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Fatty acid profiling in animal feeds and related food matrixes using a fast GC/MS method and *in situ* derivatization

Astrid Leiva[1] **Fabio Granados-Chinchilla**[1,*]

[1]Centro de Investigación en Nutrición Animal (CINA), Universidad de Costa Rica, 11501-2060 Ciudad Universitaria Rodrigo Facio San José, Costa Rica

[*]Corresponding Author: fabio.granados@ucr.ac.cr

Abstract

Fatty acid determination is used for the characterization of the lipid fraction in foods, providing essential information regarding feed and food quality. Most edible fats and oils are composed primarily of linear saturated fatty acids, branched, mono-unsaturated, di-unsaturated, and higher unsaturated fatty acids. To attain this information we developed a gas chromatography (GC) method that can separate fatty acids from C_4 to C_{24} using mass spectrometry identification. A simplified sample preparation procedure was applied so it is not time-consuming and short enough to avoid fat degradation. Additionally, one-step derivatization was applied to obtained fatty acid methyl esters in situ in the gas chromatograph injection port, using tetramethylammonium hydroxide and a high polarity polyethylene glycol-based cross-linked microbore chromatographic column was coupled to achieve the separation of 60 compounds in under 15 minutes with extreme sensibility. The versatility of the method allows fatty acid profile (including saturated [SFA], monounsaturated [MUFA], and polyunsaturated fatty acids [PUFA]) information to be gathered in different products of primary production i. raw materials commonly used in the production of animal feed, ii. profiles for balanced feed for laying hens, beef cattle and dairy cattle and iii. products of animal origin intended for human consumption, such as meat, eggs, and milk. Our data (performance parameters and fatty acid profiles) support the validity of the results; the method can be used for quality assurance both in productive species feed and feed ingredients, pet food, and related food matrices. The technique presented herein can be used as a high-throughput routine screening tool to assess fat quality as this data is paramount to improve animal nutrition and health and animal-derived products of human consumption.

Keywords: Animal feeds, Fat content and quality, Food-producing animals, GC/MS

Introduction

High-quality diets must input all necessary nutrients to maintain the animals' physical structure, biological functions, improve their physiological state and health while considering the species which the feed was meant to target (Makkar, 2016). Though, livestock feeding should also consider improvements in production (Thornton, 2010; Makkar, 2016), i.e., guaranteeing efficient growth, a persistent production without affecting the health of the animal or increasing the price of food. Therefore, their costs may limit the use of some feed ingredients. On the other hand, pet nutrition is mostly oriented toward optimizing the companion animal nutrition and health status (Di Cerbo *et al*., 2017). Thus, high-end feeding materials are preferred to formulate the latter type of feeds.

As protein and energy are considered limiting nutrients (NRC, 2001; Rostagno *et al.,* 2017), feed formulation should contemplate these requirements foremost. The use of fats and oils in animal feed, (contribution of dietary fat and fatty acids) is deemed to be essential as an energy concentrated nutrient (e.g., acylglycerols and emulsifiers), a carrier for other hydrophobic compounds (Poorghasemi *et al.,* 2013), and as feed palatability modulator, especially for cats and dogs (NRC, 2006;

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Çentingül and Yardimci, 2008; FEDIAF, 2016). Additionally, fat reservoirs supply essential fatty acid requirements. Therefore, feed materials are commonly classified by their energy input and, after that, by compatibility, digestibility, gastrointestinal functionality (Celi *et al.,* 2017), and accessibility. As such, agro by-products from local productive enterprises are exploited (Wood and Fearon, 2009; Ajila *et al.,* 2012)

However, as corn and soybean meal are versatile staple foods, they can be used in most livestock production systems. Both ingredients can represent as high as 60% of the dietary inclusion, though, corn meal is an adequate source of energy (3 294 kcal metabolizable energy kg^{-1}) whereas soybean meal is considered mostly a protein source (44 – 48 g/100 g on dry matter basis) (Rostagno *et al.,* 2017; Shepon *et al.,* 2016). Moreover, by-products in vegetable oil refining such as lecithin, soapstock, acid oil, and fatty acid distillate may also be included within diets (Kerr *et al.,* 2015).

Fat (e.g., obtained as a by-product of the rendering industry) and vegetable oil, a subgroup of lipids, production has increased as these substances are directly supplemented into livestock and poultry feed and pet foods (Kerr *et al.,* 2015). Fatty acid addition has demonstrated beneficial effects in several species such as horses (Hess and Ross-Jones, 2014), pigs (Rostagno *et al.,* 2017; Liu *et al.,* 2018), dairy cows (NRC, 2001; Harvatine and Allen, 2006), poultry (Poorghasemi *et al.,* 2013; Rostagno *et al.,* 2017) and, especially, management of several diseases and clinical problems in pets (Lenox and Bauer, 2013; Wąsik *et al.,* 2016). Of particular interest are, for example, linoleic (9c12c-$C_{18:2}$), eicosapentaenoic acid (5c8c11c14c17c-$C_{20:5}$), and docosahexaenoic acid (4c7c10c13c16c19c-$C_{22:6}$), ($C_{20:5}$) in puppy and dog food (Ahlstrøm *et al.,* 2004); relevant for cardiovascular health and nervous system development (Biagi *et al.,* 2004; Fraeye *et al.,* 2012).

On the another hand, as feed sits at the beginning of the food chain, knowledge about the fatty acid composition may improve animal nutrition (Baltić *et al.,* 2017) and enhance food products derived from such animals (Moran, 1996) (e.g., conjugated linoleic acid isomers [9c11t and 10c12t-$C_{18:2}$] and *trans* vaccenic acid [11t-$C_{18:1}$] in pasture-fed bovine meat and milk) (Daley *et al.,* 2010). In contrast, lipid and fatty acid deficiencies carry a plethora of health issues both for animals and humans alike (Sardesai, 1992).

After that, the development of fast and accurate analytical methods are necessary as all feed and food stakeholders should be able to assess lipid quality and nutritional value. Analytic approaches have historically made emphasis on food for human consumption, including gas chromatography (GC)-based Official Methods of Analysis℠ (e.g., AOAC 991.39, 975.39, 996.06, 994.15, 985.21, 963.22, 985.20, 965.49, 969.33). Several alkylation derivatization reagents have been used to generate the more volatile esters needed to perform the chromatography (Christie, 1993). Recently published research also had the same tendency and even compared transesterification or derivatization methods (Topolewska *et al.,* 2014; Topolewska *et al.,* 2015; Salimon *et al.,* 2017). However, all these methods usually rely on columns of considerable length, which results in long chromatographic runs (i.e., 60 minutes or more) to achieve an analytical separation (especially true for $C_{18:1}$, $C_{18:2}$, and, $C_{18:3}$).

Herein we report a method involving the direct extraction of fat using diethyl ether and the formation of methyl esters in the heated injection port of a GC coupled with mass spectrometry (MS) detector. The non-esterified fatty acids in a methanolic solution are pyrolyzed and suffer oxidative cleavage by the organic base catalyst. We chose this derivative formation technique as is highly practical but still able to render quantitative results. A similar approach has been applied to paint resins (West, 1975), human serum lipids (Haan *et al.,* 1979), and bacterial cells (Dworzanski *et al.,* 1990), to name a few, but, to our knowledge, never to feed or food products. Furthermore, butylated hydroxytoluene is added, during preparative stages of the method, to protect unsaturated fatty acids. Additionally, the chromatograph was equipped with a microbore short column which we applied the method successfully to a diverse group of samples that include animal feed and related matrices such as fats and oils, chicken eggs, bovine milk, and muscle tissue.

Materials and Methods

Reagents

Diethyl ether (309966, $(CH_3CH_2)_2O$, for HPLC, ≥ 99.9%, inhibitor-free), hydrochloric acid (320331, ACS reagent, 37%), 2,6-Di-tert-butyl-4-methylphenol (B1378, ≥ 99.0% purity) and tetramethylammonium hydroxide (TMAH, 334901, 25 wt. % solution in methanol) and trimethylphenyl ammonium hydroxide (TMPAH, 79266, 0.5 mol L^{-1} in methanol for GC derivatization) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol (MeOH, LiChrosolv®) was acquired from Merck Millipore (Merck KGaA, Darmstadt, Germany).

Analyzed Samples

Profiles were determined for feed ingredients such as corn meal (n = 35), soybean meal (n = 19), and peanut meal (n = 7). Fats and oils samples examined included animal fat (n = 8), palm oil (n = 8), and by-pass fat (n = 8). Feed samples analyzed encompassed layer hen feed (n = 10), beef cattle feed (n = 8), and dairy cattle feed (n = 10). Later, related food commodities tested involved chicken eggs (n = 11), bovine (n = 35), water buffalo (n = 11), and lamb (n = 11) meat tissues, and milk samples (n = 12). Also, our analysis included wet (n = 8) and dry extruded dog food (n = 20), dry extruded puppy food (n = 12), and wet (n = 6) and dry extruded cat food (n = 8). Finally, twelve forage mixtures were collected from Costa Rican northern lowlands cattle farms.

Sample fat extraction

A 100 g sample was milled and sieved to 1 mm (using a ZM 200 ultracentrifuge mill, Retsch GmbH, Haan, Germany), after that a subsample of ca. 1 gram of each feed or feed ingredient sample was set in a 50 mL glass beaker, 5 mL of diethyl ether were added and mixed using an ultrasonic shaker (USC200TH, VWR International, Center Valley, PA, USA) for 5 minutes. In the case of extruded meat samples, 2.5 mL of a 9 mol L^{-1} HCl solution in ethanol and 2.5 mL diethyl ether was added for extraction. Each egg sample was constituted by a dozen units, so four randomly chosen eggs were scrambled and freeze-dried

(LABCONCO, FreeZone 4.5 Liter, Kansas City, MO, USA), a gram of the resulting powder was used for fat extraction. Fresh forage was quartered and cut in bits and also freeze-dried before extraction. Freeze-drying was also applied to cat and dog food wet samples. Meat samples were processed using a knife mill (GM 300, Retsch) before fat extraction, a gram of minced meat was treated. Finally, bovine milk samples fatty acids extraction involved their direct mixing with a dichloromethane–ethanol solution (2:1), as described by Stefanov et al. (2010). Afterward, a 200 µL aliquot was transferred to a GC 2 mL vial (Agilent Technologies, Santa Clara, CA). Then, 800 µL of diethyl ether and 1 000 µL of a, previously prepared, 0.25 g/100 mL TMHA solution in methanol are added to the same vial. Two µL of the resulting mixture is injected into the GC system.

Additional nutritional, quality and functional assays

The total fatty acid content in oils and fats was performed using the AOCS method Ca 3a-46. Method 954.02 was used to assess fat content in by-pass fat as well. Fat content for the majority of feed and feed ingredient samples was determined using method AOAC OMA^{SM} 920.39. On the other hand, crude fat and water activity (a_w) were calculated in extruded pet foods by acid hydrolysis AOAC OMA^{SM} 954.02, and Aqualab chilled mirror methods (measurement performed at 24.50 ± 0.24 °C, Aqualab 4TE, Decagon Devices, [Pullman, WA], USA), respectively. AOAC OMA^{SM} 940.28 was used to assess free fatty acids in animal fats, palm oil, and by-pass fats; results expressed as g palmitic acid per 100 g sample. Egg, meat, and raw milk total fat content were determined by AOAC OMA^{SM} 925.32, 960.39, and 989.04, respectively.

Chromatographic conditions

Qualitative analyses of the volatile compounds were carried out using an Agilent gas chromatograph (7820, Agilent Technologies) equipped with an Agilent Technologies J&W DBWAX microbore column of 10 m length, 0.1 mm diameter, 0.1 µm film thickness and Agilent 5977E mass spectrometer (MSD). The carrier gas was helium at a constant flow of 0.3 mL min^{-1}. The GC oven temperature was kept at 50°C for 0.34 minutes and programmed to 200 °C at a rate of 72.51 °C minute^{-1}, this temperature was kept constant 0.17 minutes and then programmed to 230 °C at a rate of 8.7 °C minute^{-1}, held for 7.9 minutes for a total run time of 13.93 min. The split ratio was adjusted at 30:1. The injector, transfer line, ion source, and quadrupole temperatures were set at 250, 250, 230, and 150 °C, respectively. The mass range was 50-450 m/z. Electron energy was set at 70 eV, 150 °C. FAME mixtures GLC-486 (n = 40 analytes) and GLC-860 (n = 60 analytes, Nu-Chek Prep, Inc., Elysian, MN, USA) were used as quality control comparing retention times and mass spectra with those found in the analyzed samples (Figure 1A, B). Several compounds were used to check mass tuning including tetradecanoic (6.16 min; M^+ 227.6 m/z), pentadecanoic (6.72 min; M^+ 243.4 m/z), hexadecanoic (7.58 min; M^+ 256.3 m/z), octadecanoic (9.70 min; M^+ 285.5 m/z), *cis*-13-octadecanoic (10.21min; M^+ 285.7 m/z) and 9*Z*-octadecenoic (7.78 min; M^+ 284.1 m/z), (*Z*,*Z*)-9,12-octadecadienoic (10.86 min; M^+ 280.0 m/z) acids (Figure 1C). Constituents were identified by matching their spectra with those in NIST library 14. Only hits with a match factor above 80% were considered (Figure 1C). Enanthic acid (≥ 99%, 75190, Sigma-Aldrich) was used as an internal standard. 9c11t-$C_{18:2}$, 10c12t-$C_{18:2}$, $C_{12:0}$, 4c7c10c13c16c19c-$C_{22:6}$, 11t-$C_{18:1}$ were concurrently monitored by simultaneous ion monitoring (SIM) mode (total dwell time 100 ms and cycles 8.3 Hz. For compounds with no analytical standard injection, identification should be considered as tentative.

Results

Performance parameters and method peculiarities

From all analytes, $C_{18:0}$ showed a higher limit of detection (lower sensitivity) with 0.16 mg L^{-1}. In contrast, $C_{19:0}$ showed a higher sensitivity with 0.06 mg L^{-1}. Limits of detection determined in an extinction experiment using mix GLC-486. On feed samples, the sensitivity is calculated for corn meal, soybean meal, poultry layer feed, cattle feed, pet foods, and resulted in 0.146, 0.395, 0.066, 0.203, 0.047 g/100 g fat, respectively. On the other hand, values for $C_{18:0}$ expressed in chicken eggs, bovine milk and muscle tissue are determined to be 0.027, 0.041, and 0.011 g/100 g fat, respectively. C_{16} to C_{20} z values for rapeseed oil were found between -2.04 and 2.11, C_{18} compounds were found to be the most variable concerning the robust mean (Table 1). In a second performance test, using dry cat food, in which mostly MUFA and PUFA were assayed, z values, for $C_{16:1}$ to $C_{22:6}$, ranged from -1.34 to 1.56 (Table 1). The sum of SFA, MUFA, and PUFA were also tested. Our data were compared to a reference method (i.e., AOAC OMA^{SM} 996.06), though the reference method showed less deviation from the robust mean z values (1.14 to 1.47) for each fatty acid group (Table 1). C_{16} and 9c-$C_{18:1}$ showed the absolute difference (-37.25 and 20.54) between the methods (i.e., the proposed vs. derivatization using TMPAH vs. reference) making this a robust method (Table 2).

Feed ingredients

Data for three vegetable ingredients and fat and oils are presented. Corn meal presented a higher proportion of PUFA (544.59 ± 54.24 g kg^{-1} fat) than MUFA (282.81 ± 45.12 g kg^{-1} fat), and SFA (172.84 ± 23.78 g kg^{-1} fat). 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ predominates (i.e., 227.94 ± 123.09 and 444.07 ± 85.09 g kg^{-1} fat) (Table 3). Soybean meal showed average values for SFA, MUFA, and PUFA of 209.09 ± 29.22, 197.71 ± 40.37, and 593.81 ± 34.19 g kg^{-1} fat, respectively, with a predominance of linoleic acid (504.02 ± 122.97 g kg^{-1} fat) (Table 3). Peanut meal has a similar overall profile of SFA, MUFA and PUFA as the above two ingredients (i.e., 222.98 ± 63.93, 113.38 ± 28.81, 663.33 ± 77.56 g kg^{-1} fat) (Table 3). Regarding sample fat content, the 9c12c-$C_{18:2}$ contribution is higher for peanut than corn meal. On the contrary, in the animal fat exhibit a tendency toward SFA and MUFA (465.60 ± 11.50 and 430.90 ± 9.10 g kg^{-1} fat, respectively) which, in turn, reflects on 9c-$C_{18:1}$ concentration (i.e., 415.50 ± 15.30 g kg^{-1} fat) (Table 4 and Figure 2A). A similar profile is found in by-pass fat (i.e., 454.55 ± 55.95 and 483.95 ± 72.25 g kg^{-1} fat for SFA and MUFA, respectively) where $C_{18:0}$ is the most prominent fatty acid (i.e., 450.93 ± 57.83 g kg^{-1} fat). The same is true for palm oil (i.e., 436.83 ± 25.78 and 460.93 ± 29.81 g kg^{-1} for SFA and MUFA) (Table 4).

Compound feeds

Overall, compared to MUFA, layer hen feed have a higher PUFA ratio (i.e., 433.44 ± 43.2 and 327.59 ± 32.94 g kg^{-1} fat, respectively) (Table 5 and Figure 2B). The most significant fatty acids include palmitic, oleic, and linoleic (i.e., 170.28 ± 17.79, 314.83 ± 30.41, and 369.48 ± 55.00 g kg^{-1} fat, respectively) (Table 5). Cattle feed presents a higher concentration of SFA and MUFA (430.26 ± 134.42 and 358.09 ± 140.84 g kg^{-1} fat) with an average oleic acid input of 387.70 ± 56.57 g kg^{-1} fat (Table 5). In contrast, dairy cattle feed has a higher PUFA and MUFA (434.90 ± 34.42 and 335.18 ± 29.47 g kg^{-1} fat) with a considerable linoleic acid input (369.20 ± 26.06 g kg^{-1} fat) (Table 5).

Food samples (Feed-related matrices)

Eggs show an almost equivalent concentration of SFA and MUFA (i.e., 407.26 ± 80.64, and 427.25 ± 79.37 g kg^{-1}). In contrast, PUFA input is moderate (i.e., 165.66 ± 28.29 g kg^{-1} fat) with 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ as the predominant fatty acids (i.e., 363.20 ± 96.37 and 158.34 ± 57.95 g kg^{-1} fat) (Table 6 and Figure 2C). Interestingly, all meat tissues analyzed possess a similar range of total fat. Additionally, have a tendency toward SFA and MUFA where buffalo meat and lamb meat present the higher concentration of said fatty acids (i.e., 636.26 ± 99.44 and 462.81 ± 55.65 g kg^{-1} fat) (Table 6). Notwithstanding, 9c12c-$C_{18:2}$ levels are higher in bovine meat (405.31 ± 70.22 g kg^{-1} fat). Bovine milk presented a total fat of 32.93 ± 5.30 g kg^{-1} (Table 6). Interestingly, SFA largely predominates (i.e., 792.83 ± 84.52 g kg^{-1}). However, 9c-$C_{18:1}$ and $C_{16:0}$ are both major fatty acids present (i.e., 259.81 ± 58.22 and 268.02 ± 25.10 g kg^{-1} fat, respectively) (Table 6).

Pet foods

In the case of dog food, data are presented in adult and puppy dog food. Dry foods (less than 10 g/100 g moisture) showed, on average, a higher concentration of SFA (526.79 ± 151.31 g kg^{-1} fat) compared to wet foods (> 80 g/100 g moisture, 386.77 ± 85.88 g kg^{-1} fat) (Table 7). On the other hand, MUFA and PUFA remained in the same trend in both wet foods (382.02 ± 91.06 and 175.45 ± 37.78 g kg^{-1} fat, respectively), and dry foods (317.74 ± 84.48 and 167.40 ± 90.98 g kg^{-1} fat, respectively) (Table 7 and Figure 2D). About the presence of omega-3 and omega-6 fatty acids, higher levels of both types of fatty acids were found in wet foods (e.g., 9c12c-$C_{18:2}$ at 372.50 ± 57.03 g kg^{-1} of fat) (Table 7). In wet and dry foods, a higher concentration of 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ was found (i.e., 376.10 ± 35.48 and 194.88 ± 54.65 g kg^{-1} fat, respectively) (Table 7). Dry puppy foods have lower concentrations of MUFA and PUFA (i.e., 291.30 ± 61.62 and 132.51 ± 80.36 g kg^{-1}, a piece) compared to adult dog foods and trivial levels of 5c8c11c14c17c-$C_{20:5}$ and 4c7c10c13c16c19c-$C_{22:6}$ (Table 7). In the case of cat food, the data show a lower concentration of SFA, in wet foods when compared to dry foods (384.75 ± 105.39 and 560.92 ± 147.65 g kg^{-1} fat, respectively) (Table 7). Later, the concentration of MUFA in the wet foods is higher than in the dry ones (390.15 ± 51.15 and 287.85 ± 119.73 g kg^{-1} fat, respectively), while the PUFA presented a similar trend (225.10 ± 130.89 and 151.35 ± 53.10 g kg^{-1} of fat, respectively) (Table 7 and Figure 2D).

Forage blends fatty acid profiling

Forage mixtures used in the feeding of beef and dairy cattle show a predominance for SFA with ranging from 815.52 to 440.33 g kg^{-1} (Table 8). The concentration of MUFA and PUFA is similar in most samples. In forages with parts of PUFA less than 60.00 g kg^{-1}, there is an absence of 11c14c17c-$C_{20:3}$. On the other hand, all the forage samples have $C_{16:0}$ (Table 8). PUFA for the grass mixtures ranged from 21.25 to 375.47 g kg^{-1} fat (Table 8) with 9c-$C_{18:1}$ and 11c14c17c-$C_{20:3}$ with levels ranging from 136.45 to 262.58 and 94.15 to 164.87 g kg^{-1}, respectively.

Discussion

Performance parameters and method peculiarities

Sensitivity-wise, results are intuitive as meat samples, and pet foods have a more substantial fat content among their respective groups, which in turn, result in a lower limit of detection expressed within the matrix. Performance parameters obtained during the validation procedure (e.g., *z* values) speak toward an accurate, true, and relatively unbiased method (experimental *z* values should be between -2 and 2 to be deemed acceptable (Sykes *et al.,* 2014). The simple precision analysis demonstrated that minor modifications (i.e., using a different mass detector, derivatization agent, and conditions during the assay) do not affect the method performance considerably. A specific advantage that this method presents is the catalyst; under our experimental conditions, the transesterification occurs spontaneously, which, in turn, means a fewer step procedure. Finally, additional performance parameters such as %RSD for retention times and areas, k, α_s, N, and R_s are reported for the proposed method (Table 2) and are deemed adequate for a fit-to-purpose method (US FDA, 2015; Bhardwaj *et al.,* 2016; Borman and Elder, 2018). As a final consideration regarding the method scope, neither the column nor the mass spectra can distinguish among *cis*/*Z* and *trans*/E isomers. Such is the case for elaidic acid/oleic acid, linoelaic/linoleic, palmitoleic/palmitelaic. Fortunately, only *cis* isomerism is naturally occurring (except for ruminal fats), endogenous radical stress is the responsible mechanism *trans* isomerism in non-processed food and feed samples (Chatgilialoglu *et al.,* 2013).

Feed ingredients

Corn meal is used in more than half of animal diets, especially for poultry. However, in laying hen feeds, maximum inclusions of 65 g corn meal/100 g feed are usually achieved mostly due to economic and practical reasons. Mean crude fat contents range from 35.0 to 37.0 g kg^{-1} for this matrix, a value well in line with our experimental results (39.14 g kg^{-1}). Corn meal is considered a source of 9c12c-$C_{18:2}$ (1.78 g/100 g corn meal), a fact which is supported by our data (444.07 g kg^{-1} fat, 1.73 g/ 100 g corn meal). In contrast, 9c12c15c-$C_{18:3}$ acid exhibits higher levels (i.e., 106.61 g kg^{-1} fat, 0.42 g/100 g corn meal) than those reported (0.03 g/100 g corn meal) by other researchers (Sauvant *et al.,* 2004; Rostagno *et al.,* 2017). On the other hand, soybean meal also can be used in higher rates in animal diets. However, in laying hen feeds, inclusions of a maximum 30 g soybean meal/100 g feed are usually achieved. Soybean meal and hulls are utilized during feed formulation both by-products from the soybean oil industry (Kerr *et al.,* 2015).

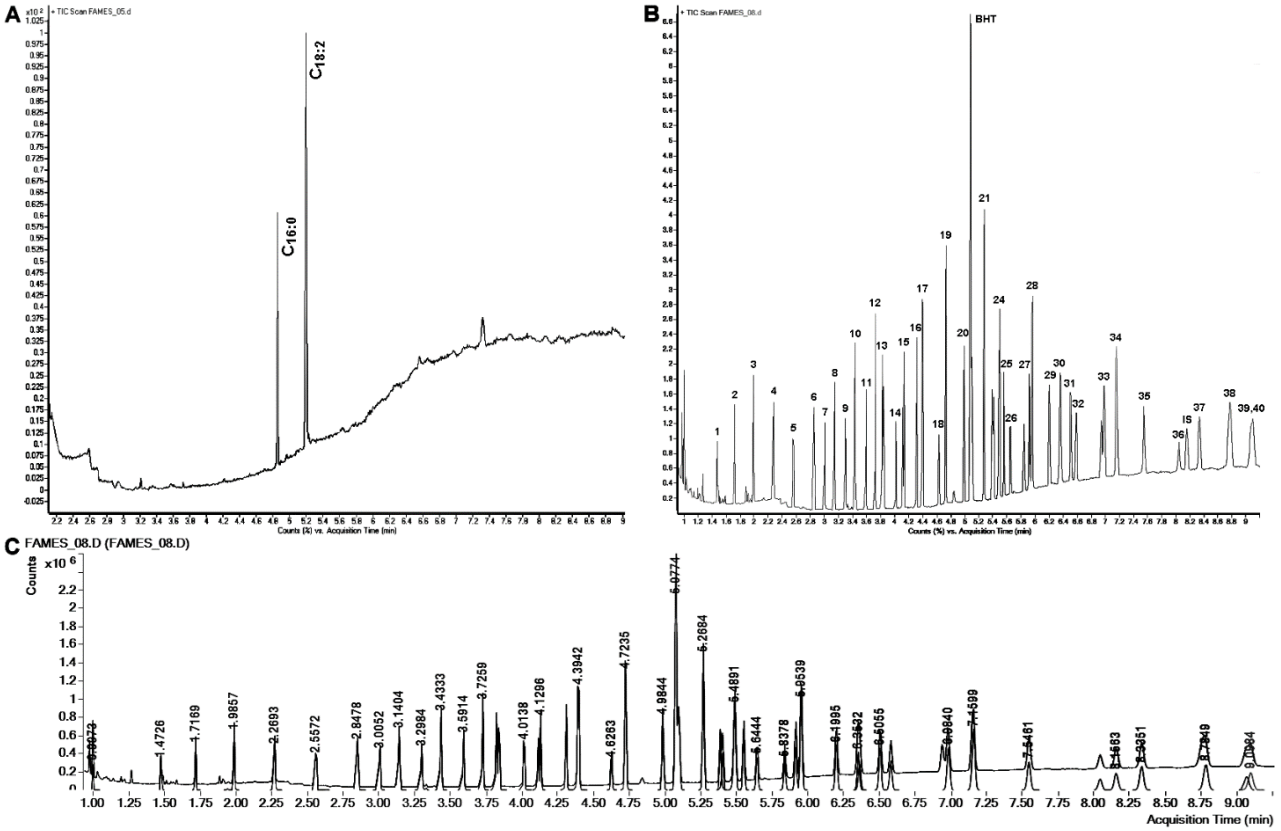

Figure 1. A. Methyl palmitate (hexadecanoic acid methyl ester) and methyl linoleate (9*Z*,12*Z*-octadecadienoic acid methyl ester) at 1.25 mg L^{-1} in diethyl ether. B. chromatographic separation of 40 fatty acid methyl esters mixture (GLC-486, from hexanoate, $C_{6:0}$, to lignocerate, $C_{24:0}$). 1 $C_{6:0,\ tr=1.47}$ 2 $C_{7:0,\ tr=1.72}$ 3 $C_{8:0,\ tr=1.99}$ 4 $C_{9:0,\ tr=2.27}$ 5 $C_{10:0,\ tr=2.56}$ 6 $C_{11:0,\ tr=2.85}$ 7 10e-$C_{11:1\ 10:1,\ tr=3.00}$ 8 $C_{12:0\ 12:0,\ tr=3.14}$ 9 $C_{1\text{-}P\text{-}12:0,\ tr=3.30}$ 10 $C_{13:0,\ tr=3.43}$ 11 7c-$C_{16:1,\ tr=3.59}$; 12 $C_{14:0,\ tr=3.73}$ 13 $C_{15:0,\ tr=4.01}$ 14 $C_{16:0,\ tr=4.31}$ 15 9c-$C_{16:1,\ tr=4.39}$ 16 $C_{17:0,\ tr=4.63}$ 17 10c-$C_{17:1,\ tr=4.72}$ 18 $C_{18:0,\ tr=4.98}$ 19 9c-$C_{18:1,\ tr=5.08}$ 20 9c12c-$C_{18:2,\ tr=5.26}$ 21 $C_{19:0,\ tr=5.39}$ 22 6c9c12c-$C_{18:3,\ tr=5.40}$ 23 10c-$C_{19:1,\ tr=5.49}$ 24 9c12c15c-$C_{18:3,\ tr=5.55}$ 25 9c11t-$C_{18:2,\ tr=5.64}$ 26 $C_{20:0,\ tr=5.84}$ 27 11c-$C_{20:1,\ tr=5.95}$ 28 11c14c-$C_{20:2,\ tr=6.19}$ 29 $C_{21:0,\ tr=6.35}$ 30 8c11c14c-$C_{20:3,\ tr=6.36}$ 31 5c8c11c14c-$C_{20:4,\ tr=6.50}$ 32 11c14c17c-$C_{20:3,\ tr=6.58}$ 33 $C_{22:0,\ tr=6.98}$ 34 11c-$C_{22:1,\ tr=7.16}$ 35 11c14c-$C_{20:2,\ tr=7.55}$ 36 7c10c13c16c-$C_{22:4,\ tr=8.04}$ 37 4c7c10c13c16c-$C_{22:5,\ tr=8.33}$ 38 $C_{24:0,\ tr=8.78}$ 39 15c-$C_{24:1,\ tr=9.07}$ 40 4c7c10c13c16c19c-$C_{22:6,\ tr=9.10}$. All analytes with a relative area sum of 2.70 g/100 g except for 15, 18, 19, 20, and, 27 at 3.8 g/100 g. C. Mass spectrometry identification based library match for mixture (GLC-486).

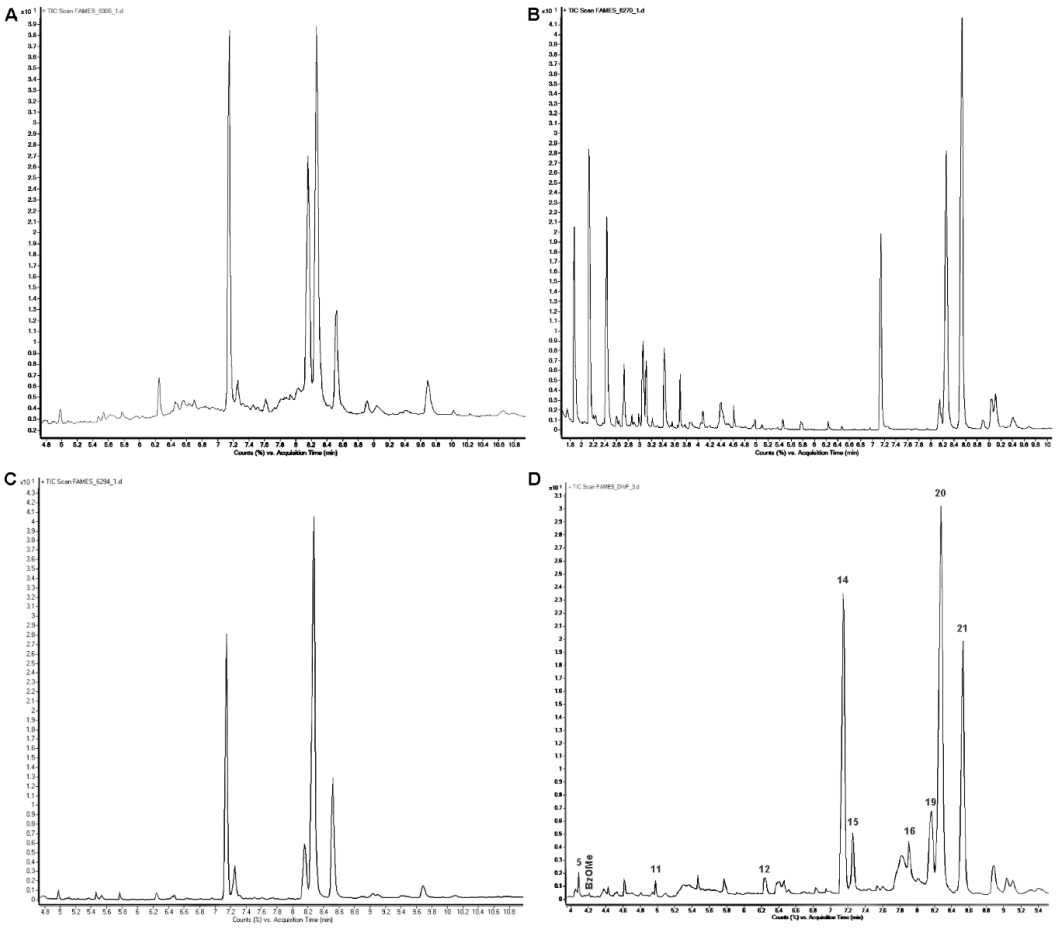

Figure 2. Fatty acid profiles (based on their methyl esters) using the proposed method of A. an animal fat. B. Layer feed. C. Chicken eggs. D. Wet dog food, more relevant acids identified were 5 $C_{10:0,\ tr=2.56}$ Benzoic acid methyl ester, 11 7c-$C_{16:1,\ tr=3.59}$; 12 $C_{14:0,\ tr=3.73}$ 14 $C_{16:0,\ tr=4.31}$ 15 9c-$C_{16:1,\ tr=4.39}$ 16 $C_{17:0,\ tr=4.63}$ 19 9c-$C_{18:1,\ tr=5.08}$ 20 9c12c-$C_{18:2,\ tr=5.26}$ 21 $C_{19:0,\ tr=5.39}$

Table 1. Method performance parameters values obtained for three different food commodities

Rapeseed oil (LGC Standards AFPS 025).						
Fatty acid shorthand	***Mean ± U_x [a]***		***Assigned value***		***Range or z value***	
	Concentration, g fatty acid/kg fat					
$C_{14:0}$	0.78 ± 0.04				0.50 to 0.78 *g fatty acid/kg fat*	
$C_{16:0}$	44.20 ± 2.07		44.20		0.00	
9c-$C_{16:1}$	1.86 ± 0.09		1.95		-0.35	
$C_{18:0}$	15.60 ± 0.73		19.00		-2.04	
9c-$C_{18:1}$	647.60 ± 30.39		583.46		2.11	
9c12c-$C_{18:2}$	201.30 ± 9.45		205.20		-1.29	
9c12c15c-$C_{18:3}$	78.60 ± 3.69		83.19		-1.05	
$C_{20:0}$	5.60 ± 0.26		5.41		0.11	
9c-$C_{20:1}$	10.01 ± 0.47				9.45 to 11.05 *g fatty acid/kg fat*	
Dry Cat Food (AAFCO Check Sample program 2018-25)						
Fatty acid shorthand	***g fatty acid/100 g fat***	**Corrected for fat content[b]**	**Reported range**	**Robust mean**	**Robust standard deviation**	***z* value**
		g fatty acid/100 g sample				
9c-$C_{16:1}$	3.46	0.543877	0.4115 - 0.535	0.45401	0.05751	1.56
9c12c-$C_{18:2}$	10.51	1.652067	1.435 - 2.19	1.8441	0.30459	-0.63
9c12c15c-$C_{18:3}$	0.018	0.282942	0.2545 - 0.37	0.31063	0.0528	-0.52
4c7c10c13c16c19c-$C_{22:6}$	1.11	0.174481	0.1325 - 0.225	0.16461	0.04116	0.24
5c8c11c14c17c-$C_{20:5}$	0.42	0.06602	0.0835 - 0.145	0.10393	0.02817	-1.34
Component	**Sum of saturated fatty acids (SFA)**		**Sum of monounsaturated fatty acids (MUFA)**		**Sum of polyunsaturated fatty acids (PUFA)**	
	Assay, *g fatty acid/100 g fat*	***z* value**	**Assay, *g fatty acid/100 g fat***	***z* value**	**Assay, *g fatty acid/100 g fat***	***z* value**
Wet cat food (Priida Round 75)						
	Robust mean = 35.83 *g fatty acid/100 g fat*[c]		**Robust mean = 49.11 *g fatty acid/100 g fat*[c]**		**Robust mean = 16.56 *g fatty acid/100 g fat*[c]**	
Proposed method	38.09	1.26	52.71	1.47	17.50	1.14
Reference Laboratory[d]	34.25	-0.88	49.26	0.06	15.89	-0.81
Canned meat (Priida Round 86)						
	Robust mean = 37.52 *g fatty acid/100 g fat*[c]		**Robust mean = 46.71 *g fatty acid/100 g fat*[c]**		**Robust mean = 16.84 *g fatty acid/100 g fat*[c]**	
Proposed method	40.10	1.40	41.10	-2.40	18.70	0.69
Reference Laboratory[d]	34.60	-1.60	46.50	-0.1	17.30	

[a]U_x calculated as the result of the fatty acid with the most variability for (i.e., $C_{18:1}$) $n = 5$ replicates, measured on five different days, using a coverage factor of 95% where $k = 2$. [b]Fat obtained by acid hydrolysis (15.72 ± 0.47) g/100 g [c]Analysis for wet cat food and canned meat-based on results by 16 and 7 laboratories, respectively. [d]Reference laboratory used method AOAC 996.06.

Table 2. Robustness assays for the selected method

Proposed method			Derivatization using TMPAH		Reference Laboratory	
	g fatty acid/kg fat				*g fatty acid/ kg fat*	
Fatty acid shorthand	***Concentration***	***Assigned value ± U_x***	***Concentration***	***Difference***	***Concentration***	***Difference***
Sunflower oil LGC Standards QFCS 246 sample 778						
2c-$C_{4:1}$	ND		0.20	-0.20	ND	0
$C_{4:0}$-diacid	0.20		ND	0.20	ND	0.20
2Me-$C_{10:0}$	0.40		ND	0.40	ND	0.40
$C_{9:0}$-diacid	0.61		ND	0.61	ND	0.61
$C_{13:0}$	ND		0.30	-0.30	ND	0
$C_{14:0}$	ND	0.80 ± 0.11	0.91	-0.91	0.73	-0.73
$C_{16:0}$	35.05	64.30 ± 0.90	49.79	-14.74	72.34	-37.29
11c-$C_{16:1}$	0.41	1.11 ± 0.05	0.81	-0.40	0.95	-0.54
$C_{18:0}$	35.96	34.33 ± 0.45	37.67	-1.71	31.97	3.99
9c-$C_{18:1}$	269.27	260.73 ± 3.07	283.31	-14.04	248.73	20.54
9c12c-$C_{18:2}$	620.34	612.95 ± 3.06	615.90	4.44	636.92	-16.58
9c11t-$C_{18:2}$	20.91		ND	20.91	ND	20.91
9c12c15c-$C_{20:3}$	1.31	1.93 ± 0.04	1.41	-0.10	2.03	-0.72
$C_{20:0}$	4.14	2.40 ± 0.07	2.83	1.31	1.91	2.23
11c-$C_{20:1}$	ND		1.72	-1.72	0	0
$C_{22:0}$	12.65	6.70 ± 0.21	11.41	1.24	4.41	8.24
$C_{24:0}$	3.63		3.54	0.09	ND	3.63
Sum of SFA	92.64		106.45	-13.81	111.37	-4.92
Sum of MUFA	269.68		286.04	-16.36	249.68	36.36
Sum of PUFA	642.56		617.31	25.25	638.95	-21.64

Fatty acid shorthand	**5977B[b]**		**5977E[b]**	
	Retention time (min)	**Area**	**Retention time (min)**	**Area**
$C_{6:0}$	1.4992	87381	1.5055	91982
$C_{16:0}$	4.0277	701584	4.0716	750947
$C_{18:1}$	4.9024	733871	4.9467	715453
$C_{18:2}$	5.1196	450546	5.1606	405774
4c7c10c13c16c19c-$C_{22:6}$	9.3794	180352	9.4452	169777

Overall	**Maximum**	**Minimum**
Retention time (min) %RSD[a]	2.707 for $C_{5:0}$	0.238 for 5c8c11c14c17c-$C_{20:5}$
Area %RSD[a]	14.101 for 5c8c11c14c-$C_{20:4}$	1.268 for 9c-$C_{18:1}$
Retention factor (k)	0.62	13.16
Selectivity (α)	1.84	1.01
Theoretical plates (N)	77108 $C_{11:0}$	2155 for $C_{24:0}$
Resolution (R_s)	14.42	0.57 between C21:0 and 8c11c14-$C_{20:3}$

[a]Analysis based on three individual samples assayed on different days. [b]A sample was analyzed using the same chromatographic conditions indicated above but using a different instrument model, different analyst, and days.

Table 3. Fat analysis and fatty acid profiling of feed ingredients using the proposed method

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat[a]			
	Corn meal (*n* = 35)			
$C_{8:0}$	21.32 ± 44.77	1.85	121.40	0.20
$C_{16:0}$	166.51 ± 45.14	151.00	260.70	87.10
11c-$C_{16:1}$	16.37 ± 22.96	6.00	75.30	3.10
$C_{18:0}$	61.63 ± 48.24	50.30	201.30	5.00
9c-$C_{18:1}$	227.94 ± 123.09	243.10	529.30	5.60
9c12c-$C_{18:2}$	444.07 ± 85.09	463.30	588.30	174.20
11c14c17c-$C_{20:3}$	14.99 ± 13.51	12.00	36.20	1.20
9c12c15c-$C_{18:3}$	106.61 ± 81.70	88.65	343.40	1.10
11c-$C_{18:1}$	156.46 ± 88.41	194.10	253.70	3.00
9t12t-$C_{18:2}$	49.15 ± 46.61	31.90	110.10	4.30
Sum of SFA	172.84 ± 23.78	166.80	222.10	121.70
Sum of MUFA	282.81 ± 45.12	276.00	377.50	196.00
Sum of PUFA	544.59 ± 54.24	546.50	659.30	409.50
Crude fat	39.14 ± 4.16	39.00	45.90	32.90
	Soybean meal (*n* = 19)			
$C_{16:0}$	186.82 ± 63.17	169.60	379.20	120.50
$C_{18:0}$	39.37 ± 13.85	37.00	87.10	27.20
9c-$C_{18:1}$	155.94 ± 65.28	157.40	235.90	21.50
9c12c-$C_{18:2}$	504.02 ± 122.97	533.90	643.60	65.80
9c12c15c-$C_{18:3}$	71.66 ± 22.19	75.55	101.30	23.30
11c14c17c-$C_{20:3}$	52.28 ± 23.67	52.60	79.50	10.10
Sum of SFA	209.09 ± 29.22	201.05	269.10	171.90
Sum of MUFA	197.71 ± 40.37	202.45	256.40	130.10
Sum of PUFA	593.81 ± 34.19	588.70	663.30	518.00
Crude fat	16.94 ± 4.18	16.95	23.30	8.40
	Inca Peanut meal/*Plukenetia volubilis* L. (*n* = 7)			
$C_{5:0}$	16.25 ± 13.25	16.25	29.50	3.00
$C_{9:0}$	16.20 ± 6.18	15.00	24.30	9.30
$C_{10:0}$	8.93 ± 10.87	1.30	24.30	1.20
$C_{12:0}$	3.00 ± 0.40	3.00	3.40	2.60
$C_{14:0}$	10.46 ± 8.01	12.80	20.40	1.00
$C_{16:0}$	119.78 ± 55.02	117.55	194.40	54.40
$C_{18:0}$	55.70 ± 17.52	60.60	74.10	31.70
9c-$C_{18:1}$	212.15 ± 123.82	187.50	450.00	98.00
9c12c-$C_{18:2}$	242.73 ± 100.26	291.65	326.90	32.20
9c12c15c-$C_{18:3}$	256.57 ± 126.94	271.60	413.80	27.80
9c11t-$C_{18:2}$	7.47 ± 3.25	6.30	11.90	4.20
11c14c-$C_{20:2}$	59.40 ± 14.58	59.90	77.00	41.30
Sum of SFA	222.98 ± 63.93	236.30	303.20	125.70
Sum of MUFA	113.38 ± 28.81	101.40	169.90	81.20
Sum of PUFA	663.33 ± 77.56	672.00	776.40	527.00
Crude fat	451.53 ± 89.90	504.60	524.50	325.50

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 4. Fat analysis and fatty acid profiling of fats and oils using the proposed method

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat[a]			
	Animal fat (*n* = 8)			
$C_{14:0}$	15.55 ± 1.45	15.55	17.00	14.10
$C_{16:0}$	345.85 ± 43.95	345.85	389.80	301.90
$C_{17:0}$	13.75 ± 3.25	13.75	17.00	10.50
$C_{18:0}$	83.35 ± 18.15	83.35	101.50	65.20
9c-$C_{18:1}$	415.50 ± 15.30	415.50	430.80	400.20
9c12c-$C_{18:2}$	100.45 ± 17.65	100.45	118.10	82.80
Sum of SFA	465.60 ± 11.50	465.60	477.10	454.10
Sum of MUFA	430.90 ± 9.10	430.90	440.00	421.80
Sum of PUFA	103.50 ± 20.70	103.50	124.20	82.80
Crude fat	875.47 ± 40.82	846.63	933.27	846.62
Free fatty acids (as oleic acid)	15.55 ± 10.49	11.70	33.20	5.60
	Palm oil (*n* = 8)			
$C_{12:0}$	12.10 ± 1.30	12.10	13.40	10.80
$C_{14:0}$	32.63 ± 18.11	26.60	57.20	14.10
$C_{16:0}$	423.00 ± 39.12	413.55	480.60	384.30
$C_{18:0}$	67.50 ± 13.12	65.20	84.60	52.70
9c-$C_{18:1}$	383.50 ± 66.42	380.55	465.70	307.20
9c12c-$C_{18:2}$	71.85 ± 29.73	84.15	97.80	21.30
9c12c15c-$C_{18:3}$	60.40 ± 9.30	60.40	69.70	51.10
9c11t-$C_{18:2}$	40.60 ± 10.50	40.60	51.10	30.10
Sum of SFA	436.83 ± 25.78	432.10	477.10	406.00
Sum of MUFA	460.93 ± 29.81	452.85	507.50	430.50
Sum of PUFA	102.23 ± 19.87	96.70	132.70	82.80
Crude fat	966.03 ± 26.38	967.12	997.82	933.25
Free fatty acids (as oleic acid)	2.09 ± 0.61	1.97	3.28	1.47
	By-pass fat (*n* = 8)			
$C_{14:0}$	24.40 ± 3.16	25.50	27.60	20.10
$C_{16:0}$	276.60 ± 0.00	276.60	276.60	276.60
11c-$C_{16:0}$	34.30 ± 0.00	34.30	34.30	34.30
$C_{17:0}$	9.50 ± 0.60	9.50	10.10	8.90
$C_{18:0}$	184.27 ± 11.13	189.00	194.90	168.90
9c-$C_{18:0}$	450.93 ± 57.83	456.70	518.70	377.40
9t12t-$C_{18:2}$	14.67 ± 6.19	10.90	23.40	9.70
9c12c-$C_{18:2}$	45.47 ± 8.90	45.60	56.30	34.50
9c12c15c-$C_{18:3}$	14.10 ± 5.00	14.10	19.10	9.10
Sum of SFA	454.55 ± 55.95	454.55	510.50	398.60
Sum of MUFA	483.95 ± 72.25	483.95	556.20	411.70
Sum of PUFA	58.20 ± 21.10	58.20	79.30	37.10
Crude fat	792.27 ± 125.58	845.60	912.30	618.90
Free fatty acids (as oleic acid)	0.48 ± 0.42	0.27	1.20	0.17

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 5. Fat analysis and fatty acid profiling of compound feed using the proposed method

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat[a]			
Layer hen feed (*n* = 10)				
$C_{16:0}$	170.28 ± 17.79	169.80	206.10	143.50
9c-$C_{16:1}$	14.35 ± 8.53	14.55	32.50	2.90
$C_{18:0}$	57.39 ± 18.95	54.00	94.80	29.80
9c-$C_{18:1}$	314.83 ± 30.41	310.30	376.50	273.70
9c12c-$C_{18:2}$	369.48 ± 55.00	348.20	509.20	307.70
9c12c15c-$C_{18:3}$	20.46 ± 6.80	21.15	28.10	9.30
9c11t-$C_{18:2}$	26.39 ± 13.08	27.35	49.90	10.60
10t12c-$C_{18:2}$	28.70 ± 5.06	29.60	37.20	22.20
Sum of SFA	235.41 ± 34.30	233.30	296.30	189.80
Sum of MUFA	327.59 ± 32.94	327.00	386.90	290.10
Sum of PUFA	433.44 ± 43.21	429.00	518.50	371.50
Crude fat	45.99 ± 8.56	44.20	68.00	37.30
Beef cattle feed (*n* = 8)				
$C_{6:0}$	11.37 ± 0.87	11.60	12.30	10.20
$C_{8:0}$	18.40 ± 6.28	16.10	28.90	12.50
$C_{10:0}$	21.35 ± 6.86	22.20	28.10	12.90
$C_{12:0}$	21.93 ± 8.88	19.45	36.00	9.80
$C_{14:0}$	12.72 ± 2.66	12.80	16.20	9.00
$C_{16:0}$	230.36 ± 20.99	234.40	261.10	206.80
9c-$C_{16:1}$	8.13 ± 3.25	8.70	11.80	3.90
$C_{18:0}$	39.97 ± 14.27	45.60	60.90	20.10
9c-$C_{18:1}$	387.70 ± 56.57	407.00	464.50	269.80
9c12c-$C_{18:2}$	98.97 ± 50.17	95.95	162.70	23.90
9c11t-$C_{18:2}$	105.22 ± 12.27	107.10	121.50	88.60
10t12c-$C_{18:2}$	48.28 ± 24.99	49.50	75.90	6.90
Sum of SFA	430.26 ± 134.42	388.10	666.30	274.30
Sum of MUFA	358.09 ± 140.84	389.90	591.70	180.10
Sum of PUFA	211.41 ± 54.37	225.50	282.70	133.80
Crude fat	93.22 ± 30.83	85.60	142.40	59.31
Dairy cattle feed (*n* = 10)				
$C_{8:0}$	19.30 ± 7.06	20.30	27.40	10.20
$C_{12:0}$	23.50 ± 26.16	11.45	67.90	3.20
$C_{14:0}$	13.10 ± 7.17	12.20	22.30	4.80
$C_{16:0}$	166.14 ± 13.17	161.30	191.20	154.50
$C_{18:0}$	30.60 ± 8.33	28.70	46.30	22.20
9c-$C_{18:1}$	335.18 ± 29.47	334.60	372.60	294.80
9c12c-$C_{18:2}$	369.20 ± 26.06	364.80	404.00	333.50
11c14c-$C_{20:2}$	47.72 ± 8.44	43.90	63.70	40.30
9c11t-$C_{18:2}$	24.17 ± 2.94	23.00	28.20	21.30
Sum of SFA	229.94 ± 39.95	212.80	305.60	190.00
Sum of MUFA	335.18 ± 29.47	334.60	372.60	294.80
Sum of PUFA	434.90 ± 34.42	414.60	477.80	399.60
Crude fat	47.21 ± 13.41	48.00	67.30	22.10

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 6. Fat analysis and fatty acid profiling of food commodities using the proposed method

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat[a]			
Chicken eggs (*n* = 11)				
Benzoic acid	15.17 ± 5.82	14.00	22.80	8.70
2-Me-$C_{4:0}$	8.70 ± 2.20	8.70	10.90	6.50
$C_{16:0}$	254.27 ± 19.68	249.10	297.30	228.50
9c-$C_{16:1}$	40.99 ± 6.13	41.90	48.60	33.00
$C_{18:0}$	140.87 ± 79.06	111.75	264.70	57.40
11c-$C_{18:1}$	16.60 ± 0.00	16.60	16.60	16.60
9c-$C_{18:1}$	363.20 ± 96.37	406.00	470.10	194.30
9c12c-$C_{18:2}$	158.34 ± 57.95	141.10	330.00	90.90
9c12c15c-$C_{18:3}$	7.43 ± 4.70	10.40	11.10	0.80
11c14c-$C_{20:2}$	12.86 ± 5.73	14.90	18.00	2.50
11c14c17c-$C_{20:3}$	59.10 ± 0.00	59.10	59.10	59.10
10t12c-$C_{18:2}$	16.55 ± 6.55	16.55	23.10	10.00
$C_{20:0}$	11.15 ± 9.15	11.15	20.30	2.00
9t11c-$C_{18:2}$	11.80 ± 6.80	11.80	18.60	5.00
5c8c11c14c-$C_{20:4}$	3,06 ± 0.49	2.80	3.80	2.50
Sum of SFA	407.26 ± 80.64	379.30	550.50	313.60
Sum of MUFA	427.25 ± 79.37	448.20	517.00	273.50
Sum of PUFA	165.66 ± 28.29	167.50	210.30	120.30
Total fat content	91.73 ± 16.13	83.60	125.60	70.30
Bovine meat tissue (*n* = 35)				
$C_{7:0}$	11.43 ± 9.77	5.20	35.10	1.70
$C_{8:0}$	6.79 ± 3.12	6.10	12.20	1.60
$C_{9:0}$	8.05 ± 3.55	6.45	15.20	3.80
$C_{10:0}$	8.94 ± 5.51	7.60	18.60	2.20
$C_{14:0}$	57.13 ± 35.05	43.85	163.10	12.40
$C_{15:0}$	9.39 ± 6.91	8.05	38.60	3.10
11c-$C_{16:1}$	32.84 ± 16.35	26.60	73.60	15.80
$C_{16:0}$	309.14 ± 75.85	309.65	607.30	48.40
9c-$C_{12:1}$	19.42 ± 27.55	11.40	113.30	5.30
9c-$C_{16:1}$	27.92 ± 19.49	24.70	73.50	3.00
$C_{17:0}$	13.80 ± 8.46	10.10	35.90	5.90
10c-$C_{17:1}$	8.77 ± 2.17	8.80	11.90	4.80
$C_{18:0}$	133.04 ± 41.75	131.70	211.40	45.10
9c-$C_{18:1}$	405.31 ± 70.22	394.10	609.20	269.70
9c11t-$C_{18:2}$	10.45 ± 4.33	9.60	19.50	5.80
9c12c-$C_{18:2}$	27.06 ± 30.14	13.85	116.80	3.00
11c14c-$C_{20:2}$	16.53 ± 6.54	16.00	24.80	8.80
Sum of SFA	526.41 ± 74.06	524.35	678.80	376.10
Sum of MUFA	437.61 ± 78.87	412.60	615.90	275.40
Sum of PUFA	33.43 ± 32.22	24.80	118.60	0.00
Total fat content	308.90 ± 66.07	310.55	486.80	158.00
Water buffalo (*Bubalus bubalis*) meat tissue (*n* = 11)				
$C_{14:0}$	35.58 ± 10.95	33.40	57.70	22.30
$C_{15:0}$	13.58 ± 4.09	12.20	20.80	8.00
15Me-$C_{16:0}$	7.30 ± 5.09	3.80	14.50	3.60
11Me-$C_{16:0}$	27.47 ± 5.41	24.10	35.10	23.20
$C_{16:0}$	270.87 ± 44.58	248.50	355.10	221.70
$C_{17:0}$	27.53 ± 6.27	30.20	35.80	14.10
9c-$C_{16:1}$	27.48 ± 8.29	23.70	44.90	18.60
14Me-$C_{16:0}$	8.68 ± 3.25	7.50	13.90	5.80
$C_{18:0}$	248.55 ± 67.47	246.10	378.10	148.40

9c-$C_{18:1}$	326.78 ± 45.84	337.75	393.90	253.50
14c17c-$C_{18:2}$	7.87 ± 1.86	7.20	10.40	6.00
9c11t-$C_{18:2}$	13.41 ± 2.25	13.50	16.80	10.70
9c12c-$C_{18:2}$	15.74 ± 10.90	14.30	36.10	4.00
Sum of SFA	636.26 ± 99.44	618.70	933.70	554.20
Sum of MUFA	335.81 ± 97.99	359.00	423.60	48.70
Sum of PUFA	27.88 ± 8.47	25.00	46.50	17.30
Total fat content	356.00 ± 130.40	332.10	548.20	106.10
Lamb meat tissue (*n* = 11)				
$C_{14:0}$	30.46 ± 5.76	32.00	3.90	1.82
$C_{15:0}$	6.34 ± 2.78	5.10	1.08	0.36
$C_{16:0}$	250.74 ± 24.62	259.80	26.99	18.3
9c-$C_{16:1}$	17.18 ± 5.70	17.45	2.62	0.48
$C_{17:0}$	16.39 ± 6.30	15.60	3.10	0.42
10c-$C_{17:1}$	8.80 ± 5.59	4.90	1.67	0.48
$C_{18:0}$	336.47 ± 54.99	360.40	39.69	23.09
9c-$C_{18:1}$	291.35 ± 60.70	266.90	40.08	21.97
9c11t-$C_{18:2}$	9.30 ± 3.90	9.50	1.36	0.12
10t12c-$C_{18:2}$	3.00 ± 0.10	3.00	0.31	0.29
Sum of SFA	523.30 ± 53.59	523.30	603.70	386.50
Sum of MUFA	462.81 ± 55.65	471.00	598.40	369.20
Sum of PUFA	16.10 ± 9.52	15.80	30.10	1.40
Total fat content	301.50 ± 110.45	355.60	406.10	131.40
Raw bovine milk (*n* = 12)				
$C_{6:0}$	20.39 ± 4.78	20.25	30.80	12.30
$C_{8:0}$	17.72 ± 7.44	18.00	36.40	7.50
$C_{10:0}$	34.41 ± 13.27	32.40	65.20	15.10
$C_{12:0}$	40.43 ± 14.04	36.95	77.70	25.80
$C_{14:0}$	116.68 ± 27.16	113.80	173.00	75.20
$C_{15:0}$	11.90 ± 1.35	12.70	13.00	10.00
$C_{16:0}$	268.02 ± 25.10	270.50	315.80	218.50
$C_{16:1}$	45.19 ± 78.44	13.30	277.40	5.00
$C_{17:0}$	18.00 ± 0.50	18.00	18.50	17.50
$C_{17:1}$	12.81 ± 6.32	10.80	26.80	3.40
$C_{18:0}$	11.12 ± 7.94	13.10	26.80	1.30
9c-$C_{18:1}$	259.81 ± 58.22	267.80	359.40	158.40
6c-$C_{18:1}$	134.77 ± 75.42	109.90	291.40	55.50
9c12c-$C_{18:2}$	16.10 ± 4.86	16.00	22.10	10.20
Sum of SFA	792.83 ± 84.52	822.70	906.30	626.80
Sum of MUFA	159.77 ± 78.74	133.70	314.60	48.50
Sum of PUFA	47.46 ± 19.92	41.20	81.60	17.70
Total fat content	32.93 ± 5.30	32.70	49.40	23.70

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 7. Fatty acid profile and water activity for commercially available pet foods

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat[a]			
Wet dog food (*n* = 8)				
$C_{14:0}$	14.93 ± 4.60	13.40	22.50	10.40
$C_{16:0}$	259.12 ± 34.90	253.20	327.80	212.40
$C_{16:1}$	45.95 ± 9.44	41.95	62.00	37.90
$C_{18:0}$	124.62 ± 37.36	104.65	188.80	90.30
9c-$C_{18:1}$	376.10 ± 35.48	394.55	403.90	301.90
9c12c-$C_{18:2}$	143.13 ± 31.37	140.00	199.80	103.40
11c14c-$C_{18:2}$	16.50 ± 5.78	15.20	27.50	11.60
5c8c11c14c17c-$C_{20:5}$	4.25 ± 0.72	4.15	5.30	3.40
4c7c10c13c16c19c-$C_{22:6}$	1.85 ± 0.93	1.80	3.20	0.60
Sum of SFA	386.77 ± 85.88	369.35	494.70	246.60
Sum of MUFA	382.02 ± 91.06	413.50	483.30	203.40
Sum of PUFA	175.45 ± 37.78	190.10	216.70	115.70
Dry dog food (*n* = 20)				
$C_{2:0}$-diacid	16.33 ± 10.66	13.10	33.80	1.40
$C_{3:0}$	11.90 ± 12.60	5.50	33.20	0.50
$C_{4:0}$-diacid	15.18 ± 11.31	10.65	36.60	1.80
$C_{5:0}$	3.68 ± 4.52	1.90	15.50	1.10
$C_{12:0}$	20.77 ± 14.82	15.80	46.70	5.90
$C_{14:0}$	15.70 ± 8.61	15.55	29.30	2.20
$C_{16:0}$	359.10 ± 94.31	341.00	547.00	224.90
9c-$C_{16:1}$	12.63 ± 1.62	13.30	14.20	10.40
$C_{18:0}$	76.24 ± 37.49	76.50	169.80	22.40
9c-$C_{18:1}$	283.57 ± 45.81	291.30	352.20	192.70
9c12c-$C_{18:2}$	194.88 ± 54.65	186.70	328.00	122.50
9c12c15c-$C_{18:3}$	7.43 ± 2.19	7.65	10.10	4.30
11c14c17c-$C_{20:3}$	8.98 ± 4.05	10.45	12.80	2.20
Sum of SFA	526.79 ± 151.31	545.90	810.60	290.70
Sum of MUFA	317.74 ± 84.48	315.35	495.30	169.70
Sum of PUFA	167.40 ± 90.98	207.40	345.30	19.60
Crude fat	125.20 ± 15.54	125.90	155.60	106.00
a_w	0.5356 ± 0.0961	0.5492	0.6790	0.3720
Dry puppy food (*n* = 12)				
$C_{2:0}$-diacid	20.27 ± 6.79	20.95	28.70	10.40
$C_{3:0}$	14.46 ± 9.25	12.30	29.30	2.00
$C_{4:0}$-diacid	35.06 ± 33.70	24.20	109.40	7.40
2-Me-$C_{6:0}$	14.23 ± 10.47	10.30	35.50	5.10
2-Me-$C_{5:0}$	45.97 ± 47.34	26.40	111.20	0.30
$C_{8:0}$	10.24 ± 6.35	12.50	18.60	2.80
$C_{11:0}$	16.90 ± 8.07	17.30	27.90	5.10
9c-$C_{12:1}$	28.10 ± 14.60	19.00	48.70	16.60
$C_{14:0}$	19.95 ± 2.79	20.90	22.60	15.40
$C_{16:0}$	378.27 ± 89.09	410.00	511.20	257.00
$C_{18:0}$	78.11 ± 23.37	81.80	105.60	40.00
9c-$C_{18:1}$	212.61 ± 37.89	206.80	309.90	173.70
9c12c-$C_{18:2}$	112.99 ± 32.99	115.10	173.80	48.70
13t-$C_{18:1}$	214.90 ± 46.30	214.90	261.20	168.60
Sum of SFA	590.88 ± 122.96	568.80	813.10	348.90
Sum of MUFA	291.30 ± 61.62	276.70	396.90	183.00
Sum of PUFA	132.51 ± 80.36	116.30	254.10	3.90
Crude fat	132.35 ± 14.14	126.85	153.70	116.80
a_w	0.5837 ± 0.0682	0.5966	0.6694	0.4339

Wet cat food (*n* = 6)				
6c-$C_{16:1}$	35.18 ± 2.96	34.95	38.70	32.10
2Me-$C_{4:0}$	8.08 ± 2.66	8.00	11.20	5.10
$C_{16:0}$	257.40 ± 81.62	248.50	379.70	152.90
$C_{18:0}$	151.08 ± 25.96	154.20	179.70	116.20
9c-$C_{18:1}$	372.50 ± 57.03	393.90	425.30	276.90
9c12c-$C_{18:2}$	172.88 ± 5.53	172.60	180.80	165.50
9c11t-$C_{18:2}$	58.35 ± 3.65	60.00	61.30	52.10
11c14c- $C_{20:2}$	64.50 ± 6.50	63.60	74.50	56.30
11c14c17c- $C_{20:3}$	44.15 ± 8.24	41.30	57.90	36.10
Sum of SFA	384.75 ± 105.39	359.95	551.80	267.30
Sum of MUFA	390.15 ± 51.15	404.80	441.70	309.30
Sum of PUFA	225.10 ± 130.89	206.55	423.40	63.90
Dry cat food (*n* = 8)				
$C_{2:0}$-diacid	19.48 ± 18.09	11.70	48.50	0.80
$C_{3:0}$-diacid	12.68 ± 8.51	12.85	24.40	0.80
$C_{4:0}$-diacid	17.51 ± 9.54	19.80	31.70	3.40
$C_{4:0}$	8.58 ± 7.66	8.10	17.90	0.20
2-Me-$C_{10:0}$	13.30 ± 4.36	12.20	19.10	8.60
$C_{11:0}$	8.50 ± 1.90	8.50	10.40	6.60
$C_{14:0}$	48.10 ± 16.78	47.45	71.30	26.20
$C_{16:0}$	368.38 ± 61.73	352.30	460.60	298.00
9c-$C_{16:1}$	29.10 ± 7.35	26.90	39.00	21.40
$C_{18:0}$	97.33 ± 14.70	106.40	109.00	76.60
9c-$C_{16:1}$	192.13 ± 89.01	212.95	289.50	36.40
9c-$C_{16:1}$	128.70 ± 67.41	128.70	227.00	44.70
Sum of SFA	560.92 ± 147.65	559.40	842.90	391.80
Sum of MUFA	287.85 ± 119.73	326.40	387.80	42.40
Sum of PUFA	151.35 ± 53.10	137.30	236.30	78.90
Crude fat	126.02 ± 15.67	129.40	144.30	107.40
a_w	0.5477 ± 0.0505	0.5332	0.6369	0.4987

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 8. Fatty acid analysis for multi-species forages found along with dairy cattle farms in Costa Rica

Botanical sample[a] (*Composition. g/100 g*)				*Major components*[b] *(Concentration. g/kg)*			
Sample 1	Ratana (57)	Tanner (18)	Brachiaria (14)	$C_{5:0}$ (118.02)	$C_{14:0}$ (145.06)	$C_{16:0}$ (437.44)	13c-$C_{18:1}$ (278.23)
Sample 2	Tanner (53)	Ratana (36)	Tropical kudzu (4)	$C_{14:0}$ (111.21)	$C_{16:0}$ (580.08)	$C_{18:0}$ (124.23)	9c-$C_{18:1}$ (141.65)
Sample 3	Ratana (44)	Aleman (33)	Tanner (19)	9:0-diacid (118.02)	$C_{14:0}$ (125.90)	$C_{16:0}$ (465.73)	13c-$C_{18:1}$ (183.31)
Sample 4	Ratana (42)	Other grasses (31)	Tanner (21)	2-Me-$C_{10:0}$ (13.21)	12-Me-$C_{13:0}$ (199.71)	$C_{16:0}$ (460.61)	9c-$C_{18:1}$ (292.52)
Sample 5	Other grasses (44)	Aleman (27)	Tanner (22)	2-Me-$C_{14:0}$ (113.40)	$C_{16:0}$ (379.41)	9c-$C_{18:1}$ (136.45)	11c14c17c-C20:3 (164.87)
Sample 6	Guinea (50)	Other grasses (32)	Poró (13)	$C_{14:0}$ (133.31)	$C_{16:0}$ (307.02)	9c-$C_{18:1}$ (189.01)	11c14c17c-$C_{20:3}$ (160.06)
Sample 7	Guinea (40)	Ratana (37)	Other grasses (17)	$C_{16:0}$ (418.45)	$C_{18:0}$ (199.71)	13c-$C_{18:1}$ (262.58)	11c14c17c-$C_{20:3}$ (107.81)
Sample 8	Other grasses (53)	Tanner (31)	Guinea (11)	$C_{14:0}$ (154.56)	$C_{16:0}$ (313.55)	$C_{18:0}$ (211.02)	$C_{18:1}$ (222.44)
Sample 9	Tanner (53)	Other grasses (33)	Aleman (9)	$C_{14:0}$ (62.38)	$C_{16:0}$ (452.40)	13c-$C_{18:1}$ (202.12)	11c14c17c-$C_{20:3}$ (114.93)
Sample 10	Other grasses (51)	Tanner (21)	Aleman (10)	$C_{14:0}$ (147.25)	$C_{16:0}$ (478.00)	$C_{18:0}$ (152.37)	14c-$C_{18:1}$ (164.78)
Sample 11	Guinea (69)	Ratana (21)	Other grasses (8)	$C_{16:0}$ (332.66)	$C_{18:0}$ (202.21)	9c-$C_{18:1}$ (173.58)	11c14c17c-$C_{20:3}$ (173.18)
Sample 12	Tanner (44)	Other grasses (33)	Ratana (21)	$C_{16:0}$ (435.36)	$C_{18:0}$ (185.50)	9c-$C_{18:1}$ (194.20)	11c14c17c-$C_{20:3}$ (94.51)

Sample	1	2	3	4	5	6	7	8	9	10	11	12
						Concentration. g/kg)						
Sum SFA	700.52	815.52	709.65	673.53	492.81	440.33	618.16	679.13	514.78	777.62	534.87	620.86
Sum MUFA	278.23	141.65	183.31	292.52	136.45	189.01	262.58	222.44	202.12	164.78	173.58	194.2
Sum PUFA	21.25	42.83	107.04	33.93	370.74	375.47	176.32	98.43	333.04	57.6	283.24	255.3

[a]Para: *Brachiaria mutica* (Forssk.) Stapf. Tanner: *Brachiaria arrecta* (Hack. ex T. Durand & Schinz) Stent. Ratana: *Ischaemum indicum* Houtt.. Tropical kudzu: *Pueraria phaseoloides* (Roxb.) Benth. Guinea: *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs. Aleman: *Echinochloa polystachya* (Kunth) Hitchc.
[b]Represented the four most abundant acids found. other fatty acids include 2-Me-$C_{5:0}$. 2-Me-$C_{6:0}$. 2-Me-$C_{7:0}$. 6-Me-$C_{7:0}$. 2-Me-$C_{8:0}$. $C_{10:0}$. $C_{11:0}$

Soybean meal inputs, nutritionally, 1.05 – 1.83 g crude fat, 0.68 g 9c12c-$C_{18:2}$, and 0.09 g 9c12c15c-$C_{18:3}$ per 100 g material (Sauvant *et al.,* 2004). Values consistent with our data (504.02 g kg^{-1} fat, 0.85 g/100 g soybean meal and 71.66 g kg^{-1} fat, 0.12 g/100 g soybean meal, respectively). Additionally, energy-wise, it can still impart 2 120 kcal metabolizable energy kg^{-1}. Notwithstanding, the primary dietary input of soybean meal lies in amino acids (e.g., 2.74 – 2.91 g lysin/100 g) (Sauvant *et al.,* 2004; de Blass *et al.,* 2010; Rostagno *et al.,* 2017). Crude fat and fatty acids obtained for the Inca peanut meal are consistent with those reported elsewhere (Pereira de Souza *et al.,* 2013). Beneficial impacts on supplementing fats and oils are based on their high-energy coefficients, their nitrogen-keeping effect in a body, positive influence on metabolism regulation and accumulation of vitamins in tissues (Janovych and Lagodyuk, 1991). For animal-derived fats, inclusion rates vary from 3 to 7 g/100 g in layer hens and up to 3 g/100 g in ruminants (NRC, 2001; de Blass *et al.,* 2010; Rostagno *et al.,* 2017).

These ingredients have a high SFA and MUFA ratio. As a result, fats usually have compact buildings; several types of fats are available for use in food, pet foods, and feed applications (Sharma *et al.,* 2013). Poultry, swine, and bovine-derived fat inputs 8 681, 8 080, and 7 401 kcal metabolizable energy kg^{-1}. Additionally, contributes 20.5, 9.2 to 9.63 and 3.1 g 9c12c-$C_{18:2}$/100 g fat (Chilliard *et al.,* 2001; de Blass *et al.,* 2010; Rostagno *et al.,* 2017). Our mean values of 9c12c-$C_{18:2}$ round up to 8.79 g/100 g fat. On the other hand, vegetable oils with high diversity in fatty acids are preferred. In this regard, palm oil has a low peroxidizability and is a good source of $C_{16:0}$, 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ (39.2, 44.0, and 10.0 g/100 g, respectively) (Kerr *et al.,* 2015). Our data concur with values reported elsewhere with 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ mean values at 37.04 (383.50 g kg^{-1} fat) and 10.86 g/100 g (112.45 g kg^{-1} fat) (Sauvant *et al.,* 2004). Finally, by-pass fat (usually calcium salts from fatty acids) are not retained in the rumen (and even can evade reticulum, omasum, and abomasum) (Chilliard *et al.,* 2001). Saponified fats derived from palm oil and tallow report mean values of 4.1 and 42.7 g $C_{18:0}$/100 g by-pass fat, respectively (NRC, 2001; de Blass *et al.,* 2010). Our data show by-pass fats with a high content of stearic acid (i.e., 35.71 g/100 g by-pass fat, 450.93 g kg^{-1} fat) which hints toward an origin from palm oil.

Incorporation of long-chain fatty acids such as $C_{16:0}$ and $C_{18:0}$, found in palm fats, greatly influence lactation efficiency (Paintoni *et al.*, 2015; Boerman *et al.*, 2017). Also, the application of supplements (as high as 4 g/100 g inclusion) (FEDNA, 2009; de Blass *et al.,* 2010) of protected fats and polyenoic fatty acids of different age and productive groups of cattle demonstrates positive metabolic and productive effects (Pavkovych *et al.,* 2015). Less palatable (e.g., low fat) foods, may result in rejection by animals (an effect mainly observed in pets). Finally, free fatty acids in fats and oils are a measurement of hydrolysis due to storage or processing (Mahesar *et al.,* 2014). These compounds are less stable than neutral oil and, thus, more prone to oxidation and rancidity (Mahesar *et*

al., 2014). Data found herein are well in line with maximum thresholds for fats and oils (i.e., 4-8 g/100 g expressed as oleic acid) (Baião and Lara, 2005; Azeman *et al.,* 2015).

Compound feeds

In the case of dairy and beef cattle feeds, dietary input has the primary purpose of providing the substrate for the ruminal microbiota. In turn, the type of substrate modifies the rumen itself and its fermentation characteristics (Duarte *et al.,* 2017). Microorganisms such as bacteria, fungi, and protozoa can degrade complex structures (e.g., forages) and free utilizable nutrients (NRC, 2001; FEDNA, 2009; Duarte *et al.,* 2017). After rumen-mediated lipolysis (where metabolisms of long-chained fatty acids and hydrogenation of unsaturated fatty acids take place), fat biohydrogenation takes place, a process responsible for the concentration and proportion of fatty acids in tissue and milk (Woods and Fearon, 2009; Castillo *et al.,* 2013). As the choice of animal feeding system influences animal products (Schmitt et al., 2018), fatty acid-rich feed ingredients are also included in ruminant diets to ensure biotransference to meat and milk which, in turn, have demonstrated to an extent to improve public health (Givens, 2015; Schmitt *et al.,* 2018). In ruminants, nutritional requirements are based on weight, age, stage of production, physiological stage, as well as physical activity. However, no minimum thresholds have been set for fatty acids (NRC, 2001; FEDNA, 2009). High-throughput dairy production has a high demand for energy (needed net energy for lactation 1.8 Mcal kg^{-1}) (NRC, 2001). Hence, feed (especially in 9c-$C_{18:1}$, 9c12c-$C_{18:2,}$ and 9c12c15c-$C_{18:3}$) are based mostly on vegetable ingredients to obtain a fatty acid-rich balanced formulation. Dairy feed analyzed herein obtained 434.90 ± 34.42 g PUFA kg^{-1} fat and 369.20 ± 26.06 g 9c12c-$C_{18:2}$ kg^{-1} fat.

In poultry breeding, especially the laying hens, their production phase requires a minimum contribution of fat of 2.5 g/100 g, which also includes 1.35 g/100 g of linoleic acid with the primary objective of increasing the egg size and production (NRC, 2006; FEDNA, 2008). In contrast, broilers require a lower contribution of linoleic acid (1.0 g/100 g) (FEDNA, 2008; Rostagno *et al.,* 2017). The above data match that obtained during our trial (i.e., 1.7 g linoleic acid/100 g; 369.48 g/kg of fat).

Pet foods

Dogs are considered facultative carnivores. As their diet is supplemented with carbohydrate sources (up to 50% input), fatty acid biosynthesis in the animal intestine increases (NRC, 2006; FEDIAF, 2016). In contrast, cats, despite being strictly carnivorous, require an essential input of 5c8c11c14c-$C_{20:4}$ (NRC, 2006). Additionally, pets need, mandatorily, feed ingredients which input 9c12c-$C_{18:2}$ (as they are not able to synthesize it from linolenic acid) (Biagi *et al.,* 2004; NRC, 2006). During their development, a minimal input of 9c12c-$C_{18:2,}$ 9c12c15c-$C_{18:3}$, 5c8c11c14c-$C_{20:4}$, 5c8c11c14c17c-$C_{20:5}$, and 4c7c10c13c16c19c-$C_{22:6}$ is required (Biagi *et al.,* 2004; NRC, 2006). Values obtained for the above mentioned fatty acids are below 1.00 g kg^{-1} fat in the dry pet foods analyzed, an expected result as only fish-based pet foods are usually rich in 5c8c11c14c17c-$C_{20:5}$ and 4c7c10c13c16c19c-$C_{22:6}$ (Biagi *et al.,* 2004). Deficiencies in omega-3 and -6 generate sight and learning issues as it concentrates in the brain and retina during gestation and subsequent development (NRC, 2006; Fraeye *et al.,* 2012; Cherian, 2017). After 6 to 8 weeks of birth, pups start feeding on compound feed, which must provide twice as much maintenance energy (FEDIAF, 2016). However, when compared, adult dog food and puppy food show similar mean values in crude fat. Hence, the difference in energy requirement, mentioned above, is not being satisfied with fats.

Water activity in pet foods

AAFCO check sample dry cat food 2018-25 sets a 0.444 ± 0.031 as robust mean and standard deviation. Experimental data for the same sample was 0.4373 and a *z* value of -0.22. Water activity obtained for a dry extruded adult dog, puppy, and cat foods were 0.5356 ± 0.0961, 0.5837 ± 0.0682, and 0.5477 ± 0.0505, respectively. These values are relatively higher than those reported elsewhere for the cat (0.30-0.50) and dog food (0.30-0.54) (Baser and Yalçin, 2017). However, according to international guidelines, these values still rank local feeds as low-moisture animal food (US FDA, 2018). Also, these values are well below the threshold for bacterial pathogen growth (i.e., $a_w \geq 0.92$) (US FDA, 2018). Increased water activity may have a severe impact on pet food shelf life (US FDA, 2018). However, as a cost management strategy, the Costarican feed industry usually maintains moisture contents between 8 and 10 g/100 g. a_w has demonstrated to be a functional alternative to moisture content analysis (Van der Hoeven-Hangoor *et al.,* 2014) and is related to lipid quality and has proved to influence lipid oxidation (Choe and Oh, 2013) lipid modification (Lee and Parkin, 2001) mycoflora and fumonisin B_1 accumulation (Marín *et al.,* 2001).

Food samples (Feed-related matrices)

The composition of the acids can vary concerning the animal's diet, as such, there are differences between the grazing, and strictly stabled animals are observed (Woods and Fearon, 2009; Cabrera and Saadoun, 2014). In Costa Rica, cattle are grazed and, as such, considerable concentrations of 9c11t-$C_{18:2}$ (which has anti-carcinogenic activity) in meat tissue and bovine milk is observed. $C_{18:0}$ inhibits the activity of $C_{14.0}$ and $C_{16:0}$ since they are responsible for the hearts' health (Cabrera and Saadoun, 2014). Besides, the figures reported in Table 6, on lamb and beef, coincide with other published data (Woods and Faeron, 2009). Though meat tissue is not usually considered a good source of linoleic/linolenic acids (Cabrera and Saadoun, 2014), in meat tissue samples tested, said fatty acids are the predominant PUFA; which are not synthesized by humans and are, therefore, essential (Cabrera and Saadoun, 2014). Additional reports indicate that milk, another by-product of bovine production, has on average 3.5 – 4 g/100 g of fat (NRC, 2001; FEDNA, 2006), which is composed approximately from 50% of fatty acids of chains of 4 to 16 carbons (from acetic acid and butyric acid from ruminal fermentation), while the other 50% is composed of fatty acids of 16 to 18 carbons (from intestinal absorption) (FEDNA, 2006; Castillo *et al.,* 2013). On the other hand, bovine milk is usually characterized by providing a more significant proportion of palmitic acid (on average 28 g/100 g), oleic acid (21.2 g/100 g) and myristic acid (10.8 g/100 g) (Woods and Faeron, 2009; Markiewicz-Kęszycka *et al.,* 2013).

The egg, a high-consumption staple food, has relatively low production costs and a high nutritional value (e.g., crude protein 12.5 g/100 g, energy 150 kcal/100 g, also has all the vitamins, except for vitamin C) (Moreiras *et al.,* 2013; Khan *et al.,* 2015). According to other literature, eggs contain 30 - 35 g/100 g of SFA, while the main MUFA is oleic acid (22 - 26 g/100 g), and palmitic acid (8 - 10 g/100 g) (both on average 42 - 46 g/100 g of MUFA). On the other hand, is considered a food rich in oleic acid (42.7 g/100 g), linoleic acid (17.2 g/100 g), and in PUFA such as docosahexaenoic acid and arachidonic acid (Woods and Faeron, 2009; Khan *et al.,* 2015; Cherian, 2017). All of the above coincides with our data. As a point of interest, in poultry farms it is common to supplement fatty acids in poultry feeds, intending to modify the fatty acid profile in the eggs and in the poultry meat, to transfer the benefits towards human health (Woods and Faeron, 2009; Fraeye *et al.,* 2012; Khan *et al.,* 2015).

Forage blends fatty acid profiling

Costa Rican farming systems dairy cattle are grass-fed; forages are incorporated into full rations that are complemented compound feed. Often, forages are nutritional relevant as they can include PUFA to animal diet (Woods and Faeron, 2009; Glasser *et al.,* 2013). 9c12c15c-$C_{18:3}$ was reported elsewhere (Glasser *et al.,* 2013) as a prominent fatty acid was not found in our survey at significant levels. Though the forages assayed here are considered of relatively low nutritional quality, 9c-$C_{18:1}$ and 11c14c17c-$C_{20:3}$ are among the most abundant in the grass blends which depending on their concentration in the diet of ruminants, can furthermore modify the profile of fatty acids in milk and meat (Castillo *et al.,* 2013).

Conclusions

Fatty acid profiles from economically essential feed ingredients, such as soybean and corn meal, can be used for feed formulation and energy balance. In addition, the use of fat and oils in animal feed contributes to an increase in patability (as in the case of pet food), or to increase the energetic density (use of by-pass fat in bovine feeding).

Possible associations can be drawn from the fatty acids composition found in compound feed and the related matrix obtained from the food-producing animal (e.g., poultry feed vs. eggs). Up to some extent, fatty acid profiles can be useful data to trace the source and origin of feed ingredients. Additionally, it can be used as routine quality control to ensure lipid sources meet specifications and the requirements; to this aim, a high-throughput, and accurate methods, like the one used herein, should be developed. In this regard, FAME profiling should be included within national-wide feed/food monitoring programs, especially for those fatty acids considered as essential.

Compliance with Ethical Standards

Conflict of Interest

Astrid Leiva and Fabio Granados-Chinchilla declare that they have no conflict of interest.

Author Contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethics committee approval

This article does not contain any studies with human or animal subjects. Ethics committee approval is not required.

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Data availability

Not applicable.

Informed Consent

Not applicable.

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