Project: A method for the determination of dietary fructans in animal feed.

Method Needs Statement and Validation Criteria

1. Method Needs Statement

Dietary fructans are a class encompassing oligosaccharide and polysaccharide carbohydrates that is comprised predominantly of fructose molecules and containing no more than one-third glucose molecules. They are water-soluble. Based on the bonding among the monosaccharides, fructans do not have potential to be digested by enzymes in the small intestine, and can only be fermented by microbes in the gastrointestinal tract. Accordingly, they are counted as dietary fiber. Fructans are nutritionally and compositionally distinct from other carbohydrates such as sugars, starch, and other forms of dietary fiber. Fructans can comprise a substantial (20%) or minor (<1%) portion of animal feedstuffs of plant or blended origin. Primary sources of fructans are cool season grasses, chicory, and onions. Fructans have been added to animal and human foods for "prebiotic effects" (inclusion of a carbohydrate that is fermented in the gut to potentially maintain desired microflora and production of desired microbial products) that may have positive impacts on animal health. Fermentation of fructans by gut microbes in monogastric animals can yield short chain fatty acids that can be used by the animal for energy, whereas, in ruminants, ruminal fermentation of fructans can provide high quality microbial protein to the animal, as well. Because of the potential nutritional and claimed prebiotic effects of fructan, there is interest in being able to accurately measure fructan in feedstuffs for use in diet formulation. There are two AOAC methods for fructan evaluation in foods (999.03 and 997.08), however, researchers with cool season grasses report that the use of acid hydrolysis is more effective than use of inulinase (effective on inulin) for analysis of fructans in cool season grasses. Therefore, another approved method to assess the fructan content of animal feeds is needed if this information is to be included on the nutritional labeling of feeds. The desired method should apply to feed and feed ingredients of animal and plant origin. When added to complete feeds, purified fructans are often added at a ratio of 1 to 4 lbs per ton.

2. Performance Characteristics

The following performance characteristics must be demonstrated by the method.

2.1 Selectivity (Specificity)

The method should be capable of detecting dietary fructan in animal feed, pet food and feed ingredients. The method must be capable of distinguishing this compound exclusive of free glucose, fructose, or sucrose and within the 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:
    0.01% to 100% (100 mg/kg to 1000 g/kg) of dry matter

2.3 Accuracy (see recovery):
    0.01% to 0.1% (100 mg/kg to 1 g/kg) of dry matter: 85% - 110%
    0.1% to 1% (1 g/kg to 10 g/kg) of dry matter: 90% - 108%
    1% to 10% (10 g/kg to 100 g/kg) of dry matter: 92% - 105%
    10% to 100% (100 g/kg to 1000 g/kg) of dry matter: 95% - 102%

2.5 Precision Repeatability
    0.01% to 0.1% (100 mg/kg to 1 g/kg) of dry matter: CV_r = or < 8 %
    0.1% to 1% (1 g/kg to 10 g/kg) of dry matter: CV_r = or < 6 %
    1% to 10% (10 g/kg to 100 g/kg) of dry matter: CV_r = or < 4 %
    10% to 100% (100 g/kg to 1000 g/kg) of dry matter: CV_r = or < 3 %

2.5 Precision Reproducibility:

0.01% to 0.1% (100 mg/kg to 1 g/kg) of dry matter: CV_R = or < 16 %
0.1% to 1% (1 g/kg to 10 g/kg) of dry matter: CV_R = or < 12 %
1% to 10% (10 g/kg to 100 g/kg) of dry matter: CV_R = or < 8 %
10% to 100% (100 g/kg to 1000 g/kg) of dry matter: CV_R = or < 6 %

2.6 Detection Limits:
0.003% (30 mg/kg) of dry matter

2.7 Determination Limits:
0.01% (100 mg/kg) of dry matter

2.8 Recovery:
0.01% to 0.1% (100 mg/kg to 1 g/kg): 85% - 110%
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
Method must be usable with moist as well as dried and ground feedstuffs.

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
At this time, a modified method of McCleary et al. (2000) with an acid hydrolysis step rather than the use of inulinase may serve as the basis for prospective technology. This assay has shown itself to be fairly robust and devoid of some common analytical defects that reduce fructan recovery in other methods.

Method Performance:

Table 1. Recommended Method Performance Characteristics:

	Method LOQ, %	Operational concentration range, %	Accuracy at 2x LOQ	Accuracy at midrange	Repeatability (CV_r) at Midrange	Repeatability (CV_r) at 2xLOQ
Fructan (as dry matter)	0.01%	0.01% – 100%	85% - 110%	95% - 102%	= or < 3%	= or < 8%

Fitness for Purpose Review:

Fitness for Purpose Statement: