Rapid Method for Determination of Total Fatty Acid Content and Composition of Feedstuffs and Feces

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Methods currently used for quantitating total fat and fatty acid composition in feedstuffs require solvent extraction, purification, and esterification followed by gas chromatographic analysis. A 2-h, one-step extraction–transesterification procedure using 5.0 mL of solvent mixture consisting of methanol–benzene (or chloroform)–acetyl chloride (20:27:3) for 50–500 mg of sample containing 10–50 mg of fatty acids is described, which gave reproducible results for fatty acid content and composition of many feedstuffs, oilseeds, calcium soap, milk fat, and feces samples. The procedure also was useful for quantitating fatty acids of oil extracted by Soxhlet and of lipid classes separated by thin-layer chromatography. The one-step extraction–transesterification procedure is simple, rapid, convenient, and quantitative; fatty acids are determined specifically, providing a precise estimate of nutritive value of fats. The method is useful both for research and commercial purposes.

Total oil content of feedstuffs is generally estimated by Soxhlet (AOAC, 1980) or by cold extraction methods (Bligh and Dyer, 1959; Folch et al., 1957) using organic solvents; and the fatty acid composition of the extracted oil is determined by gas–liquid chromatography after transmethylation. Oil extraction followed by esterification is cumbersome, time-consuming, and often uneconomical (Browse et al., 1986; Lepage and Roy, 1988). Further, oil content estimated by Soxhlet or by cold extraction procedures is not precisely related to feed value of many feedstuffs. Nonnutritive waxes and pigments that are also extracted, or soaps that are not extracted, present an inaccurate estimation of the energy value; therefore, it is more appropriate to estimate the total fatty acid content rather than the amount of the total oil ("ether extract") of the sample. Use of calcium soaps as a feed supplement has gained interest; procedures recommended for estimation of their fat content are similarly cumbersome (AOAC, 1980). Accurate determination of milk fatty acid composition requires preservation of short-chain fatty acids, usually by detailed procedures (Smith, 1961).

Recently, emphasis has been placed on one-step digestion, extraction, and esterification of samples by many workers: MacGee and Allen (1974) in biological tissues, Outen et al. (1974, 1976) in feeds, Shimasaki et al. (1977) in mammalian tissues, Haan et al. (1979) in human tissues, Lepage and Roy (1986, 1988) in plasma, and Browse et al. (1986) in leaves.

The combined one-step extraction and esterification method (one-step) described by Outen et al. (1976) has been used since 1980 in this laboratory. Recently, we have applied modifications to this method for a variety of samples and compared the oil content and fatty acid composition with those determined by other methods. In this paper, we describe the procedure and demonstrate its usefulness for quantitative estimation of fatty acids in feeds, forages, feces, and whole oilseeds and qualitative estimation (relative percent) of fatty acids in milk samples.

MATERIALS

The forages, feed mixtures, feces, and grain samples were freeze-dried and finely ground (1-mm screen, Wiley mill). Where indicated, samples were oven-dried at 55 °C. Whole oilseeds were cut into small pieces with a sharp blade, taking necessary precautions to prevent loss of free oil. Calcium soap of palm fatty acid distillate was from Church and Dwight, Inc., Princeton, NJ. The soap sample was ground finely in a mortar and pestle and was thoroughly mixed.

Organic solvents were either of analytical grade or were redistilled. Nonadecanoic acid (NuCheck Prep, Inc., Elysian, MN) was used as an internal standard; acetyl chloride was from Mallinkrodt Chemicals, St. Louis, MO.

Apparatus. One-step methylation was performed in 15 cm × 2.5 cm or 12 cm × 1.5 cm Teflon-lined screw-cap culture tubes. A firm seal of cap and tube was essential for success. Methyl esters in organic phase were stored in 10 cm × 1.0 cm screw-cap culture tubes. Thin-layer chromatographic plates, 5 cm × 20 cm coated with silica gel G, 250 μm (Analtech, Inc., Newark, DE), were used to check completeness of esterification and for preparative separation of various lipid classes. Hydrolysis (HCl) of calcium soap samples was carried out in 100-mL round-bottomed flasks fitted with condensers having ground-glass joints.

For ether extraction, Allihn Condenser Soxhlet extraction apparatus was used.

METHODS

Internal standard: Nonadecanoic acid (19:0), 4 mg/mL in benzene, chloroform, or petroleum ether (PE).

One-Step Methylation Using Benzene. Samples (50–500 mg) of freeze-dried feces, forage, grain; 100 mg of the oilseed; and 10–50 mg of the soap and other fatty samples were accurately weighed and transferred into the culture tubes. The amount of the sample was selected to contain 10–50 mg of fatty acids. Fluffy material such as forages and feces samples required 15 cm × 2.5 cm culture tubes; for other samples 12 cm × 1.5 cm tubes were more convenient. To each of the tubes were added 1 mL of benzene containing internal standard, 1 mL of benzene, and 3 mL of freshly made 5% methanolic HCl (prepared by slowing adding 10 mL of acetyl chloride to 100 mL of anhydrous methanol) slowly so that the solvents fell over the material without touching the side walls of the tube. After being tightly capped, the culture tubes were vortexed for 1 min with a slow speed so that the material remained 2–3 cm from the bottom. Though vortexing is essential, it should be undertaken with caution because transesterification may remain incomplete due to incomplete extraction if the sample spreads over the walls. The tightly capped tubes were heated for 2 h in a water bath at 70 °C; if solvent escaped, 2 mL of benzene was added after cooling, and the tube was returned to the water bath to ensure complete methylation. After the contents were

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cooled to room temperature, 5 mL of 6% K_2CO_3 was added, followed by 2 mL of benzene. Acetyl chloride in the reaction mixture reduces the pH to 1–2; increasing the pH to neutrality is necessary to prevent degradation of the thin coating of the polyester stationary phase of GLC capillary columns (Rogiers, 1977).

The contents of the tube were vortexed for $^1/_2$ min at medium speed, followed by centrifugation for 5 min at 1500 rpm. The upper organic phase (benzene) of the tube was transferred with a Pasteur pipet to a 10 cm × 21 cm screw-capped culture tube. To the benzene extract in the culture tube were added 1 g of anhydrous sodium sulfate and 1 g of activated charcoal (in case of presence of pigments), and the sample was vortexed for $^1/_2$ min and allowed to stand for 1 h. The culture tubes were centrifuged for 5 min at 1500 rpm, and the clear benzene (upper) layer containing methyl esters was transferred to a 10 cm × 1 cm culture tube until analysis by GLC.

In order to assure that esterification was complete, samples were examined by thin-layer chromatography (TLC) using benzene–diethyl ether–ethyl acetate–acetic acid (80:10:10:2, v/v) as developing system, and the single spot representing fatty acid esters was visualized with iodine vapors.

One-Step Methylation Using Chloroform. The procedure was as for benzene except that chloform replaced benzene for extraction–methylation. After extraction and methylation, the tubes were cooled to room temperature and 5 mL of 6% K_2CO_3 and 4 mL of chloroform were added. The tubes were vortexed for $^1/_2$ min at medium speed and centrifuged at 2500 rpm for 10 min. The methyl esters were in the bottom chloroform layer, and the residue formed a plug between the upper water and the lower chloroform layer. The lower layer was removed with a Pasteur pipet by piercing the floating residue at the edge without disturbing the upper or the lower layers. Another washing with 4 mL of chloroform completely removed the methyl esters. An aliquot of the combined chloroform portion was dried under nitrogen and redissolved in benzene. The purity of the esters was tested by TLC as above.

Transesterification of Milk Fat. Milk fat was obtained by detergent extraction of milk (Stine et al., 1954). Samples were transesterified as for other materials; after methylation, tubes were kept on ice and samples were transferred quickly with immediate capping to prevent loss of highly volatile methyl esters of short-chain fatty acids.

Soxhlet Extraction and Esterification. A sample (ca. 1 g) was accurately weighed and transferred to a cotton thimble (22 × 80 mm). Petroleum ether (2 mL; bp 40–60 °C) containing 4 mg of internal standard was put over the sample in the thimble with care so that it did not fall over the sides of the thimble. Petroleum ether (100 mL) with or without 10% glacial acetic acid was added to the Soxhlet extraction flask, and the sample was extracted for 6 h. The flasks containing ether extract were dried at 40 °C under nitrogen, and the weight of the crude lipids was noted. The whole crude lipid residue was redissolved in 5–10 mL of benzene and transferred to 15 cm × 2.5 cm screw-capped culture tubes. The contents of the tubes were concentrated to 2 mL, and transesterification was performed as described above.

Soap Hydrolysis and Esterification. A 1-g portion of calcium soap was acidified with 6 N HCl, and the total fatty acids were extracted 3× with hexane, dried, and weighted (AOAC, 1980).

The soap samples (50–100 mg) were also extracted in culture tubes as follows: 2 mL of benzene containing 4 mg of C_{19} acid, 3 mL of water, and 3 mL of 6 N HCl was added, and the tubes were capped. The contents were heated on a dry heating block at 90 °C for 1 h and cooled to room temperature. The upper nonaqueous layer was transferred with Pasteur pipets into culture tubes. The esterification was carried out as described above.

Extraction of Total and Free Oil and Solvent Partition. Total lipids were extracted by the method of Folch et al. (1957) and free lipids by that of Kartha (1961). Free fatty acids were removed by washing the lipid extract with 0.02 N K_2CO_3 and subsequently reextracting them into PE after acidification of the aqueous wash to pH <2 with concentrated H_2SO_4.

Separation of Total Lipids into Lipid Classes and Their Esterification. Total lipids were separated on TLC plates coated with silica gel G (250 μm) with hexane–diethyl ether–acetic acid (90:10:1, v/v) and visualized by iodine vapors as described by Sukhija and Bhatia (1970). Iodine-stained lipid classes adsorbed to the silica gel were scraped from the TLC plates and transferred to 10-mL culture tubes. The spots containing mono- and diacylglycerides were pooled as partial glycerides. Transesterification was performed using benzene solvent system as described above.

Gas–Liquid Chromatography and Quantification of Fatty Acids. Fatty acid analysis was done on a Hewlett-Packard 5890 gas–liquid chromatograph fitted with automatic sampler 7673A, integrator 3392A, and FID detector. Conditions: SP-2340 fused silica capillary column (0.32 mm × 3 mm) (Supelco, Inc., Bellefonte, PA), temperature programmed from 160 to 180 °C at 3 °C/min for feed fats and from 90 to 180 °C at 4 °C/min for milk fat. Gas flows: carrier nitrogen, 1 mL/min; hydrogen, 30 mL/min; air, 400 mL/min. Fatty acids were identified by comparison of their retention times with that of the internal standard and with retention times reported in the literature. Total fatty acids (milligrams/gram of dry sample) were calculated as follows:

$$\frac{\text{(total area under peaks)} - \text{(area under int std)}}{\text{area of int std}} \times \frac{4\text{ mg}}{\text{dry wt of sample (g)}}$$

Statistical Analysis. Data were analyzed by analysis of variance. Means with significant F ratio were separated by Duncan's multiple-range test.

RESULTS AND DISCUSSION

One-step extraction and esterification of fatty acids was developed for routine fatty acid analysis of a large number of samples from a variety of sources. In most analyses in common use, gas chromatographic separation of fatty acid methyl esters is the final step after extraction with organic solvents, purification, and esterification. These procedures are lengthy, and sometimes a portion of the lipid is lost during the multistep procedure. We have used the method of Outen et al. (1976) after suitable modifications for fatty acid content and composition of feedstuffs, calcium soap, milk, and feces.

When three methods of fat extraction of four feed fats were compared (Table I) with the one-step method, the values were generally highest by Soxhlet. Fat content by Folch, Kartha, and Soxhlet methods was determined by weighing total extracted fat. Soxhlet and Folch procedures extract pigments, waxes, etc., as well as fat, and thus, higher values for fat are normally expected. On the other hand, the Kartha procedure extracts mostly free lipids; thus, any bound lipids not extracted would decrease the value. The one-step method determines total fatty acid

Table I. Fat Content (mg/g) of Feed Fats by Four Extraction Methods[a]

extraction method	fat source (n)			
	1 (3)	2 (3)	3 (3)	4 (3)
Folch[b]	780[a]	790[a]	818[a]	758[a]
Kartha[b]	690[b]	646[b]	690[b]	680[b]
Soxhlet[b]	834[c]	840[c]	820[a]	745[a]
one-step[c]	763[a]	765[d]	803[c]	822[c]
SE	11.36	6.64	2.74	8.15

[a] Various commercial feed fats adsorbed onto dry carriers. Values in a column with similar superscripts do not differ ($P > 0.05$). [b] Total lipids determined gravimetrically. [c] Fatty acids/0.9.

Table II. Fatty Acid Content (mg/g) by Gas-Liquid Chromatography after Soxhlet Extraction or One-Step Extraction with Benzene or Chloroform[a]

extraction method	fat source (n)		
	alfalfa pellets (3)	whole cottonseed (3)	whole soybean (3)
Soxhlet	36.20	146.7[a]	96.83[a]
one-step			
benzene	44.87	150.0[a]	117.1[a]
chloroform	44.33	185.9[b]	175.0[b]
SE	3.08	3.05	7.79

[a] Values in a column with similar superscripts do not differ ($P > 0.05$).

Table III. Comparison of Chloroform and Benzene as Solvent in One-Step Procedure (Fatty Acids, mg/g)

solvent	fat source (n)			
	hay silage (4)	alfalfa hay (3)	grain mix (4)	calcium soap (4)
benzene	19.05	15.27	38.40	794
chloroform	19.52	13.00	36.90	807
SE	0.557	0.984	1.11	12.6

Table IV. Fatty Acid Content (mg/g) by Gas-Liquid Chromatography after Soxhlet-Acid Extraction and One-Step Extraction with Benzene or Chloroform

method	fat source (n)		
	feces (3)	corn silage (3)	hay (3)
Soxhlet–acid	32.67	38.60	13.20
one-step			
benzene	34.37	31.73	15.27
chloroform	32.00	39.50	13.00
SE	1.19	2.51	1.42

content, the nutritionally useful and major component of fat, and thus values lower than Folch or Soxhlet may be expected because glycerol and nonfatty acid components may form 10–50% of the total ether-soluble material in various feedstuffs (Palmquist and Jenkins, 1980). For comparison with gravimetric determination in Table I, weight of fatty acids determined by gas–liquid chromatography was divided by 0.9 to provide triacylglycerol equivalent.

The one-step procedure was then compared with Soxhlet extraction for estimation of fatty acids in alfalfa pellets, whole cottonseed, and soybean seed. In this experiment, efficacy of extraction with benzene was compared with extraction by more polar chloroform (Table II). In the case of oilseeds (whole cottonseed and soy seed), total fatty acid content was higher ($P < 0.05$) with chloroform than with benzene or Soxhlet extraction. For alfalfa pellets benzene and chloroform were equal, and the one-step procedure tended to be higher than Soxhlet. On the other hand, benzene and chloroform were equal as solvents in the one-step method for other feedstuffs (Table III). Fatty acid content of oilseeds by Soxhlet extraction was lower probably because of incomplete extraction of fatty acids. However, extraction of feces, corn silage, and hay by Soxhlet with PE–glacial acetic acid gave fatty acid content similar to that by the one-step method (Table IV). Thus, our data support acidification of PE as described by Andrews and Lewis (1970).

For quantitative estimation of fatty acids from soaps, AOAC has recommended hydrolysis of soaps with 6 N HCl followed by diethyl ether extraction of fatty acids and weighing after drying (AOAC, 1980). The procedure involves multiple steps and requires considerable technical expertise to gain precision. When we used calcium soap to compare the one-step procedure with the procedure using HCl hydrolysis followed by esterification and quantification by GLC, we found no difference between the methods, either in recovery of fatty acids or in fatty acid composition (Table V). Thus, during extraction at 70 °C for 2 h, complete dissociation of the soap and esterification of fatty acids took place. [We have found subsequently that smaller samples of soap (10–50 mg) and higher temperature (90 °C) improve precision of soap fatty acid analysis.]

No differences in fatty acid composition between samples prepared by the one-step method or extracted by Soxhlet followed by esterification and fatty acid analysis by GLC were detected. High amounts of polyunsaturated fatty acid (18:3) were observed in soybeans, suggesting that oxidation during extraction did not occur. Cooke (1986) indicated about 10% higher values for fat content in wheat and other feedstuffs with acid hydrolysis followed by PE extraction than with PE extraction alone. On the basis of these results, acid hydrolysis prior to PE extraction has been accepted in Great Britain as the official method for ruminant feeds containing soaps (Sanderson, 1986).

Ether extraction does not always indicate the true energy value of feedstuffs and sometimes may be misleading because nonnutritive pigments and waxes are also extracted, or alternatively, fatty acids of calcium soaps will not be extracted unless glacial acetic acid is added to ether (Andrews and Lewis, 1970). We have compared the fatty acid content (mg/g) in hay, corn silage, hay–grain mixtures, total mixed ration, and feces extracted by Soxhlet using PE or PE–acetic acid with the PE extract estimated gravimetrically, and the results are in Table VI. The fatty acid content was significantly higher with extraction by PE–glacial acetic acid than by PE alone, indicating that the latter is inadequate for fat determination. Gravimetric determination gave much higher values in all the samples. Consistent and reproducible results obtained by the one-step method demonstrate its usefulness for routine analysis in research and the feed industry. Further, fat value may

Table V. Fatty Acid Composition (Weight %) of a Calcium Soap Prepared by Acid Hydrolysis, Solvent Extraction, and Transesterification or by the One-Step Method

method	n	total fatty acid, mg/g	fatty acids					
			14:0	16:0	18:0	18:1	18:2	18:3
one-step	6	799 ■ 5.4[a]	1.3 ± 0	49.1 ± 0.2	4.2 ± 0	35.2 ± 0.2	7.8 ± 0	0.3 ± 0
hydrolysis	6	796 ± 3.6	1.3 ± 0	49.0 ± 0	4.2 ± 0	35.0 ± 0.2	7.8 ± 0.1	0.3 ± 0

[a] Mean ± SE.

Table VI. Fat Content (mg/g) by Soxhlet Extraction: Comparison of Solvent and Quantitation Methods[a]

method	source (n)					
	hay (3)	corn silage (3)	hay–grain mix[b] (3)	hay–grain mix[c] (3)	TMR[d] (3)	feces (3)
Soxhlet–GLC[e]	5.50[a]	21.25[a]	7.84[a]	13.76[a]	9.97[a]	15.77[a]
Soxhlet–acid–GLC[e]	13.20[b]	38.60[b]	17.89[b]	33.87[b]	18.68[b]	32.67[b]
Soxhlet–gravimetric	26.38[c]	28.66[a]	26.39[c]	38.83[b]	30.04[c]	25.86[c]
Soxhlet–acid–gravimetric	32.37[c]	53.00[c]	37.56[d]	54.51[c]	50.51[d]	46.47[d]
SE	1.83	2.82	1.46	1.11	2.76	2.02

[a] Values in a column with similar superscripts do not differ ($P > 0.05$). [b] Alfalfa hay–concentrate mix, 1:1. [c] Alfalfa hay–high-fat concentrate mix, 1:1. [d] Total mixed ration: alfalfa silage–concentrate mix–corn silage, 1:2:1, DM. [e] Fatty acids.

Table VII. Fatty Acid Content (mg/g) of Several Feed Fats by Solvent Partition and Esterification or by Thin-Layer Chromatography (TLC) Followed by One-Step Extraction and Esterification (All Quantities by Gas–Liquid Chromatography)

fat	total oil (Folch)	separated by TLC					solvent partition	
		TG[a]	partial glycerides	complex lipids[c]	esterified FA[b]	nonesterified FA	esterified FA	nonesterified FA
1	780 ± 31[d]	606 ± 26	86 ± 6	79 ± 14	771 ± 55	12 ± 2	737 ± 18	20 ± 5
2	806 ± 9	400 ± 50	126 ± 26	16 ± 1	542 ± 42	83 ± 9	582 ± 10	78 ± 3
3	818 ± 16	565 ± 5	90 ± 16	13 ± 2	668 ± 6	90 ± 5	656 ± 9	86 ± 3
4	760 ± 10	408 ± 24	183 ± 14	58 ± 6	649 ± 18	83 ± 5	460 ± 14	76 ± 6

[a] Triacylglycerol. [b] Fatty acid. [c] Phospho- and glycolipids. [d] Mean ± SE ($n = 3$).

Table VIII. Comparison of One-Step (A) and Smith (B) Procedures for Determination of Fatty Acids in Milk Fat (Weight %)

fatty acid	sample							
	1		2		3		4	
	A	B	A	B	A	B	A	B
4:0	2.53	2.04	1.80	1.50	2.10	2.56	2.14	1.85
6:0	1.57	1.38	0.88	0.75	1.86	2.09	1.55	1.31
8:0	0.83	0.77	0.41	0.37	1.30	1.40	0.87	0.80
10:0	1.48	1.41	0.82	0.71	2.80	2.99	1.71	1.62
12:0	1.57	1.37	0.92	0.72	3.09	3.28	1.89	1.63
14:0	6.32	6.18	4.55	4.24	10.73	11.05	6.17	6.01
16:0	24.71	24.49	26.41	25.30	25.50	26.14	25.97	25.79
16:1	2.16	2.18	2.66	2.54	1.38	1.45	2.29	2.27
18:0	16.36	16.53	18.08	17.67	14.01	14.12	13.72	13.78
18:1	34.91	34.31	36.26	36.37	26.06	24.20	34.27	35.97
18:2	2.68	2.89	2.53	2.91	2.75	2.15	4.04	4.16
18:3	0.67	0.60	0.51	0.58	0.76	0.72	0.42	0.47

be estimated on the basis of total fatty acid content and composition rather than crude ether extract.

The one-step method as used in our laboratory uses 5.0 mL of a solvent system consisting of benzene–methanol–acetyl chloride in the ratio of 2.0:2.7:0.3 (v/v/v), which can extract 0.5 g or smaller amounts of sample containing 10–50 mg of fatty acid. We used more acetyl chloride and benzene than was used by Lepage and Roy (1986); this did not affect recovery of fatty acids. The greater amount of solvent was useful for conveniently transferring the sample with the Pasteur pipet.

As recommended by Outen et al. (1976), we have used 70 °C for 2 h, though the same results were obtained when reaction time was reduced to 1 h. Haan et al. (1979) have recommended a lower temperature during digestion because they anticipated some oxidation of polyunsaturated fatty acids at higher temperature. However, oxidation should not occur with purified solvents in the absence of air. Some polyunsaturated fatty acids may be lost by oxidation if the solvent evaporates during the esterification process, and, therefore, precautions must be taken to ensure air-tight reaction tubes. As noted above, higher temperature (90 °C) improves precision of soap analysis.

Outen et al. (1976) indicated that samples must be dried over P_2O_5 for about 12 h prior to extraction and methylation. We have found that freeze-dried (about 95% dry matter in our hands) or oven-dried (55 °C) samples give reproducible results with the method. Indeed, the methanol in the system adequately absorbed up to 100 mg of water (20% of the sample weight) in samples during development of the procedure. We routinely freeze-dry all samples for fatty acid analysis; however, oven-drying (55 °C) yielded similar results. Oxidation of polyunsaturates may be of concern with oven-drying of some samples.

The original procedure of Outen et al. (1976) utilized benzene as extraction solvent and has proven to be satisfactory in our hands with the exception of whole oilseeds as noted above. However, benzene is a recognized biohazard, which may prevent its use in some laboratories. We found that toluene was an acceptable substitute. The contents of total fatty acids (mg/g) in hay silage with benzene and toluene extraction were 19.40 ± 0.74 and 19.45 ± 0.93, means ± SE, $n = 3$.

The one-step method was also used for fatty acid analysis of lipids adsorbed to thin-layer chromatographic silica gel scrapings. Preparative TLC followed by this procedure quantitated esterified and nonesterified fatty acids in feed fat samples (Table VII). The results compared quite closely with those obtained by solvent partition. The recovery of various lipid fractions was lower when these were first extracted from the silica gel and then transesterified. Lepage and Roy (1986) used the one-step procedure for lipids adsorbed to silica gel and reported that up to 200 mg of silica does not hinder the transesterification process.

We have previously used the method of Smith (1961) to measure milk fatty acid composition, as this allows estimation of the lower chain fatty acids. We have now

standardized the conditions of the one-step method to prevent loss of methyl esters of short-chain fatty acids during the esterification and subsequent processes. The fatty acid compositions of four fat samples esterified by the one-step and Smith techniques were not different (Table VIII). We have also found that the one-step procedure will quantitatively transmethylate fatty acids in cream skimmed from milk, eliminating need for prior extraction with detergent.

Considering the wide application of the one-step method for quantitation of fatty acids in feedstuffs and feces, and qualitative estimation of milk fatty acids, the procedure is appropriate for commercial use as well as research. We recommend benzene (or toluene) rather than chloroform as a solvent for convenience in extraction of esters. The unique determination of fatty acids provides a more precise estimate of nutritive value of feedstuffs than other recently recommended modifications of the Soxhlet extraction.

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