

FEED INGREDIENTS EFFECTIVENESS ASSESSMENT TECHNICAL EFFECT

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It is recommended for the companies willing to submit applications/dossiers for pre-market authorization, to contact the jurisdictions of the countries concerned 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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FEED INGREDIENTS EFFECTIVENESS ASSESSMENT – TECHNICAL EFFECT

1. INTRODUCTION

1.1. Objective of the guidance

The objective of this guidance document is to describe the information necessary to assess the technical effect of a feed ingredient under the proposed conditions of use. This guidance document should be read in connection with the ICCF guidance document ‘[Feed Ingredients Effectiveness Assessment – General Recommendations](#)’. While this guidance document describes the recommendations for providing information on the technical effect of the feed ingredient on feed, applicants are advised to consult the relevant authority or jurisdiction where the feed ingredient is intended to be marketed, and, if applicable, their guidance document(s) during the development phase of new feed ingredients, or new uses of a previously approved or authorized feed ingredient.

Although the guidance document has been developed with the purpose of submitting information to obtain the authorization or approval of feed ingredients, it might also be used to substantiate claims made about feed ingredients having a technical effect on feed, even if they are not subject to an authorization process.

1.2. Definitions

The following definitions apply solely in the context of this guidance document:

Batch: An identified quantity of a feed ingredient or intended matrix having uniform characteristics, with specified limits and being produced from the same cycle of manufacturing production

Experimental Unit: An entity subjected to an intervention independently of all other units for statistical purposes.

Feed (Feedingstuff)ⁱ: any single or multiple material, whether processed, semi-processed or raw, which is intended to be fed directly to animals.

ⁱ Adapted from Codex Alimentarius, Code of Practice on good animal feeding (CAC/RCP 54-2004)

Feed Ingredient¹: a component part or constituent of any combination or mixture making up a feed, whether or not, it has nutritional value in the animal's diet. Ingredients are of plant, animal, microbial or aquatic origin, or other organic or inorganic substances.

Feed matrix: The material, in which the feed ingredient under assessment is incorporated, to assess the effect of the feed ingredient. The material could be a feed ingredient, ingredient market formulation, premix and feed, and water.

In vitro effectiveness study: The study performed in a laboratory without recourse to intact animals to support or to demonstrate the feed ingredient's intended effect.

Ingredient market formulation: The commercial form containing the feed ingredient under assessment with carrier(s) and/or other feed ingredient(s) used to facilitate its incorporation into premix, feeds or water.

Premix (Premixture): A uniform mixture of one or more feed ingredients with (or without) carriers, not intended for direct feeding to animals. It is used to facilitate uniform dispersion of the feed ingredients within/in a larger mix.

Reference feed ingredient: A feed ingredient known to have the same effect as the feed ingredient under evaluation, when used under the same conditions of use and serving as a standard/, used to validate the test.

1.3. Scope of the Guidance

This guidance document is applicable to all types of feed ingredients, whose purpose is to improve or maintain the technological, microbiological and organoleptic properties of feed matrices during manufacturing, transport, storage and further use. It proposes relevant endpoints and test methods to assess the technical effect of the feed ingredient (see Table 1) under its proposed conditions of use.

2. FUNCTIONS AND ENDPOINTS TO BE MEASURED

During production, storage, distribution and use of feed, various potential impacts on the feed nutritional or physical quality may occur (e.g. oxidation, development of molds, particle sizes separation), which could be controlled with the use of suitable feed ingredients. The guidance document covers the non-exhaustive list of potential functions of feed ingredients, accompanied by examples of endpoints and parameters to be evaluated. This list is provided in Table 1. It should be

noted that the applicant may choose one or more endpoints and parameters for evaluating the feed ingredient under assessment.

If evidence concerning the mode of action of the feed ingredient under assessment is available, this may be included in the submission package, to support the evaluation of its effectiveness. This may facilitate the proper classification of the feed ingredient under assessment, as defined by the relevant jurisdiction.

3. EVALUATION OF THE FEED INGREDIENT'S EFFECT

The expected technical effect of the feed ingredient relates to the presence of the active substance(s). Hence, the test item(s) used in the effectiveness study(ies) should be the feed ingredient under assessment, as used in practice in accordance with its proposed conditions of use. If multiple ingredient market formulations will be used in practice, the extrapolation of the results obtained with the test item(s) to other ingredient market formulations should be considered.

In line with the approach outlined in the ICCF guidance document '[Feed Ingredients Effectiveness Assessment – General Recommendation](#)', the evaluation of the technical effect of a feed ingredient should consider all available scientifically valid information (bridging information and effectiveness studies).

Effectiveness studies should be conducted based on a protocol, developed in line with Section 3.1.1 of the guidance document '[Feed Ingredients Effectiveness Assessment – General Recommendation](#)'. Specific attention should be given to the following aspects:

- Description of the test itemⁱⁱ:
 - The test item should be representative of the feed ingredient under assessment
 - If certain physical characteristics of the feed ingredient (e.g., particle size) may influence its effectiveness, it should be provided in the identification and characterization of the feed ingredient
 - A certificate of analysis of the batch(es) of the feed ingredient used in the effectiveness study, establishing the identity and purity, should be provided, in accordance with the recommendations of the ICCF guidance document '[Identification and Characterization of feed ingredients](#)'.

ⁱⁱ Based on the information indicated in the ICCF guidance document on '[Identification and Characterization of feed ingredients](#)'

- Study design should include:
 - A description of the tested feed matrix and its form (e.g. granulated, pelleted, mash),
 - The composition of the negative control (feed matrix without the test item) and, if appropriate, the composition of the positive control feed matrix which contains a reference feed ingredient (e.g., to validate the study design and establish the range, magnitude and/or significance of the effect),
 - The inclusion level of the test item in the feed tested, according to the proposed conditions of use,
 - The test methods should be used to test the study relevant endpoints considering the matrix and study conditions. The test methods referred to [Table 1](#) are provided for information (as examples),
 - The study design should be representative of the proposed conditions of use and application,
- The endpoints and corresponding parameters used for the study should be appropriately selected to assess the proposed technical function of the feed ingredient under assessment (see [Table 1](#) for more information)
 - The list of proposed endpoints in [Table 1](#) is not exhaustive, and the use of other endpoints should be scientifically justified.
 - The endpoint(s) and parameter(s) that are employed to assess the proposed function should be relevant in practice.
- The size of the study should be adequately justified and defined, including:
 - the number of independent replicate tests (e.g. technical replicates, experimental replicates) for each treated sample
 - the sample size/subsample size plan for each batch
 - the number of feed types and batches tested, according to the proposed conditions of use.

The conditions of effectiveness studies (scale, environmental conditions) should allow conclusion to be set on the effectiveness of the feed ingredient under the proposed conditions of use. If necessary, the applicant should justify the conditions used.

For verifying consistency of results, it is recommended to test feed ingredients on independent batches of the matrix(es) covering the proposed conditions of use. The choice of the feed matrices should consider their composition, based on the expected technical effect (i.e., emphasize the presence or absence of a specific feed ingredient or nutritional content of the feed matrix, rather the feed of a target species.

It may not be possible to conduct effectiveness studies on all types of feed matrices and conditions. Therefore, bridging information (see Section 2 of the ICCF guidance document ‘[Feed Ingredients Effectiveness Assessment – General Recommendation](#)’) may be used to complement, analyze and discuss the results obtained in the effectiveness studies. One of the objectives of the discussion may be to extrapolate the results of the effectiveness studies on the representative feed to other feeds, and the conditions that should be applied to such extrapolation.

4. DATA EVALUATION AND STATISTICAL ANALYSIS

Data obtained in effectiveness studies could be qualitative (e.g., color, taste, or smell) or quantitative (e.g., pH reduction) and therefore a standard approach for data evaluation and statistical analysis cannot be universally defined.

The statistical model used for the evaluation of the data and reporting of data should follow the requirements of the ICCF guidance document ‘[Feed Ingredients Effectiveness Assessment – General Recommendation](#)’ (Section 3.1.2). Based on the expected results, the size of the study, sample sizes, number of replicates and the endpoints/parameters considered with its expected distribution of data, the applicant should choose and justify the statistical methodology that is most suitable for the effectiveness study.

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Table 1 – Non exhaustive list of potential functions of feed ingredients and their relevant endpoints

Functions	Definition (effect expected on ingredient market formulation, premix, other feed ingredient or feed)	Relevant endpoints (examples)	Test methods (examples)
Anti-caking	Prevent the particles from adhering/to adhere.	Flowability. Moisture absorption. Caking tendency.	Flowability tests ^{1, 2} (angle of repose ^{3, 4} , ^{5, 6} , flow rate ^{5, 6} , Carr's compressibility Index ⁶). Moisture absorption tests (moisture content ⁷ , hygroscopicity ⁸). Caking tendency tests ⁹ (compression, storage stability).

Functions	Definition (effect expected on ingredient market formulation, premix, other feed ingredient or feed)	Relevant endpoints (examples)	Test methods (examples)
Antioxidation	Prevent degradation by oxidation.	Antioxidant activity. Rancidity testing (for feed with high fat content, especially unsaturated fatty acids). Prevention of oxidation degradation of chemical compounds (e.g. vitamins).	Oxidative Stability tests^{10, 11, 12} (Peroxide value¹³ and p-anisidine value¹⁴, Thiobarbituric Acid Reactive substances, Oxidative Stability Index, Total Oxidation Valueⁱⁱⁱ, Acid value^{15 iv}, Hexanal analysis). Sensory Evaluation¹⁶. Antioxidant Activity Assays^{17, 18} (2,2-diphenyl-1-picrylhydrazyl Assay, Oxygen Radical Absorbance Capacity Assay¹⁹). Spectrometry methods (Ion trap mass, Cupric reducing antioxidant capacity, 2,2-azino-<i>bis</i>-3-ethylbenzothiozoline-6-sulphonic acid, 2-2-diphenylpicrylhydrazyl, etc¹⁸). Fluorometric analysis¹⁸. Electrochemical techniques¹⁸. Chromatography (Gas chromatography/Flame ion detector or thermal detector, and High-performance liquid chromatography coupled with different detectors¹⁸).

ⁱⁱⁱ TOTOX is calculated as (2 x Peroxide Value) + Anisidine Value

Functions	Definition (effect expected on ingredient market formulation, premix, other feed ingredient or feed)	Relevant endpoints (examples)	Test methods (examples)
Binding	Increase the capacity of the particles to adhere.	Pellet durability. Pellet stability, including in water. Dusting potential/Presence of fine particles.	Pellet breakage ^{20, 21} (ASAE drum ²² , Önorm M 7135 ²³). Pellet Durability Tester (Holmen equipment) .
Coloring	Add or restore color.	Color.	Measurement by colorimeter or spectrophotometer.
Emulsifying / Gelling / Thickening	Allow the formation and maintenance of homogeneous mixture of two or more immiscible phases, or Form a gel, or Increase the viscosity.	Durability/Stability of emulsion. Density of the emulsion. Volumetric density. Viscosity. Visual presentation.	Overview of measurement methods for emulsion stability ²⁴ . Overview of viscosity measurement methods ²⁵ .

^{iv} Acid value is measured by the number of mg of potassium hydroxide required to neutralize the free acid in 1 g of the substance.

Functions	Definition (effect expected on ingredient market formulation, premix, other feed ingredient or feed)	Relevant endpoints (examples)	Test methods (examples)
Silage fermentation regulator	Facilitate the silage fermentation process, by e.g. stabilizing, reducing losses.	Silage pH. Concentration of volatile fatty acids. Aerobic stability. Effluent/Nitrogen/Energy losses comparison. Ammonia-N and DM losses. Fermentation quality. Microbial parameters. Volatile Organic Compounds (e.g., ethanol, 1,2-propanediol) .	Volatile Fatty Acids Measurement²⁶. Evaluation of aerobic stability²⁷. Sensory evaluation²⁸. Evaluation of fermentation quality²⁹. Enumeration of microbial contaminants and comparison to reference value³⁰. Volatile Organic Compound measurements³¹.
pH regulator	Regulate the pH.	pH value change. Buffering capacity. Acid binding capacity.	pH measurement in accordance with OECD Guidelines ³² .

Functions	Definition (effect expected on ingredient market formulation, premix, other feed ingredient or feed)	Relevant endpoints (examples)	Test methods (examples)
Reducing microbiological load / Controlling microbial load Mitigating microbial load	Prevent the growth of harmful microorganisms impacting the feed hygiene and security, or Prevent the growth of microorganisms responsible for the deterioration of feed.	Microbial pathogen load (e.g. <i>Salmonella</i> spp., <i>Clostridium perfringens</i> , African Swine Fever Virus, etc.) to ensure feed safety. Microbial load of spoilage microorganisms, indicators of food spoiling (e.g. water activity, microbial respiration and metabolites).	Guidelines for efficacy ³³ . Enumeration methods and comparing results with reference values for the specific microbial contaminant ^{18, 28, 29, 30} .
Smell preservation and enhancement	Add or restore the smell	Olfactory sensory analysis. Chemical analysis.	Description tests (Quantitative: ranking tests; qualitative: description with a list of descriptors on which testers are trained (“panelists”). Discrimination tests (as triangular tests). Hedonic tests (“I like” or “I don’t like”). Volatile Organic Compound measurements ³⁴ . Gas Chromatography coupled with Mass Spectrometry.

Functions	Definition (effect expected on ingredient market formulation, premix, other feed ingredient or feed)	Relevant endpoints (examples)	Test methods (examples)
Taste preservation and enhancement	Add or restore the taste	Gustatory sensory analysis. Determination of key markers. Sensory test receptors activation.	Palatability Test. Discrimination tests (as triangular tests). Hedonic tests (“I like” or “I don’t like”). Liquid Chromatography coupled with Mass Spectrometry, High Performance Liquid Chromatography, Gas Chromatography coupled with Mass Spectrometry). Cell reporter systems (pT1r2/pT1r3 – sweet taste; pT1r1/pT1r3 – umami taste) 35, 36, 37 .
Tracing	Allow the identification of the origin.	Color. Presence of the feed ingredient, which is a result of the detection of the tracer (qualitative and/or quantitative).	Lab analysis of the feed ingredient.