

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

Method Needs Statement and Validation Criteria

1. Method Needs Statement

Dietary starch is an α -linked-glucose carbohydrate of or derived from plants, animals and microbes from which glucose is released through the hydrolytic actions of purified α -amylases and amyloglucosidases that are specifically active only on α -(1-4) and α -(1-6) linkages in samples that have been gelatinized in heated, mildly acidic buffer. Its concentration in feed is determined by enzymatically converting the α -linked-glucose carbohydrate to glucose and then measuring the liberated glucose. This definition encompasses plant starch, glycogen, maltooligosaccharides and maltose/isomaltose.

Dietary starch is nutritionally and compositionally distinct from other carbohydrates such as sugars, 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. Dietary starch can comprise a substantial (70%) or minor (0.8%) portion of animal feedstuffs of plant or blended origin. Dietary starch has 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 animal health. Accordingly, there is interest in being able to accurately measure dietary starch in feedstuffs for use in diet formulation. Discontinued production of the enzyme Rhozyme-S required for AOAC method 14.075 invalidated the only approved method for starch in animal feeds. Therefore, another approved method to assess the dietary starch 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.

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 starch in animal feed, pet food and feed ingredients. The method must be capable of distinguishing this compound exclusive of free glucose and glucose from non-starch sources 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:

1.0% to 100% (10 g/kg to 1000 g/kg) of dry matter

2.3 Accuracy (see recovery):

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

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:

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:

0.3% (3.0 g/kg) of dry matter

2.7 Determination Limits:

1.0% (10 g/kg) of dry matter

2.8 Recovery:

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

Select enzymes and run conditions to avoid the release of glucose from acid-labile substances (such as sucrose) and non-starch carbohydrates.

Method must be usable with moist as well as dried, ground, extruded and pelleted feedstuffs.

The method must be applicable to products containing up to 40% crude fat.

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 Bach Knudsen (1997) is the selected, prospective technology. This assay has shown itself to be fairly robust and devoid of some common analytical defects that reduce starch 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 2x LOQ
Dietary starch (as dry matter)	1.0%	1.0% – 100%	92% - 105%	95% - 102%	= or < 3%	= or < 4%

Fitness for Purpose Review:

Fitness for Purpose Statement: