

SCIENTIFIC OPINION

Guidance on the assessment of microbial biomasses for use in animal nutrition¹

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)^{2,3}

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BACKGROUND

Directive 82/471/EEC⁴ regulated the use of certain products used in animal nutrition, which acted as direct or indirect protein sources. Included in these certain products were biomasses obtained from the fermentation of different substrates by microorganisms. The assessment of the safety and nutritional value of these products was done according to the guidelines fixed in Directive 83/228/EEC.⁵

In 2009, the European Parliament and Council adopted Regulation (EC) No 767/2009⁶ on the placing on the market and use of feed, which repeals both Directives 82/471/EEC and 83/228/EEC as of 1 September 2010. Biomasses produced from non-genetically modified organisms do not need a formal authorisation/assessment to be placed on the market. However, Regulation (EC) 1829/2003⁷ applies to those biomasses which are produced from a genetically modified organism.

In recent years EFSA has received several dossiers related to applications for the placing on the market of biomasses obtained from the production of amino acids or other molecules. Most of these applications fall under the scope of Regulation (EC) No 1829/2003, since they are produced by genetically modified microorganisms. Applicants have been requested to follow the guidance provided by the GMO Panel⁸ for the assessment of the genetic modification and the guidelines detailed in Directive 83/228/EEC for the assessment of the product itself. However, these guidelines, which have now been repealed, are more than 25 years old and the requirements are not always in line with the assessment practices of the FEEDAP Panel.

Therefore, there is a need for an up-to-date guidance document to help applicants in the preparation of dossiers for the assessment of biomasses for use in animal nutrition. This guidance will focus on the nutritional value and safety of the product itself but not on the aspects related to the genetic modification, which are covered by the guidance of the GMO Panel.

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⁴ OJ L 213, 21.7.1982, p. 8.

⁵ OJ L 126, 13.5.1983, p. 23.

⁶ OJ L 229, 1.9.2009, p. 1.

⁷ OJ L 268, 18.10.2003, p. 1.

⁸ <http://www.efsa.europa.eu/en/scdocs/scdoc/374.htm>

TERMS OF REFERENCE

The FEEDAP Panel is requested to produce a guidance document for the assessment of the safety and nutritional value of microbial biomasses intended to be used in animal nutrition.

Scope of the document

This document provides guidance to applicants on the preparation of dossiers related to biomasses for which a formal assessment is requested to EFSA by the European Commission, the European Parliament or a Member State. For biomasses containing, consisting of, produced from or produced with genetically modified microorganisms (GMM), this document complements the '[Guidance document of Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use](#)' produced by the GMO Panel'.⁹

For the purpose of this document the term 'product' refers to 'microbial biomass' and includes:

- The total product (cells with/without spent medium) of a microbial fermentation/cultivation process.
- The residue of a microbial fermentation/cultivation process left after the primary product(s) has(have) been removed (e.g. the residue left after enzyme or amino acid extraction).

General considerations

The dossier must allow the assessment of the biomasses based on the current state of knowledge.

The studies to be submitted and their extent will depend on the nature of the biomass, the target animals and the conditions of use.

The dossier shall include original reports of all the studies performed, presented in accordance with the numbering system proposed in this guidance document. Reasons must be given for the omission from the dossier of any data prescribed there.

The dossier shall include references and copies of all the published scientific data mentioned and the copies of any other relevant opinions which have already been produced by any recognised scientific body. Where those studies have already been evaluated by a European scientific body following the legislation in force in the European Union, a reference to the result of the evaluation should be sufficient and a copy should be provided. Data from studies that have been conducted and published previously or coming from a peer review shall clearly refer to the same biomass as the one subject to the application for authorisation.

Studies, including those that have been conducted and published previously or coming from a peer review, shall be performed and documented according to appropriate quality standards (e.g. Good Laboratory Practice (GLP) in accordance with [Directive 2004/10/EC](#) or International Organization for Standardization (ISO)). Where *in vivo* or *in vitro* studies are carried out outside the Community, the applicant shall demonstrate that the facilities concerned comply with the Organisation for Economic Cooperation and Development (OECD) [Principles of Good Laboratory Practice](#) (GLP) or ISO standards.

Studies involving animals should respect the rules on animal welfare laid down by European Union legislation and should not be repeated if not necessary. It is encouraged to use *in vitro* methods or methods which refine or replace the usual tests performed with laboratory animals or reduce the number of animals used; such methods should be of the same quality and provide the same level of assurance as the method they aim to replace.

1. General information

1.1. Administrative data

The applicant should provide the following:

⁹ Currently under revision

- name and address of the applicant;
- name and qualification of the contact person;
- scope of the application.

1.2. Scientific summary

A scientific summary addressing each part of the dossier submitted to support the application should be provided. It should include the conclusions reached by the applicant(s).

2. Characterisation of the product and production process

The product has to be fully identified and characterised. The studies described in this section must be based on the final product(s) for which authorisation is sought. In-house identifiers should be avoided unless they are embedded in third-party documents. In this case, a statement is required to confirm that the identifier(s) refer(s) to the product for which authorisation is sought.

Any data from other known uses of the product and/or production strain (e.g. use in food, human or veterinary medicine, agriculture and industry) should be provided.

2.1. Production organism

For all microorganisms (taken as including archaea, bacteria, filamentous fungi, yeasts, protozoa and microalgae), the origin should be provided and, where relevant, any history of modification (selection/mutagenesis) should be indicated if not part of the mandatory information required for genetically modified microorganisms (GMM). For GMM, the description of the genetic modifications should be provided in accordance with the most recent version of the [‘Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use’](#).

The identity and taxonomic classification of each microorganism should be provided according to the latest published information in the International Codes of Nomenclature (ICN). Microbial strains should be deposited in an internationally recognised culture collection (preferably in the European Union). A certificate of deposition from the collection, which should specify the accession number under which the strain is held, must be provided. In addition, all relevant morphological, physiological and molecular characteristics necessary to provide the unique identification of the strain and the means to confirm its genetic stability should be described.

For microorganisms qualifying for the qualified presumption of safety” (QPS) approach to safety assessment, the requirements listed in the most recent version of the [QPS opinion](#) should be followed. For non-QPS microorganisms, the potential occurrence of toxins, genotoxic substances, virulence factors, antibiotics or other pharmacologically active substances should be reported based on the present knowledge of the metabolism of the species/strain.

- Known toxins (including endotoxins) or virulence factors should be demonstrated to be absent or of no concern. Strains of microorganisms belonging to a taxonomic group that includes members known to be capable of producing toxins or other virulence factors should be subject to appropriate tests to demonstrate at a molecular and, if necessary, cellular level the absence of any cause for concern. As an example on how to assess the potential for toxin production, see the technical [guidance on toxin production in *Bacillus* spp.](#)
- Strains of microorganisms intended for use as biomasses should not contribute further to the reservoir of antibiotic resistance genes already present in the gut microbiota of animals and the environment. Consequently, all strains of bacteria should be tested for resistance to antibiotics in use in human and veterinary medicine unless evidence of substantial degradation of DNA has been provided. Where resistance is detected, the genetic basis of the resistance should be established to allow a conclusion to be drawn on the likelihood of transfer of resistance to other gut-inhabiting organisms. See [technical guidance on antibiotic resistance](#).

2.2. Manufacturing process

Details of the fermentation/cultivation process should be provided including the composition of the media and down-stream processing (including harvesting, the method of sterilisation and drying).

The HACCP procedure for avoiding physical, chemical and biological contamination should be described.

Evidence should be provided that the product does not contain viable cells from the fermentation/cultivation process (e.g. no outgrowth from samples of 25 g from three batches). The presence of viable but non-culturable cells should be considered. For products for which multiple industrial production scale batches are not available, ten samples of one batch of a large scale fermentation/cultivation should be analysed.

2.3. Characterisation of the product

The following information is required to describe the nutritional value of the product, its purity and its physical properties.

2.3.1. Composition

- Proximate analysis based on at least five batches¹⁰ (identified by manufacturing date) of the product.
- Characterisation of the different fractions based on at least three batches:
 - ✓ Nitrogen fraction: Total N, amino acid N, amino acid composition, biogenic amines, ammonia N, purine and pyrimidine N, nitrate and nitrite N
 - ✓ Lipids: fatty acid composition, free fatty acids, phospholipids, non-saponifiable matter
 - ✓ Composition of the carbohydrate fraction
 - ✓ Vitamins, including provitamins.
 - ✓ Minerals: Ca, P, Mg, Na, K, Cl, S
 - ✓ Trace minerals: Fe, Zn, Mn, Cu, I, Co, Se, Mo
 - ✓ Pigments
- Depending on the production organism, further characterisation of these fractions may be required (e.g. D/L forms of amino acids, *cis/trans* fatty acids, branched chain fatty acids). This data may be generated from a single batch of the product.

2.3.2. Microbial and chemical impurities

The applicant should identify and quantify microbial and chemical (including residual solvents) impurities, substances with toxic or other undesirable properties that are not intentionally added. If a primary product has been removed, its residual concentration should be measured. The product should be essentially free of antimicrobial activities relevant to the use of antibiotics in humans or animals.

The following should be considered depending on the nature of the biomass: microbiological contamination (e.g. *Enterobacteriaceae* including *Salmonella* spp. and *Escherichia coli*, aerobic and anaerobic spore-formers, yeasts and filamentous fungi), mycotoxins, marine biotoxins, heavy metals (Pb, Hg, Cd), arsenic and fluorine.

The protocol used for the routine screening of production batches for contaminants and impurities should be described and appropriate action levels should be defined. These should be consistent with existing legislation (e.g. [Directive 2002/32/EC](#)) and recommendations from internationally

¹⁰ For continuous fermentation, sampling over time may replace 'batches'.

recognised sources, when available (e.g. [Commission recommendation on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding](#)).

Data should be provided from at least three production batches showing that levels of identified contaminants are below the action limits set.

2.3.3. Physical properties

The physical properties of the product (three batches) should be described, including for dry products particle size distribution and dusting potential expressed as g/m^3 .

2.4. Shelf life of the product

The expected shelf life of the product as marketed (three batches) should be proposed, based on at least two model situations covering the likely range of use conditions (e.g. 25 °C, 60 % relative air humidity (RH) and 40 °C, 75 % RH).

Endpoints to establish the shelf life are:

- Appearance, water content.
- Microbiological quality (total aerobic and anaerobic bacteria, yeasts, filamentous fungi and the product organism).
- Nutritive stability (crude protein content, ammonia N, concentration of biogenic amines, lipid hydrolysis and oxidation).

2.5. Conditions of use

The intended use in animal nutrition, including target species/categories, should be described.

3. Studies on target species

The primary objective of the studies in target species is to define a safe use level.

Studies should be performed and documented according to appropriate quality standards. Trials should be compliant with the criteria established by a recognised, externally-audited, quality assurance scheme (e.g. Good Laboratory Practice (GLP) in accordance with Directive 2004/10/EC).

It is recommended that such studies should consist of four treatment groups (a group without dietary biomass and three groups with graded levels of biomass inclusion). Diets should be formulated for both control and treated groups to match nutritional requirements and avoid nutritional imbalances that might distort results. It is suggested that a control mixture of feed materials (and nutritional additives) should be formulated so that the product can replace this mixture in equal (graded) amounts without any other changes of the basal diet. The nutritional aspects to be considered are: amino acid N, total N for ruminants, limiting amino acids (for which a supplementation of the product may be necessary), minerals, trace elements, nitrogen-free extracts (rough proportion of starch:fibre), gross energy. For products with a high protein content, sources for protein in the control mixture may be soy-protein concentrate, casein, whey protein, wheat gluten; for products with a lower protein content, soybean meal or other solvent-extracted oilseed meals. The control mixture in the control diet should be replaced by the product to one-third, two-thirds and three-thirds in the treatment diets, as exemplified in Table 1. The maximum incorporation level should reflect the intended maximum incorporation rate of the product in the complete diet/daily ration, without producing any negative effect. The diets should be provided in a form that minimises fine dust particles (e.g. pelleted, extruded). An example for an experimental design is given in Table 1.

Table 1: Example of an experimental design with 15 % maximum incorporation rate of the product

	Control	Treatment 1	Treatment 2	Treatment 3
Control mixture (%)	15	10	5	0
Product (%)	0	5	10	15
Basal diet (%)	85	85	85	85

Test animals should be routinely monitored for visual evidence of clinical effects, morbidity/mortality, performance parameters, haematology and routine blood chemistry, and for other parameters likely to be related to the biological properties of the product. Any critical endpoints known from the toxicological studies in laboratory animals should be considered. Unexplained deaths should be investigated by necropsy and, if appropriate, histology. For meat-producing animals, necropsy (organ weights, gross pathology with histological follow-up if indicated) should be performed in animals from at least the control and highest dose group.

Food product composition/quality (including nutritional and sensory characteristics) should be examined in the control group and the highest product dose group. For meat-producing animals, those studies could be combined with necropsy.

In principle, one study should be performed for each animal category for which the product is intended to be used. If the application is for poultry, ruminants, pigs and salmonids, including all relevant species/categories, a single study should be done in each case in the most sensitive category (chickens for fattening for poultry, dairy cows for ruminants, piglets for swine and rainbow trout (*Oncorhynchus mykiss*) for salmonids).

Group size (replicates × animals per replicate) should be chosen, in general, so that a difference of 5 % in any measured parameter would be detected as significant at $P < 0.05$ (α -level of 0.05 and a β -level of 0.20), in ruminants at $P < 0.1$. As a guide, the minimum group size in Table 2 should be followed. The minimum duration of the studies is given in Table 2.

Table 2: Minimum duration of studies and recommended group size (minimum number of replicates and animals per replicate) for different animal species/categories

	Age/weight at start	Duration (weeks)	Replicates	Animals per replicate
Chickens for fattening	1 day	5	6 per sex	10
Piglets	6-8 kg	6	6 per sex	2
Dairy cows	*	8	10	1
Rainbow trout	10-50 g	13	8	15

* Experiments should not be done during the last third of lactation period.

The experimental unit for the statistical evaluation is the replicate (pen/tank), except for dairy cows.

Reporting of the studies should be done following the provisions of the [Technical guidance for tolerance and efficacy studies in target species](#). It should include the completion of the 'Trial protocol data sheet' given in Appendix A of that guidance.

4. Assessment of consumer safety

For products based on/derived from a microorganism recognised by EFSA to qualify for QPS approach to the assessment of safety, no further assessment of consumer safety is required provided that the qualifications for QPS are satisfied and:

- no substances that raise safety concerns are used in the production process.
- for GMM, the molecular characterisation of the event does not give rise to concern.

For all the other products:

- A set of genotoxicity studies¹¹ should be performed using, for example, suitable extract(s); and
- a subchronic (90-day) oral toxicity study should be provided.
- Residue/metabolism studies are only required when known metabolites of the fermentation process or compounds resulting from the further treatment of the product give rise to safety concern.

Depending on the outcome of these studies, further toxicological studies (e.g., chronic toxicity, reproduction toxicity) may be required.

For details, refer to the [Technical guidance on consumer safety](#).

5. Assessment of user safety

In general, an assessment of user safety should be based on data on skin/eye irritation, skin sensitisation and inhalation exposure, together with any information on systemic toxicity.

It should be noted that many of those products have a high protein content and have a potential for sensitisation, particularly via respiratory route. In this respect, data on particle size distribution and dusting potential should be taken into account in assessing risk. In addition, the allergenic potential of many microorganisms is unknown.

For details on how to assess user/worker safety, refer to the [Technical guidance on user safety](#).

6. Assessment of safety for the environment

Since most of the product consists of nutrients, an assessment of the safety for the environment is normally not necessary, particularly because the product will substitute for other feed materials. However, if metabolites/substances of toxicological concern are excreted in more than trace amounts, an assessment should be performed for those metabolites/substances according to the [Technical guidance for the environment](#).

¹¹ The subject of genotoxicity testing is currently under consideration by the Scientific Committee of EFSA with the aim of harmonising approaches. The current requirements of the Technical guidance for consumer safety may be revised.