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NGFA Industry Guidance

Considerations for Testing Animal Feed or Feed
Ingredients for Salmonella

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Editor's Note

The National Grain and Feed Association (NGFA) has developed this guidance for the animal feed and feed ingredient industry to assist in making determinations on whether and how to implement a program for testing for Salmonella and other pathogens. It was developed with the assistance of the NGFA's Feed Legislative and Regulatory Affairs Committee and Feed Manufacturing and Technology Committee. Questions about the content and subject matter of this document should be directed to – and additional information is available from – NGFA Vice President for Feed Services David Fairfield at 712-243-4035, or by email at dfairfield@ngfa.org.

Introduction

Animal feed companies may choose to use microbiological testing to evaluate animal feed or feed ingredients for pathogens, such as *Salmonella*.

The purpose of this guidance developed by the National Grain and Feed Association (NGFA) is to provide basic information to assist companies in deciding whether to use *Salmonella* testing (or other microbiological testing) as part of their overall feed safety system. It is recommended strongly that companies consult a trained microbiologist when considering *Salmonella* testing. In addition, competent legal counsel should be consulted on legal and regulatory issues.

Related to the potential relevance of such product testing, the Food and Drug Administration (FDA) in 2012 published an article⁽⁸⁾ in the *Journal of Foodborne Pathogens and Disease* that presented surveillance data from the agency's testing during 2002-2009 of 2,058 samples of complete animal feeds, feed ingredients, pet foods, pet treats and supplements for pets for the presence of *Salmonella*. Of the 2,058 samples tested, 257 were positive for *Salmonella* (12.5 percent). The positive samples underwent further testing to identify the *Salmonella* serotype. Of the top 25 most common serotypes identified through the additional testing, none found in complete animal feeds and feed ingredients consisted of serotypes that FDA has deemed to be significantly pathogenic to poultry, swine, sheep, horses or cattle.

Meanwhile, regarding pet foods, pet treats, and supplements for pets, FDA's surveillance data indicated that 7.4 percent of the 972 samples of such products tested positive for the presence of *Salmonella*. FDA views the presence of any *Salmonella* serotype in such products as adulteration unless the product will undergo a subsequent process, such as heat treatment, that will eliminate the *Salmonella*.

Quick Facts on Microbiological Testing

The following are several facts about microbiological testing:

- *You cannot test safety into the product.* Microbiological testing of product should be used only as verification that the company's feed safety system is working. But it is not the only form of verification. Obtaining a negative result when testing for a pathogen does not necessarily mean that the pathogen is not there at some level.
- *Under FDA policy,⁽²⁾ a product that has tested and been confirmed positive for a known pathogen **may not be retested and released** based upon subsequent negative results.* Unless there is a confirmed laboratory error that resulted in the initial positive result, retesting cannot be used to offset a positive result.
- *If confirmation of a presumptive positive result from a rapid method is not performed, the presumptive result is considered a true positive.* In this case, a company that chooses not to confirm a presumptive result should take all necessary actions on the subject feed material as if the test were a confirmed positive.

- *When testing the environment for a pathogen (e.g. swabbing), results from the product contact surface are considered to have the same implications as testing the product itself.*
 - If a product sample or product contact surface tests positive for a pathogen and *the product already has been released from your control, you may have created regulatory consequences that will need to be addressed (see following section). Conversely, if you test a feed ingredient for a pathogen upon receipt, you may have created regulatory consequences for your supplier and any other manufacturers that have received the same ingredient lot from that supplier.*
 - *Poor sample collection practices can contaminate an otherwise clean sample. Aseptic techniques always should be used to collect samples of feed or feed ingredients.*
 - All testing laboratories and testing methods *do not perform the same*. Selection of properly accredited laboratories and validated methods can increase confidence that the results are accurate.
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Determining if Microbiological Testing is Appropriate

Before a testing program is started on feed or feed ingredients, the question “Why am I testing this material?” should be answered. Understanding what question you are trying to answer will help determine if testing is appropriate and, if so, what type of testing should be done.

Microbiological testing may be appropriate if the results will provide sufficient information to help answer the pertinent question. Some questions cannot be answered by microbiological testing, such as “Is the feed or feed ingredient free of a given pathogen, such as *Salmonella*.” As will be explained below, testing a feed or feed ingredient for a pathogen cannot ensure that the material does not contain the pathogen at some level. Given that many feeds or feed ingredients are minimally processed agricultural products, it is not reasonable to expect them to be completely free of *Salmonella*.

Examples of when testing for *Salmonella* may be appropriate in feed or feed ingredients are to: 1) verify that a feed safety system designed to reduce or eliminate *Salmonella* from the feed is working; or 2) determine if a feed or feed ingredient is compliant with a desired specification. If neither of these criteria applies, it is inadvisable to conduct *Salmonella* testing on a feed or feed ingredient. *Salmonella* testing can never be used to prove the safety of a feed or feed ingredient. If it is desired to understand the presence of *Salmonella* in the processing environment, an environmental monitoring program (e.g. swabbing) would be more appropriate than product testing.

Feed or feed ingredients also may be tested for indicator microorganisms (e.g. total aerobic bacteria, total coliforms, *Enterobacteriaceae* or mold). Testing for indicator microorganisms can provide information concerning the microbiological quality of the feed material and/or the conditions under which it was held or processed. For example, excessive levels of mold or *Enterobacteriaceae* in feed or

feed ingredients may indicate that the material was not dried sufficiently or was exposed to moisture during storage. Depending upon the purpose for microbiological testing, indicator testing may be more appropriate than pathogen testing.

After establishing the purpose of testing and concluding that testing will provide meaningful, useful information, the testing program may be developed. Although the remainder of this guidance focuses on *Salmonella* testing, the information on sample collection, selecting methodologies and selecting testing laboratories applies to indicator testing, as well. It is recommended strongly that companies consult a trained microbiologist when considering *Salmonella* testing of feed or feed ingredients. In addition, competent legal counsel should be consulted on legal and regulatory issues.

Limitations of Microbiological Testing

Microbiological testing cannot ensure that *Salmonella* is not present in an animal feed or feed ingredient at some level. To have such confidence through testing, one would need to test all of the material in a given lot. Since microbiological testing is destructive (meaning the sample being tested is destroyed by the testing), this approach would leave no material remaining to use or sell. For this reason, microbiological testing usually is performed on a specific number of samples collected from a finished product or ingredient lot. The number and frequency at which samples are collected is referred to as a *sampling plan*.

Sampling plans vary in their ability to detect a pathogen based upon a number of factors. The distribution of contamination within a lot, the number of samples collected, and the sensitivity and specificity of the test all play a role in the ability of a sampling plan to detect *Salmonella*. Pathogens, such as *Salmonella*, often are present in feed materials at low incidence rates (meaning *Salmonella* is there, but will not be found in every sample collected).

Table 1 below demonstrates the limitations of sampling plans to discriminate between clean and contaminated lots of feed, based upon the number of samples collected and contamination rate (percent of samples contaminated) within the feed material. According to the table:

- If one (1) sample is collected from a feed material that has *Salmonella* in 1 percent of samples, there is a 99 percent probability that *Salmonella* will **not** be detected.
- If ten (10) samples are collected from a feed material that has *Salmonella* in 5 percent of samples, there is a 60 percent probability that *Salmonella* will **not** be detected.

Table 1: Probability of Accepting a Contaminated Lot Depending on the Number of Samples Tested and Distribution of Contamination*

Percent of Samples Contaminated	Number of Samples Tested					
	1	5	10	15	30	60
1	0.99	0.95	0.90	0.86	0.74	0.55
2	0.98	0.90	0.82	0.74	0.55	0.30
5	0.95	0.77	0.60	0.46	0.21	0.05
10	0.90	0.59	0.35	0.21	0.04	<0.005
20	0.80	0.33	0.11	0.04	<0.005	<0.005

* adapted from the ICMSF⁽⁶⁾

Sample Collection and Preparation

To test animal feed or a feed ingredient for *Salmonella*, samples must be collected and submitted to a laboratory. If improper procedures or poor techniques are used to collect samples, an otherwise clean sample may become contaminated with *Salmonella*. Examples of poor sample collection practices include the use of dirty utensils (such as knives or scoops), touching the sample or sampling utensil with unclean hands, or the collection of samples in a place with excessive dust in the air.

Samples should be collected in a manner that prevents contamination of the sample. Cups or scoops used to collect samples should be clean and sterile. The use of disposable cups or scoops is best practice when each sample is collected using a new cup or scoop. Collected samples should be placed in a clean, sterile bag or other sterile container that can be closed adequately to prevent contamination of the sample during handling, storage and shipping. During the process of collecting the sample, hands should not touch any of the clean, sterile surfaces that the sample may touch (including the inside of the bag or other container used to hold the sample). The use of sterile gloves is best practice when collecting and handling the sample prior to placing it into a sterile bag or other sterile container. If animal feed or feed ingredient bags are to be opened and sampled, the cutting instrument used to open the bag must be clean and sterile. The process of cutting the bag can transfer contamination from either the cutting instrument or the outside of the bag into the material inside in exactly the location where the sample will be collected. When bagged material is to be sampled for *Salmonella*, best practice is to disinfect the outside of the bag and the cutting instrument immediately before cutting the bag. For more information on proper sampling techniques, please refer to the FDA’s Inspections Operation Manual⁽⁴⁾ or contact a qualified microbiology testing laboratory.

CASE STUDY (adapted from Jones, F.T. 2011)⁽⁷⁾

“At one feed mill, facility personnel were instructed to collect samples and researchers collected samples from many of the same locations. A total of 43.75 percent of the samples collected by mill personnel were positive for *Salmonella*, while only 7.32 percent of samples collected by researchers were positive. Therefore aseptic sample collection is essential to have confidence in the results of *Salmonella* testing.”

It is common to composite (or mix together) several samples collected from a lot into one sample for testing purposes to reduce testing costs. The use of a compositing approach should be evaluated by a trained microbiologist to ensure it is consistent with accepted practices. Only samples from the same lot of feed or feed ingredient should be composited together. If samples from multiple lots are composited together, the results of the testing should be applied to all lots represented in the composite.

Selection of Methodologies and Testing Laboratories

Salmonella testing requires specialized equipment and a properly trained microbiologist. “In-house” testing is not recommended unless the company has a laboratory facility that is properly designed and equipped for *Salmonella* testing (minimum biosecurity level 2), adequately segregated from the processing environment and staffed by properly trained personnel. Testing laboratories should maintain appropriate certifications (e.g. A2LA, ISO 17025) and use only methods that have been properly validated for use with animal feed and feed ingredients. There are many third-party laboratories (TPL) that offer *Salmonella* testing for animal feed and feed ingredients.

Many of the rapid test methods for *Salmonella* can provide a presumptive or negative result within two to three days. Some rapid test methods may provide a presumptive result even though the sample is negative, particularly if the sample was obtained after being subject to a process or treatment to eliminate *Salmonella*. If a presumptive result is obtained, the result always should be confirmed, which will take several more days. If *Salmonella* is confirmed in a feed or feed ingredient, it may be useful to have the isolate serotyped, which may take additional time. Having serotype information may help determine if there are any regulatory considerations (described below). If confirmation of a presumptive result is not performed, the presumptive result is considered to be a true positive. A company that chooses not to confirm a presumptive result should be prepared to take all necessary actions on the feed material, as if the test were a confirmed positive.

For more information on interpreting results of *Salmonella* testing, please refer to the FDA guidance document.⁽²⁾

Positive Release Programs (Test and Hold Programs)

If finished product and/or product contact surfaces are tested for *Salmonella*, all material represented by the sample (that is, all the material that would be implicated if the result comes back positive) should be placed on hold until final results are obtained *in writing* (i.e. positive release)⁽⁵⁾. Many companies are limited by their ability to hold the subject material for the length of time required for *Salmonella* testing, which may take between two to 10 days. However, the release of material for shipment outside of company control before final results are obtained increases the risk of regulatory consequences – such as reporting obligations associated with FDA’s Reportable Food Registry (RFR)⁽³⁾ – and potential product liability issues.

Testing incoming feed or feed ingredients for *Salmonella* creates additional risk of regulatory consequences for the supplier of the feed or feed ingredient, as well as any other manufacturers that have received the same material from that supplier. If testing of an incoming feed or feed ingredient from an outside supplier is determined to be appropriate for the company's feed safety system, it is recommended strongly that the firm's suppliers be consulted so they are aware of and understand your program, and can take any appropriate actions to control their risk should one of the tests on your products yield a positive result. Some suppliers may be willing to provide pre-shipment samples for you to test. Or they may offer to conduct the testing themselves and provide you with the results to maintain positive release of their material. These arrangements with suppliers should be considered if testing incoming feed or feed ingredients is appropriate.

Regulatory Considerations

Companies that choose to test animal feed and/or feed ingredients for *Salmonella* should be knowledgeable about FDA's current policy on *Salmonella* in animal feed, as well as FDA's RFR requirements and recall procedures.

FDA's policy on *Salmonella* in animal feed can be located in its Compliance Policy Guide (CPG), section 690.800⁽¹⁾. Although the CPG was in draft status at the time this NGFA guidance was prepared, FDA has been basing its regulatory action using the criteria set forth in the draft policy.

FDA's CPG describes a risk-based policy for *Salmonella* in animal feed that differentiates between feed likely to come in direct contact with humans and other feeds intended to be fed to livestock and/or poultry. For animal feeds likely to come into direct contact with humans (e.g. pet food, pet treats and pet chews), FDA's policy states the presence of any *Salmonella* serovar in such products is cause for FDA to consider the feed to be adulterated, unless the feed will undergo a process that will eliminate the *Salmonella*. For other animal feeds, FDA's policy states such feeds will be considered to be adulterated if specified *Salmonella* serovars are present that CVM considers significantly pathogenic to the animal species to which the feed is to be fed, unless the feed will undergo a process that will eliminate the *Salmonella*. If an animal feed or feed ingredient tests positive for *Salmonella*, the information within this FDA CPG will be helpful to determine the next steps a company should take. Such actions may include destroying the feed or feed ingredient, processing the material using a heat treatment sufficient to eliminate the *Salmonella*, or returning the material to the supplier from which it was purchased. For more information on this CPG, please contact NGFA.

FDA's RFR is an electronic portal that industry is to use to report to the agency when there is a reasonable probability that an article of food or feed will cause serious adverse health consequences or death to animals and/or humans. The RFR applies to all FDA-regulated categories of food and feed, except dietary supplements and infant formula. If an animal feed or feed ingredient tests positive for *Salmonella*, a company would need to determine if the criteria for a reportable food have been met. If so, the company would be required to file a report through the RFR webpage. For more information on the RFR and how it has been applied to animal feed and feed ingredients, please contact NGFA.

Companies may voluntarily initiate a recall when feed products present a risk of causing adverse health consequences to animals and/or humans. In addition, the Food Safety Modernization Act of 2010

provides FDA with authority to issue a mandatory recall when a company fails to voluntarily recall unsafe food or feed after being asked to do so by FDA. If an animal feed or feed ingredient tests positive for *Salmonella*, a company would have to determine if a recall should be conducted. If so, the company would need to work with FDA to establish the appropriate recall classification and implement necessary recall procedures. For more information on FDA's recall policies, please contact NGFA.

Conclusion

The decision on whether to test animal feed or feed ingredients for *Salmonella* should be considered carefully. If such testing is incorporated into an overall feed safety system, the purpose for testing should be well understood, and the sampling plan and sample collection techniques should be designed appropriately. The testing program should consider how to handle a positive result and how to maintain control of the feed or feed ingredient during the testing process (i.e., positive release). It is recommended strongly that companies consult a trained microbiologist when considering *Salmonella* testing of feed or feed ingredients. In addition, companies should consider legal and regulatory issues pertaining to *Salmonella* testing with the assistance of competent legal counsel.

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