AOAC Agricultural Materials Task Force **January 30, 2008**
Sub-Committee on Feed Additives and Contaminants Final Draft

Project: A method for the determination of free disaccharides and monosaccharides (sucrose, lactose, maltose glucose, fructose and galactose) in animal feed.

Method Needs Statement and Validation Criteria

1. Method Needs Statement

Sugars are a class of low molecular weight carbohydrates that tend to be readily digested and absorbed in the small intestine. Sugars have been noted as a desirable energy source in some animal species, but in other species, or at high dietary levels has been associated with undesirable effects on health and elevation of blood glucose levels. This class of carbohydrates is nutritionally and compositionally distinct from other carbohydrates such as starch, fructans, and dietary fiber. As such, it is listed as a separate carbohydrate category in USDA and FAO carbohydrate fractionation schemes that address carbohydrates in human nutrition. FAO and USDA include monosaccharides and disaccharides potentially digestible by small intestinal enzymes (sucrose, maltose, lactose) as sugars. Problematically, current "sugar" analyses typically rely on extraction, acid hydrolysis of solubilized carbohydrates, and their measurement as reducing sugars, or measurement of total extracted carbohydrates using condensation reactions. Use of either detection analysis inappropriately leads to inclusion of oligosaccharides and other solubilized carbohydrate in the sugar fraction. The magnitude of the error is dependent upon the amount of the interfering carbohydrates present. An accurate measurement of sugars will need to be analyte specific, and is not presently available, except for specific matrices (e.g., molasses).
The desired method should apply to feed and feed ingredients of animal and plant origin, excluding inorganic mineral mixes.

2. Performance Characteristics

The following performance characteristics must be demonstrated by the method.

2.1 Selectivity (Specificity)

The method should be capable of detecting as sucrose, lactose, maltose glucose, fructose and galactose in animal feed, pet food and feed ingredients. The method must be capable of distinguishing these compounds from each other as well as from other substances within grains, forages and feedstuffs. 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 Operational range:

Sucrose: 0.1 to 100% (1 g/kg to 1000 g/kg)
Lactose: 0.1 to 100% (1 g/kg to 1000 g/kg)
Maltose: 0.1 to 100% (1 g/kg to 1000 g/kg)
Glucose: 0.1 to 100% (1 g/kg to 1000 g/kg)
Fructose: 0.1 to 100% (1 g/kg to 1000 g/kg)
Galactose: 0.1 to 100% (1 g/kg to 1000 g/kg)

2.3 Accuracy (see Recovery):
The method should demonstrate the following accuracy for each of the compounds listed in 2.2.

0.1 to 1% (1 g/kg to 10 g/kg): 90% - 108%
1% to 10% (10 g/kg to 100 g/kg): 92% - 105%
10% to 100% (100 g/kg to 1000 g/kg): 95% - 102%

2.4 Precision Repeatability:
The method should demonstrate the following accuracy for each of the compounds listed in 2.2.

0.1 to 1% (1 g/kg to 10 g/kg): CV_r = or < 5 %
1% to 10% (10 g/kg to 100 g/kg): CV_r = or < 4 %
10% to 100% (100 g/kg to 1000 g/kg): CV_r = or < 3 %

2.5 Precision Reproducibility:
The method should demonstrate the following accuracy for each of the compounds listed in 2.2.

0.1 to 1% (1 g/kg to 10 g/kg): CV_R = or < 10 %
1% to 10% (10 g/kg to 100 g/kg): CV_R = or < 8 %
10% to 100% (100 g/kg to 1000 g/kg): CV_R = or < 6 %

2.6 Detection Limits:

Each component listed in 2.2: 0.03% (300 mg/kg)

2.7 Determination Limits:

Each component listed in 2.2: 0.1% (1000 mg/kg)

2.8 Recovery:

0.1 to 1% (1 g/kg to 10 g/kg): 90% - 108%
1% to 10% (10 g/kg to 100 g/kg): 92% - 105%
10% to 100% (100 g/kg to 1000 g/kg): 95% - 102%

2.9 Linearity of standard curve:

$r \geq 0.999$, and 95 % confidence limit of the y intercept includes zero.

3. Special consideration criteria:
Different detector technologies may impact on the results, thus this possibility should be investigated.

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.

5. Prospective technologies:
Multiple technologies are being explored at this time that may be able to address the method needs.

Method Performance:

Table 1. Recommended Method Performance Characteristics:

	Method LOQ, %	**Operational concentration range, %**	**Accuracy at LOQ**	**Accuracy at midrange**	**Repeatability (CV_r) at Midrange**	**Repeatability (CV_r) at 2xLOQ**
Each compound	0.1%	0.1% – 100%	90% - 108%	92% - 105%	= or < 4%	= or < 5%

Fitness for Purpose Review:

Fitness for Purpose Statement: