Guidance Document #01
Stability Testing of Feed ingredients
March 2019
At Step 7: Steering Committee Endorsement

STABILITY TESTING OF FEED INGREDIENTS

Endorsed by the Steering Committee in
March 2019

It is recommended for the companies willing to submit applications/dossiers for pre-market authorization, to contact the jurisdictions of the countries concern to confirm their acceptance of the current guidance document.

The International Cooperation for Convergence of Technical Requirements for the Assessment of Feed Ingredients (ICCF) was launched in 2017 and aims to develop and establish common guidance documents to provide technical recommendations for the assessment of feed ingredients, including new uses of existing feed ingredients.

This guidance document has been developed by the appropriate ICCF Experts Working Group and was subject to consultation by the Parties, in accordance with the ICCF Process.

The founding members of the ICCF include the Canadian Food Inspection Agency (CFIA), the European Commission (DG SANTE), the U.S. Food and Drug Administration (FDA), as well as the American Feed Industry Association (AFIA), the Animal Nutrition Association of Canada (ANAC), the EU Association of Specialty Feed Ingredients and their Mixtures (FEFANA) and the International Feed Industry Federation (IFIF).

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STABILITY TESTING OF FEED INGREDIENTS

1. INTRODUCTION

1.1 Objective of the Guidance

This document provides guidance regarding the stability testing approaches and data to be included in a pre-market approval or authorization application for feed ingredients.

Considerations in the document are provided for the assessment of feed ingredients throughout the feed chain, from ingredient production to the use in the intended feed. Guidance has been developed with an international team of experts and considers the best practices for the provision of meaningful results.

While the guidance provided supports the acceptability of the study protocol, applicants are advised to consult the appropriate regulatory authorities or guidelines during the development phase of new feed ingredients or a new use of an authorized ingredient, for further determination whether the study protocol is acceptable, or the study is needed for pre-market assessment.

1.2 Definitions

The Following definitions apply solely in the context of this guidance document.

Active substance[1]: Any substance in a feed ingredient to contribute to the intended effect.

Extract: A feed ingredient containing the active substance(s) originally found in an animal, botanical, or microbial material obtained by a mechanical and/or solvent extraction process.

Feed (Feedingstuff) [2]: Any single or multiple materials, whether processed, semi-processed or raw, which is intended to be fed directly to animals.

Feed Ingredient[2]: A component part or constituent of any combination or mixture making up a feed, whether or not it has a nutritional value in the animal's diet. Ingredients are of plant, animal, microbial or aquatic origin, or other organic or inorganic substances.

[1] Active substance includes microorganisms that contribute to the intended effect.
[2] Note adapted from Code of practice on good animal feeding (CAC/RCP 54-2004)

Flavoring agent: A feed ingredient added to feed for the purpose of enhancing aroma and taste.

Ingredient market formulation: The feed ingredient under assessment formulated with carrier(s) and/or other feed ingredient(s). It is the commercial product used to incorporate the feed ingredient under assessment into premixtures, feeds or water.

Intended matrix: A matrix in which the feed ingredient is added and is used to supply the feed ingredient to the animals. It may include the ingredient market formulation, premixture, feed, feed supplement, and water.

Label Guarantees: The specifications listed on the product label for parameters that can be tested to ensure that the feed ingredient contained in the product meets the intended effect.

Liquid Feed Supplement: Fluid or suspension supplements, including those containing molasses, colloidal materials, or gums to provide necessary nutrients (e.g., protein, vitamins, minerals, fat) to animals. Liquid feed supplement does not include drinking water for animals. In the context of this guidance, liquid feed supplement also includes the solidified blocks/tubs, where the manufacturing processes uses significant amount of molasses or other liquid or suspension ingredients.

Mineral Source: A feed ingredient added to feed to provide micro- and macro-mineral(s).

Premixture (Premix): A uniform mixture of one or more micro-ingredients/feed ingredients with diluent and/or carrier, not intended for direct feeding to animals. It is used to facilitate uniform dispersion of the micro-ingredients/feed ingredients in a larger mix.

Stability/Shelf-life: The time point at which the product no longer meets one or more of the label guarantees within acceptable analytical method variations.

1.3 Scope of the Guidance

This guidance addresses the storage stability testing of the feed ingredient as produced and in packaged ingredient market formulation under the intended storage conditions.

This guidance also addresses the in-process stability of a feed ingredient while producing premixtures and feeds (e.g., pelleting, extrusion, or canning), as well as the stability of the feed ingredient in premixtures and feeds during the storage of these feed matrices under the intended storage conditions.

The types of feed ingredients covered by this guidance in each regulatory jurisdiction are determined by each region's relevant statutes and regulations.

2. GENERAL PRINCIPLE

A stability study could include, as appropriate, physical, chemical, biological, or microbiological parameters that are relevant to the intended effect of a feed ingredient. The same parameter(s) should be measured over the entire study period.

Stability testing should be conducted under the intended conditions of use of the feed ingredient over its anticipated lifetime in its commercial packages. If the container used in the stability study is not the same material and not sealed in the same manner as the commercial package for the test article, justification should be provided to support that the container used in the study does not impact the stability of the ingredient in a manner different from the commercial package under the intended storage conditions.

When the feed ingredient contains more than one active substance, stability should be assessed for each active substance. If the intended physical or technical effect depends on a particular form of the feed ingredient (e.g., chelation), the integrity of that particular form should be demonstrated throughout the shelf life. For some complex chemical mixtures and extracts, stability may be assessed by monitoring the concentration/activity of one or more appropriate marker substances. For enzyme products, the stability should be demonstrated by testing the enzyme activity. When the stability of a feed ingredient is expected to be affected by potential microbial contaminants, the levels of these microbes should be tested throughout the shelf life of the product.

The stability study should be conducted on three independent production batches and contains sufficient data points during the specified storage period to facilitate appropriate statistical analysis. For each batch, repeated analysis at least in duplicate is recommended preferably at each time point to assess variabilities over time and from batch to batch for the length of the stability study. Where appropriate, potential degradation or decomposition products should be characterized.

Analytical method used in a stability study should be a regulatory or commonly accepted method (e.g., compendial) or validated in all the intended matrices. The method validation

should follow the protocols recommended by relevant international standards or guidance documents.

3. STABILITY TESTING

3.1 Stability of the Feed Ingredient

Where appropriate, the stability of the feed ingredient should be assessed as produced and as packaged for market (e.g., as an ingredient market formulation) using conditions mimicking recommended storage conditions for marketing. If more than one ingredient market formulation will be marketed, and the differences in the formulation may impact the stability, the stability of the ingredient should be assessed in each ingredient market formulation.

Data should be generated using at least three independent batches of feed ingredient over its anticipated lifetime. When appropriate, expected shelf-life of the feed ingredient should be based on at least two model situations covering the likely range of the conditions of storage, for example, 25 °C, 60% relative air humidity (HR) and 30 °C, 65% HR, or 40 °C, 75% HR for products recommended for storage at ambient temperature.

Where pilot batches are used in place of production batches, justifications should be provided to support that the stability data obtained from pilot batches reflects the stability of the production batches.

3.2 Stability of the Feed Ingredient in Premixture

The stability of feed ingredients in premixtures should be demonstrated when the feed ingredient is intended to be used in a premixture. The stability study in a premixture should be conducted at intended inclusion level, under intended storage conditions, in the commercial packaging material for at least 6 months. The quantitative and qualitative composition of the premixtures should be provided. The compositions of the premixtures should reflect the formulations commonly used in the regulatory region in which the approval is sought and be representative for the target animal species (e.g., poultry, swine, ruminant, aquatic animals, etc.). When different premixture formulations exist that are likely to impact the stability of the ingredient, each formulation should be tested separately.

The study should investigate three batches of premixtures made using one to three independent batches of the ingredients (three independent batches of ingredient are recommended by the USA).

3.3 Stability of the Feed Ingredient in Feed

The stability study in feed should be conducted at the intended use level as presented on the product label. The study should be conducted under intended storage conditions in commercial packaging over the anticipated lifetime, typically at least 3 months of storage time. The quantitative and qualitative composition of the feed should be provided. The composition of the feed(s) used for the stability study should reflect the formulation commonly used in the regulatory region in which the approval is sought and be representative for the target animal species (e.g., poultry, swine, ruminant, aquatic animals, etc.). If the formulations for different feeds are likely to impact the stability of the ingredient, each formulation should be assessed separately. For aquaculture feed, the stability study should also demonstrate that the ingredient does not leach out of the fish or shrimp pellet before it is consumed.

The study should investigate three batches of feed made using one to three independent batches of ingredients (three independent batches of ingredient are recommended by the USA).

If relevant, the effect of certain processing (e.g., pelleting, extrusion, or canning) on the stability of the feed ingredient should be demonstrated. The conditions of each step of processing should be provided. The relevant conditions include but are not limited to, time and temperature of thermal process, mixing duration, and style of mixer used for blending operations. If data show problems with stability, a proposal for a label declaration of the ingredient and premixture containing the ingredient should be included in the dossier, for example, "Do not pellet", “Do not pellet above XX [temperature (Fahrenheit or Celsius)]”, "Apply post-pelleting", or “Pelleting may negatively affect the effectiveness of this product."

The stability study of a feed ingredient in liquid feed supplements should be conducted to ensure the ingredient is chemically and rheologically stable (in the case of suspensions) in the intended matrices. The studies should be conducted at the proposed inclusion levels under the conditions simulating field storage and use over the anticipated lifetime of the supplement. For liquid and suspension supplements, samples should be taken from the top, middle, and bottom of the container to demonstrate the ingredient remains chemically stable and evenly dispersed throughout the anticipated lifetime.

3.4 Stability of the Feed Ingredient in Animal Drinking Water

The stability of the feed ingredient intended to be distributed via animal drinking water should be studied at the recommended inclusion level and under specified water conditions simulating practical use (e.g., pH, mineral contents, water temperature, time, microbial contents, dispersion/suspension of the feed ingredient, etc.). These data should also take into consideration the presence of excipients that could affect the stability of the ingredient.

The study should investigate three batches of animal drinking water made using one to three independent batches of ingredients (three independent batches of ingredient are recommended by the USA).

4. SPECIAL CONSIDERATIONS

4.1 Mineral Source

The stability studies for feed ingredients in packaged form, in premixtures, and in feed for a chemical substance intended to be used as a mineral source are generally not necessary. However, if the bioavailability of the mineral depends on the feed ingredient's chemical structure, the integrity of the chemical structure should be determined for the feed ingredient itself and for the feed ingredient in its intended matrices (premixtures, feed, liquid feed supplement, animal drinking water) throughout the anticipated storage time under recommended storage conditions, if practical.

4.2 Flavoring agent

The stability study for a flavoring agent as produced, and a flavoring agent in the premixtures should be demonstrated to ensure the chemical integrity of the flavoring agent. When practical, the stability of a flavoring agent in feed should also be tested to ensure the palatability of the feed over the anticipated storage time.

If the flavoring agent is a crude extract/oil, the predominant compound(s) providing the flavoring should be identified and analyzed to demonstrate the stability of the flavoring agent.

Where it is not practical to identify the predominant compound(s), one or more marker compounds should be identified to demonstrate the stability of the ingredient.

4.3 Silage Ingredient

For feed ingredients intended to be used in silage to provide certain technical effects (e.g., to control pH or undesirable microbes), which result in the decrease of the feed ingredients levels in silage over time, the stability study of such feed ingredients in silage is not necessary. However, the stability of these feed ingredients as produced should be demonstrated.

For those silage ingredients intended for application through an aqueous suspension/solution, short term stability (48 hours) should be demonstrated.

5. SAMPLING FOR A STABILITY STUDY

As described previously in this document, a stability study for a feed ingredient as produced and as packaged for market should be conducted on three independent batches of the feed ingredient. A stability study for a feed ingredient in each type of its intended matrix (e.g., premixtures, feed, liquid feed supplement, animal drinking water) should be conducted on three batches of the intended matrix made using one to three independent batches of the feed ingredient (three independent batches of feed ingredient are recommended by the USA). For each batch of feed ingredient or its intended matrix, repeated analysis at least in duplicate is recommended preferably at each time point to assess variabilities over time and from batch to batch for the length of the stability study. It is recommended to consult each region's regulatory agency for more specific recommendations.

The sample(s) of the feed ingredient used in the stability study should be representative of commercial production. Samples from commercial production size batches are preferred to be used in the stability studies. If pilot or laboratory scale samples are used, justification should be provided to support that the stability data obtained from these pilot batches reflect the stability of the production batches. To help ensure that the samples used are representative, an appropriate sampling plan based on well accepted sampling methodology should be employed and provided to the regulatory agency. Samples should be analyzed as soon as possible after they are manufactured to establish an initial analysis point (i.e., the time zero in the stability study) and then be stored in their respective stability testing environments. If the initial analysis cannot be performed right after the production, the samples should be kept under conditions that have minimal effect on their stability. Storage for an extended period of time prior to the initiation of the stability study should be avoided. Samples that are shipped to sites other than the

manufacturing site for stability testing should be properly packaged to minimize the effect from the transportation. These samples should be tested as soon as possible upon receipt to establish the initial analysis and then be stored in their respective stability testing environment.

6. DATA EVALUATION AND STATISTICAL ANALYSIS

A stability study could include, as appropriate, physical, chemical, biological, or microbiological parameters that are relevant to the intended effect of the feed ingredient. A systematic approach should be used to evaluate the stability testing data.

In general, regression analysis is considered an appropriate statistical approach to analyze certain quantitative parameters (e.g., enzyme activity assay, substance degradation). The estimated shelf life is the earliest time point at which the 95 percent confidence limit for the mean intercepts the proposed acceptance criterion. Linear regression is often suitable but other quantitative parameters (e.g., pH value) and microbiological tests may require a different model. In some cases, a non-linear regression analysis can provide better fit. Other statistical approaches can also be used when demonstrated to be appropriate. Using a statistical method to demonstrate stability is preferred but, when it can be justified, may not be necessary.

With sufficient data and in compliance with the requirements from the regulatory region to which the pre-market approval application is sought, an applicant can request extrapolation of stability data beyond the tested time duration before approval or extension of an approved shelf life.

The following is an example of a two-step linear regression approach to evaluate stability data for batches formulated with the same level of the feed ingredient:

Step 1: Testing for Poolability of Batches

A preliminary statistical test should be performed to determine whether the regression lines from different batches have a common slope.

If there is a significant difference in slopes among batches or the batches are formulated with different levels of the feed ingredient, it is not appropriate to combine the data from all batches for further analysis. The data from each batch should be analyzed individually using the approach described in Step 2 below. The shortest estimated shelf life among batches should be established as the shelf life for all batches.

If there is no significant difference in slope among batches, the data from all batches can be combined for further analysis as described in Step 2 below.

Step 2: Data Analysis for a Single Batch or Pooled Data from all Batches

For a parameter known to decrease with time, the lower one-sided 95 percent confidence limit of the regression line should be compared to the acceptance criterion. For a parameter known to increase with time, the upper one-sided 95 percent confidence limit should be used. For a parameter that can either increase or decrease, or whose direction of change is unknown, two-sided 95 percent confidence limits should be calculated and compared to the acceptance criterion.

7. DATA REPORTING

The stability study report should include a description of the study, analytical data and the proposed shelf life under the intended storage conditions based on the analytical test results.

7.1 The Description of a Stability Study Should Include:

1. Identity of the feed ingredient under test
   Note: Documents (e.g., Certificates of Analyses) should be provided to demonstrate the name, batch numbers, manufacturing dates and contents of the feed ingredient under test.
2. Analyte(s) and parameter(s) that are tested for, including feed ingredient, active substance, marker compound, microbial levels, pH, moisture, etc. where necessary
3. Formulations of the intended matrices (premixtures, feed, liquid feed supplement, animal drinking water) used in the test
4. Proposed inclusion levels of the feed ingredient in the intended matrices
5. Conditions of storage
6. Type of container(s) and size for the test samples
7. Sampling protocol and number(s) of samples and replicates for each batch of test articles (feed ingredient, intended matrices) at each time point
8. Testing schedule
9. Name and address of the testing facility.

7.2 The Analytical Data Should Include:

1. Actual test date of each time point
2. Individual analytical result with measurement units including replicates for each sample tested with a reference to the replicate number, lot number, and time point
   Note: Original analyst worksheets, spectra, chromatograms, certificates of analyses, charts, or other pertinent information should be submitted to support and verify reported analytical results. When applicable, the coefficients of variation or standard errors of each time point should be provided to support data precision. When providing instrument/computer printouts, explanations should be included to clarify information such as sample ID, method code, etc. It is recommended to consult the regional regulatory authorities to determine whether the original data are necessary for a specific submission.
3. Description of test method(s)
   Note: If a test method is not a commonly accepted standard method for the intended analysis, method validation information may be needed to support the use of the method in the stability study.
4. Evaluation (e.g., statistical analysis) of the data and summarized data presentation (tables, charts, etc.)
   Note: The data points excluded from the statistical analysis (i.e., outlier) should be included in the raw data and a justification of the exclusion should be given.
5. Sample of calculations, where necessary.

8. BIBLIOGRAPHY

8.1 CODEX Alimentarius

1. Codex Alimentarius Commission Code of Practice on Good Animal Feeding CAC/RCP 54-2004 http://www.fao.org/3/i1379e/i1379e06.pdf
2. Codex Alimentarius Commission General Guideline on Sampling CAC/GL 50-2004 http://www.fao.org/uploads/media/Codex_2004_sampling_CAC_GL_50.pdf

8.2 ISO

3. International Organization for Standardization (ISO) International Standard 6498-2012, Animal Feeding Stuffs – Guidelines for Sample Preparation
https://www.iso.org/standard/52285.html

8.3 VICH

4. International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) GL3(R) – Stability Testing of New Veterinary Drug Substances and Medicinal Products (Revision)
https://vichsec.org/component/attachments/attachments/136.html?task=download

5. International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) GL51 – Statistical Evaluation of Stability Data
https://vichsec.org/component/attachments/attachments/330.html?task=download

8.4 United States of America

6. Association of American Feed Control Officials (AAFCO), GOOD Samples: Guidance on Obtaining Defensible Samples
https://www.aafco.org/Publications/GoodSamples

7. Association of American Feed Control Officials (AAFCO), GOOD Test Portions: Guidance on Obtaining Defensible Test Portions
https://www.aafco.org/Publications/GoodTestPortions

8. U.S. Code of Federal Regulations Title 21 Part 571 – Food Additive Petitions
https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=571

9. U.S. Food and Drug Administration, Center for Veterinary Medicine Guidance for Industry #221 - Recommendations for Preparation and Submission of Animal Food Additive Petitions
https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM367746.pdf

8.5 Canada

10. Canada Feeds Regulatory Guidance RG-1 Chapter 2 – Data Requirements for Single Ingredient Approval and Feed Registration
http://www.inspection.gc.ca/animals/feeds/regulatory-guidance/rg-1/chapter-2/eng/1329298059609/1329298179464

8.6 European Union

11. European Food Safety Authority Guidance on the Identity, Characterisation and Conditions of Use of Feed Additives
http://www.efsa.europa.eu/en/efsajournal/pub/5023

12. Union Commission Regulation (EC) No 429/2008 of 25 April 2008 on Detailed Rules for the Implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as Regards the Preparation and the Presentation of Applications and the Assessment and the Authorization of Feed Additives
https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1551960527181&uri=CELEX:32008R0429

13. Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on Additives for Use in Animal Nutrition
https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1551960555114&uri=CELEX:02003R1831-20151230