AOAC Agricultural Materials Task Force **August 2, 2009**
Feed Additives and Contaminants

Project: A method for the determination of fatty acids in animal feed, feed ingredients, forage, grain and pet food.

Method Needs Statement and Validation Criteria draft v2

1. Method Needs Statement

The feedstuffs used in the animal feed industry are diverse and often inconsistent in quality. In many feeds, especially forages, fatty acids make up not more than half of the ether extractable material. Of 132 attendees, 39 international from 12 countries, at a Discover Conference (sponsored by the American Dairy Science Association) in May 2008 on lipid metabolism in dairy cattle, more than 80 were industry personnel, representing 61companies. The attendees set a high priority on a need to develop an AAFCO-approved method to quantify fatty acids in feeds; D.L. Palmquist, Prof. Emeritus, The Ohio State University, was charged to lead such efforts.

Distillers grains have become increasingly important in feeding, because so much is being generated by the ethanol industry. Distillers grains are notoriously variable in fatty acid content. Research and industry have come a long way to define nutrient requirements of animals, using the information to develop sophisticated computer programs to model nutrient requirements and to predict animal responses to nutrient inputs. With the present AOAC accepted systems for measuring the lipid content of feedstuffs, information is not adequate to fully use the power of the models available.

Finally, proper analysis of fatty acid content and composition will allow development of more accurately assessing value of feedstuffs, allowing development of more economical feeding systems and greater efficiency of nutrient utilization.

The desired method should apply to feed and feed ingredients of animal and plant origin, excluding inorganic mineral mixes.

[This section explains the background information and statement of need. I copied from several paragraph from your emails which can be reworded or elaborated upon.]

2. Performance Characteristics

The following performance characteristics must be demonstrated by the method.

2.1 Selectivity (Specificity)

The method should be capable of detecting total fatty acids and identifying as many as possible those listed in table 1. The method must be capable of distinguishing these compounds from each other as well as from other substances within the feeds, feed ingredients, forage, grain and pet foods. It must be demonstrated to be free of interference from the other analytes included in the method over the concentration ranges of the method.

2.2 Limit of Quantitation (LOQ) Levels:

The method should aim to quantify as many of the specified fatty acids in feeds, feed ingredients, forage, grain and pet foods as possible at or below the LOQ levels. The LOQ is listed in table 1 as the lowest value in the "Operational Range". It is recognized that these LOQ values are to be used as target quantitation levels and may not be achievable for all the fatty acids.

2.3 Operational range:

The method should be capable of detecting and quantifying as many of the specified fatty acids as possible over the ranges indicated in Table 1.

[This would be the sensitivity desired for each fatty acid. It should be based on what is important nutritionally. For example, is it important to know each to 1 ppm, to 0 .1% or to 1%, etc. Does a method need to have more sensitivity for some fatty acids than others]. We need to add a row where the values are expressed on a percent of feed material basis.

Table 1. Recommended Method Performance Characteristics:

	Target Concentration	Accuracy, %		Repeatability, % (CV_r)		Reproducibility, % (CV_R)	
Fatty Acids	**Operational Range (%)[1]**	**at 2x LOQ**	**at midrange**	**at 2x LOQ**	**at midrange**	**at 2x LOQ**	**at midrange**
*Arachidonic Acid 20:4n-6 (AA)	0.01 – 15	85-110	95-102	< 8	< 3	< 16	< 6
Arachidic Acid 20:0	0.01 – 5.0	85-110	92-105	< 8	< 4	< 16	< 8
Behenic Acid 22:0	0.01 – 1.0	85-110	90-108	< 8	< 5	< 16	< 10
*Butyric Acid 4:0	0.01 – 5.0	85-110	92-105	< 8	< 4	< 16	< 8
*Capric Acid 10:0	0.01 – 10	85-110	92-105	< 8	< 3	< 16	< 6
*Caproic Acid 6:0	0.01 – 3.0	85-110	92-105	< 8	< 4	< 16	< 8
Caprylic Acid 8:0	0.01 – 3.0	85-110	92-105	< 8	< 4	< 16	< 8
Dihomo-gamma-linolenic Acid 20:3n-6 (DGLA)	0.01 – 0.30	85-110	90-108	< 8	< 6	< 16	< 12
*Docosahexaenoic Acid 22:6n-3 (DHA)	0.01 – 30	85-110	95-102	< 8	< 3	< 16	< 6
*Docosapentaenoic Acid 22:5n-3 (DPA)	0.01 – 15	85-110	95-102	< 8	< 3	< 16	< 6
*Eicosapentaenoic Acid 20:5n-3 (EPA)	0.01 – 15	85-110	95-102	< 8	< 3	< 16	< 6
Elaidic Acid 18:1*trans*-9	0.01 – 2.0	85-110	92-105	< 8	< 4	< 16	< 8
Erucic Acid 22:1n-9	0.01 – 35	85-110	95-102	< 8	< 3	< 16	< 6
Gamma-linolenic Acid 18:3n-6 (GLA)	0.01 – 30	85-110	95-102	< 8	< 3	< 16	< 6
Heptadecanoic Acid 17:0	0.01 – 1.0	85-110	90-108	< 8	< 5	< 16	< 10
Lauric Acid 12:0	0.01 – 5.0	85-110	92-105	< 8	< 4	< 16	< 8
Linoleic Acid 18:2n-6	0.01 – 80	85-110	95-102	< 8	< 3	< 16	< 6
Linolenic Acid 18:3n-3	0.01 – 60	85-110	95-102	< 8	< 3	< 16	< 6
Myristic Acid 14.0	0.01 – 15	85-110	95-102	< 8	< 3	< 16	< 6
Myristoleic Acid 14: 1n-5	0.01 – 0.30	85-110	90-108	< 8	< 6	< 16	< 12
Oleic Acid 18:1n-9	0.01 – 80	85-110	95-102	< 8	< 3	< 16	< 6
Palmitic Acid 16:0	0.01 – 50	85-110	95-102	< 8	< 3	< 16	< 6
Palmitoleic Acid 16:1n-7	0.01 – 10	85-110	92-105	< 8	< 3	< 16	< 6
Pentadecanoic Acid 15:0	0.01 – 2.0	85-110	92-105	< 8	< 4	< 16	< 8
*Rumenic Acid 18:2*cis*-9, *trans*-11	0.01 – 8.0	85-110	92-105	< 8	< 3	< 16	< 6
Stearic Acid 18:0	0.01 – 40	85-110	95-102	< 8	< 3	< 16	< 6
Stearidonic Acid 18:4n-3	0.01 – 25	85-110	95-102	< 8	< 3	< 16	< 6
*Vaccenic Acid 18:1*trans*-11	0.01 – 10	85-110	92-105	< 8	< 3	< 16	< 6

[1] Note that operational range values are listed as % of total fatty acids.

2.4 Accuracy:
The method should demonstrate accuracy as specified in Table 1. This accuracy requirement must be met by measuring naturally incurred or fortified fatty acids in feeds, feed ingredients, and pet foods, at the midpoint of the operational range as well as 2X the LOQ.
These requirements are taken from the AOAC's Single Lab Validation document which notes, however, that "These limits may be modified as needed in view of the variability of individual results or which set of regulatory requirements are referenced.

AOAC's Single Lab Validation document recommends that accuracy be measured at "1x or 2x the expected concentration". For the elements of study, the concentration ranges may be very great. For the purposes of this document, the middle of the operational range and 2x the LOQ may be considered the "expected concentrations". Therefore, accuracy measurements should be made at both of these concentration levels (see Table 1).

2.5 Repeatability
The coefficient of variation will depend upon the target quantitation level. The repeatability coefficient of variation (CV_r) will vary depending on the concentration of the fatty acid in the feed matrix. The method should demonstrate repeatability as specified in Table 1 and Figure 1.

Concentration	CV_r (HCV)	RSD = $C^{-0.15}$
10%	<3	1.5
5%	<3	
1 %	<4	2
5000 mg/kg (0.5%)	<5	
1000 mg/kg (0.1%)	<6	3
500 mg/kg (0.05%)	<7	
100 mg/kg (0.01%)	<8	4
50 mg/kg	<9	5
10 mg/kg	<12	6
5 mg/kg	<13	7
1 mg/kg	<16	8

Figure 1. Repeatability coefficients for specified concentrations

The repeatability shall be measured by multiple analyses of naturally incurred or added fatty acids in feeds, feed ingredients, forage, grain and pet foods, at both the midrange as well as 2x the LOQ.

2.6 Reproducibility
As with repeatability, the variation will depend upon the target quantitation level and the reproducibility coefficient of variation (CV_R) will vary depending upon the concentration of the fatty acid in the feed matrix. The reproducibility shall be measured by analysing several naturally incurred or added fatty acids at both the midrange and at 2x the LOQ. The reproducibility should be approximately twice the repeatability as specified in Figure 1 (CV_R = 2x HCV).

3. Special consideration criteria

Are there other special considerations? Calculations for the concentration of fatty acids need to be provided on both a % of fatty acids basis and on a % of feed material basis (both as received and dry matter).
In addition to LOQs below the lower limit of the operational range, as described in Table 1, candidate methods will also be evaluated against subjective criteria including method simplicity, method costs, use of commercially available consumables and common laboratory instrumentation, and existence of in-house, single-laboratory validation.

It is important that pet foods and high fat supplements be included as sample matrices to any validation study. The study should also attempt to determine fatty acids in difficult to extract matrices such as extruded products, high urea products, fermentation by-products and high-mineral supplements.

4. Method validation protocol

A validation protocol specific to the proposed method of analysis will be developed by the project team, through consultation with the method's author or sponsor, and approved by the sub-committee as a whole. The method is to be rugged and robust and critical parameters are to be identified and controlled. The method performance criteria are to be defined. A familiarization plan is to be suggested which will demonstrate that the laboratory analyst can capably perform the method prior to analyzing samples. In addition, a quality control plan is to be suggested along with warning and out of control limits.

5. Prospective technologies

Preferred methods and standards for fatty acid analysis have been developed. The preferred method is a one-step quantitative extraction/methylation of fatty acids in feedstuffs, followed by quantitative analysis using gas-liquid chromatography (GLC). Direct one-step extraction and methylation of fatty acids in feedstuffs, for quantitative analysis by gas-liquid chromatography, has been documented (Sukhija and Palmquist, *J. Agric. Food* Chem**.** 36:1202-1206, 1988; Palmquist and Jenkins, J. Anim. Sci., 81: 3250-3254, 2003), and is used widely in research on fat in feedstuffs. After Palmquist was charged to initiate procedures, it was learned that Dr. Steve Hansen of Cargill had undertaken a similar project for AOCS. AOCS has taken the approach to separate methods for extraction and chromatography (see the following table).

Gina to fill in table

We have sent samples analyzed by the Sukhija and Palmquist procedure to Dr. Hansen; results from the methods are not different ***(are they statistical not different? "Identical" is probably too strong language)***. Inasmuch as Dr. Hansen´s protocol is developed to meet AOCS standards, and is flexible in nature and adaptable for routine use in the feed industry, it is proposed to document that procedure for AAFCO approval.