CHAPTER 7

Recommended Laboratory Analytical Procedures for DDGS

Introduction

LABORATORY ANALYSIS OF FEED INGREDIENTS IS A COMMON PRACTICE in the feed industry to verify the ingredient meets guaranteed specifications, determine nutrient composition for use in animal feed formulation, and determine the presence and concentration of potential contaminants. Therefore, the accuracy of measurement of various chemical compounds in feed ingredients including DDGS is essential.

Analytical procedures can be categorized based on the level of validation of a specific laboratory method (Thiex, 2012). A single laboratory validation applies to a specific laboratory, technician, and equipment, whereas, a multi-laboratory validation involves validating a procedure in two to seven laboratories to provide information on how well the results of a method are reproduced outside of the original laboratory. A full harmonized protocol collaborative study validation occurs when at least eight laboratories provide acceptable data using the same procedure. An excellent summary of recommended analytical procedures for DDGS has been published by Thiex (2012) and key points are summarized in this chapter.

Recommended Analytical Methods for Meeting DDGS Trading Standards (AFIA, 2007)

Analyte	Method	Method Description
Moisture	NFTA 2.2.2.5	Lab Dry Matter (105°C/3hr)
Crude protein	AOAC 990.03	Protein (Crude) in Animal Feed
Crude protein	AOAC 2001.11	Protein (Crude) in Animal Feed and Pet Food Copper Catalyst
Crude fat	AOAC 945.16	Oil in Cereal Adjuncts (Petroleum Ether)
Crude fiber	AOAC 978.10	Fiber (Crude) in Animal Feed and Pet Food (F.G. Crucible)

Recommended Methods for Nutrient Analysis of DDGS for Diet Formulation

Analyte	Method	Method Description
Acid detergent fiber (ADF)	AOAC 973.18	Fiber, Acid Detergent and Lignin, H_2SO_4 in Animal Feed and ISO, 2008 are equivalent
Acid detergent lignin (ADL)	AOAC 973.18	Fiber, Acid Detergent and Lignin, H_2SO_4 in Animal Feed and ISO 13906:2008 are equivalent
Amylase-treated neutral detergent fiber (NDF)	AOAC 2002.04	AOAC 2002.04 Amylase Treated Neutral Detergent Fiber in Feeds and ISO 16472:2006 are equivalent
Starch	No official method	AOAC 920.40 is no longer valid because of discontinued production of the enzyme needed for the assay, **AOAC 996.11** is most commonly used but has deficiencies.
Amino acids	AOAC 995.12 ISO 13903:2005	AOAC 994.12 for all amino acids except tyrosine and tryptophan
Tryptophan	AOAC 988.15	
Ash	AOAC 942.05 ISO 5984:2002	AOAC 942.05 and ISO 5984:2002 are equivalent. Note: If the ash contains unoxidized carbon, the sample should be re-ashed

Analyte	Method	Method Description
Chlorine	AOAC 969.10 AOAC 943.01 ISO 6495:1999	AOAC 969.10 is the Potentiometric Method and AOAC 943.01 is the Volhard Method
Chromium	No official method	No methods have been validated
Fluorine	Microdiffusion technique (Mineral Tolerances of Animals, 2005)	No methods have been validated
Iodine	ICP-MS technique (Mineral Tolerances of Animals, 2005)	No methods have been validated
Phosphorus	AOAC 965.17 ISO 6491:1998 ISO 27085:2009	AOAC 965.17 Phosphorus in Animal Feed, Photometric Method, ISO 6491:1998 Determination of Total Phosphorus Content – Spectrophotometric Method, and ISO 27085:2009 can be used
Selenium	AOAC 996.16 AOAC 996.17	AOAC 996.16 Selenium in Feeds and Premixes, Fluorometric Method and AOAC 996.17 Selenium in Feeds and Premixes, Continuous Hydride Generation Atomic Absorption Method are acceptable
Sulfur	AOAC 923.01 ISO 27085:2009	AOAC 923.01 Sulfur in Plants and ISO 27085:2009 are comparable
Trace minerals	AOAC 968.08 ISO 6869:2000 ISO 27085:2009	Solubilization involves either dry ash followed by dissolving in acid, or wet ash using various acids depending on the elements being measured. Detection includes gravimetric techniques, visible spectrophotometry, flame and graphite furnace atomic absorption spectrophotometry (AOAC 968.08; ISO 6869:2000), or atomic mass spectroscopic detection (ICP-MS; ISO 27085:2009)

Recommended Procedures for Measuring Possible Contaminants in DDGS (Caupert et al., 2012)

Mycotoxins

Since the 1960s, many analytical methods have been developed for analysis of mycotoxin content in human foods and animal feeds due to the concern of toxicity for human health (Trucksess, 2000). Among them, the methods of thin-layer-chromatography (TLC), enzyme-linked immunosorbent assay (ELISA) and immunosensor-based methods have been widely used for rapid screening, while high-performance liquid chromatography (HPLC) with fluorescence detection (FD) and mass spectrometry detection (MS) have been used as confirmatory and reference methods (Krska et al, 2008). However, due to the need for rapid, accurate, and low cost on-site methods for mycotoxin determinations, test kits have been developed and approved by the Grain Inspection, Packers and Stockyards ADMInistration (GIPSA) of the United States Department of Agriculture, and are specific for use with DDGS (**Table 1**; http://www.gipsa.usda.gov/GIPSA/webapp?area=home&subject=lr&topic=hb).

These methods are for detection of a single mycotoxin, are relatively simple to use, are quantitatively sensitive and allow high sample throughput. There are six GIPSA approved methods for testing mycotoxins in DDGS (four methods for aflatoxin, one method for fumonisin and one method for zearalenone).

Table 1. GIPSA approved mycotoxin test kits for DDGS (adapted from Zhang et al., 2009)

Brand Name	Manufacturer	Test Range	Test Format	Extraction	Clean-up
Aflatoxin					
Veratox Aflatoxin	Neogen Corporation	5–50 ppb	Microtiter Well Plate Assay	Methanol/water (70 + 30)	ELISA
Ridascreen FAST SC	R-Biopharm	5–100 ppb	Microtiter Well Plate Assay	Methanol/water (70 + 30)	ELISA
Aflatest	Vicam	5–100 ppb	Immunoaffinity Column	Methanol/water (80 + 20)	Affinity column
FluroQuant® Afla IAC	Romer	5–100 ppb	Fluorometry	Methanol/water (80 + 20)	Affinity column
Fumonisin					
AgraQuant Total Fumonisin 0.25/5.0	Romer	0.5–5 ppm	Direct Competitive ELISA	Methanol/water (70 + 30)	ELISA
Zearalenone					
ROSA® Zearalenone	Charm Sciences, Inc.	50–1000 ppb	Lateral Flow Strip	Methanol/water (70 + 30)	

When considering analysis of DDGS samples for possible mycotoxin contamination, it is essential to use approved analytical procedures to get accurate results. High performance liquid chromatography (HPLC) is the preferred method to determine the presence and concentration of mycotoxins in animal feeds. By using HPLC and a variety of detectors, most of the mycotoxins in animal feeds can be separated and detected (Krska et al, 2008). The methods used by major commercial laboratories in the U.S. are listed in **Table 2** and have been validated by individual labs and recently published in peer-reviewed scientific journal articles.

Table 2. Recommended methods for mycotoxin analysis in animal feed (adapted from Zhang et al., 2009)

Target	Testing	Detection Range	Reference
Aflatoxin			
Corn, almonds, Brazil nuts, peanuts and pistachio nuts	HPLC – FD	5 – 30 ppb	AOAC 994.08
Deoxynivalenol			
Cereals and cereal products	HPLC – UV	ppm (detection limit)	MacDonald et al., 2005a
Fumonisin			
Corn and corn flakes	HPLC – FD	0.5 – 2 ppm	AOAC 2001.04
Corn and corn-based feedstuffs	Thin layer chromatography (TLC)	ppm (detection limit)	Rottinghaus et al., 1992
T-2			
Food and feed	Thin layer chromatography (TLC)	ppm (detection limit)	Romer Labs, 1986

Table 2. Recommended methods for mycotoxin analysis in animal feed (adapted from Zhang et al., 2009)			
Zearalenone			
Corn, wheat and feed	Microtiter Well Plate Assay	0.8 ppm (detection limit)	AOAC 994.01
Barley, maize and wheat flour, polenta and maize-based baby foods	HPLC – FD	0.05 ppm (detection limit)	MacDonald et al., 2005b
Aflatoxins, Deoxynivalenol, Fumonisin, T-2, Zearalenone			
Food and feed	LC/MS/MS	Aflatoxins (1 – 100 ppb); Deoxynivalenol, (1, 1000 ppb) Fumonisin (16 – 3,200 ppb) T-2, (2 – 1,000 ppb) Zearalenone (20 – 1,000 ppb)	Sulyok et al., 2007

Antibiotic residues

The CVM of the U.S. Food and Drug ADMInistration has used a liquid chromatography and ion trap tandem mass spectrometry procedure (Heller, 2009) to determine to presence and concentrations of residues from 13 antibiotics in DDGS including:

- Ampicillin
- Bacitracin A
- Chloramphenicol
- Chlortetracycline
- Clarithromycin
- Erythromycin
- Monensin
- Oxytetracycline
- Penicillin G
- Streptomycin
- Tylosin
- Virginiamycin M1

Extraction efficiency of this procedure ranged from 65 percent to 97 percent with quantitation limits from 0.1 to 1.0 µg/g. Accuracy ranged from 88 to 111 percent with coefficients of variation from 4 to 30 percent. The only FDA approved method for detecting virginamycin residues is a bioassay procedure Phibro (QA@Phibro.com), and is recommended for accurate determination of the presence of virginiamycin residues. The Phibro bioassay accounts for possible biological activity which can only occur with the presence of both subunits of the virginiamycin molecule, compared with the LC-MS method of Heller (2009) which only detects one subunit and can lead to a high percentage of false positive readings.

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