of extraction. The results follow:

	Maximum	Minimum	Average
12 Concentrates, 18 determinations	.06%	.01%	.02%
12 Hays and excrements, 12 determinations	.02%	.01%	.01%

Five extractions with petroleum ether seem to be sufficient to extract practically all of the material soluble in that medium, under the conditions of the method.

The method as given differs slightly from that on which the above tests were made. The change was made for convenience in manipulation and does not affect the accuracy of the results.

The Saponified Residue: Under this heading we include ether-soluble saponifiable matter, which is not dissolved by petroleum ether after acidifying with acetic acid. The reason acetic acid is used instead of hydrochloric acid, is that the latter renders chlorophyll more readily soluble in petroleum ether than the former. Petroleum ether is recognized as a solvent for fats, and is said to be more desirable than ether under certain conditions. All fatty acids are more or less readily soluble in petroleum ether, and since the ratio of solvent to solute in our work is about 1000 to 1, and the weight of the fifth extraction usually less than 2 milligrams, it does not seem likely that any fatty acids remained undissolved after five successive extractions with petroleum ether. The petroleum ether extracts a fraction of the saponifiable matter which is relatively free from chlorophyll, and, for that reason, we know that, in the case of the samples containing that substance, the fraction of the ether-soluble plant material designated by us as "saponified residue" consists, in part, of a substance which is not a fatty acid. This does not make up a very great percentage of the fraction, however, and we find the latter in considerable amount in samples free from chlorophyll.

A sample of lard compound weighing 30 milligrams yielded only 4 milligrams of the "saponified residue," which would be equivalent to 0.04 per cent. on a 10-gram sample. Since the sample contained commercial fats of only average purity, it is possible that pure fats would be entirely free from the fraction designated "unsaponified residue."

Completeness of Extraction of Saponified Residue: The fourth extraction of this fraction was dried and weighed separately. The results follow:

	Maximum	Minimum	Average
12 Concentrates, 24 determinations	.04%	.00%	.02%
12 Hays and excrements	.02%	.01%	.02%

Four extractions appear to be sufficient.

Saponified Residue in Fatty Acid Fraction: The fatty acids may be contaminated with petroleum-ether soluble non-fatty acids. The only data we have on this point is qualitative. The fatty acids were all tinged with green in samples where chlorophyll was present, and the depth of color was apparently related to the amount of chlorophyll present. Compared with the saponified residue fraction, however, they were very lightly colored. Chlorophyll constitutes a part only of the latter fraction, and its coloring power is very great. We believe the amount of chlorophyll, at least, in the fatty acid fraction, is relatively small.

Saponified Residue in the Unsaponified Fraction: This is indicated by green color and by quantitative tests. We have already discussed the amount of acids in the unsaponified. We have assumed these to be fatty acids. Our reason for this is that, in every case where chlorophyll is present in the sample, the unsaponified fraction was entirely free from green color.

Washing Required to Remove Hydrochloric Acid After Liberating Acids in Unsaponified: The method of liberating the acids in the unsaponified as used by Fraps and Rather is somewhat long; we substituted a process in which the acids were liberated in ethereal solution before evaporation, and the excess of mineral acid washed out with water. Later it was found that the washing was unnecessary when the unsaponified matter is evaporated to dryness. Samples of unsaponified matter equivalent to one or more per cent. in the sample yielded an acid value of zero by the process as finally adopted in the method. Fatty acids are, however, always found in the unsaponified matter of concentrates. Comparative determinations are given below:

	Per cent Acid in Unsaponified.	
	20 c.c. 2N HCl washed 5 times	20 c.c. 2N HCl not washed
1	.06	.04
2	.04	.04
3	.03	.06
Average	.04	.05

There is practically no difference in the results by the two processes, and as the one in which no washing is done is much shorter, it is to be recommended. This process reduces the time required for the correction to a negligible amount. The process has additional confirmation in a large number of determinations not reported here.

Checking of Duplicate Determinations: All determinations were made at least twice. The results given in the tables in this bulletin are

averages. A few duplicate determinations, none run at the same time, are given here to show the accuracy possible with the method.

		Unsaponified	Fat Acids	Non-fat Organic Acids
12996	Corn Chops	.17	2.96	.43
		.24	3.05	.44
		.23	3.14	.29
		.31	3.17	.....
		.21	.....	.....
13030	Milo Maize Chops	.22	.....	.59
		.23	.....	.69
		.22	.....	.....
		.18	.....	.....
		.20	.....	.....
13045	Cold Pressed Cottonseed	.....	6.66	.....
		.....	6.47	.....
		.....	6.33	.....
13023	Rice Bran	.....	.....	.77
		.....	.....	.72
		.....	.....	.68

Results on the hays and on both concentrates and roughages by the Alcoholic Soda Method checked equally as well.

A NEW METHOD FOR THE EXTRACTION OF TOTAL FATTY ACIDS AND OTHER ETHER-SOLUBLE CONSTITUENTS OF FEEDING STUFFS AND EXCREMENTS.

This process was devised with the intention of securing a substitute for the method of determining fats as fatty acids in feed stuffs. The results obtained are in every case much higher than the fatty acids of the ether extract, and do not justify its use as a substitute for the latter. The method is valuable in another direction, which will be discussed later.

The method consists in the digestion of the sample for 1 hour with 2/N alcoholic soda, removal of the alcohol, addition of water and shaking the acidified mixture of ethyl ether. The ether solution is fractionated, as described in the Precipitation Method above. The method in detail is given below.

Digest 10 gm. of the sample, or 5 gm. if it is bulky, or contains more than 4 per cent, ether extract, with 50 c.c. of approximately 2/N alcoholic soda, for 1 hour under a reflux condenser. Filter with the aid of suction through asbestos in a carbon funnel of suitable size and wash 10 times with the boiling alcohol. Transfer to a dish with water and evaporate to about 10 c.c. Transfer with hot water to a pear-shaped separatory funnel. Acidify with 10 c.c. glacial acetic acid and extract the warm solution 5 times with 50 c.c. portions of ethyl ether (redistilled), removing the bulk of the suspended matter in the manner described under "Determination of Fatty Acids" in the Precipitation Method. Separate the constituents of this extract as described under the Precipitation Method, beginning with the addition of the 1:2 sodium hydroxide solution. Discard the insoluble matter remaining after the determination of the saponified residue.

Most of the factors affecting the accuracy of this method are the same as those discussed under the Precipitation Method. Two additional factors require notice. They are discussed below.

Completeness of Saponification: The percentage of fatty acids obtained under different conditions is an indication of the completeness of saponification. Six samples of hays and excrements were used. The fatty acids were determined (A) in an extract prepared by digesting 10 grams of the sample 1 hour with 20 c.c. N alcoholic soda and 100 c.c. alcohol, (B) in an extract prepared as above except 40 c.c. 2/N alcoholic soda and 60 c.c. alcohol were used, (C) in an extract prepared by digesting 5 grams of the sample 1 hour with 50 c.c. 2/N alcoholic soda. The results follow:

	Percentage Fatty Acids.		
	(A)	(B)	(C)
6288	.19	.67	.54
6290	.26	.89	.91
6724	.32	1.15	1.06
7763	.37	1.05	1.00
7799	.21	1.49	1.48
7970	.85	1.49	1.72
Average	.37	1.12	1.12

There is a great difference between the results by methods (A) and (B), but very little between those by (B) and (C). We have included the process (C) in the method as finally used.

Completeness of Extraction with Ethyl Ether: In extracting the aqueous mixture of fatty acids, etc., with ethyl ether to separate it from ether insoluble matter, the fifth extract was dried and weighed separately. The results follow:

	Maximum	Minimum	Average
12 Concentrates	.06%	.01%	.02%
12 Hays and excrements	.02%	.01%	.01%

Five extractions seem to be sufficient.

The number of washings of the sample, after digestion with the alcoholic soda, was ten. This number was entirely arbitrary. The entire amount of chlorophyll is probably not removed by this number of washings, but we believe all fatty acids are removed.

The Solubility of Calcium Salts in Alcoholic Soda: A number of methods for the determination of fat in human feces have been proposed which aim to extract, in addition to glycerides and free fatty acids, the fatty acids present in the sample as lime soap. Acids are usually used for this purpose, either in connection with or before the use of ether. In order to see what effect our alcoholic soda process would have on lime soaps, we prepared lime salts of mixed fatty acids and determined the fatty acids soluble and insoluble in alcoholic soda under the conditions of the method, with and without an excess of lime ($CaCO_3$). The results follow:

	Dissolved	Insoluble	Solubility
1 Without lime	121 mg.	12 mg.	91%
2 With lime	85 mg.	4 mg.	95%

The amount dissolved is equivalent to 1.7 per cent. to 2.4 per cent. on a 5-gram sample, which is about the amount of fatty acids present in the alcoholic soda extract in excess of those of the ether extract. We believe that lime soap would be dissolved nearly completely from excrements under the conditions of the Alcoholic Soda method.

A STUDY OF FEED STUFFS WITH THE NEW METHODS.

Twelve samples of concentrated feeding stuffs, six samples of hays and six samples of sheep excrements from the hays were used. The concentrates consisted of some of the more common unmixed feeds on the Texas market, and were products or by-products of the seeds of corn, cotton, rice, wheat, milo maize, and kafir corn. They were fresh and in good condition. With the exception of one sample of "wheat shorts," No. 12996, which appeared to be ground wheat bran, the materials were true to name. The hays were prairie hay, and tabosa grass, both native Texas hays, sorghum, Sudan grass, and moth-bean hay, and Sudan straw. The Sudan straw was made from fully matured Sudan grass with the heads removed. All of these samples were in good condition.

Determinations of the ether-soluble constituents of the samples were made by the methods given above. In addition, determinations were made as follows: Ether Extract, by the A. O. A. C. Method; Constituents of the Ether Extract, by the Digestion Method of Fraps and Rather; and Fatty Acids in the Chloroform and Alcohol Extract Following Ether Extraction.

The Digestion Method is given in detail in Bulletin No. 162 of the Texas Experiment Station. It consists essentially in digesting the aqueous solution of the saponified ether extract in the warm, with successive portions of petroleum ether followed by ethyl ether, to remove the unsaponifiable matter, and treatment of the residue as described in the Precipitation Method given above.

Fatty acids were determined in the chloroform and alcohol extracts following ether extraction to ascertain whether ether, followed by chloroform, followed by alcohol, would extract a total of fatty acids equal to that extracted by alcoholic soda alone. The process was as follows:

Extract the dried residue from the ether extraction with chloroform for 16 hours. Remove solvent from extract and residue, and extract further in the same flask with alcohol for 16 hours. Determine the total fatty acids by the Precipitation Method, bringing the soap into solution as described under Alcoholic Soda Method.

In the case of the samples of hays and excrements, the fatty acids were determined in the chloroform extract alone, and the Digestion Method was used.

The ether, chloroform, and alcohol used in this work were redistilled in this laboratory.

THE TOTAL FATTY ACIDS.

In Table 1 are shown the results of the determination of fatty acids by the various methods.

The total fatty acids represent the free fatty acids in the original sample, the fatty acids from the glycerides, from saponifiable waxes, and lecithin. All feeding stuffs contain free fatty acids in appreciable amounts, and the free acids often make up the bulk of the fat. Likewise the glycerides may not be all triglycerides. For these reasons, and since fats are valuable as a food only because of their fatty acid content, we believe that it is better to express fat in terms of fatty acids.

TABLE 1.

Percentage of Fatty Acids in Feedstuffs and Excrements by Various Methods.

Laboratory No.		Ether extract.	Fatty acids in ether extract.		Fatty acids in chloroform and alcoholic extracts.	Total (A)	Fatty acids in alcoholic soda extract. Total (B)	Difference (B–A)
			Digestion Method.	Precipitation Method.				
12996	Wheat Shorts	3.79		3.08	0.37	3.45	4.43	0.98
12999	Corn chops	4.31		3.77	0.16	3.93	4.34	0.41
13021	Cottonseed meal	15.23		13.82	0.69	14.51	14.46	-0.05
13023	Rice bran	7.75		6.21	0.39	6.60	8.10	1.50
13030	Milo maize chops	3.22		2.05	0.37	2.42	2.92	0.50
13045	Cold pressed cottonseed	7.26		6.49	0.46	6.95	7.32	0.37
13091	Kaffir chops	3.20		2.57	0.34	2.91	3.18	0.27
13140	Corn bran	8.59		5.50	0.36	5.86	8.13	2.27
13150	Red rice	1.64		1.04	0.44	1.48	1.81	0.33
13166	Wheat bran	4.10		3.50	0.55	4.05	4.41	0.36
13172	Wheat shorts	2.65		2.22	0.27	2.49	2.84	0.35
13190	Rice polish	10.38		8.53	0.45	8.98	10.29	1.31
6288	Tabosa grass	0.92	0.15	0.30	0.08	0.38	0.61	0.23
6290	Excrement from tabosa grass	1.06	0.23	0.25	0.11	0.36	0.90	0.54
7724	Prairie hay	2.30	0.54	0.65	0.09	0.75	1.11	0.36
7763	Sudan grass	1.46	0.71	0.63	0.06	0.69	1.03	0.34
7799	Excrement from prairie hay	2.88	0.43	0.90	0.17	1.07	1.49	0.42
7970	Excrement from Sudan grass	1.90	0.29	0.25	0.27	0.52	1.61	1.09
7980	Sudan straw	1.44	0.45	0.67	0.06	0.73	1.29	0.56
7991	Sorghum hay	1.82	0.63	0.89	0.07	0.96	1.17	0.21
7999	Excrement from Sudan straw	1.71	0.35	0.39	0.14	0.53	1.64	1.11
8002	Moth bean hay	1.55	0.35	0.62	0.15	0.77	1.70	0.93
8011	Excrement from sorghum hay	1.78	0.40	0.62	0.05	0.67	1.71	1.04
8013	Excrement from moth bean hay	3.03	0.26	0.62	0.06	0.68	2.28	1.60
	Average for concentrates	6.01		4.90	0.40	5.30	6.02	0.72
	Average for hays and excrements	1.82		0.57	0.11	0.69	1.38	0.70

The fatty acids in the concentrates varied from 13.82 per cent. in cottonseed meal to 1.04 per cent. in red rice. They average 4.90 per cent., about 19 per cent. less than the average for the corresponding total ether extracts. The difference, however, in individual cases varies from 36 per cent. less with red rice to 16 per cent. less with wheat shorts, No. 13172.

The fatty acids in the hays and excrements varied from 0.25 per cent. in the excrement from tabosa grass and the experiment from Sudan grass to 0.96 per cent. in excrement from prairie hay. They

average 0.54 per cent., which is about 56 per cent. of the average of the corresponding total ether extracts.

Chloroform and alcohol extracted from the concentrates, which had previously been extracted with ether, from 0.16 to 0.69 per cent. of fatty acids, averaging 0.40 per cent.

The fatty acids in the acoholic soda extract of the concentrates varied from 14.46 per cent. in cottonseed meal, to 1.81 per cent. in red rice, with an average of 6.02 per cent. The difference between the sum of the fatty acids obtained with ether, chloroform and alcohol, and the fatty acids by the Alcoholic Soda Method of extraction varied from 0.05 per cent. in the cottonseed meal to 2.27 per cent. in corn bran. The average is 0.72 per cent.

The sum of the fatty acids obtained by successive extractions with ether, chloroform, and alcohol is less than the acids extracted from the concentrates by alcoholic soda alone.

It will be noted that the fatty acids by the Digestion Method are lower than by the Precipitation Method. This may be explained as follows: When the Precipitation Method was being developed for the concentrates, it was found that 5 extractions with *violent* shaking were required to remove the fatty acids. For the sake of uniformity this was applied to the hays and excrements. The only difference between the two processes consists in one additional extraction and more vigorous shaking by the Precipitation Method. It appears that the separation of the two saponifiable fractions is not entirely satisfactory.

The fatty acids extracted by chloroform only, after ether extraction, were determined in the hays and excrements. The fatty acids extracted by this solvent after previous extraction of the sample with ether varied from 0.05 per cent. in excrement from sorghum hay to 0.27 per cent. in excrement from Sudan grass, and averaged 0.11 per cent.

The percentage of fatty acids as determined by the Alcoholic Soda Method in the hays and excrements was in every case higher than the sum of the fatty acids extracted by ether followed by chloroform. The fatty acids varied from 0.61 per cent. in excrement from sorghum hay to 2.28 per cent. in excrement from moth-bean hay, and averaged 1.38 per cent. This is about 250 per cent. of the fatty acids in the ether extract and about 210 per cent. of the fatty acids extracted by both ether and chloroform.

The fatty acids extracted by the Alcoholic Soda Method from the 24 feeds and excrements were higher in every case than the fatty acids in the ether extract of these samples, averaging 123 per cent. as much for the concentrates and 250 per cent. as much for the hays and excrements from them.

The Alcoholic Soda Method proposed in this Bulletin gives higher results for the total fatty acids in feeding stuffs than does the method involving extraction of the sample with ether.

The higher results may be due in part to ether insoluble, alcohol soluble, incrustations on the fats and in part to fatty acids combined as lime or other ether-insoluble salts. This may be especially true of the excrements.

An Alcoholic Soda Method has been devised for the determination

of total fatty acids and other ether-soluble constituents of plants, by which process are extracted more fatty acids from plant products than ether and, in hays and excrement, twice as much as fatty acids as can be obtained by successive extraction of the sample with ether, chloroform, and alcohol.

An improved method of determining the total fatty acids and other constituents of ether extracts has been developed which is believed to be very accurate and rapid. Six determinations each of unsaponified matter, total fatty acids, and saponified residue, in ether extracts may be determined in about 24 hours, and require only about 3 or 4 hours of work. This method is believed to meet a need for a convenient analytical process for the determining fats as fatty acids in ether extracts when more than approximations or comparative results are required.

THE UNSAPONIFIED MATTER.

In Table 2 are collected the determinations of ether extract, unsaponified matter in the ether extract by the Precipitation and Digestion Methods, and unsaponified matter in the alcoholic soda extract. The unsaponified matter in the ether extract varied from 0.15 per cent. in red rice, to 0.60 per cent. in rice bran, and averaged 0.30 per cent. The ether extract in the concentrates varied from 1.64 per cent in red rice, to 15.23 per cent. in cottonseed meal, and averaged 6.01 per cent. Expressed in terms of percentage of ether extract, cottonseed meal is lowest in unsaponified matter, with 3.1 per cent; and red rice is highest with 9.2 per cent. The average is 5.8 per cent. Stellwaag finds the unsaponified matter in the ether extract of corn grain to be 3.7 per cent.; in cottonseed cake, 1.1 per cent.; and in wheat bran 7.5 per cent. Our results on corn chops, cottonseed meal, and wheat bran are 3.7, 3.1, and 6.1 per cent., respectively.

The unsaponified matter in the alcoholic soda extract of the concentrates is essentially the same in amount as that in the ether extract. It averages 0.32 per cent. of the sample.

It appears that, while the unsaponified matter in the ether extracts of the concentrates does not make up a very large percentage of the extract, it is large enough, when expressed in percentage of sample, to deserve attention in the case of rice bran, cottonseed meal, and rice polish.

In considering the results with the hays and the excrements therefrom, we find the unsaponified matter present in relatively large amounts. The ether extract of these products varies from 0.92 per cent. to 3.03 per cent., and averages 1.82 per cent. The unsaponified matter in the ether extract varies from 0.49 per cent. in sorghum hay to 1.99 per cent. in excrement from moth-bean hay, and averages 0.97 per cent. Expressed in percentage of extract, the unsaponified matter varies from 26.9 per cent. in sorghum hay to 74.2 per cent. in excrement from Sudan grass, and averages 52.7 per cent. On an average, more than half of the ether extract of these hays and excrements consists of non-fats. These results are similar to those already reported from this Station (Fraps and Rather, *loc. cit.*). They emphasize the conclusion made in the publication referred to, that "it is not correct

to use the term 'fats' or 'oils' to designate the ether extract of hays and fodders."

The unsaponified matter in the alcoholic soda extract is about the same as that in the ether extract in some cases, but averages 0.09 per cent. higher. The results by the former method of extraction, however, are considerably higher in the case of moth-bean hay and excrement from Sudan straw and excrement from moth-bean hay. The sum of the averages of determinations of unsaponified matter in the ether extracts of these samples, and that in the chloroform extracts following ether is 1.10 per cent., and the average for the alcoholic soda extracts is 1.06 per cent.

These results show that in order to determine the fats of hays and excrements by extractions with ether, with anything more than the merest approximation of the truth, there must be some method incorporated in the analytical process to eliminate the unsaponified matter. Likewise, the determination of fats in concentrates by extraction with ether would have a more positive value if the unsaponifiable were removed in the process of determination.

TABLE 2.

Unsaponified Matter in Some Feed Stuffs and Excrements by Various Methods.

Laboratory No.		Ether extract.	Unsaponified matter in Ether Extract. (Digestion Method.)	Unsaponified matter in ether extract (Precipitation)		Unsaponified matter in Alcoholic Soda Extract— In per cent of sample.
				In per cent of sample.	In per cent of ether extract.	
12996	Wheat shorts (bran) No. 1	3.79	0.23	0.23	6.1	0.24
12999	Corn chops	4.31	0.25	0.16	3.7	0.18
13021	Cottonseed meal	15.23	0.28	0.47	3.1	0.31
13023	Rice bran	7.75	0.18	0.60	7.7	0.65
13030	Milo maize chops	3.22	0.13	0.21	6.5	0.21
13045	Cold pressed cottonseed	7.26	0.16	0.29	4.0	0.23
13091	Kafir chops	3.20	0.56	0.26	8.1	0.26
13140	Corn bran	8.59	.....	0.29	3.4	0.34
13150	Red rice	1.64	0.14	0.15	9.2	0.21
13166	Wheat bran	4.10	0.18	0.25	6.1	0.25
13172	Wheat shorts	2.65	0.07	0.17	6.4	0.23
13190	Rice polish	10.38	0.19	0.51	4.9	0.70
6288	Tabosa grass	0.92	0.33	0.56	60.9	0.44
6290	Excrement from tabosa grass	1.06	0.50	0.70	66.0	0.75
7724	Prairie hay	2.30	1.05	0.84	36.5	0.78
7763	Sudan grass	1.46	0.53	0.55	37.7	0.49
7799	Excrement from prairie hay	2.83	1.78	1.68	59.4	1.74
7970	Excrement from Sudan grass	1.90	1.32	1.41	74.2	1.50
7980	Sudan straw	1.44	0.43	0.55	38.2	0.65
7991	Sorghum hay	1.82	0.58	0.49	26.9	0.55
7999	Excrement from Sudan straw	1.71	1.18	1.09	63.7	1.29
8002	Moth bean hay	1.55	0.73	0.66	42.6	1.03
8011	Excrement from sorghum hay	1.78	0.80	1.08	60.7	1.24
8013	Excrement from moth bean hay	3.03	1.76	1.99	65.7	2.20
	Average for concentrates	6.01	0.22	0.30	5.8	0.32
	Average for hays and excrements	1.82	0.92	0.97	52.7	1.06

Comparison of the Digestion and Precipitation Processes for the Separation of the Unsaponified Matter: The determination of the unsaponified matter in the ether extract of the concentrates by the Precipitation Method and by the Digestion Method gave results which

were, in most cases, in fair agreement. The averages of the determinations on the concentrates were 0.30 per cent by the Precipitation Method and 0.22 per cent by the Digestion Method. The results tend to be lower by the Digestion Method than by the Precipitation Method. In a few cases the differences were very great. We believe that the low results are due to incomplete extraction of the unsaponified. The Digestion Method, which was devised for roughages, proved very tedious on the concentrates on account of the formation of emulsions. In one case it was necessary to include the emulsion in the unsaponifiable and to extract the latter again the same number of times, after evaporation of the ether and dilution with water, in order to separate it with reasonable completeness from the soap solution. In the case of No. 13140, Corn Bran, the determination was not completed because of the formation of a very persistent emulsion.

No difficulty was experienced with emulsion in determinations by the Precipitation Method, and we believe it is a much more convenient, rapid and accurate process for the determination of unsaponifiable matter in fats and oils than has heretofore been proposed.

With the hays and excrements, the Precipitation Method gave, in some cases, higher results than the Digestion Method. The averages are, however, 0.97 per cent. for the former and 0.92 per cent. for the latter. We have shown that these higher results are not due to incomplete saponification, or to soap in the unsaponified, and it appears that even the vigorous treatment of the soap solution to remove unsaponified matter as directed in the Digestion Method was not entirely successful. So far as we know, the Digestion Method is the most vigorous treatment to remove unsaponifiable matter from the soap solution ever reported. It seems much more desirable, both from a standpoint of speed and of accuracy, to remove the fatty acids from the unsaponifiable, by the precipitation process, than vice versa.

We believe that the Precipitation Method described in this Bulletin is sufficiently accurate, speedy, and simple to allow it to be used as an analytical as well as a research method.

THE SAPONIFIED RESIDUE.

The results of the determinations of saponified residue in the ether extract and by the alcoholic soda method are shown in Table 3, together with the percentage of ether extract in the samples for comparison.

TABLE 3.

Percentage of Saponified Residue in Feed Stuffs and Excrement by Various Methods.

Laboratory No.		Ether extract.	Saponified residue in ether extract.		Saponified residue by alcoholic soda method.
			Precipitation method.	Digestion method.	
12996	Wheat shorts	3.79	0.39		0.71
12999	Corn chops	4.31	0.44		0.27
13021	Cottonseed meal	15.23	0.18		0.38
13023	Rice bran	7.75	0.72		0.91
13030	Milo maize chops	3.22	0.64		0.19
13045	Cold pressed cottonseed	7.26	0.24		0.26
13091	Kafir chops	3.20	0.60		0.24
13140	Corn bran	8.59	1.70		0.59
13150	Red rice	1.64	0.25		0.34
13166	Wheat bran	4.10	0.14		1.01
13172	Wheat shorts	2.65	0.12		0.31
13190	Rice polish	10.38	0.55		0.50
6288	Tabosa grass	0.92	0.18	0.37	1.57
6290	Excrement from tabosa grass	1.06	0.17	0.28	1.85
7724	Prairie hay	2.30	0.44	0.71	2.00
7763	Sudan grass	1.46	0.23	0.24	1.71
7799	Excrement from prairie hay	2.83	0.36	0.34	3.02
7970	Excrement from Sudan grass	1.90	0.34	0.34	2.62
7980	Sudan straw	1.44	0.16	0.43	1.75
7991	Sorghum hay	1.82	0.29	0.41	1.60
7999	Excrement from Sudan straw	1.71	0.20	0.15	2.44
8002	Moth bean hay	1.55	0.27	0.35	1.25
8011	Excrement from sorghum hay	1.78	0.25	0.46	2.87
8013	Excrement from moth bean hay	3.03	0.46	0.86	2.53
	Average for concentrates	6.01	0.50		0.48
	Average for hays and excrements	1.82	0.28		2.10

By the saponified residue, we mean the substances which were not dissolved by petroleum ether after treatment with acetic acid, but were dissolved by ether after further addition of hydrochloric acid.

The saponified residue in the concentrates, by the ether extraction method, varied from 0.12 per cent. in wheat shorts to 1.70 per cent. in corn bran. They averaged 0.50 per cent.

The individual variations in the percentage of the saponified residue in the ether extract in percentage of the latter are very great. The percentage in the cottonseed meal ether extract is about 1.1 per cent., while that in the corn bran is 19.8 and in milo maize chops 19.9 per cent.

The average is about 8 per cent. of the ether extract. Add to this the average amount of unsaponified matter in the same samples, and we have approximately 14 per cent of non-fats in the ether extracts of the concentrates examined. From these figures it appears that the use of the word "crude" in connection with fat obtained by extraction with ether is fully justified.

The saponified residue in the ether extract in the hays, and excrements from them, varies from 0.16 per cent, in Sudan straw to 0.46 per cent. in excrement from moth-bean hay, and averages 0.28 per cent. This is a little more than half as much as the average of the fatty acid content of the same samples and about 15 per cent of the ether extract. This fraction consists, in part, of chlorophyll derivatives.

The results show that the saponifiable matter of the ether extract of feeding stuffs may consist to a considerable extent of substances which are not fatty acids. This fact has already been brought out in regard to the chloroform extract of hays and fodders (Fraps and Rather, Texas Bulletin No. 162).

By the Alcoholic Soda Method the saponified residue in the concentrates varies from 0.19 per cent., in milo maize chops to 1.01 per cent. in wheat bran. The average is 0.48 per cent., practically the same as the same fraction in the ether extract of the concentrates. The individual variations are considerable in some cases, however; in milo maize chops, kafir chops, and corn bran it is much lower, and in wheat shorts, wheat bran, and cottonseed meal, it is much higher than the non-fat organic acids of the ether extract. The low results appear to be abnormal, but all figures are the averages of two or more concordant determinations. It may be that some of the ether soluble matter was rendered insoluble by the alcoholic soda treatment. Lack of time and the spoiling of the samples prevented further study of this discrepancy.

In the hays, and excrements from them, the saponified residue by the Alcoholic Soda Method are in every case much higher than the same fraction in the ether extract. It varies from 1.25 per cent. in moth-bean hay to 3.02 per cent. in excrement from prairie hay, and averages 2.10 per cent., over seven times higher than the average for the saponified residue of the ether extract.

After deducting the amount of the same fraction in the ether extract and the chlorophyll calculated as "crude protein" the remainder of this group of substances falls into the group designated in ordinary proximate analysis of feeds as "nitrogen-free extract." This will be discussed later.

It will be noted that the saponified residue by the Precipitation Method is lower in some cases than by the Digestion Method. Since this fraction and the fatty acids make up the saponified matter, it follows that the variation of one will cause an inverse variation in the other, in the same sample. The reasons already given under the heading Total Fatty Acids, in regard to similar variations in the fatty acids by the two methods, will, therefore, apply to these differences.

These results show that hays and excrements from them may contain relatively large amounts of ether-soluble, saponifiable material which is apparently not fatty acids.

THE NATURE OF THE ACIDS SOLUBLE IN PETROLEUM ETHER.

The substances considered as fatty acids in this Bulletin are the constituents of the saponifiable material of the ether extract, or of the saponifiable ether-soluble material of the alcoholic soda extract, which are soluble in petroleum ether after acidifying with acetic acid. In this section, we will summarize the evidence in favor of this view and present further data in support of it.

Two samples of excrement and one each of moth-bean hay and rice

polish were extracted with ether, and the fatty acids separated by the Precipitation Method. The residues from the ether extraction were treated with alcoholic soda and the fatty acids obtained by the Alcoholic Soda Method. The saponification values of the dried acids were determined, nitric acid being used to titrate back the excess of alkali. Silver salts of the acids were then prepared, dried in air, in the dark, powdered, dried in a vacuum over sulphuric acid, and the percentage of silver determined. Because of the small amounts available (less than 0.5 gm.) the saponification values are less accurate than the silver determinations, and will not be given here. The calculated mean molecular weights of the acids agreed fairly well, however, with those calculated from the silver determinations.

The results on the silver salts are shown in Table 4.

TABLE 4.

Mean Molecular Weights of Fatty Acids Not in Ether Extract.

Laboratory No.		Per cent silver.		Mean molecular weight.	
		Acids in ether extract.	Not extracted by ether but extracted by alcoholic soda.	Acids in ether extract.	Not extracted by ether but extracted by alcoholic soda.
7970	Excrement, Sudan grass	17.48	24.73	510	329
8002	Moth bean hay	24.23	26.57	338	299
8013	Excrement, moth bean hay	17.57	26.91	507	294
13190	Rice polish	27.33	29.01	288	265

The mean molecular weights are higher in every case for the fatty acids from the ether extract than for the acids extracted by alcoholic soda after ether. The difference is very great with the two excrements. It will be noted that the mean molecular weight of the fatty acids in the ether extract of moth-bean hay is 308, and 507 for the corresponding acids of the excrement from this hay. This will be discussed below.

The acids from the alcoholic soda fraction vary in mean molecular weight from 265 to 329. The molecular weights of fatty acids of similar magnitude are: linolenic, 276; oleoic, 283; stearic, 284; ricinoleic, 298; archidic, 312, and erucic, 338.

Nearly all of the acids of similar molecular weight and melting between 140 and 100° C., listed in Mulliken's Identification of Organic Compounds, Vol. 1, under the group which these acids would come, are saturated or unsaturated fatty acids. While Mulliken's list is undoubtedly far from complete, this is evidence that the acids are fatty acids.

Melting point determinations and physical examinations were made on the acids obtained from six samples of the concentrates, and no difference was noted between the two fractions of the acids, either in appearance or melting point.

The evidence in favor of the assumption that the organic acids, soluble in petroleum ether, extracted by alcoholic soda and not by ether, are fatty acids may be summarized as follows:

1. The acids are physically similar to fatty acids obtained from the ether extract.

2. They have mean molecular weights of about the same magnitude as several of the most common fatty acids.

3. They are soluble in petroleum ether, ethyl ether and alcohol, and are insoluble in water. All fatty acids are considered soluble in petroleum ether. The ether extract of plant and animal products is accepted as "fat" in ordinary analytical processes, on the single fact of solubility in ether.

4. They are separated by the analytical process from a relatively large amount of other saponified matter which is known to consist in part of substances which are not fatty acids.

5. The fatty acids of a commercial fat (lard compound) were nearly completely soluble under the conditions of the method.

While this evidence is not sufficient to enable one to say with certainty that the amount of acids in the alcoholic soda extract in excess of the fatty acids of the ether extract are really fatty acids, we believe that they are probably fatty acids of lower mean molecular weights than those of the corresponding ether extract. Lack of time prevents the continuation of the study at present.

The nature of the relatively large amounts of the fraction designated saponified residue, we hope to study further. At present the evidence that they are not fatty acids is that they consist in the hays and excrement from them, in part of chlorophyll products, and that fatty acids are soluble in petroleum ether under the conditions of the method. The relatively large amounts of that solvent employed and the repeated extractions in the warm, together with the slightly green color of the fatty acids fractions, make it appear more probable that the saponified residue contaminates the fatty acids than vice versa.

THE DIGESTIBILITY OF THE VARIOUS ETHER-SOLUBLE FRACTIONS.

The data for the calculation of the digestibility of the various ether-soluble fractions, described above, was available from digestion trials made for another purpose, and calculations were made to find what value the products have as food for the animal body. The figures are especially interesting as a comparison of the food value of the total fatty acids of the ether extract and of the alcoholic soda extract.

Only hays were used in this work. The digestion trials were made with sheep, with two periods of 8 days each, the manner of conducting the experiment being essentially the same as that described in Bulletin No. 147 of this Station. The results are shown in Table 5. The digestibility of the unsaponified matter is not given, because the ether-soluble and chloroform-soluble unsaponified in a large number of hays has already been studied at this Station (Fraps and Rather, *loc. cit.*).

TABLE 5.

Percentage Digestibility of the Various Ether-Soluble Acids.

	Ether extract.	Fatty acids.			Saponified residue.		
		In ether extract.	Alcoholic soda extract.	Extracted by alcoholic soda not by ether.	Ether extract.	Alcoholic soda extract.	Extracted by alcoholic soda not by ether.
Tabosa grass	40.7	57.1	24.3	0.0	51.4	39.4	37.8
Prairie hay	32.9	24.4	26.8	30.2	55.7	17.8	6.9
Sudan grass	45.4	83.8	34.5	0.0	37.4	31.1	31.7
Sudan straw	35.1	68.1	32.3	0.0	32.3	23.9	23.0
Sorghum hay	59.5	71.2	39.4	0.0	63.0	25.8	18.0
Moth bean hay	19.3	58.5	44.8	36.7	30.8	16.6	12.6
Average	35.5	60.5	33.7	11.2	45.1	25.8	21.7

The digestibility of the ether extract was, in every case but one (prairie hay) much lower than that of the total fatty acids of the ether extract, averaging 35.5 per cent. for the former and 60.5 per cent. for the latter. This is in accordance with the conclusion of Fraps and Rather (*Texas Bulletin,* 150) in regard to the digestibility of the *total* saponifiable material of the ether extract.

The digestibility of the fatty acids in the alcoholic soda extract was less in every case but one (prairie hay) than the corresponding fraction of the ether extract. The average is 33.7 per cent. for the former and 60.5 per cent. for the latter.

The fatty acids not extracted by ether but extracted by alcoholic soda had a digestibility of zero in the case of tabosa grass, Sudan grass, Sudan straw, and sorghum hay. The fraction in prairie hay was digested 30.2 per cent. and that in moth-bean hay was digested 36.7 per cent. The average digestibility for the six hays is 11.2 per cent. This is less than one-fifth of the average digestibility of the total fatty acids of the ether extract.

Two explanations may be offered for the above results: First, the acids not extracted by ether may be radically different from the fatty acids extracted by that solvent, and of lower digestibility. Second, the acids may be in the feeds in such combination as to resist the action of the digestion juices, or so encrusted with indigestible material as to prevent the access of the juices. The fact that alcoholic soda dissolves lime soap lends plausibility to this view. Whichever explanation may be correct, the fact remains that the fatty acids not extracted by ether but extracted by alcoholic soda, have little or no digestibility in the case of the hays investigated.

The molecular weight determinations throw some light on this point. The mean molecular weights of the fatty acids not extracted by ether but extracted by alcoholic soda are 299 and 294, respectively, for moth-bean hay and the excrement therefrom. Since this fraction was digested 36.7 per cent. in this case, the above figures indicate that the various fatty acids in the mixture are digested to the same extent. Fatty acids of similar molecular weights are: stearic acid, 284; ricinoleic acid, 298; and archidic acid, 312. The glycerides of these acids are readily digested.

The fatty acids in the ether extract of the moth-bean hay have a

mean molecular weight of 338, which is the same as that of erucic acid, while the fatty acids in the ether extract of the corresponding excrement have a mean molecular weight of 507, somewhat higher than that of melissic acid (452). The fatty acids of high molecular weight are less easily digested than those of lower molecular weight (see also Fraps and Rather, *Texas Bulletin* 150, p. 15.)

The fatty acids extracted by ether are higher in molecular weight, in this case, than those not extracted by ether but extracted by alcoholic soda.

While these results are too few to admit of definite conclusions being drawn, it appears probable that the low digestibility of the fatty acids extracted by alcoholic soda but not extracted by ether, is due to the form of combination of the acids in the feed or to difficultly soluble incrustations rather than to inherent unavailability.

The digestibility of the saponified residue extracted by ether is less in every case than that of the total fatty acids of the ether extract. The average is 45.1 per cent. for the former and 60.5 per cent. for the latter. Since both are fractions of the same extract this is evidence of a difference in their nature.

The digestibility of the saponified residue soluble in alcoholic soda is less in every case than the corresponding fraction of the ether extract, averaging 25.8 per cent. for the former and 45.1 per cent. for the latter.

The digestibility of the saponified residue not extracted by ether but extracted by alcoholic soda is slightly less in every case than that of the total non-fat organic acids of the alcoholic soda extract. The average is 21.7 per cent. as against 25.8 per cent. for the total non-fat organic acids.

Since the total saponified residue is present in the hays studied in percentage varying from 1.25 to 2.00, and are equal in amount and nearly in digestibility to the total ether extract, we believe that the fraction is important both theoretically and practically.

ETHER SOLUBLE MATTER IN THE NITROGEN-FREE EXTRACT OF FEED STUFFS

We have collected in Table 6 for comparison, the determinations of the ether-soluble matter by several methods. The ether extract requires no explanation and the alcoholic soda extract has already been discussed. The chloroform extract, alcohol extract, and nitrogen in alcoholic soda extract require some explanation. The sample, after 16 hours extraction with ether, was extracted 16 hours with chloroform, and then for 16 hours with alcohol. The constituents of the chloroform extract were determined by the method given in *Texas Station Bulletin* 162, and the sum of the ether soluble constituents are shown in the table. The alcohol extracts were treated with calcium carbonate and the ether-soluble matter determined by shaking out. The work on the chloroform and alcohol extracts form part of another investigation as yet unpublished.

The nitrogen determinations were made on the ether soluble material of the alcoholic soda extract of samples previously extracted with ether. The factor 6.25 is used, not because it is thought that any protein

was present, but in order to find what part of these extracts lies within the group "Nitrogen-free extract."

TABLE 6.

Percentage Ether-Soluble Matter by Various Methods.

Laboratory No		Ether Extract.	In chloroform following ether.	In alcohol following chloroform.	Total.	Alcoholic soda extract.				
						In alcoholic soda.—Total.	In excess of ether extract.	Less ether-soluble. N x 6.25	In excess of ether chloroform and alcoholic extract.	Excess in per cent nitrogen free extract.
12996	Wheat shorts	3.79	.....	.....	.....	5.38	1.59	.....	.....	2.76
12999	Corn chops	4.31	.....	.....	.....	4.79	.48	.....	.....	.71
13021	Cottonseed meal	15.23	.....	.....	.....	15.15	.08	.....	.....	.....
13023	Rice bran	7.75	.....	.....	.....	9.66	1.91	.....	.....	3.84
13030	Milo maize chops	3.22	.....	.....	.....	3.32	.10	.....	.....	.15
13045	Cold pressed cottonseed	7.26	.....	.....	.....	7.81	.55	.....	.....	1.74
13091	Kafir chops	3.20	.....	.....	.....	3.68	.48	.....	.....	.70
13140	Corn bran	8.59	.....	.....	.....	9.06	.47	.....	.....	.77
13150	Red rice	1.64	.....	.....	.....	2.36	.72	.....	.....	1.07
13166	Wheat bran	4.10	.....	.....	.....	5.67	1.57	.....	.....	3.07
13172	Wheat shorts	2.65	.....	.....	.....	3.38	.73	.....	.....	1.14
13190	Rice polish	10.38	.....	.....	.....	11.49	1.11	.....	.....	.92
6288	Tobosa grass	0.92	.24	.67	1.83	2.62	1.70	1.51	.79	3.27
6290	Excrement from tobosa grass	1.06	.28	.55	1.89	3.50	2.14	1.92	1.61	4.46
7724	Prairie hay	2.30	.29	.83	3.42	3.89	1.59	1.35	0.47	2.82
7763	Sudan grass	1.46	.32	.94	2.72	3.23	1.77	1.61	.51	3.34
7799	Excrement from prairie hay	2.83	1.12	.75	4.70	6.25	3.42	3.18	1.55	7.35
7970	Excrement from Sudan grass	1.90	.81	.86	3.57	5.73	3.83	3.62	2.16	7.64
7980	Sudan straw	1.44	.28	1.46	3.18	3.69	2.25	2.01	.51	4.77
7991	Sorghum hay	1.82	.24	.....	.....	3.32	1.50	1.26	.....	2.72
7999	Excrement from Sudan straw	1.71	.40	1.13	3.24	5.37	3.66	3.42	2.13	7.81
8002	Moth bean hay	1.55	.45	1.15	3.15	3.98	2.43	2.15	.83	6.17
8011	Excrement from sorghum hay	1.78	.25	1.12	3.15	5.82	4.04	3.80	2.67	8.74
8013	Excrement from moth bean hay	3.03	.30	1.26	4.59	7.01	3.98	3.61	2.42	12.39
	Average concentrates	6.01	.....	.....	.....	.81	6.80	.....	.....	1.49
	Average roughages	1.82	.4.	.97	3.22	4.53	2.71	.....	1.42	5.97

The results on the concentrates show that 2 N Alcoholic soda extracted more ether soluble material than ether in every case but one (cottonseed meal), the variation of the difference is from —.08 per cent. in cottonseed meal to 1.91 per cent. in rice bran. The average is 0.80 per cent.

Alcoholic soda extracted much more ether-soluble matter from the hays and excrements from them, than ether. The difference varies from 1.50 per cent in sorghum hay to 4.04 per cent. in excrement from sorghum hay, and averaged 2.71 per cent. This is about 150 per cent. as much as the average for the corresponding ether extracts.

Alcoholic soda extracted from these samples of hays and excrements, more ether-soluble material than the sum of the ether-soluble constituents of successive extractions with ether, chloroform, and alcohol. The difference varies from 0.79 per cent. in tabosa grass to 2.67 per cent. in excrement from sorghum hay, and averages 1.42 per cent. as compared with an average of 3.42 per cent. for the ether-soluble matter of the other extracts.

The ether-soluble matter in excess of the ether extract of the concentrates was assumed to contain no nitrogen. On this basis, the ether-soluble matter in the nitrogen-free extracts of the concentrates varied

from zero in cottonseed meal to 3.84 per cent. in rice bran and averaged 1.49 per cent.

Nitrogen determinations were made on the alcoholic soda extract of six of these samples of hays and excrements which had previously been extracted with ether. This nitrogen calculated as protein (for comparative purposes only) averages 0.24 per cent. for the samples examined. Subtracted from the corresponding figures representing the ether-soluble material not in the ether extract, the remainder varies from 1.26 per cent. in sorghum hay to 3.80 per cent. in excrement from sorghum hay. Assuming that this material contains no ash, the figures represent *ether-soluble constituents of the nitrogen-free extract.* Calculated in percentage of nitrogen-free extract instead of percentage of feed figures vary from 2.72 per cent. with sorghum hay to 12.39 per cent. with excrement from moth-bean hay and average 5.97 per cent. That is to say that, on an average, *6 per cent. of the nitrogen-free extract of these hays and excrements consists of material soluble in ether.*

The data reported in this Bulletin and other data already reported from this station (Fraps and Rather, Bulletin 162) throw considerable light on the nature of this material. It consists of about 4 per cent. of unsaponifiable matter, probably wax alcohols, about 33 per cent. of acids which are probably fatty acids, and about 63 per cent. of saponified residue containing chlorophyll and related products. The figures are averages for the 12 hays and excrements given in the table. The exact nature of these products is reserved for future study in this laboratory.

DATA OF DIGESTIVE WORK.

TABLE 7.

Nutrients Fed, Excreted and Digested in Grams per Period.

	Ether extract.	Fatty acids.			Non-fat organic acids.		
		In ether extract.	In alcoholic soda extract.	Extracted by alcoholic soda but not by ether.	In ether extract.	In alcoholic soda extract.	Extracted by alcoholic soda and not by ether.
Period No. 24, feed tobosa grass. Sheep No. 4, feed No. 6288.							
Fed 4000 gm	36.8	11.0	24.4	.....	7.2	62.8	.....
Residue 20 gm, No. 6288	0.2	0.1	1.0	.....	0.0	0.3	.....
Eaten	36.6	11.9	24.3	12.4	7.2	62.5	55.3
Excreted 2049 gm, No. 6290	21.7	5.1	18.4	13.3	3.5	37.9	34.4
Digested	14.9	6.8	5.9	−0.9	3.7	24.6	20.9
Percentage digested	40.7	57.1	24.3	0.0	51.4	39.4	37.8
Period No. 38, feed prairie hay. Sheep No. 3, food No. 7724.							
Fed 4000 gm	92.0	26.0	44.4	.....	17.6	80.0	.....
Residue 97 gm, No. 7724	2.3	0.6	1.1	.....	0.2	1.9	.....
Eaten	89.7	25.4	43.3	17.9	17.4	78.1	60.7
Excreted 2127 gm, No. 7799	60.2	19.2	31.7	12.5	7.7	64.2	56.5
Digested	29.5	6.2	11.6	5.4	9.7	13.9	†4.2
Percentage digested	32.9	24.4	26.8	30.2	55.7	*17.8	6.9
Period No. 39, feed Sudan grass. Sheep No. 1, food No. 7763.							
Fed 4000 gm	58.4	26.2	41.2	.....	9.2	68.4	.....
Residue 25 gm, No. 7763	0.4	0.2	0.3	.....	0.1	3.8	.....
Eaten	58.0	26.0	40.9	14.9	9.1	64.6	55.5
Excreted 1662 gm, No. 7970	31.6	4.2	26.8	22.6	5.7	43.6	37.9
Digested	26.4	21.8	14.1	−7.7	3.4	21.0	17.6
Percentage Digested	45.5	83.8	34.5	0.0	37.4	31.1	31.7
Period No. 42, feed Sudan grass. Sheep No. 1, feed No. 7980.							
Fed 4000 gm	57.6	26.8	51.6	.....	6.4	70.0	.....
Residue 122 gm, No. 7980	1.8	0.8	0.2	.....	0.2	2.1	.....
Eaten	55.8	26.0	51.4	25.4	6.2	67.9	61.7
Excreted 2119 gm, No. 7999	36.2	8.3	34.8	26.5	4.2	51.7	47.5
Digested	19.6	17.7	16.6	−1.1	2.0	16.2	14.2
Percentage digested	35.1	68.1	32.3	0.0	32.3	23.9	23.0
Period No. 43, feed sorghum hay. Sheep No. , food No. 7991.							
Fed 4000 gm	72.8	35.6	46.8	.....	11.2	64.0	.....
Residue 133 gm, No. 7991	2.4	1.2	1.6	.....	0.4	2.1	.....
Eaten	70.4	34.4	45.2	10.8	10.8	61.9	51.1
Excreted 1600 gm, No. 8011	28.5	9.9	27.4	17.5	4.0	45.9	41.9
Digested	41.9	24.5	17.8	−6.7	6.8	16.0	9.2
Percentage digested	59.5	71.2	39.4	0,0	63.0	25.8	18.0
Period No. 44, feed moth bean hay. Sheep No. 1, food No. 8002.							
Fed 3000 gm	46.5	18.6	51.0	.....	8.2	37.5	.....
Residue 154 gm, No. 8002	2.4	1.0	2.6	.....	0.4	1.9	.....
Eaten	44.1	17.6	48.4	30.8	7.8	35.6	27.8
Excreted 1174 gm, No. 8013	35.6	7.3	26.8	19.5	5.4	29.7	24.3
Digested	8.5	10.3	21.6	11.3	2.4	5.9	3.5
Percentage digested	19.3	58.5	44.8	.....	30.8	16.6	12.6

SUMMARY AND CONCLUSIONS.

1. An improved method for the determination of the total fatty acids and other constituents of ether extracts has been developed.

2. A new method has been developed for extracting the total fatty acids and other ether-soluble constituents of feeding-stuffs and excrements, which, by means of alcoholic soda, removed more fatty acids from feeding-stuffs than did successive extractions with ether, chloroform and alcohol.

3. Ether extracts of the concentrates contained saponifiable material which does not appear to be fatty acids, averaging about 8 per cent. and unsaponifiable matter averaging about 6 per cent., a total of approximately 14 per cent. of non-fats in the ether extract of concentrates. Ether extracts of hays and excrements from them contain saponified material which does not appear to be fatty acids, averaging about 15 per cent. of the ether extract. Together with the unsaponifiable matter, they made a total of approximately 68 per cent. of non-fats in the ether extract of roughages.

4. Molecular weight determinations and other evidence indicate that the ether soluble, petroleum-ether soluble acids in the alcoholic soda extracts of feed stuffs are probably fatty acids.

5. The digestibility of the various ether-soluble fractions was determined in six hays with sheep. The fatty acids are digested on an average of 60.5 per cent. in the ether extract; the fatty acids in the alcoholic soda extract were digested 33.7 per cent.

6. The digestibility of the fatty acids extracted by alcoholic soda but not by ether had an average digestibility of 11.2 per cent. The digestibility in four cases was zero.

7. The saponified residue of the ether extract were digested, on an average, 45.1 per cent., and in the alcoholic soda extract 25.8 per cent.

8. The nitrogen-free extract of feed stuffs contains considerable material soluble in ether, which can be extracted by alcoholic soda. This ether-soluble matter consists of unsaponfiable matter, fatty acids, and, principally, of non-fat organic acids, in the case of hays and excrements from them. It made up from 2.72 to 12.39 per cent. of the nitrogen-free extract of those samples, and averaged 5.97 per cent. In the concentrates it make up from zero to 3.84 per cent. of the nitrogen-free extract, and averaged 1.49 per cent. of the nitrogen-free extract.