APPROVED: 06 June 2024

Annex A

Public consultation on the draft guidance on the assessment of the efficacy of feed additives

European Food Safety Authority (EFSA)

Abstract

The European Food Safety Authority (EFSA) carried out a public consultation to receive input from the scientific community and all interested parties on the draft guidance on the assessment of the efficacy of feed additives prepared by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) and endorsed by the Panel for public consultation at its Plenary meeting on 16 November 2023. The written public consultation for this document was open from 1 December 2023 to 9 February 2024. EFSA received a total of 164 comments from 21 interested parties. EFSA and its FEEDAP Panel wish to thank all stakeholders for their contributions. The current report summarises the outcome of the public consultation and includes the comments received and brief descriptions of how the comments were addressed. The FEEDAP Panel prepared an updated version of the Guidance on the assessment of the efficacy of feed additives, considering the questions/comments received. This guidance was discussed and adopted at the FEEDAP Plenary meeting on 6 June 2024 and was published in the EFSA Journal.

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Introduction

The European Food Safety Authority (EFSA) asked the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) to update the guidance document on the assessment of the efficacy of feed additives. The FEEDAP Panel endorsed the draft guidance on 16 November 2023. In line with EFSA's policy on openness and transparency and in order for EFSA to receive comments from the scientific community and stakeholders on its work, EFSA engages in public consultations on key issues. The draft guidance on the assessment of the efficacy of feed additives, which the FEEDAP Panel endorsed on 16 November 2023, was released for public consultation from 1 December 2023 to 9 February 2023. Stakeholders were informed and invited to submit comments. All comments were subject to evaluation and assessment. Where considered appropriate, the guidance document has been modified to take account of the comments. EFSA received 164 comments from 21 interested parties: 4 industry associations, 11 private companies, 1 consultant organisation, 3 national public health organisations, and 2 individuals in their personal capacity. The comments received (after the removal of empty comments and duplicates) are listed in Table 1, together with answers or comments from EFSA. EFSA and its FEEDAP Panel wish to thank all stakeholders for their contributions.

Table 1 - Comments received and answers/comments provided by EFSA

#	Chapter	Organisation	Comment	Answer/comment by EFSA
1	1 Introduction	Federal Office of Consumer Protection and Food Safety	General Remarks: Double spaces or uneven word spacing are noticeable in several parts of the document. Table text is either left-aligned or justified. Bulleted lists are indicated by dashes, dots or letters. Spaces between < or > characters are sometimes present, sometimes missing. Standardization of the formatting is recommended.	Editorial comment. Comment not related to risk assessment.
2	1 Introduction	AFCA-CIAL	In order to foster innovation by industry, it is considered that feed additives applications and their assessment should focus on the primary function(s) of the additives and that benefits of feed additives that are a consequences of the primary functions should not require pre-market authorisation. As for feed materials and compound feeds, scientific substantiation should be available for national competent authority.	Comment not related to risk assessment.
3	1 Introduction	Chr. Hansen A/S	Thank you for the opportunity to comment on this draft. We hope that EFSA will take our comments in the following into account and that applicants are given a sufficient transition period to adjust to new requirements when implemented. We also hope that the files are uploaded as expected, but it is not possible to see the files and check if they are all attached as intended. We are relying on the green OK from the system. Kind regard,	EFSA confirms that a transitional period is envisaged after the adoption of all Guidance documents of the FEEDAP Panel.
4	1.2 Scope of the guidance	FEFANA asbl	Comment 1: Establishing an appropriate transition period for the implementation of the updated guidance document is crucial to ensure compliance with the revised rules. This transition period must consider the ongoing or completed trials and the efforts invested in generating results. Applicants should not be retroactively required to meet criteria that were not available at the time of their planning (e.g., because they were not included in the 2018 Guidance - such as the newly proposed veterinary health certificates, documents on ethical committee decisions etc.). If the new rules are enforced too quickly, the necessity to plan, schedule, and conduct new trials would arise (replacing those with planning faults due to uncertainty). This would demand several months or even over a year, potentially causing delays in the submission process and potentially wasting animal lives - contrary to the 3R principles. The substantial number of comments and questions received from FEFANA members during this open consultation exercise underscores the significance and	The FEEDAP Panel acknowledges the fact that not all new requirements of the updated Guidance could be implemented in trials designed and performed before the endorsement of this document. A transitional period is envisaged after the adoption of all Guidance documents of the FEEDAP Panel.

			complexity of the guidance for applicants. During the transition period trials conducted according to the previous guidance (2018) should remain acceptable and be assessed according to that guidance. This approach ensures a smooth transition without compromising the integrity of ongoing research or jeopardising the welfare of animals.	
			Comment 2: The guidance indicates that applicants should provide justifications for any omission of data or deviations from the outlined requirements. While Lines 98 and 102 suggest that this document is designed to assist and guide applicants, Lines 111-112 introduce the term "requirements". If the requirements are not met, justifications have to be provided. It is our understanding that EFSA guidance aim at assisting applicants rather than establishing (legal?) requirements. The wording may need revision to be compliant with the letter of the law (i.e., Art 7 para 6 of 1831/2003) and the intended purpose of EFSA guidance documents in contrast to guidelines, i.e., Regulation (EC) No 429/2008. As already expressed in different occasions EFSA often does not engage with the applicant during the assessment process if the data provided are deemed insufficient or inadequate to conclude on the efficacy. As a result, inconclusive opinions are adopted without further communication with the applicant. Such practice increases the application timeline and introduces unnecessary burdens on the EC, MS, applicants, and EFSA due to the need to create a new mandate for a post-opinion submission and at the same time, it may result in additional animal testing and food waste (in the form of discarded products of animal origin) which is contrary to the principles of the 3Rs in animal testing, as reaffirmed by EFSA's 2027 strategy. Addressing efficacy issues during the risk assessment process could prevent this additional burden and align more effectively with EFSA's objectives.	Comment not related to risk assessment.
5	1.2 Scope of the guidance	Pen & Tec Consulting, SLU trading as Argenta ®	Lines: 108-109 Text in the GD: The requirements for efficacy demonstration for the different categories of additives are listed in Section 2. Comment: The requirements for efficacy demonstration for the different categories of additives are listed in (should say:) Section 3.	The text was modified to address the comment.
			Lines: 109-110 Text in the GD: Section 3 provides information on the number of efficacy studies required for those additives for which in vivo studies are needed.	The text was modified to address the comment.

			Comment: Section 4 (should say) provides information on the number of efficacy studies required for those additives for which in vivo studies are needed.	
			Line: 111 Text in the GD: Sections 4 and 5 describe the principles for in vivo and in vitro studies, while sections 6 and 7... Comment: it shoud say: Sections 5 and 6 describe the principles for in vivo and in vitro studies, while sections 7 and 8...	The text was modified to address the comment.
			Lines: 105-107 Text in the GD: This document is intended to guide applicants in assessing the efficacy of additives intended to be used in animal feed to demonstrate compliance with the requirements of Article 5.3 of Regulation (EC) No 1831/2003. Comment: Given the inherent complexities in assessing the diverse array of animal feed applications, we acknowledge the clear benefits of EFSA guidelines as a tool to support effective study design. Guidance by nature is non-binding, it is not designed to regulate applicants but instead to assist them. Promotion of important issues such as animal welfare (e.g., the 3Rs principle aiming to reduce the use of animals in research), should not preclude assessment of a study on the basis of its own scientific merit, even if it is not wholly aligned with an updated guidance. It would benefit the entire community to clarify here that, like line 633, flexibility is provided to allow scientific discretion in design and conduct of studies, and that deviation from the guidance is possible providing that the scientific rational and justification are supplied.	The Guidance aims to assist the applicants in preparing and presenting their application for the authorisation of a feed additive. The requirements and information provided in the Guidance are intended to cover most of the situations scope of the applications. In the current document it is said that "No single design is recommended; flexibility is provided to allow for scientific discretion in the design and conduct of the studies". Therefore, it is the responsibility of the applicant to decide the strategy to follow when preparing and submitting an application for the authorisation of the feed additives, including the design and conduct of studies submitted to support the efficacy.
			Lines: 114-115 Text in the GD: Applicants should justify the omission from the dossier of any data or any deviations from the requirements detailed in this guidance. Comment: Consider rewording (as per comments for lines 105 - 107). We think it would be useful that it is clarified in the text that the aim of EFSA guidance is not to regulate, and it is always possible to deviate from the guidance provided that the scientific rationale for such deviation is described and justified.	Comment not related to risk assessment.
6	1.2 Scope of the	AFCA-CIAL	line 101-102 : The guidelines of regulation 429/2008 represent the regulatory framework. The EFSA Guidance is not a regulatory text and it is voluntary for the applicants. The EFSA Guidance must, as a priority, focus on result demonstration	Comment not related to risk assessment.

	guidance		rather than a standardized obligation of means for the obtention of the said result. A standardized obligation of means slows down innovation or access to innovation for a company.	
			Line 114-115 : Line 101 says that this document is prepared to assist applicants, Line 105 says that it is intended to guide applicants. However, in lines 114-115, it is mentioned that the guidance establishes "requirements". If the requirements are not met, justifications have to be provided. It is our understanding that EFSA guidance aim at assisting applicants not at establishing (legal?) requirements. The wording should probably be revised in order to be compliant with the letter of the law (ie Art 7 para 6 of 1831/2003) and the objective of the EFSA guidance documents vs the guidelines, ie Regulation 429/2008 Proposed reformulation : Applicants should justify the omission from the dossier of any data or any deviations from this guidance. Any omission or any deviations from this guidance on the basis of Commission Regulation No 429/2008 should not require additional justification.	Comment not related to risk assessment.
7	2 General principles of efficacy assessment	FEFANA asbl	Line 132-134: The guidance should be improved by consistently establishing a connection with Regulation (EC) No 429/2008 when applicable. This paragraph serves as a clear example, and we propose an amendment to accurately reflect the provisions outlined in the first paragraph of 3.4 in Section IV concerning studies on the efficacy of the additive.	Unclear comment. Comment not related to risk assessment.
			Lines 140-143: We believe that the assessment of efficacy through in vitro studies should extend beyond additives intended only to affect the characteristics of feed. For instance, functional groups such as substances which favourably affect the environment or physiological condition stabilisers are severely underrepresented in the EU and their assessments are burdened with uncertainty due to lack of established criteria and lack of sectors experience in dossier building for such additives. In light of this we propose that EFSA reconsider this stance and allows at least one in vivo study for those additives to be replaced by an in vitro study, if and when specific in vitro validated systems are available and suitable for the demonstration of the intended effect on the animal (so using the exact same approach as proposed for enzymes [chapter 4.3, lines 602-611]). We would appreciate if this point could be considered, and amendments introduced accordingly.	The Guidance already foresees that a combination of in vitro and in vivo studies can demonstrate efficacy in specific circumstances. In any case, if the applicant considers that some in vitro studies can be used to support the efficacy of the additive, these can be submitted with appropriate justifications and will be assessed by the FEEDAP Panel on a case-by-case. At present, it is not possible to provide more details, and it is up to the applicant to propose adequate methods to demonstrate the efficacy according to the intended effect.

			Line 142: The text refers to the impact on the animal (here and in various parts throughout the draft guidance (e.g., lines 307, , 603). However, in other cases, it discusses the effect in the animal (e.g., lines 299, 305). Could you please provide clarification on whether the intention is to specifically address an effect "on" or "in" the animal? We would appreciate it if this could be clarified or amended for consistency.	The text was modified to address the comment.
			Line 143: Could examples, illustrating "specific circumstances," be provided to clarify the concept? Including specific situations where a combination of both types of studies is deemed acceptable would enhance the clarity of this section and offer the expected guidance to applicants. Reference in this paragraph to the specific sections in which this is further developed would be also a positive amendment.	This section is intended as a general introduction and does not address all specific aspects of the efficacy assessment, which are described in the following sections. Examples of additives for which a combination of in vitro and in vivo tests would be acceptable are substances for the reduction of the contamination of feed by mycotoxins.
			Line 145-146: This sentence is a repetition (already mentioned on lines 128-129) and could potentially be removed.	The text was modified to address the comment.
			Line 149 "Efficacy should be supported by positive results in independent studies.": We would appreciate clarifications in the guidance concerning the outcomes considered as "positive results".	The text was modified to address the comment.
			Line 149-150: The mention of only feed materials seems too limiting, particularly considering that an additive can exert its effect in compound feeds or within preparations of feed additives (e.g., a technological additive used in feed additive preparations, such as antioxidants for vitamin formulations) or in premixtures. It may be more fitting to use the legal term "feed," which covers feed additives, premixtures, complete feed, and feed materials.	The text was modified to address the comment.
			Line 150 "...different batches of feed materials.": Please consider providing clarification regarding the "different batches of feed materials". It would be beneficial to clarify the criteria that distinguish one batch from another - whether it pertains to feed material sourced from a distinct silo, delivered separately, harvested from different fields, or any other relevant factor. Unless it is meant to refer to different batches of compound feed?	The text was modified to address the comment.

			Lines 150-152: It is appreciated that guidance on expectations on independence is given. However, this paragraph would require further development to make the expectations of FEEDAP as clear as possible and unambiguous. Please consider the following points: 1. We suggest deleting "formulations of diets and". It will be difficult to define "how different", should this be ingredients and/or nutrient level. In commercial setting often an almost identical diet composition is used throughout the feed season, this also is an argument for not have the "formulation of diets" mentioned. Alternatively, a clear definition of "different formulations of diets" should be added. In this case, please also consider that the term diet is not defined in the feed regulation. It would be better to use a term from the feed regulation, so this is clearer for applicants. Is diet referring to "compound feeds"? 2. Also, the wording "batches of feed materials" can be confusing as studies are not only performed on feed materials. Or should this refer to compound feeds? 3. Does the sentence mean that if the same animals are used for two consecutive trials, but different batches of the same feed material are used the two studies will be considered as independent? A study would still have a different result if it were done with the same batches of feed materials but a different diet composition or different animals and the same formulation of diets. The text should probably read: "In vivo studies are considered independent when performed with different formulations of diets or batches of feed materials or with different animals." 4. We seek clarification from EFSA on whether the intention is to deem two trials conducted simultaneously as non-independent. Applicants have observed that a different trial start in the same facility is often not enough for EFSA to deem it an independent study, even if the batch of animals is a different one and the trials were specifically planned and conducted separately, if the trial time slots overlap (e.g., by 2 of 4 weeks). 5.It would be highly beneficial for applicants to have explicit criteria to be applied in trial facilities to minimize the risk of rejection based on the perceived lack of independence. For instance, by citing corresponding EFSA opinions where the reasons for not considering the studies "independent" are described.	The text mentioned in the comment has been removed from this section. The Panel's considerations regarding the performance of multiple trials are included in sections 5 (for in vivo efficacy studies) and 6 (for in vitro efficacy studies).
			Line 157: As in other places in the guidance, we would consider it more appropriate to use terms that are defined in the feed regulations. The term diet is	This specific wording comes from Regulation (EC) No 429/2008.

			not defined and while it likely refers to compound feed, it is considered appropriate to use the adequate term instead. Also, what is meant by components of the diet?	
			Line 163-164: The same exact sentence is already included in Section 5. We wonder if this repeated text is needed in the guidance.	Editorial comment. Comment not related to risk assessment.
8	2 General principles of efficacy assessment	FEDIAF European Pet Food Association	Lines 152-155 (page 5): FEDIAF would like to request further clarification on the practical interpretation/implementation of the definition of "independent" in in vivo studies, especially in cases where the guidance requires three in vivo studies in one pet/non-food-producing species. For example, if 3 studies are intended to be conducted in dogs, and each one will use different batches of feed materials and will be performed with different animals or at different times: is it necessary to add the additional request for 3 different formulations for the same species? We would suggest limiting the requirement, at least for pet/non-food-producing species, to studies performed with different batches of feed materials and with different animals or at different times.	The text mentioned in the comment has been removed from this section. The Panel's considerations regarding the performance of multiple trials are included in sections 5 (for in vivo efficacy studies) and 6 (for in vitro efficacy studies).
9	2 General principles of efficacy assessment	Orion	Efficacy studies on pets (especially cats and dogs) Guidance is mainly focusing on production animals, which normally live in similar conditions and are fed the same diet. But as the efficacy guidance is also applicable to pets, it causes some difficulties to find pet animals which are living in the same conditions and are fed the same diet. To get enough subjects, the studies with dogs and cats need to be carried out in shelters/kennels/ laboratories. This might not be ideal as animals living in shelters/kennels/laboratories do not represent very well the target users of the commercial feed additive, which are pets at home environment. Conditions between homes and shelters/kennels/laboratories can be very different. The requirement of having the same study conditions and same diet rules out the possibility to carry out efficacy studies on dogs and cats with client owned animals, in their natural environment. It is proposed to clarify in the guidance the requirements of the conditions for efficacy studies on pets (especially cats and dogs) and open up the possibility to conduct the efficacy studies on pets with client owned dogs.	The Guidance does not exclude the possibility of performing trials with client-owned animals. However, the experimental design should be justified based on the conditions of use and the intended effect of the additive.
			Section 2 General principles of efficacy assessment (rows 149-152) There is no mention if different batches of feed additive are to be used in the efficacy studies, or should it be the same feed additive batch in every study.	It is the responsibility of the applicant to design the studies considering all possible factors that may affect the outcome of the trials in order to minimise bias.

10	2 General principles of efficacy assessment	Erawan Consulting SARL	Lines 144-145 / Comment: The term "in specific circumstances" should be clarified.	See reply to #7.
			Line 152 / Comment: It seems that the term "positive" is not appropriate. Do you mean significant results?	See reply to #7.
			Lines 159-160 / Comment: It seems that the term "diet" is not appropriate. We usually use feedingstuffs.	See reply to #7.
11	2 General principles of efficacy assessment	Federal Office of Consumer Protection and Food Safety	Page 3 LL 120-122 Please add "as well as animal welfare" : Moreover, such studies must permit the evaluation of the efficacy of the additive according to common feed manufacturing, animal husbandry and farming practices as well as animal welfare in the European Union (EU).	The text was modified to address the comment.
			Page 3 LL 132-133 Please delete "and flavouring compounds": However, the Panel considers that there are some additives for which efficacy is recognised (e.g. many nutritional additives). Explanation: To exclude flavouring compounds per se seems to be not optimal because the palatability of feed may differ from the humans one for food.	These are general principles that are further developed in the section on Sensory additives. Indeed, for most flavouring compounds, efficacy demonstration is unnecessary as the feed effect is assumed to be the same as in food.
			Page 4 LL 133-134 Please add "but data from metastudies and literature research": These additives do not require further demonstration of efficacy but data from metastudies and literature research.	When an additive is already authorised for use in food, and its intended use of the additive in feed is the same, no further demonstration of efficacy is generally necessary, provided that the effect seen when the additive is used in

				food could reasonably be expected to be seen when it is used in feed at the recommended concentration and that food and feed matrices are comparable. For those additives, no further information in the form of a literature search is needed.
			Page 4 LL 146-148 Please add "in feedingstuffs or drinking water": Efficacy should be investigated by comparison of the lowest recommended use level in feedingstuffs related or also calculated to the complete feedingstuff with a moisture content of 12 % or drinking water with a control group and designed to allow statistical evaluation.	The text was modified to address the comment.
			Page 4 LL 156-159 Please add "medicated feed[1]" including the following footnote "[1]Regulation (EU) 2019/4 of the European Parliament and of the Council of 11 December 2018 on the manufacture, placing on the market and use of medicated feed, amending Regulation (EC) No 183/2005 of the European Parliament and of the Council and repealing Council Directive 90/167/EEC": Attention should also be paid to known or potential biological or physico-chemical interactions between the additive, other additives and/or veterinary medicines, medicated feed[1] , and/or components of the diet, where this is relevant to the efficacy of the additive concerned, e.g. compatibility of a microbial additive with coccidiostats and histomonostats or organic acids.	The proposed addition is considered unnecessary as the interactions should be evaluated at the level of the veterinary medicines.
			Page 4 LL 161-162 Please " delete the link in the text to the guidance , " add "EFSA" and " add: "(EFSA FEEDAP Panel, 2018)" " add as footnote a literature reference: 2EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE,Wallace RJ, Wester P, Glandorf B, Herman L, Karenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206 For details on how to perform compatibility studies between microbial additives and other additives showing antimicrobial activity, see the EFSA guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).	The text was modified to address the comment.

12	2 General principles of efficacy assessment	Pen & Tec Consulting, SLU trading as Argenta ®	145 - 146 The studies should be based on the additive(s) for which authorisation is sought. It would be useful that the text clarifies whether generally studies based on the active substance would be acceptable considering that: (1) Once an active agent has been authorised as a feed additive, different formulations can be placed on the market with reference to that authorisation. (2) In some cases, the final formulation of an additive has not been established when performing the studies. In other cases, the additive is presented in different formulations, e.g. liquid or solid, but the proposed dose (active substance in feed) is the same.	The text was modified to address the comment.
			146 - 148 Efficacy should be investigated by comparison of the lowest recommended use level with a control group and designed to allow statistical evaluation. It would be useful that refers to both, positive and negative controls since some studies are performed with a positive control (e.g. bioequivalence studies assess the expected in vivo biological equivalence of two additives).	The text was modified to address the comment.
			149 - 150 In vitro studies are considered independent when performed with different batches of feed materials. Question 1: Please confirm if different location would be sufficient to consider the studies independent (even if performed at "same time") Question 2: Some in vitro studies use compound feeds - would different batches of compound feed be acceptable, even if the feed materials used are of the same batch? Question 3: We understand that in the case of in vitro studies it is acceptable that they are run at the same time provided that "different batches" of feed are used e.g. of feed materials or compound feed - please confirm.	It is the responsibility of the applicant to design the studies considering all possible factors that may affect the outcome of the trials in order to minimise bias. For in vitro studies, those are required to be designed to cover a representative range of materials to which the additive will be applied. The Panel's considerations regarding the performance of multiple trials are included in sections 5 (for in vivo efficacy studies) and 6 (for in vitro efficacy studies).
			150 -152 In vivo studies are considered independent when performed with different formulations of diets and batches of feed materials and with different animals or at different times. Consider re-phrasing for clarity: In vivo studies are considered independent when they are performed at different times or locations, or when all of the following differ between studies: animals, diet formulation and batches of feed material used in diets.	The text mentioned in the comment has been removed from this section. The Panel's considerations regarding the performance of multiple trials are included in sections 5 (for in vivo efficacy studies) and 6 (for in vitro efficacy studies).

			Please clarify what do you mean by "different animals", since all animals are different unless they are clones.	
			150 -152 In vivo studies are considered independent when performed with different formulations of diets and batches of feed materials and with different animals or at different times. Question 1: Please confirm if different location would be sufficient to consider the studies independent (even if performed at "same time")	The text mentioned in the comment has been removed from this section. The Panel's considerations regarding the performance of multiple trials are included in sections 5 (for in vivo efficacy studies) and 6 (for in vitro efficacy studies).
			Question 2: For studies run in the same location: please clarify the scientific rationale for considering studies with same formulation/batches of feed materials (1) "independent" when performed at "different times", and (2) "not-independent" when performed at the "same time". The setting is the same, and based on the definition of "independent" in biostatistics, the studies are independent even if run at the same time since the outcome of one study does not affect the outcome of the other. If data show efficacy of a feed additive, the studies should be accepted independently on whether (or not) they are run at the same time. Now that the transparency regulation is in place, studies are notified, hence there is sufficient evidence that studies are planned and managed as separate studies in all aspects from the start.	See reply to #7.
			Question 3: For "different times" - would one day difference be sufficient?. And 1 day difference but sampling at the same days?	See reply to #7.
			149-152 "Efficacy should be supported by positive results in independent studies. In vitro studies are considered independent when performed with different batches of feed materials. In vivo studies are considered independent when performed with different formulations of diets and batches of feed materials and with different animals or at different times". We would like further clarification on the practical interpretation/implementation of the definition of "independent" in vivo studies, especially in cases where the guidance requires three in vivo studies in one pet/non-food-producing species. For example, if 3 studies are intended to be conducted in dogs, and each one will use different batches of feed materials and will be performed with different animals or at different times ? is it necessary to add the additional request for 3 different formulations for the same species? We would suggest limiting the requirement, at least for pet/non-food-producing species, to studies performed	See reply to #7.

			with different batches of feed materials and with different animals or at different times.	
			150 -152 In vivo studies are considered independent when performed with different formulations of diets and batches of feed materials and with different animals or at different times. Please clarify what "different formulations" means ? i.e. what would be the required variation introduced in the diets between studies to be considered as sufficient. In our view, even small variations provide sufficient evidence that the study was planned &/or performed as a separate study. Would inclusion of a marker (e.g. microtracer, titanium dioxide or other appropriate marker) in one of the feeds be sufficient?. Furthermore, now that the transparency regulation is in place, studies are notified, hence there is evidence that studies are planned and managed as separate studies in all aspects from the start.	See reply to #7. The inclusion of a marker in the feed is not considered evidence of a different formulation. Moreover, the notification of two studies separately would not necessarily mean that they are independent.
			153 - 155 Reference can be made to published studies to fulfil the requirements listed in the guidance provided that the active substance/agent in literature studies is identical to that under application or, if not, would still allow conclusions on the additive under application to be made. We welcome the possibility of using published references. The text refers to the fulfilment of the requirements listed in the guidance. Published studies are often not performed by the applicants, so in most of the cases it will not be possible to provide raw data. Will EFSA accept these studies?	Published studies will be accepted provided that they report the data necessary to perform an assessment under the requirements of the guidance.
13	2 General principles of efficacy assessment	Nor-Feed SAS	Lines 149-152 : In vitro studies may be possible for other additives than technological additives, having an effect on the feed. However, the description for "independent in vitro studies" only focusses on different batch of "feed materials". - What about in vitro studies that will not involve feed materials or feed ingredients?	It is not clear what other in vitro studies the comment refers to. In any case, if the applicant considers that some in vitro studies not involving feed materials can be used to support the safety of the additive, these can be submitted with appropriate justifications and will be assessed by the FEEDAP Panel. It should be noted that the Guidance already foresees that a combination of in vitro and in vivo studies can be used to demonstrate efficacy in specific

				circumstances. At present, it is not possible to provide more details, and it is up to the applicant to propose adequate methods to demonstrate the efficacy according to the intended effect.
			- For in vivo studies, do we have to understand that if multiple trials are launched in the same facility, the applicant will have to check every single batch of feed materials used in the formulation of the complete feed used for the trial ?	See reply to #7.
14	2 General principles of efficacy assessment	AFCA-CIAL	§2 Line 132-134 : This consideration of the panel potentially goes beyond the requirement of Regulation 429/2008 (See 3.4 section IV) and may need to be revised in order to comply with the guidelines - It is considered important to refer to 429/2008 as often as appropriate in order to establish a clear link between the EFSA guidance document and the requirements established in the Regulation 429/2008	See reply to #7.
			§2 Line 142 : The text speaks about effect on the animal (here but also at other places in the draft guidance: line 307, 33, 603); elsewhere it speaks about effect in the animal. Is the intention to speak about an effect ON or IN the animal (line 299,305 for example) ? Could that be clarified/amended?	See reply to #7.
			§2 Line 143 : Could it be indicated, via examples, what is meant by "specific circumstances"? Would it be possible to include examples of such situations where a combination of the two types of studies is acceptable? That would facilitate the understanding of this part of the text and provide the expected assistance to applicants	See reply to #7.
			§2 Line 145-146 : Repetition: already mentioned on lines 128/129	See reply to #7.
			§2 Line 149-150 : It appears too restrictive to mention only feed materials when an additive can have an effect in compound feeds or in preparations of feed additive (eg a technological additive for use in feed additive preparations : antioxidants for vitamin preparations) or in premixtures. It is probably more appropriate to speak about "feed" which is the legal term that covers Feed Additives, Premixtures, Feed materials and compound feeds.	See reply to #7.

			Rewording proposed : In vitro studies are considered independent when performed with different batches of feeds	
			§2 Line 151-152 : What is the definition of "independent studies" ? The term diet is not defined in the feed regulation. It would be better to use a term from the feed regulation so this is clearer for applicants. It is understood that "compound feeds" is meant here when diet is used? Different batches of Feed Materials : does that mean that if the same animals are used for two consecutive but different batches of the same feed material are used the two studies will be considered as independent ? Also here, is it meant feed materials or compound feeds ? Different times: here as well it is not clear what would be accepted as independent studies ? what about if a study is made with sows, would two studies with the same compound feed/same feed materials (from two different batches) but at different period in the year be considered as independent? This part of the text would require revision to provide clarity for readers, especially regarding the expectations in terms of "different diets/compound feeds", different feed materials, different animals and/or different times. Would it be possible to provide examples of situations where studies are not considered independent; for example by citing corresponding EFSA opinions where the reasons for not considering the studies "independent" are described.	See reply to #7.
			§2 Line 157 : As in other places in the guidance, we would consider it more appropriate to use terms that are defined in the feed regulation. The term diet is not defined and while it likely refers to compound feed, it is considered appropriate to use the adequate term instead. Also, what is meant by components of the diet ?	See reply to #7.
			§2 Line 163-164 : The exact same sentences are also repeated in lines 643-644: is it needed to have this mentioned twice in the guidance	See reply to #7.
15	2 General principles of efficac	Chr. Hansen A/S	Lines 150-151, page 5 of 33 pages "In vivo studies are considered independent when performed with different formulations of diets and batches..." Comment: Definition of "different" is missing (how different, and in ingredients and/or nutrient level)? We suggest deleting "formulations of diets and". It will be	See reply to #7.

	y assessment		difficult to define "how different", should this be ingredients and/or nutrient level. In commercial setting often an almost identical diet composition is used throughout the feed season, this also is an argument for not have the "formulation of diets" mentioned. The most important text is "different batches of feed materials and with different animals or at different times". Alternatively, a clear definition of "different formulations of diets" should be added. Proposal: Consider deleting "formulations of diets and"	
16	2 General principles of efficacy assessment	Oy Medfiles Ltd	Dear EFSA, L150-152: Could you give some examples please what you mean by different formulations and feed material batches, please. In the recent Feedap open plenary you mentioned that feed materials from neighboring countries would not be different enough. Furthermore, in the same meeting, you mentioned that the stuides could be conducted with the same animals and locations provided that the time is different, Thus, should the end of the sentence read: ....or with same animals at different times. Thank you.	See reply to #7.
17	2 General principles of efficacy assessment	G. Bertin	Lines 144-145 Draft content: "In contrast, for those intended to have an effect on the animal, efficacy should be assessed via in vivo studies or, in specific circumstances, by a combination of in vitro and in vivo studies". Comment: The term "in specific circumstances" should be clarified.	See reply to #7.
			Line 152 Draft content: "Efficacy should be supported by positive results in independent studies". Comment: It seems that the term "positive" is not appropriate. Do you mean significant results?	See reply to #7.
			Lines 159-160 Draft content: "Attention should also be paid to known or potential biological or physicochemical interactions between the additive, other additives, veterinary medicines and/or components of the diet, where…" Comment: It seems that the term "diet" is not appropriate. We usually use feedingstuffs.	See reply to #7.

18	3.1.1 Technological additives which exert their function in feed	FEFANA asbl	Line 175 "Appropiate control feed": For clarity, could it be indicated whether the term intended in that sentence is "negative control"?	The term "appropriate control" is considered to cover both "positive" and/or "negative" control.
			Line 176-177: Please clarify: 1. What is considered the minimum number of different feeds/feedingstuffs (feed in general? Or compound feeds or feed materials?) to be tested.	At least three studies should be designed to cover a representative range of feeds to which the additive will be applied. The selection of the feeds will depend on the conditions of use of the additive and the target species scope of the application.
			2. What types of studies are meant? Three studies in feed and one in water? Or two studies in feed and one in water? Since water is not a feed, it would be probably more appropriate to modify the sentence as it may create confusion in its present form and applicants may, ultimately, not provide the expected number of studies. For example: ?The studies (at least three) should be designed to cover a representative range of feeds to which the additive will be applied. If the additive is intended to be used in water, its efficacy should also be assessed when applied in water for drinking. In this case two studies in feed and one in water should be conducted?.	The text was revised to remove water. Technological additives are not authorised to be used in water.
			Table 1 ? stabilisers "Maintenance of the physico-chemical state of feedingstuffs, including use of coating agents." Feed and feedingstuffs are synonymous terms. For coherence across the guidance, we would like to ask if it is possible to refer only to FEED (=feed additive, premixture, compound feed, feed material) or to list the intended types of feed to be targeted (e.g., using the term compound feed and/or feed material as appropriate). In table 1 feedingstuffs is mentioned three times and should be revised.	The text was modified to address the comment and harmonised throughout the document.
			Table 1 ? silage additives. "Improved production of silage (better preservation of nutrients)." Demonstration of efficacy for silage additives through ?Improved production of silage (better preservation of nutrients)? goes beyond	The Guidance foresees at least four different claims regarding the efficacy of silage additives: (i) improvement of

			Reg.429/2008, where ?improved production of silage? is required, not better preservation of nutrients. Improved silage production can be achieved through different benefits and is not restricted to better preservation of nutrients. For example, through the fermentation profile of the silage: pH, lactic acid, NH3-N. The two parameters that are looked at, in priority, for assessing silage quality/silage preservation are pH and ammonia nitrogen. Hence, it may be considered as too restrictive, especially for experts in silage making, to speak about nutrient preservation only. For clarity and to help applicants understand how the efficacy of silage additives is assessed, it would be beneficial to clarify how assessment is performed and how the other parameters required in the guidelines are assessed versus nutrients only.	silage production/preservation of nutrients; (ii) aerobic stability; (iii) reduction of effluents; (iv) reduction of specific microorganisms (e.g., clostridia, Listeria spp). Regarding the improvement of silage production/nutrient preservation, the Panel considers that at least an improvement of the dry matter loss or the ammonia-N content is necessary to demonstrate the efficacy.
			Line 181-182: For clarity, we would suggest including the functional group: ?other technological additives? in the Table with the text as proposed in the draft guidance. As presented now, it may not be perceived that the guidance refers to an existing functional group.	The text has been modified to address the comment.
			Line 184-185: What about the possibility to extrapolate between categories of silage types? Actually, depending on the endpoints and expected benefits of a silage additive, extrapolation may be possible for example from difficult to easy/moderately difficult to ensile category. As an example, an additive with an effect on pH in difficult to ensile forage is expected to be efficient as well in easy/moderately easy to ensilage materials and extrapolation should be possible as long as three significant studies are provided. We would appreciate if the FEEDAP could consider a modification according to this.	The Panel considers that the proposed extrapolation is not adequate for silage additives.
			Line 193-194: Please consider replacing the term feedingstuffs by feed materials or fresh forage material, as appropriate	The provision in that paragraph is meant for fresh materials not from plant origin. The text has been modified to improve the clarity.
			Line 197-198 : The recommendation to work with 1kg of fresh material (unless it is meant 1kg Dry Matter) may de facto exclude some research facilities working with for example glass jar which contains only 0.7kg of FM. That may also exclude to work according to DLG protocol which recommend typically working with lower size (see page 18 of DLG guidance where it refers to 800g). Please consider an amendment of the 1kg recommendation based on the above.	The guidance states that it should be approximately 1 kg. The applicant may justify the use of smaller silos in the trials.
			Line 198: Extend ?laboratory silo with the potential to vent gas? with vacuum packed bags. Plastic wrapped silage bunkers keep gas built in the silage bunker,	The text was modified to address the comment.

			so vacuum bags mimic silage bunkers more compared to containers with regulated gas release. We would appreciate if the following amendment could be considered: ?laboratory silo with the potential to vent gas or vacuum packed bags?	
			Line 202-204 and 222-225: The duration provided in the EFSA guidance corresponds to the requirements outlined in Regulation (EC) No 429/2008 regarding silage additives. However, the regulation explicitly acknowledges the potential for deviations, provided they are duly justified. While EFSA guidance documents, in principle, permit deviations when appropriately justified in the dossier, it would be beneficial to explicitly align the wording of these paragraphs with Regulation 429/2008. This adjustment would facilitate that applicants fully understand the potential flexibility in shortening the study duration, as permitted by the regulation. We kindly request the consideration of the above points and propose adapting the text to cover the possibility of deviations, consistent with the provisions of Regulation 429/2008.	The Guidance already foresees the possibility of applicants deviating from the requirements as long as justification is provided. These deviations will be judged on a case-by-case basis by the Panel.
			Line 212 "calculated dry matter losses during ensiling" + footnote 1: It is understood that any equation can be used to assess DM losses as long as the reference is provided. Could this be confirmed?	The procedure used to estimate the silage DM content corrected for the loss of volatiles should be duly referenced and justified considering the method used for DM determination (e.g., drying temperature) and the type of silage. The calculations performed should be made available.
			Line 215 "alcohols": Regulation 429/2008 requires for Ethanol to be measured, could you please clarify if is it expected to look at other types of alcohols?	The Panel acknowledges that ethanol is the most relevant alcohol to be measured. However, other alcohols may be relevant for some specific additives depending on the claim.
			Line 219-221: In contrast to Table 1, this text shows that improvement of silage production and preservation of nutrients are different ways of demonstrating the efficacy of silage additives. It reinforces the relevance to revise the text in Table 1 and to provide further assistance in the guidance in terms of recommendations for the efficacy data for silage additives. In case of microbial silage additives, DM loss is a difficult parameter to look at for heterofermentative bacteria: actually, DM loss is known to increase during fermentation when such silage additives are used. Also, heterofermentative bacteria are not aimed at reducing NH3-N which	The text included in Table 1 provides examples of endpoints considered to assess the efficacy of silage additives. The use of pH as a standalone parameter is not considered sufficient to conclude on the efficacy of silage additives. The DM loss is a parameter to be monitored

			is linked to rapid acidification (rapid pH drop) that is typical to homofermentative bacteria and not to heterofermentative ones. For heterofermentative bacteria, aerobic stability is a more relevant parameter. We fully understand that reduced DM losses makes sense from an application and final user stand-point but it is a very tricky parameter to study at experimental level (lab/in vitro scale) as the accuracy of measurement is often smaller than then analytical error=> depicting with confidence an effect on DM is scientifically questionable and certainly challenging with mini-silos. Depending on the experimental set-up, DM losses may even be negative due to the lack of accuracy of the measurements linked to the sum of analytical errors. Is DM loss a parameter to be studied during fermentation only or also during aerobic stability studies? The proposed parameters cannot satisfy heterofermentative and homofermentative bacteria-based silage additives since they are opposed in terms of end-points/expected benefits-claims => improved acidification pattern instead? (satisfactory for homofermentative only, besides more acetic, less yeast and molds...) pH is a key parameter for assessment of silage preservation/efficacy directly related to good fermentations (lowering pH is also key to reduce growth of spoilage microorganisms). It is considered important to include pH as a parameter that can be used to demonstrate efficacy of silage preservation.	but not used to conclude when the claim is to improve aerobic stability.
			Line 226-228 : Could it be specified in the guidance if aerobic stability can be demonstrated only after a 90 days study or if it would also be acceptable to demonstrate an effect at "early silage opening" (e.g., after 7, 15 or 30 days), provided that the study lasts for 90 days? Regarding aerobic stability and the "seven days after exposure": is it expected to have an effect after that period (i.e., a two days longer duration) or is it expected that the efficacy is shown during that time frame? Actually, an opening of seven days is rather long and may not permit to show a difference since during such a long period, spoilage organisms are expected to grow even in the treated silage and pH will increase. Could you please clarify of aerobic stability is to be studied within a time frame of seven days or after seven days (which is not practically/technically realistic)?	The studies on aerobic stability are intended to assess the stability after the silage has been opened, so they should be performed at a time in which it would be reasonable to expect that ensiling has been completed. Measurements should be done after exposure to air, and for a minimum of 7 days.
			Line 231-233: We kindly request clarification on the use of "supportive" information by applicants, as well as the Panel's methodology for assessing it to determine the efficacy of a silage additive in relation to other aerobic stability parameters. Additionally, we seek guidance on whether pH could be considered a	The Panel considers the measurement of temperature increase as the key endpoint to assess the aerobic stability in silage. Any other parameter may be

			viable indicator for assessing aerobic stability, potentially serving as supportive evidence. On this aspect, the text could be modified as follows: Temperature measures may be complemented by the measurement of CO2 production or changes in pH. The assessment of dry matter loss and direct counts of aerobic spoilage organisms could also be employed as supportive evidence of improved stability.	supportive, but it alone will not be considered enough to draw conclusions on aerobic stability. The text was amended to clarify this aspect.
			3.1.1.2 Hygiene condition enhancers and/or preservatives Lines 234-298 General comment: The combination of two functional groups in one paragraph potentially creates various sources of confusion, e.g., The specificity of the effect; where hygiene CE would work against specific (families of-) species according to its authorization, preservative authorizations would typically not mention specific (families of) species which is logical from its broad effect and the requirement to prevent deterioration of feeds from whatever microbial species. What would be the exact purpose of this point? Maintain microbial numbers (preservatives) or reduce (hygiene CE)? Related to this, the minimum study duration may be of higher relevance (preservatives, where increases can be observed after initial stasis) or of lower relevance (hygiene CE, where reductions are achieved within a short period after which the microbial agent is absent or usually remains at a low level) The need for assessing the microbial quality of the feed at the end of the study (lines 290-293) only seems to apply to preservative studies. Proposal: Please consider splitting the paragraph such that a general introduction mentions the aspects that both functional groups have in common after which specific instructions for either functional group are provided	The effects of the two functional groups are considered to be largely overlapping; therefore, the requirements were considered to be applicable to both functional groups and grouped in one section.
			Lines 234-298 - General comment: The guidance seems to bring efficacy requirements of preservatives more in line with zootechnical functional groups where the conditions under which an additive is effective must be more detailed than before. This seems to be implied from the indirect notions in the paragraph where 'studies shall reflect the claimed conditions of use' (e.g., duration of studies, line 279-280; mention of minimum required use level, line 258-260; possibly also other criteria like microbial species of choice for which the effect is claimed etc.). However, we would like to bring to your attention that this is not how microbial growth works in practice, but instead, it is a multifaceted phenomenon: feed	The choice of the reference strain(s) will depend on the target microorganism(s). To prove the efficacy of the microbial additive, a representative number of studies targeting relevant reference strains are needed. The studies should include a sufficient number of strains covering the variability of the target organism(s). Therefore, the broader the range expected, the larger the number of studies/reference strains will be

			matrix, pH, moisture, water activity, temperature, presence of other microbial species etc. can all cause variation in the readiness of microbial species to show growth, which then also affects the effective preservative dosage and duration of the effect. The exact conditions of effective use of preservatives therefore need to be defined for each concrete practical situation by the operator in a quantum satis approach. This cannot be mimicked in full by the choice of efficacy studies (although a sufficient wide choice of conditions and microbes in these studies may help to arrive at a general conclusion about efficacy). We therefore strongly recommend continuing to offer the applicant the choice to claim a general preservative effect, without minimum dosage, choice of feedingstuffs or duration of claimed effect. It would be good to have clear what then is the minimum set of study designs that would facilitate the assessment by EFSA of the efficacy of such general use, where the studies themselves are considered as examples of the practical situation. We would like to highlight another crucial aspect – that the current "requirements" should not go beyond what has been mandated thus far for this functional group. Essentially, if the "new" requirements would be more rigorous than the previous ones, it implies that future preservatives would be evaluated based on different criteria than before. This imbalance is disproportionate and may not establish the expected level playing field for products within a given functional group. This becomes even more critical when considering that efficacy is to be assessed only once, i.e., at the initial application for authorisation, not during subsequent renewals. We would appreciate an amendment in line with the above considerations. See also our other general comment to this section on the apparent number of studies when applying all mentioned criteria like different required moisture levels, feeds, microbial species etc.	needed. The numbers reported in this section are just an example.
			Lines 234-298 - General comment: While the text in general reflects in our view the relevant aspects of efficacy testing of additives inhibiting microorganisms, the various required dimensions of a study design make the setup too heavy for execution in practice (capacity of	See the reply to the comment above. Regarding the moisture content, the different levels should be represented by the different (at least 3) feeds. It is not required that for each feed chosen, different moisture levels are tested.

			incubators etc.), so choices need to be made. The text now indicates the following dimensions: Minimum of 3 studies One or more effective supplemented levels (although we strongly recommend multiple dosages, e.g. 5, see our comment to line 261-264) 'a range of' feed materials to be tested (4?) Various pathogenic resp. food spoiling microorganisms to be tested (4?) Minimum of 3 replicates Various moisture levels (4?) Based on above chosen (and realistic) number of levels per criteria, this would make a total of 2880 (!) groups, that also have to be measured at least at 3 moments in time. We also ask ourselves whether this extensive amount of data might exceed what is necessary to draw conclusions regarding the potential efficacy. We suggest making a choice for the most relevant aspects. For instance: The 3 studies may entail different microbial species, feedingstuffs and/or moisture levels (e.g., study 1 is on various liquid food coproducts where yeasts and enterobactericaea are the food spoiling MO, next to study 2 where various dry compound feeds are tested and where moulds are the typical food spoiling MO). When testing multiple serotypes of the same microbial species, the testing of 3 replicates may not be strictly necessary, especially when the different serotypes already show the same sensitivity; these could then be considered replicates.	
			Line 239-240 - Evidence of efficacy should be demonstrated using a minimum of three independent laboratory-based studies. Comment: In case of silage additive, a minimum sample size of 1kg is recommended (we have suggested a revision of this requirement in the corresponding section), what about here, is a minimum sample size also recommended?	The sample size should be enough to represent practical use conditions. The sampling is part of the study protocol and should be fully described.
			Line 241-244 - should be measured in the feedingstuffs containing the additive in comparison with a control feed. The choice of the feedingstuffs and the target microorganism(s)/contaminant(s) against which the additive will exert its function should be justified and reflect the proposed conditions of use. For additives intended to be used in all feedingstuffs,	The text was modified to address the comment and homogenised throughout the document.

			Comment: As elsewhere in the guidance, it would be appropriate to use the same term across the guidance: either feed or feedingstuffs. And also to clarify if when "feed" is used, it refers to FA, FM, premixture and compound feeds.	
			Lines 244-247- For additives intended to be used in all feedingstuffs, efficacy should be demonstrated in a representative range of feed materials and dry matter content according to the intended use (i.e., covering a range of approximately 10-80% DM). The matrix's pH, dry matter content, and water activity should be provided for each study. Comment: Preservatives (and probably also hygiene CE) are typically meant for all feedingstuffs. It would be advised, also for the sake of keeping practical size of the studies to be therefore more explicit; e.g., one feed material and one compound feed (mind that not only feed materials may require preservation). Besides, moisture levels shall not be prescribed but depend on intended use. 'i.e.' better be changed into 'e.g.'. Please consider the following amendment: "For additives intended to be used in all feeds, efficacy should be demonstrated in a representative range of three trials with at least one feed material and one compound feed at dry matter content levels according to the intended use (e.g., covering a range of approximately 10-80% DM). The matrix's pH, dry matter content, and water activity should be provided for each study."	The choice of feeds depends on the applicant. However, evidence should be provided that the additive is efficacious over the range of materials for which applications are made. In order to cover "all feeds", the Panel considers that the DM range should be from 10 – 80%.
			Line 246 Comment: Analysis of pH of the matrix should not be mandatory to assess the efficacy of additives in this functional group, especially when used in dry feeds. We would appreciate if reference to pH could be removed from this section: "The matrix's pH, dry matter content and water activity should be provided for each study"	The Panel considers pH to be an essential parameter for microbial growth and should monitor it.
			Line 250 Comment: The term "Clonality" in this passage is ambiguous, and we would welcome further clarification for better understanding.	This requirement aims to exclude that closely related strains are used, which would indicate a common ancestor.
			Line 250-253 - At least three replicates for each strain of the target microorganism(s) tested should be included in each experiment. This would also	Three independent studies with different feeds; three replicates per feed and strain.

			apply to preservatives when the feed material is inoculated with spoilage micro-organisms Comment: Could you please clarify if it refers to 3 different batches on the same matrix or from the same batch on 3 different matrices?	
			Lines 254-256 - For hygiene condition enhancers, different molecular (sero)types relevant for humans and the target animals should be tested (e.g., for Salmonella spp. at least four serovars to reach a minimum of five strains Comment: At least three serovars to reach three strains for assessing the efficacy of the additive against Salmonella spp. should be considered as representative especially if the mechanism of action and the use conditions of the additive can be considered as similar by the applicant. Please consider the following proposal: (e.g. for Salmonella spp., at least three serovars to reach a minimum of three strains, one of which should be a reference or well-known strain; […])	The studies should include a sufficient number of strains covering the variability of the target organism(s). Therefore, the broader the range expected, the larger the number of studies/reference strains will be needed. The numbers reported in this section are just an example.
			Lines 258-261 – The experimental design should include at least two groups: one with the feedingstuff contaminated with the target microorganism(s) (control) and another with the same contaminated feedstuff supplemented with the additive at the minimum use level. If appropriate, other groups with different levels of the additive may be included in the design. Comment: An alternative to the classical control vs. minimum dosage group would fit this functional group better, due to the multifaceted nature of microbial growth (feed matrix, pH, moisture, water activity, temperature, presence of other microbial species etc. can all cause variation in the effective dosage). It is thus recommended to add here the possibility to test various additive levels and apply regression analysis to the data cloud to conclude on general efficacy (also see the methodology of the ACIAC efficacy studies from 2012 for formic acid - EFSA Journal 2014;12(10):3827, and formate salts – EFSA Journal 2015;13(5):4056. Please consider the following amendment: "The experimental design should include at least two groups: one with the feedingstuff contaminated with the target microorganism(s) (control) and another with the same contaminated feedstuff supplemented with the additive at the minimum use level. If appropriate, other groups with different levels of the	The Guidance foresees that at least the minimum inclusion level proposed is tested. Apart from this, no single design is recommended, and flexibility is provided to allow for scientific discretion in the design and conduct of the studies.

			additive may be included in the design. In the latter case, regression analysis is accepted to define the effectiveness of the additive."	
			Line 258-260 - The experimental design should include at least two groups: one with the feedingstuff contaminated with the target microorganism(s) (control) and another with the same contaminated feedstuff supplemented with the additive at the minimum use level. Comment: As elsewhere in the guidance, it would be appropriate to use the same term across the guidance: either feed or feedingstuffs. And also to clarify if when "feed" is used, it refers to FA, FM, premixture and compound feeds.	The text was modified to address the comment and homogenised throughout the document.
			Lines 266-269 – On the contrary, for preservatives, the additive should be present in the feed when the target spoilage microorganism(s) is added. The use of naturally contaminated feeds is preferred in the case of preservatives. Comment: It is not very clear to us why food-spoiling microorganisms cannot be added to the feed before the additive. Why this difference between preservatives and hygiene CE? Could you please explain why such a difference is made between the two functional groups?	Studies can be done with naturally or artificially contaminated feeds. In the case of artificially contaminated feed, the additive and target microorganism(s) may be added simultaneously for both hygiene condition enhancers and preservatives. The text was modified to address the comment.
			Line 267 - Hygiene condition enhancer could be also present in the feed before contamination by the target microorganism(s), such as preservatives. This functional group shouldn't be excluded from the sentence. Please consider the following amendment: On the contrary for preservatives or hygiene condition enhancers, the additive should be present in the feed when the target microorganism(s) spoilage is added.	According to the legal definition, hygiene condition enhancers should not be present in feed before the target microorganism(s).
			Lines 271-272 The levels and growth phase of the microbial strain(s) at the beginning of the experiment should be described. Comment: What are EFSA's expectations about the description of the growth phase of the microbial strain(s)? We would appreciate clarifications on this aspect.	This sentence was removed from the Guidance. The comment is not relevant anymore.

			Line 279-280 - The duration of the study should reflect actual farming conditions and cover the period for which an effect is claimed according to the proposed conditions of use. Comment: The intended use of such feed additives is not restricted to the farm as they may also be used in feed mills. This should be reflected in the text as such expectations would de facto restrict the use of some FA and the possibility to submit applications. As a proposal, the text could say: "storage conditions" / "typical conditions of use of the feeds intended to be treated with the additive".	The text was modified to address the comment.
			Line 288-289 page 8 - Changes in microbial counts below 0.5 log are considered within the normal variation of the methods and will not be taken as proof of an effect. Comment: A difference below 0.5 log is considered within the normal variation of the method and would not support efficacy. Does it mean that a difference above or equal to 0.5 log between control and treated groups is sufficient to consider the efficacy of the additive? Could you please clarify this aspect in order for applicants to have a clear understanding of EFSA's expectations?	A difference above 0.5 log indicates a difference, but per se, it might not be biologically relevant and, therefore, not supportive of the efficacy.
			Line 291-292 - (e.g., pH, temperature, counts of total aerobic bacteria, Enterobacteriaceae, total yeasts and filamentous fungi). Comment: pH and temperature are not microbial quality criteria such as total bacteria/Enterobacteriaceae... In addition, pH is more relevant for the functional group "acidity regulators" and temperature (of the feed?) shouldn't be required if the temperature during the trial is reported. Please consider removal of these two parameters: (e.g., pH, temperature, counts of total aerobic bacteria, Enterobacteriaceae, total yeasts and filamentous fungi)	The Panel considers that changes in pH and temperature are relevant parameters for the monitoring of microbial growth.
			Lines 292-293 – For target microorganisms producing toxic compounds, the presence of these compounds should be analysed in the feed samples at the end of the study.	It is challenging to establish a general link between the toxin production and CFU counts. Therefore, the Panel considers that it is not possible to establish a fixed threshold.

			Comment: Related to the need to measure toxic compounds produced by target microorganisms, in our view this is of less relevance when microbial numbers remain low. It is recommended to introduce a CFU threshold for this. We recommend revising the text to include a provision for granting a waiver when the microbial numbers of the target species are consistently low. Additionally, if deemed applicable, consider incorporating a threshold for CFU.	
19	3.1 Technological additives	EASY BIO, Inc	We do disagree with the details given in lines 254 to 257, Such a level of prescription is not equally applied to efficacy studies of other feed additives and the inclusion of such limitations on the trials has the effect of limiting the type of additives to broad-range additives while interfering with the approval of narrow-range, specific additives which may have a targeted effect on specific microorganisms. In addition, the current requirements are over-burdensome and impractical by requiring five serovars per strain x triplicate per strain x three independent trials. Additionally, it is unclear what the reference strain is by types of microorganisms.	See reply to #18.
20	3.1 Technological additives	Federal Office of Consumer Protection and Food Safety	Page 4 L 170 Please add the following sentence at the end of the text: When the additive is already authorised for use in food and the intended use of the additive in feed is the same, no further demonstration of efficacy is generally necessary, provided that the effect seen when the additive is used in food could reasonably be expected to be seen when it is used in feed at the recommended concentration and that food and feed matrices are comparable. *The fact, that food and feed matrices are comparable should be demonstrated and taken into account by the applicant*.	The text was modified to address the comment.
21	3.1 Technological additives	Pedersen Nutrition Ltd	Testing at constant temperature is not relevant, a change in temperature is more like commercial circumstance for bales.	The studies to test silage additive efficacy should be done in silos in controlled conditions; temperature is one of the conditions that should be monitored and controlled.
22	3.1 Technological additives	Chr. Hansen A/S	Lines 231-233, pages 7-8 Temperature measures may be replaced by measurement of CO2 production. Measurement of dry matter loss and direct counts of aerobic spoilage organisms may be used as supportive evidence of improved stability. We are of the opinion that it should also be possible to use pH to assess aerobic stability, at least as supportive evidence.	See reply to #18.

			We suggest changing the text to: "Temperature measures may be complemented by measurement of CO2 production *or changes in pH*. Measurement of dry matter loss and direct counts of aerobic spoilage organisms may be used as supportive evidence of improved stability."	
			Lines 249-250, page 8 Data should be provided to confirm taxonomic/serotype identification and to exclude clonality. We would appreciate further explanation on the word "Clonality".	See reply to #18.
23	3.1.1 Technological additives which exert their function in feed	Erawan Consulting SARL	Lines 237 -301 / Comment: Having two functionalities in one paragraph creates a confusion This paragraph should be split in 2 sub-sections, one for preservatives, the other for hygiene conditions enhancers	See reply to #18.
24	3.1.1 Technological additives which exert their function in feed	Federal Office of Consumer Protection and Food Safety	Page 5 L 177 Please delete "including water for drinking if appropriate" because technological feed additives can only be used in feed, not in water for drinking due to the existing rules of Regulation 1831/2003 at that time: The studies (at least three) should be designed to cover a representative range of feeds to which the additive will be applied.	The text was modified to address the comment.
			Page 5 L 178 Please replace "appropriate endpoints for the various functional groups are" by "demonstration of efficacy is": The demonstration of efficacy is indicated in Table 1.	The text was modified to address the comment.
			Page 5 Line 179; Table 1, Line "Preservatives": It is noted, that preservatives are used in hygienically flawless feed (Delimitation to hygiene condition enhancers).	Preservatives are meant to be used in feeds contaminated with spoilage microorganisms (not pathogens).

			Page 5 L 179 Table 1, Line Thickeners, column Demonstration of efficacy: Please delete "feed materials or" because they are included in feedingstuffs: Viscosity of the feedingstuffs.	The text was modified to address the comment.
			Page 5 L 179 Table 1, Line Acidity regulators, column Demonstration of efficacy: Please ? delete in the cell "and/or water" and ? add in the same cell the following sentence at the end: pH and/or buffering capacity in feedingstuffs. Duration of the study should cover the period for which an effect is claimed.	The text was modified to address the comment.
			Page 5 L 179 Table 1, Line silage additives, column Demonstration of efficacy: Please replace production by quality: Improved quality of silage (better preservation of nutrients).	The text was modified to address the comment.
			Page 5 LL 186-188: Please note, that dry matter content, crude ash and crude protein content have a significant influence on the ensilability of a feed and should ideally be categorized when it comes to classifying silage as "easy", "moderately difficult" and "difficult to ensile". DLG e.V.; DLG-Information 2/2006, ?Grobfutterbewertung, Teil B - DLG-Schlüssel zur Beurteilung der Gärqualität von Grünfuttersilagen auf Basis der chemischen Untersuchung?; https://www.dlg.org/fileadmin/downloads/landwirtschaft/themen/ausschuesse_facharbeit/tier/futtermittel/grobfutterbewertung_B.pdf	The text was modified to address the comment.
			Page 5 LL 190-192 Please add "in-vitro" for better understanding: Three in-vitro tests should then be made with material representative of the claimed range, where possible using examples of different botanical origins.	The proposed addition was not considered necessary.
			Page 5 LL 216-217 Please add an additional hyphen at the end of the list after line 217: - content of hydro-soluble carbohydrates	This endpoint is considered relevant to define the categories of feed materials, not to measure the silage additive's influence on the ensiling process results.
			Page7 LL 244-246 Please and an example for feed material in the following sentence: For additives intended to be used in all feedingstuffs, efficacy should be demonstrated in a representative range of feed materials and dry matter content according to the intended use (i.e., covering a range of approximately 10-80% DM, for example XXX)	The choice of the feed materials to be tested is the responsibility of the applicant and is dependent on the claimed effect of the additive.

			Page 7 LL 246-247 Please add "protein content": The matrix's pH, dry matter content, protein content and water activity should be provided for each study	The protein content of the feed materials is not considered a fundamental parameter which could affect the growth of the target spoilage/pathogen (and of the additive, if relevant) microorganisms.
			Page 7 L 257 Please add the following, new sentences at the end of the passage: The applicant should demonstrate that the serotypes investigated are of current relevance to humans or animals. A classification should be made regarding the frequency of occurrence of the serotypes in the feed or the relevance with regard to animal health or food safety.	The need to indicate the relevance for humans and target animals is already considered within the current text. No further clarification is considered necessary by the Panel.
			Page 8 L 283 Please add the following sentences: In the case of hygiene condition enhancers, feed samples should be monitored for the viable counts of the specific target microorganism(s). In the case of preservatives, microbial groups (e.g., total numbers of yeasts, fungi, and aerobic bacteria) should be analysed using cultivation-based methods. For certain microorganisms, the type of sampling is crucial in order to be able to make reliable statements about decontamination due to the formation of 'nests' (e.g. salmonella). The type and scope of sampling should be scientifically sound. Efficacy is demonstrated (?)	The description of the sampling is part of the description of the study protocol.
25	3.1.1 Technological additives which exert their function in feed	Pen & Tec Consulting, SLU trading as Argenta ®	179 Table 1: Demonstration of efficacy for technological additives exerting their effect in feed Comment: Consider re-wording to "in feed and/or water"	Technological additives are not authorised to be used in water.
			193-194 Claims restricted to or including feedingstuffs other than plant material require tests specific to those feedingstuffs. Comment: It would be very useful if the text includes a definition of "plant material" and examples of "feed materials other than plant material" that EFSA considers appropriate for the production of silage.	The provision in that paragraph is meant for fresh materials not from plant origin. The text has been modified to improve the clarity.

			248-249 For hygiene condition enhancers, the choice of the target microorganism(s) should cover several unrelated reference/well-known and field strains? Comment: Please clarify the meaning of "several unrelated reference/well-known strains"	Reference (i.e., type) strains are preferable, but in their absence, well-known strains can also be used. These reference/well-known strains should belong to the same taxonomic unit (species/(sero)type) as the defined target microorganisms.
			249-250 Data should be provided to confirm taxonomic/serotype identification and to exclude clonality. Comment: Please include examples of methods accepted by EFSA to address this requirement.	It is up to the applicant to use the most appropriate methods as long as they are duly justified and validated. For example, fingerprinting molecular methods or microorganisms from different origins may allow for clonality to be excluded.
			263-269 In the case of artificially contaminated feed, the additive and target microorganism(s) can be added simultaneously for both hygiene condition enhancers and preservatives. Otherwise, for hygiene condition enhancers, the target microorganism(s) can be present in the feed when the additive is added. On the contrary, for preservatives, the additive should be present in the feed when the target spoilage microorganism(s) is added. The use of naturally contaminated feeds is preferred in the case of preservatives. Comment: *Question 1*: please clarify whether in the case of preservatives the additive and the target microorganism can be added at the same time (the initial sentence says it can, but then it stated that for preservatives, the additive should be present in the feed when the target spoilage microorganism(s) is added).	See reply to #18.
			Question 2: "The use of naturally contaminated feeds is preferred in the case of Preservatives" - in practice, this would imply that feed additive is added after contamination. Please confirm that this would be acceptable. Suggestion: in the case of hygiene condition enhancers it should also be possible to add the target microorganism after the additive since contamination of the feed can happen at any stage.	See reply to #18. The text was modified to address the comment.
26	3.1.1 Technologica	Pederse n	It is a major concern that the lack of enzymes that reduced the issues with mycotoxins. It should be possible to use the method of sampling manure	Comment not related to risk assessment.

	l additives which exert their function in feed	Nutrition Ltd	samples for demonstration of efficacy in losses of nitrogen at farm level. It is the only way to bring the cost down and more product will enter the market.	
27	3.1.1 Technological additives which exert their function in feed	AFCA-CIAL	p.4 Line 175 : For clarity could it be indicated if it is meant "negative control" in that sentence	See reply to #18.
			p.5 Line 176-177 : Three studies in feed and one in water ? Or two studies in feed and one in water ? Since water is not a feed, it would be probably more appropriate to modify the sentence as it may create confusion in its present form and applicants may, ultimately, not provide the expected number of studies Rewording proposed : "The studies (at least three) should be designed to cover a representative range of feeds to which the additive will be applied. If the additive is intended to be used in water, its efficacy should also be assessed when applied in water for drinking. In this case two studies in feed and one in water should be required"	See reply to #18.
			p. 5 Line 179 table 1 :Feed and feedingstuffs are synonymous terms. For coherence across the guidance, we would like to ask if it is possible to refer only to FEED (= FA, PREMIXTURE, FM, CF) or to list the intended types of feed to be targeted. This recommendation goes beyond the guidelines : Regulation 429/2008 requires for ?improved production of silage?, not for better preservation of nutrients. Improved silage production can be achieved through different benefits and is not restricted to better preservation of nutrients. For	See reply to #18.

			example through the fermentation profile of the silage: pH, lactic acid, NH3-N. The two parameters that are looked at, in priority, for assessing silage quality/silage preservation are pH and ammonia nitrogen. It is too restrictive to speak about nutrient preservation. For clarity and to help applicants understand how the efficacy of silage additives is assessed, it would be beneficial to clarify how assessment is performed and how the other parameters required in the guidelines are assessed versus nutrients only.	
			p. 5 Line 181-182 : For clarity the functional group: ?other technological additives? should be included in the Table with the text as proposed in the draft guidance. As presented now, it may not be perceived as the guidance refers to an existing functional group	See reply to #18.
			p. 5 Line 184-185 : What about the possibility to extrapolate between categories of silage types? Actually, depending on the endpoints and expected benefits of a silage additive, extrapolation may be possible for example from difficult to easy/moderately difficult to ensile category. As an example, an additive with an effect on pH in difficult to ensile forage is expected to be efficient as well in easy/moderately easy to ensilage materials and extrapolation should be possible as long as three significant studies are provided.	See reply to #18.
			p. 5 Line 193-194 : The term feedingstuffs should be replaced by feed materials or fresh forage material	See reply to #18.
			p. 6 Line 197-198 : The recommendation to work with 1kg of fresh material (unless it is meant 1kg Dry Matter) may de facto exclude some research facilities working with for example glass jar which contains only 0.7kg of feed material. That may also exclude to work according to DLG protocol which recommend typically working with lower size (see page 18 of DLG guidance where it refers to 800g).	See reply to #18.
			p. 6 Line 212 : It is understood that any equation can be used to assess DM losses as long as the reference is provided ? Could this be clarified if any equation is acceptable ?	See reply to #18.
			p. 6 Line 215 : Regulation 429/2008 requires for Ethanol to be measured, is it expected to look at other types of alcohols?	See reply to #18.
			p. 6 Line 219-221: In contrast to Table 1, this text shows that improvement of silage production and preservation of nutrients are different ways of demonstrating the efficacy of silage additives. It reinforces the need to amend	See reply to #18.

			the text in Table 1 and to provide further assistance in the guidance in terms of recommendations for the efficacy data for silage additives. In case of microbial silage additives, DM loss is a difficult parameter to look at for heterofermentative bacteria: actually, DM is known to increase during fermentation when such silage additives are used. Also, heterofermentative bacteria are not aimed at reducing NH3-N which is linked to rapid acidification (rapid pH drop) that is typical to homofermentative bacteria and not to heterofermentative ones. For heterofermentative bacteria, aerobic stability is a more relevant parameter. We fully understand that reduced DM losses makes sense from an application and final user stand-point but it is a very tricky parameter to study at experimental level (lab/in vitro scale) as the accuracy of measurement is often smaller than then analytical error=> depicting with confidence an effect on DM is scientifically questionable and certainly challenging with mini-silos. Depending on the experimental set-up, DM losses may even be negative due to the lack of accuracy of the measurements linked to the sum of analytical errors. Is DM loss a parameter to be studies during fermentation only or also during aerobic stability studies? The proposed parameters cannot satisfy heterofermentative and homofermentative bacteria based silage additives since they are opposed in terms of end-points/expected benefits-claims => improved acidification pattern instead ? (satisfactory for homofermentative only, besides more acetic, less yeast and molds...) pH is a key parameter for assessment of silage preservation/efficacy directly related to good fermentations (lowering pH is also key to reduce growth of spoilage microorganisms). It is considered important to include pH as a parameter that can be used to demonstrate efficacy of silage preservation.	
			p.6 Line 226-228 : Could it be specified in the guidance if aerobic stability has to be demonstrated only after 90days of study or if it would also be acceptable to demonstrate an effect at ?early silage opening? (eg after 15 or 30 days), provided that the study lasts for 90 days ? Regarding aerobic stability and the ?seven days after exposure?: is it expected to have an effect after that period (ie a two days longer duration) or is it expected that the efficacy is shown during that time frame? Actually, an opening of seven days is rather long and may not permit to show a difference since during such a long period, spoilage organisms are expected to grow even in the treated silage and pH will increase. Is aerobic stability to be studied within a time frame of seven days or after seven days (which is not practically/technically realistic)	See reply to #18.
			p. 6/7 Line 231-232 : We would appreciate to receive clarifications on how such ?supportive? information can be used by applicants and how it assessed by the	See reply to #18.

			Panel to conclude on the efficacy of a silage additive versus the other aerobic stability parameters? Also, could pH be used to assess aerobic stability, at least as supportive evidence ?	
			p.7 Line 239-241 : In case of silage additive, a minimum sample size of 1kg is recommended, what about here, no minimum sample size?	See reply to #18.
			p.7 Line 241-244 : As elsewhere in the guidance, it would be appropriate to use the same term across the guidance: either feed or feedingstuffs. And also to clarify if when ?feed? is used, it refers to Feed additive, Feed Material, premixture and compound feeds.	The text was modified to address the comment and harmonised throughout the whole document.
			p.7 Line 246-247 : Analysis of pH of the matrix shouldn?t be mandatory to assess the efficacy of additives in this functional group, especially when used in dry feeds. Rewording proposed : The matrix?s dry matter content and water activity should be provided for each study.	See reply to #18.
			p.7 Line 250-253 : From 3 different batches on the same matrix or from the same batch on 3 different matrices?	See reply to #18.
			p.7 line 255-257 : At least three serovars to reach three strains for assessing the efficacy of the additive against Salmonella spp. should be considered as representative especially if the mechanism of action and the use conditions of the additive can be considered as similar by the applicant rewording proposed : (e.g. for Salmonella spp., at least three serovars to reach a minimum of three strains, one of which should be a reference or well-known strain; [?])	See reply to #18.
			p.7 Line 258-260 : As elsewhere in the guidance, it would be appropriate to use the same term across the guidance: either feed or feedingstuffs. And also to clarify if when ?feed? is used, it refers to Feed additive, Feed Material, premixture and/or compound feeds	The text was modified to address the comment and harmonised throughout the whole document.
			p.7 Line 266-269: Hygiene condition enhancer could be also present in the feed before contamination by the target microorganism(s), similarly as preservatives. This functional group shouldn?t be excluded from the sentence. rewording proposed : On the contrary for preservatives or hygiene condition enhancers, the additive should be present in the feed when the target microorganism(s) is added.	See reply to #18.

			p.7 Line 271-272 : What do EFSA experts expect about the description of the growth phase of the microbial strain(s)? To be clarified please	See reply to #18.
			p.8 Line 279-280 : The intended use of such Feed Additive are not restricted to the farm as they may also be used in feed mills. This should be reflected in the text as such expectations would de facto restrict the use of some Feed Additive and the possibility to submit applications.	See reply to #18.
			p.8 Line 288-289 : A difference below 0.5 log is considered within the normal variation of the method and would not support efficacy. Does it mean that a difference above or equal to 0.5 log between control and treated groups is sufficient to consider the efficacy of the additive? To be clarified please.	See reply to #18.
			Line 291-292 : pH and temperature are not microbial quality criteria such as total bacteria/Enterobacteriaceae... In addition, pH is more relevant for the functional group ?acidity regulators? and temperature (of the feed?) shouldn?t be required if the temperature during the trial is reported. Rewording porposed : (e.g., counts of total aerobic bacteria, Enterobacteriaceae, total yeasts and filamentous fungi)	See reply to #18.
28	3.1.1 Technological additives which exert their function in feed	FPS Public Health, Food Chain Safety and Environment	3.1.1.2 Hygiene condition enhancers and/or preservatives Lines 254 - 261 These are tests to be carried out with pathogenic bacteria and therefore potentially dangerous for laboratory workers. The requirements are not clear on the number of strains to be tested, the strains of importance for the target species and/or for humans. A table explicitly describing the tests to be carried out would be useful. An authorization for a single strain such as "Salmonella typhimurium" will likely be misinterpreted as allowing salmonella reduction by farmers. It is preferable to require a reduction of all significant species of the genus for a target species.	It is up to the applicant to define and describe the intended effect(s) of the additive.
29	3.1.1 Technological additives which exert	Chr. Hansen A/S	3.1.1.1 Silage additives Lines 231-233, pages 7-8: Temperature measures may be replaced by measurement of CO2 production. Measurement of dry matter loss and direct counts of aerobic spoilage organisms may be used as supportive evidence of improved stability. Comment: We are of the opinion that it should also be possible to use pH to assess aerobic stability, at least as supportive evidence.	See reply to #18.

	their function in feed		Proposal: We suggest changing the text to: "Temperature measures may be complemented by measurement of CO2 production or changes in pH. Measurement of dry matter loss and direct counts of aerobic spoilage organisms may be used as supportive evidence of improved stability."	
			3.1.1.2 Hygiene condition enhancers and/or preservatives Lines 249-250, page 8: Data should be provided to confirm taxonomic/serotype identification and to exclude clonality. Comment: We would appreciate further explanation on the word "Clonality".	See reply to #18.
30	3.1.1 Technological additives which exert their function in feed	BERTIN	3.1.1.2 Hygiene condition enhancers and/or preservatives Lines 237 -301: Draft content: "3.1.1.2 Hygiene condition enhancers and/or preservatives" paragraph Comment: Having two functionalities in one paragraph creates a confusion This paragraph should be split in 2 subsections, one for preservatives, the other for hygiene conditions enhancers	See reply to #18.
31	3.1.2 Technological additives which exert their function in the animal	FEFANA asbl	Lines 300-303: The use of in vitro or in vivo studies depends on the intended effect of the additive. The additives "substance for control of radionuclide contamination" and "substance for the reduction of contamination of feed by mycotoxins" are not always expected to exert their effect after digestion. The sentence can be confusing. Please consider the following change: If "substances for control of radionuclide contamination" [?] are expected to exert their intended effect until after their digestion by the animal, demonstration of efficacy should be based on in vivo studies.	The Guidance does not refer to digestion but to ingestion. The effects of these additives can only be seen in the animals by measuring the appropriate endpoints by means of in vivo studies.
			Line 307-308: As for table 1, for clarity, it would be better to have the functional group in the table or at least to indicate: For the functional group of ?other technological additives?	The text was modified to address the comment.

			Line 311-312: What about a product having an in vitro effect only (e.g. such as those working in silage), so not on the animal? In this case, it is understood that only in vitro studies are required. Could this be reflected in the guidance?	The text was modified to address the comment. A footnote was added to clarify.
			Line 313-314: At least 3 in vivo studies with significant results should be provided for these additives. EFSA considers that in vitro studies are not sufficient for the demonstration of efficacy. It seems not appropriate to require in vitro studies in addition to in vivo trials. Moreover, it is unclear what does ?a battery? mean and we would appreciate clarifications on this term. Please consider the removal of: A battery of in vitro studies should be submitted to provide evidence of the intended effect of the additive. However, Line 317-319: Could you please clarify what is expected by two locations? Is our understanding correct that as long as the address is different this qualifies as two locations?	In vitro studies are necessary to support the additive's mode of action. However, only in vivo studies could be used to support the efficacy of additives in this functional group. The text was amended to address the comment.
			Line 320: Could you please clarify whether this covers all terrestrial species including pets or only livestock species?	For this category of additives, the term “all terrestrial species” includes pets and other non-food-producing animals.
			Line 321 "in three major species (at least one study in each) representing different digestive systems": It would be appreciated to open the opportunity to conduct trials in major or minor species representing different digestive systems for these additives. Please consider the following amendment: For additives intended to be used in all terrestrial species, efficacy should be demonstrated in vivo in three categories/species (at least one study in each) representing different digestive systems (a poultry species, a non-ruminant mammal and a ruminant).	The Panel considers that when additives are intended to be used in all terrestrial species, the studies should be performed in major species.
			Line 335-337 : For clarity, we would propose to use the term feed (compound feed or feed material in this case) as it may create confusion when the term diet is used in some place and for the same topic the term feed is used.	The text throughout the Guidance has been harmonised to address the comment.
			Line 343-345 : Here, taken as an example, the text says "target species" or in other places, line 371 for example, it says "target species/categories" - is this differentiation made on purpose? Or should we read/understand species/categories everywhere? In the second case, we would appreciate if the wording throughout the guidance could be harmonised.	The text was modified to address the comment.
			Lines 348-350, Table 3: Suggestion to add rumen fluid for ruminants as a matrix for zearalenone & a-/ b-zearalenol since absorption into plasma is relatively low. We would appreciate an adaptation according to this suggestion.	Table 3 reflects the most relevant endpoints/biomarkers for substances reducing the contamination of feed by

				mycotoxins. Alternative endpoints can be proposed and justified by the applicant. These situations will be assessed on a case-by-case.
			Footnote 5, page 9: The limit of quantification should be added in addition to the limit of detection to take into consideration the data from the analytical methods used. Please consider the following amendment: 5. Below or at least close to limit of detection or quantification.	The limit of detection, generally at least 1/3 of the limit of quantification, is considered the relevant parameter.
32	3.1.2 Technological additives which exert their function in the animal	Federal Office of Consumer Protection and Food Safety	Page 9 L 327 Please add the following words to ensure that the analytical determination of the mycotoxin is given: The target mycotoxin content in feed used in studies should not exceed the values given in Directive 2002/32/EC but specific requirements for analytical detection should be given for aflatoxin B1 and in Commission Recommendation 2006/576/EC for deoxynivalenol, zearalenone, ochratoxin A and fumonisins B1+B2 for complete feedingstuffs for the respective animal species/category and in Commission recommendation 2013/165/EU for T-2 and HT-2. Page 9	The analytical confirmation should be provided using official methods or equivalent internationally validated methods.
			L 334 Please add the following sentence at the end of the passage: For each trial, a quantitative analysis of mycotoxins present in feed should be provided. The formation of "nests" is also possible in the case of mycotoxins. When demonstrating efficacy, it must therefore be shown that the type and scope of sampling reflect a possible uneven distribution of mycotoxins in the feed.	The text was modified to address the comment.
			Page 9 L 346 Please note, that zootechnical parameters can be used when a two by two complete factorial design would be used. This is generally advisable in order to demonstrate that the additive does not exert any unspecific effect (an effect that does not act on the mycotoxin; e.g. adsorbing agents that not only adsorb mycotoxins but also essential nutrients!). Such unspecific effects cannot be detected as suggested here (incomplete design, i.e. two or three groups!). The evidence that the additive is ineffective in case of absence of mycotoxins should be given. Further in general is suggested to reflect the study design of the "Guideline for the validation of the efficacy of detoxifying agents intended for the detoxification of mycotoxin contaminated feedstuff" published by Society for Mycotoxin Research (see attached Annex Guideline detoxification mycotoxin contaminated feed.pdf)	The potential of the additive to exert unspecific effects is under the scope of the risk assessment of the safety for the target species. The relevant Guidance already covers this.

			Page 10 L 349 Table 3 line "Zearalenone" column "Most relevant species" It is noted, that for ZEA faecal excretion alone does not provide sufficient evidence of a reduced absorption due to the additive. It is recommended to specify this. Additionally, please add ?urine and faeces excretion?: Excretion of zearalenone/metabolites via urine and faeces excretion ?	The text was amended to address the comment.
			line "Ochratoxin A", column "Ochratoxin in kidney (or blood serum)" It is noted, that a longer-term exposure for Ochratoxin is neccessary.	Comment noted.
33	3.1.2 Technological additives which exert their function in the animal	Pen & Tec Consulting, SLU trading as Argenta ®	316 - 319 A minimum of three independent in vivo studies (generally short-term) performed in at least two different locations showing significant effects should be provided to demonstrate efficacy at the lowest recommended dose. Comment: Please clarify if 3 studies in total or per target species are required. E.g., if the application is for poultry and pigs, would 2 studies in poultry and 1 in pig be sufficient?	The text was amended to address the comment.
			326 - 334 The target mycotoxin content in feed used in studies should not exceed the values given in [...] Commission Recommendation 2006/576/EC [...] and in Commission recommendation 2013/165/EU [...] Naturally contaminated feed materials are preferred as source of mycotoxins. Alternatively, feed spiked with mycotoxins could be used if properly justified. For each trial, a quantitative analysis of mycotoxins present in feed should be provided. Comment: The EU Commission Recommendations are non-binding and levels in the field may exceed these values. It would be useful to allow some flexibility considering that naturally contaminated feeds may exceed the values stated in the recommendations. Also, even if spiked feeds are used, the limits set may result in extremely low levels present in biological samples (e.g. blood serum, plasma) that prevent quantification of certain mycotoxins. It will be useful if the Guidance document could refer to these considerations and how to address these issues.	Even though the levels reported in the recommendations are not binding, those are considered a relevant threshold to guarantee animal safety. Therefore, efficacy should be demonstrated at such levels or below.

			345 Recommendations on the endpoints are given in Table 3. Comment: Since these are recommendations, we suggest that the text clarifies clearly that it is optional to use these endpoints, and other endpoints can be used if the scientific rationale is described and justified.	The Guidance already foresees the possibility for applicants to deviate from the requirements as long as an adequate justification is provided. These deviations will be judged on a case-by-case basis by the Panel.
			346 Zootechnical parameters should be reported but cannot be used to demonstrate efficacy. Comment: In relation to feed additives for the reduction of contamination of feed by mycotoxins under (SRCM): Mycotoxins affect animal performance (e.g. by reducing appetite, gut integrity, decreasing digestion/absorption). Zootechnical performance is an indirect marker. Zootechnical performance is used also as indirect markers for certain zootechnical feed additives (e.g., gut flora stabilisers, coccidiostats). If the applicant performs long term studies showing improved performance ? would EFSA accept SRCM under the zootechnical feed additive group - i.e. using challenge studies with mycotoxins (feed spiked with mycotoxins)?	For additives in the functional group "Substances for reduction of the contamination of feed by mycotoxins", an indirect demonstration of efficacy based on zootechnical performances is not considered adequate. Direct evidence of the reduced exposure of the animals to mycotoxins and/or reduced deposition in foods is considered necessary.
34	3.1.2 Technological additives which exert their function in the animal	AFCA-CIAL	p.8 Line 300-303 : The use of in vitro or in vivo studies depends on the intended effect of the additive. The additives "substance for control of radionuclide contamination" and "substance for the reduction of contamination of feed by mycotoxins" are not always expected to exert their effect after digestion. The sentence can be confusing. Rewording proposed : If "substances for control of radionuclide contamination" [?] are expected to exert their intended effect until after their digestion by the animal, demonstration of efficacy should be based on in vivo studies.	See reply to #31.
			p.8 Line 307-308 : As for table 1, for clarity, it would be better to have the functional group in the table or at least to indicate: For the functional group of "other technological additives"	The text was amended to address the comment.
			p. 9 Line 311-312 : What about product having an in-vitro effect only (eg such as those working in silage), so not on the animal. In this case, it is understood that only in-vitro studies are required? Could this be reflected in the guidance ?	See reply to #31.

			p. 9 Line 313-314 : At least 3 in vivo studies with significant results should be provided for these additives. EFSA considers that in vitro studies are not sufficient for the demonstration of efficacy. It seems not appropriate to require in vitro studies in addition to in vivo trials. Moreover, what does "a battery" mean? Proposal to remove : A battery of in vitro studies should be submitted to provide evidence of the intended effect of the additive. However,	See reply to #31.
			p.9 Line 317-319 : What is expected by two locations ? Two farms in the same country or at least two studies in two different countries ? Would three studies in the same country but in different breeding facilities or farms be acceptable ?	The reference to the two locations was removed from this section. The Panel's considerations regarding the performance of multiple in vivo trials are included in section 5.
			p.9 Line 320-322 : Would that cover all terrestrial species including pets or only livestock species ? It could be appreciated to open the opportunity to conduct trials in minor species representing different digestive systems for these additives. Rewording proposed : [?] efficacy should be demonstrated in vivo in three categories/species (at least one study in each) representing different digestive systems (a poultry species, a non-ruminant mammal and a ruminant).	See reply to #31.
			p.9 Line 335-337 : For clarity, we would propose to use the term feed (compound feed or feed material in this case) as it may create confusion when the term diet is used in some place and for the same topic the term feed is used.	The text was modified to address the comment and harmonised throughout the document.
			p.9 Line 343-345 : Here, taken as an example, the text says "target species" or in other places, line 371 for example, it says "target species/categories" - is this on purpose ? Or should we read/understand species/categories everywhere ?	The text was amended to address the comment.
			p.9 Footnote 5 : Depending on the analytical method used, the limit of quantification should be taken into consideration in addition to the limit of detection in this guidance rewording proposed : Below or at least close to limit of detection or quantification	See reply to #31.
35	3.2 Sensory additives	FPS Public Health, Food Chain Safety	Line 391 Reference to literature: In line 356, it is specified "When the additive is already authorized for use in food and the intended use of the additive in feed is the same" The literature must therefore relate exclusively to publications relating to animal feed or to publications relating to foodstuffs such as the international nomenclature (FLAVIS, COE), reference works (Fenaroli's handbook of flavor ingredients) or other scientific publications clearly demonstrating the aromatic	It is the practice of the FEEDAP Panel to conclude on efficacy only when there is evidence that the additive has flavouring properties.

		and Environment	qualities of the substance studied. References to pharmacopoeia or traditional medicine which simply provide information on the taste of the medicine must be excluded.	
36	3.2.1 Substances that add or restore colour in feedingstuffs	FEFANA asbl	Line 366 "The studies (at least three)"? It is not clear what is meant by three. Please clarify what types of studies are needed.	The type of studies required is described in the sentences above.
			Line 367-368 The additive should not adversely affect feed quality: How is this expected to be demonstrated? What criteria are required to demonstrate that the quality of the feed is not affected? We would appreciate if this could be clarified	The quality of the feed can be monitored by analysing many different parameters (e.g., nutrient profile, microbial quality, physical form). The specific endpoints to be monitored will depend on the nature of the additive, its conditions of use, and its selection justified by the applicant.
37	3.2.1 Substances that add or restore colour in feedingstuffs	Federal Office of Consumer Protection and Food Safety	Page 10 L 368 Please add "and food": (?) affect feed and food quality.	This sentence is not restricted to this category of additives. Therefore, the modification suggested is already foreseen in the general section: "Any potential impact on the distinctive features of animal products should also be investigated during animal efficacy trials (e.g., off-flavour, colour changes)".
38	3.2.1 Substances that add or restore colour	AFCA-CIAL	p.10 Line 367-368 : How is this expected to be demonstrated ? What criteria are required to demonstrate that the quality of the feed is not affected?	See reply to #36.

	in feedingstuffs			
39	3.2.2 Substances which, when fed to animals, add colour to food of animal origin	Federal Office of Consumer Protection and Food Safety	Page 10 L 375 Please delete "long- or short-term": (ii) in vivo studies	The text was modified to address the comment.
40	3.2.2 Substances which, when fed to animals, add colour to food of animal origin	FEFANA asbl	Line 370 "A minimum of three independent in vivo studies showing significant effects should be provided": What is the definition of "independent study"? Please include a definition or refer to any relevant guidance section	The Panel's considerations regarding the performance of multiple in vivo trials are included in section 5.
41	3.2.2 Substances which, when fed to animals, add colour	Pen & Tec Consulting, SLU trading as Argenta ®	370 - 375 A minimum of three independent in vivo studies showing significant effects should be provided to demonstrate efficacy for the relevant target species/categories. Evidence of efficacy can be provided by: i) reference to published studies, where the relationship between a particular substance and the colour of animal tissues/products is well documented, or ii) in vivo long or short-term studies. Comment: Please clarify if the in vivo studies are mandatory or not for this functional group. The text is confusing because it starts saying that applicants	The text was modified to address the comment.

	to food of animal origin		need the 3 independent in vivo studies and, immediately afterwards, indicates that there are 2 options: reference to publish studies or in vivo studies.	
42	3.2.4 Flavouring compounds	FEFANA asbl	Line 394-395 "For iii), a minimum of three independent studies showing significant effects should be provided for each target species/category for which the application is made": Similar to "mycotoxin products" (and in line with the 3R principles), it would be appropriate to propose a reduction in terms of the number of studies if "all species" is targeted. In this case, it could be sufficient to provide one study per main species/category.	The text was modified to address the comment.
43	3.2.4 Flavouring compounds	Nor-Feed SAS	The requirement for a minimum of three independent studies should be in line with "Table 5 Minimum number of independent studies and target species required for the assessment 586 of efficacy in applications covering multiple species/categories."	The text was modified to address the comment.
44	3.2.4 Flavouring compounds	AFCA-CIAL	p. 11 Line 394-395 : What is expected by "independent" studies? Similarly as for "mycotoxin products" (and in line with the 3R principles), it would be appropriate to propose a reduction in terms of the number of studies if "all species" is targeted. In this case, it could be sufficient to provide one study per main species/category. Rewording proposed : iii), a minimum of one independent study for each target species/category. For additives intended to be used in all terrestrial species, efficacy should be demonstrated in three categories/species (at least one study in each) representing different digestive systems (a poultry species, a non-ruminant mammal and a ruminant)."	The text was modified to address the comment.
45	3.3 Nutritional additives	Comitato Nazionale Sicurezza Alimentare (CNSA; Food Safety National Committee)	Overall, this Efficacy guidance is a good document. As only comment, more detail should be provided on the key endopint "bioavailability" for those nutritional additives for which demonstration of efficacy is required (e.g., amino acid analogues and new forms of compounds of trace elements. chemically well-defined substances having similar effects to vitamins). Bioavailability is systemic absorption in a biologically active form, or in a form that has biological value. Therefore, bioavailability (distinct from simple absorption/deposition) has to be measured by appropriate biomarkers for the relevant nutrients. These biomarkers can be readily retrieved from the literature as well as from the relevant FEEDAP opinions (just as an example, specific biomarkers were used by FEEDAP to assess the bioavailability of Cu, Mn and Zn aminoacidic cheletes - FEEDAP opinions on Cu, Mn and Zn Mintrex issued on 2008-9)	The Panel agrees with the comment and considers that the text of the Guidance already considers the reference to use appropriate biomarkers for the demonstrations of bioavailability of nutritional additives.

46	3.3 Nutritional additives	Federal Office of Consumer Protection and Food Safety	Page 11 L 410 Please replace "at least one" by "at least three": For other (novel) nutritional additives at least three long-term efficacy study should be provided. Page 11 L 411 please note, that only one study is a very soft recommendation! The results could be random and the using of the substance is for at least 10 years or longer!	The Panel considers that for other nutritional additives, at least one long-term efficacy study is enough to demonstrate the efficacy.
47	3.3 Nutritional additives	FEFANA asbl	Lines 397-399: It is our understanding that the exemption provided applies at active substance level. However, the text as it is may lead to misunderstanding. We suggest the following addition: No evidence of efficacy is necessary for active substances of the following additive groups: amino acids naturally occurring in proteins of plants and 399 animals and their salts, urea and vitamins, pro-vitamins and compounds of trace elements already 400 assessed and authorised under Regulation (EC) No 1831/2003.	The Panel considers that the current text adequately defines the requirements. For instance, for new compounds of trace elements, evidence for efficacy should be provided for the specific compound and not for the trace element itself (which could be considered the active substance).
			Line 403 Regulation 429/2008 specifies that only one short term study is required: "A short term study is required to support efficacy for urea derivatives, amino acid salts and analogues not already authorised as feed additives, compounds of trace elements not already authorised as feed additives and for vitamins, pro-vitamins and chemically well-defined substances having similar effect not already authorised as feed additives.? We see the need for amending the text in order to be in line with the guidelines: Evidence can be provided by reference to literature or by one short-term in vivo study.	The current text adequately reflects the appropriate requirements of the Panel.
			Line 411 Please specify what would be an acceptable minimum study duration of a long-term efficacy study in laboratory animals.	Details on the duration of long-term efficacy studies are included in section 5.2.2. For the species not included in Table 6, the text indicates: "The minimum duration for all other species/categories should be 42 days for growing animals and 56 days for adult animals".
48	3.3 Nutritional	Pen & Tec Consulting, SLU	396 Section 3.3 Nutritional additives Comments: With respect to nutritional additives/functional group amino acids, their salts and analogues, EFSA stated in a recent opinion that in their view this functional group describes substances which finally enter the metabolism of the	Unclear comment. According to the Regulation (EU) 2015/722, taurine is authorised under the functional group of "vitamins, provitamins and chemically

	additives	trading as Argenta ®	body as amino acids and as such take part in the protein synthesis pathways. Some amino acids (or amino acids analogues) are not incorporated into proteins and are involved in physiological processes. An illustrative example is taurine, which is authorised under this group according to Regulation (EU) 2015/722. Please confirm that amino acids that are intended to satisfy the nutritional needs of animals are under the scope of the nutritional group independently on whether they are (or not) incorporated into proteins.	well-defined substances having a similar effect".
			397- 399 No evidence of efficacy is necessary for [...] urea and vitamins, pro-vitamins and compounds of trace elements already assessed and authorised under Regulation (EC) No 1831/2003. Comments: *Question 1*: Please clarify the meaning and include examples. Nutritional feed additives are non-holder specific, and if they are already authorised, they would not need an application and assessment.	An application for urea and vitamins, pro-vitamins and compounds of trace elements might be needed for different reasons (e.g., different manufacturing processes or production methods). However, the efficacy of the additive might not always need to be demonstrated.
			Question 2: where an amino acid is authorised, is the amino-acid analogue exempt from efficacy studies.	No, the amino acid analogues need a separate assessment.
			403-406 Where evidence from the literature is insufficient to reach a conclusion, one bioavailability study or one bioequivalence study is considered adequate to demonstrate efficacy for amino acid analogues and new forms of compounds of trace elements. Comments: *Question 1*: 2018 guidance stated ["a bioequivalence study considered adequate to demonstrate efficacy for amino acid analogues, new forms of compounds of trace elements and urea derivatives"] Can you confirm that bioavailability/bioequivalence studies are also applicable to urea and its derivatives? If so, please include it again.	The text was modified to address the comment.
			Comment: It would be useful if the text cross-refers to other parts of the guidance that are important for this group, e.g. Section 5.2.1.1 Bioavailability/bioequivalence studies; Section 5.1.3 (sets requirements for the control group: "Where studies are required to demonstrate that the additive contributes to the animals' nutritional requirements, the feed of the control group should contain the nutrient at concentrations marginally below the animals' requirements"; and Section 5.1.6.2 (sets requirements for statistical analysis:	The text was modified to address the comment.

			tests for non-inferiority should be done for experiments aiming at demonstrating non-inferiority between treated groups and a positive control (e.g., bioequivalence study)).	
			411 - 414 Generally, one study in a single animal species or category, including laboratory animals, will be sufficient to demonstrate efficacy. For additives specifically designed to be efficacious in a particular animal species/category (e.g., protected amino acids for ruminants), the same target species should be selected. Comments: Some feed additives may not be designed in particular for an animal species category, and the application may be restricted to animal species for other reason (e.g. target market, availability of tolerance studies, avoid studies in dogs/cats?). In the case of additives proposed for one pet species (e.g. cats) for these reasons, can efficacy be also shown with laboratory animals?	For additives specifically designed to be efficacious in a particular animal species/category, the same target species should be selected.
49	3.3 Nutritional additives	AFCA-CIAL	p. 10 Line 403 : Regulation 429/2008 specifies that only one short term study is required: "A short term study is required to support efficacy for urea derivatives, amino acid salts and analogues not already authorised as feed additives, compounds of trace elements not already authorised as feed additives and for vitamins, pro-vitamins and chemically well-defined substances having similar effect not already authorised as feed additives." Rewording proposed : Evidence can be provided by reference to literature or by one short-term in vivo studies.	See reply to #47.
			p. 11 Line 411 : Could it be specified what would be an acceptable minimum study duration for a study in laboratory animals?	See reply to #47.
50	3.4 Zootechnical additives	Federal Office of Consumer Protection and Food Safety	Page 11 LL 417-418 Germany would like to see a standardized statement regarding the performance of in vivo efficacy studies that these should ideally be carried out in the European Union (EU). In vivo studies that are not carried out in the EU must comply with European standards, which includes feed production, animal husbandry and farming practices. Various sections in the guideline (e.g. section 3.4 Zootechnical additives; L 415-418) lead to contradictory statements and should be deleted. In the opinion of Germany, a clear statement at the beginning of the guideline is most meaningful. Therefore, please delete ", one of which should be in the EU": These should be carried out at least at two different locations.	The Panel agrees that the studies should reflect EU farming practices and conditions, and this is reflected in the general principles of the guidance: "Moreover, such studies must permit the evaluation of the efficacy of the additive according to common feed manufacturing, animal husbandry and farming practices in the European Union (EU). Studies performed outside the EU must permit conclusions to be drawn on the efficacy of the additive when used in

				the EU". However, in view of the 3Rs principle, it seems appropriate that studies performed outside the EU are accepted if they allow an assessment of the efficacy under EU-like conditions.
51	3.4 Zootechnical additives	FEFANA asbl	Line 415ff AND 421-424 (General Comment): It is somewhat counterintuitive that chapter 3.4 on zootechnical additives addresses the effects of the additives according to Article 5.3 (e-f) in the subtitles while for all other additives in chapter 3 the actual additive categories are used. It is quite plain that 3.4.1 addresses 4(a) and 4(b) additives, 3.4.2 addresses 4(c) additives, 3.4.4. are supposed to reflect 4(e) additives and 3.4.3 and 3.4.5 would address what would commonly be regulated as ?other? (4(d) additives. The link between functional group and mode of action is therefore given and there is little reason why the nomenclature should be different from all other chapters. This would help applicants (especially applicants new to the process) to find what they are looking for. If a change of subtitles is unwanted, a comment in the first line of each subchapter could indicate which functional groups are most likely to be affected by the subchapters. Please consider harmonising the wording for consistency.	As mentioned in the introduction of the chapter, the Panel considers that for zootechnical additives, in contrast with the other categories of additives, there is no direct link between the functional group and the effects of the additive. For some functional groups, it could be argued that the mode of action is proposed (digestibility enhancers and arguably, gut flora stabilisers), while for others, it is an effect (substances which favourably affect the environment). Therefore, it is considered more transparent and predictable to detail how the assessment is done based on the expected effect of the additive.
			Lines 416-417: Slight contradiction with chapter 4.3, lines 602-611? since in vitro studies will be acceptable in certain conditions for enzymes, the phrasing of lines 416-417 sounds too prescriptive. Please adjust wording.	The text was modified to address the comment.
			In addition, please delete "relevant and" as the EFSA guidance already mentions endpoints defined as relevant. Other used endpoints should already be justified.	The Panel disagreed with the deletion of "relevant and" as significant results per se may not be indicative of efficacy. Effects should be on relevant endpoints and show a benefit.
			Lines 417-418: Both facilities may be in the EU. Please consider the following amendment: "These should be carried out at least at two different locations, at least one of which should be in the EU." In addition, could it be clarified what is expected in terms of locations: two different EU countries, two different farms in the same country or, when not	The reference to the number of studies was removed from this section. The provisions for the in vivo studies are included in Section 5.

			performed in an EU country, representative of EU farming conditions? And what about studies in pets: is it acceptable to perform all studies in the same country?	
			Line 418-420: The incorporation level could also be expressed in CFU per kg in case of microbial based feed additives or IU for enzymes. For such FA, an incorporation level in mg/kg or per head is not appropriate. The use of zootechnical additives through the water should be also considered. Also, the way the text is worded appears unclear. Please consider the following suggestion: Efficacy studies should be performed with feed containing the minimum recommended use level proposed by the applicant (expressed in mg/kg complete feed and/or mg/head day and/or mg/ l water or mg/day or CFU or IU where appropriate).	The text was modified to address the comment.
			Lines 421-422: Please update for accuracy: ?.link between the functional group(s) and the mode(s) of action?.	The text was modified to consider the comment.
			Line 423-424: The used reference to Article 5.3 (e) [(additives that) favourably affect the environmental consequences of animal production], and (f) [(additives that) favourably affect animal production, performance or welfare (?)] would exclude certain physiological condition stabilisers, efficacy of which would not be proven by showing improvement of animals welfare (but via other specific endpoints/parameters not necessarily connected or attributed to welfare by the scientific community). In other words, the current wording suggests (in contradiction to other chapters of the draft Guidance, where the topic is elaborated and certainly seems to imply that the assessment is intended to be flexible and open for unique endpoints and approaches) that some physiological condition stabilisers - those that would not directly impact welfare - would not be subject to assessment. This undermines the idea behind the functional group and limits novelty. Please indicate that additives under Article 5.3. (f) will be assessed as physiological condition stabilisers, in line with the full definition mentioned in Regulation 1831/2003. Alternatively, the reference to article 5.3 (e) and (f) could be removed and replaced by: based on the effect(s) expected of the additive in line with functional groups defined by the Regulation 1831/2003.	The Panel's intention is not to be restrictive in the efficacy assessment. For additives under the functional group "physiological condition stabilisers", the demonstration of efficacy is not restricted in any way, as the effects can fall under either of those listed in the Guidance. Moreover, the text was modified to address the comment.
52	3.4 Zootechnical	Pen & Tec Consulting, SLU	415 Section 3.4 Zootechnical additives Comment 1: In human foods, the use of microorganisms as a "probiotic" is accepted without the need to support the efficacy with studies. Using the same principle and based on the definition of gut flora stabiliser (feed regulation) –	According to Regulation (EC) No 1831/2003, gut flora stabilisers are considered zootechnical additives, which, by definition, are "additives used to

	additives	trading as Argenta ®	"gut flora stabilisers: micro-organisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora" - we believe that EFSA should also allow to support efficacy by providing evidence that the species of microorganism lives naturally in the gastrointestinal tract of the target species (e.g. scientific publications, sampling of faeces..) or by showing increase in beneficial microorganisms in the gut after supplementation (with the microorganism/feed additive). Also, where a strain has been isolated from an animal species and evidence is provided, this should be accepted to support efficacy without the need of additional in vivo efficacy studies.	affect favourably the performance of animals in good health or used to affect favourably the environment". The FEEDAP Panel considers that the demonstration of the colonisation of the gut by the strain is not enough evidence of efficacy and that demonstration of effects as described in the definition of zootechnical additives is needed.
53	3.4 Zootechnical additives	AFCA-CIAL	p. 11/12 Line 418-420 : - Could it be clarified what is expected in terms of locations: two different EU countries, two different farms in the same country or, when not performed in a EU country, representative of EU farming conditions ?	The reference to the number of studies was removed from this section. The provisions for the in vivo studies are included in Section 5.
			- And what about studies in pets: is it acceptable to perform all studies in the same country ?	The reference to the number of studies was removed from this section. The provisions for the in vivo studies are included in Section 5.
			- The dose could also be expressed in CFU per kg in case of microbial based feed additives or IU for enzymes. For such feed additives, a dose in mg/kg or per head is not appropriate. Also, the way the text is worded appears unclear: Efficacy studies should be performed with feed containing the minimum use level proposed by the applicant (expressed in mg/kg complete feed and/or mg/head day, where appropriate).	The text was modified to address the comment.
			- Proposal : "one of which should be in the EU" replace by : "at least one should be in the EU"	The reference to the number of studies was removed from this section. The comment is not relevant anymore.
			p.12 Line 421-424 : We understand that beside the definition of each functional group a link with Art 5.3 e and f is expected in terms of benefits of the additives. However, we would like to receive clarifications on the types of acceptable benefits when applications are made for non-food producing animals. For example in case of cats and/or dogs, zootechnical performance are not targeted and rather benefits in line with the definition of the functional groups are more relevant. It is not considered as relevant to strictly/exclusively refer to art. 5.3 (e)(f) of Regulation 1831/2003 and this restriction may better be removed	For effects not considered under sections 3.4.1 to 3.4.4 of the Guidance, please see section 3.4.5 Other additives. The applicant should specify the effects expected from the additive and justify the selection of endpoints.

			and/or revised. Efficacy of feed additives for which an application is submitted shall be linked to functional group(s) as defined in the Regulation 1831/2003.	
54	3.4 Zootechnical additives	Chr. Hansen A/S	Lines 416-417, page 12 A minimum of three independent in vivo studies showing relevant and significant effects should be provided to demonstrate efficacy for the relevant target species/categories. Please adjust the wording. "…in vivo studies showing ~~relevant and~~ significant effects" as EFSA guidance already mention endpoints defined as relevant. Other used endpoints should already be justified. Delete "relevant and"	See reply to #51.
			Lines 418-420, page 12-13 Efficacy studies should always include the lowest incorporation level (mg/kg complete feed)/lowest daily level (mg/head per day) proposed by the applicant. We miss the mentioning of CFU and IU in this sentence. Please add CFU and IU	The text was modified to address the comment.
			Line 443, page 13 "For additives which favourably affect the environment by direct or indirect means,…" What is meant by "..by direct or indirect means.."?. By mentioning indirect means, would it be acceptable to show an indirect environmental impact by for example improved feed efficiency, thereby reducing emission of greenhouse gases? Please explain further the consequence of these means.	"Direct means" refers to additives which have a direct effect on an endpoint, resulting in a benefit for the environment. "Indirect means" refers to that the effect of additives on the environment depends on, e.g., an adjustment in the diet. An example is phytases, which allow the reduction of P excretion by allowing the use of feeds with a lower content of inorganic P.
55	3.4.1 Additives affecting animal production or	Federal Office of Consumer Protection and Food Safety	Page 12 L 428 Please replace "(see Section 4.2.2.1)" by "(see Section 5.2.2.1)".	The text was modified to address the comment.

	perfor mance			
			Page 12 LL 434-438 It is noted, that balance trials place high demands on the consideration of animal welfare. As already mentioned above (Line 116 General principles of efficacy assessment), such studies should always be conducted in the EU or demonstrate compliance with EU standards.	Compliance with the EU standards is a general requirement.
			Page 12 L 440 Please note that for Germany this sentence is not clear. Please describe what is meant.	The text was modified to address the comment.
56	3.4.1 Additiv es affecti ng animal produc tion or perfor mance	FEFANA asbl	Line 425 (General comment): As already indicated, the guidance would gain in clarity if the functional groups which are concerned would be mentioned (with specific subtitles or with a clear sentence at the beginning of the subsection) as there is a lack of connection between functional groups and this paragraph. This is creating confusion for applicants.	See reply to #51.
			Lines 431-438 On the possibility to apply short-term (balance) studies, it is recommended to create an extra possibility for innovative categories of enzymes that degrade other substrates than indicated. Please consider the following amendment: "Only in the case of enzymes affecting the utilisation of phytate phosphorus or the digestibility of polysaccharides or proteins or other nutrient substrates, can short-term (balance) studies substitute long-term studies, provided that adequately defined and specific methods are applied: - For phytases: improved utilisation of dietary phosphorus by total P retention in balance trials or P digestibility plus partial P (bone) retention. - For polysaccharidases: increased metabolisable energy in balance trials. - For proteases: improved protein utilisation by nitrogen retention or by ileal digestibility of amino acids. - For other enzymes: improved utilisation of specified nutrients, according to scientifically approved methods"	The Panel considers that exogenous phytases, polysaccharidases and proteases are currently the most relevant and common enzymes used in animal nutrition. However, the applicant can design short-term studies to address the efficacy of other enzymes justifying the endpoints measured.
			Line 434-435 For phytase balance trials including urine analyses (e.g. in pigs) do not seem appropriate as there are no relevant excretions via urine when feeding	The Panel considers that the urine excretion of, for example, P, is not

			low P diets. Also, raising animals in balance crates is often rejected by ethical committees and so there are limited trial sites where this can be run ? and the availability of trial sites is becoming a significant bottleneck. Ileal digestibility is scientifically the best but as slaughtering is often criticized it should be a preference for apparent total tract digestibility (ATTD) but also other possibilities. It remains unclear why bone analyses are required also. If P is retained that is more due to the level of dietary P fed than the efficacy of the phytase why full retention should not be required. Please consider the following amendment: For phytases: improved utilization of dietary phosphorus by P digestibility (apparent or true ileal digestibility, where possible total tract digestibility is preferred).	negligible, and some nutrients in the diet (e.g., Ca) can have an effect on the excretion rate between faeces and urine. Therefore, the most accurate evaluation of the overall phytase efficacy can be achieved through balance/retention studies.
			Line 436 "For polysaccharidases: increased metabolisable energy in balance trials" Improved nutrient digestibility for NSPases not necessarily only affects metabolizable energy improvement? improved nutrient and DM digestibility should also be considered. Balance trials should not be the only option. Some effects are seen in the hindgut which is why apparent total tract digestibility is favoured. Please consider? Improved nutrient or energy digestibility (apparent or true ileal and total tract digestibility, where the latter is preferred).?	The Panel considers that the current requirements for the evaluation of the efficacy of polysaccharidases are the most adequate and accurate.
			Line 437-438 "For proteases: improved protein utilisation by nitrogen retention or by ileal digestibility of amino acids." Please consider the following amendment: For proteases: improved dietary protein utilisation by total nitrogen retention in balance trials or by ileal digestibility of amino acids.	The text was modified to address the comment.
			Line 439-440 For clarity, it would be appreciated to amend the sentence and detail what technical/ethical reasons could be acceptable to perform AFD studies. In addition, high quality ME measurement requires to immobilize animals (piglets, pigs, sows, cows?) in crates to ensure separation of faeces and urine. This cannot be considered as a welfare process. That's why the faecal digestibility measurement for these species and stages should be preferred and not restricted to sows and cows. Please consider this point and amend the text or even delete the sentence.	The text was modified to address the comment.

57	3.4.1 Additives affecting animal production or performance	Pen & Tec Consulting, SLU trading as Argenta ®	Lines Guidance Text Comments 428 Link states section 4.2.2.1 Link directs to section 5.2.2.1	The text was modified to address the comment
58	3.4.1 Additives affecting animal production or performance	AFCA-CIAL	p. 12 Line 425 : For clarity, the functional groups which are concerned should be indicated as there is a lack of connection between functional group and this paragraph. This is creating confusion for applicants. It is understood that it applies to 4a, 4b and 4d (other zootechical additives that may in some case be related to performance or to other benefits)	See reply to #51.
			p. 12 Line 428 : ?see Section 4.2.2.1 » No update on the section number. Replace by « see Section 5.2.2.1 »	The text was modified to address the comment.
			p.12 Line 435 : To be precise we should add that it is meant here is "Ileal digestibility of P" (distinguish from total tract digestibility) Therefore, propose to update to "Ileal digestibility of P" Proposal : Instead of "P digestibility", we suggest: "ileal digestibility of P" ?.	For the demonstration of the efficacy of phytases, the improved utilisation of dietary phosphorus needs to be assessed by total P retention in balance trials or P digestibility plus partial P (bone) retention. The P digestibility could be measured at ileal or faecal level.
			p.12 Line 437 : As for the bullet point of phytases, it is said Improved utilization of dietary P. To be consistent with above propose "Improved utilization of protein" This refers to consistency how it is written "utilization" for the different nutrients.	The text was modified to address the comment.
			p.12 Line 439-440 : For clarity, it would be appreciated to amend the sentence and detail what technical/ethical reasons could be acceptable to perform apparent faecal digestibility studies	The text was modified to address the comment.

59	3.4.1 Additives affecting animal production or performance	Oy Medfiles Ltd	L432; It would be helpful if the reference to the balance method section with its respective number is made in this sentence. Thank you.	The reference to section 5.2 regarding the typology of long- and short-term in vivo trials is already indicated at the beginning of this section. Further references are not considered necessary.
60	3.4.2 Additives favourably affecting the environmental consequences of animal production	FEFANA asbl	Line 443 "For additives which favourably affect the environment by direct or indirect means": We consider that the inclusion of the terms "direct or indirect means" in the guidance document goes beyond the scope of the regulatory framework for feed additives. It introduces concepts that are not regulatory defined and may require further analysis and a harmonised understanding. This proposal sets an unpredictable scenario for applicants, posing a significant burden to fostering innovation and sustainability, as highlighted in the Green Deal and explicitly stated as an objective in the September 2021 EFSA self-mandate for updating the guidance document. To address this issue, we recommend eliminating the wording "by direct or indirect means" and proposing the following amendment: For additives whose principal function(s) is to favourably affect the environment This adjustment aligns the text with the terminology outlined in Regulation (EC) No 1831/2003 ? Article 6, which specifies that an additive should be categorised into a specific functional group "according to their principal function or functions". The defined function is to be provided by the applicant within the authorisation dossier.	Comment not related to risk assessment but to the authorisation process and legal requirements, which are out of the scope of EFSA. It should be noted that this section is intended to provide advice for additives which may claim a beneficial effect on the environmental consequences of animal production, either by a direct or an indirect effect (e.g., by allowing the use of diets with reduced P or N content). However, EFSA will only assess those claims for which an application has been made. The applicant has the right to apply for the functional group/effect which is considered appropriate.
			Lines 443-445 "...in vivo studies showing significant effects should be provided to demonstrate efficacy for the relevant target species/categories.": Currently, this group of additives is notably underrepresented in the EU, and their assessment is burdened by uncertainties arising from the absence of established criteria and the limited experience in preparing dossiers. In light of this we would appreciate if EFSA could reconsider this proposal and allows at least one of the in vivo studies to be replaced by an in vitro study, if and when specific in vitro validated systems are available and suitable for the demonstration of the intended effect on the animal (mirroring the approach outlined for enzymes [chapter 4.3, lines602-611]).	The Panel considers that for additives affecting the environmental consequences of animal production, 3 *in vivo* studies are required to demonstrate the efficacy.

			Line 446-459 (Comments on the three ?groups? of additives favourably affecting the environmental consequences of animal production): Comment 1: We think it is crucial to avoid presenting a restrictive list of possible functional attributes. Therefore, we recommend emphasizing that the provided list is non-exhaustive. For instance, the current list does not include the reduction of ammonia emissions, which could be a viable deliverable for a feed additive. We suggest clearly mentioning that the functionalities described in the bullet points are a non-exhaustive list. Please consider adding before the bullet points that ?A non-exhaustive list of examples is herewith provided:?	The text was modified to address the comment.
			Comment 2: The guidance provided for the three sub-groups of additives does not align with the requirements outlined in Regulation (EC) No 429/2008. The regulation explicitly indicates that "for these additives which favourably affect the environment (e.g. reduced nitrogen or phosphorus excretion or reduced methane production, off-flavours), evidence of efficacy for the target species can be given by three short term efficacy studies with animals showing significant beneficial effects. The studies shall take into consideration the possibility of an adaptive response to the additive." In our view, the guidance shall be revised and amended to bring the guidance into alignment with the established regulatory framework. References to long-term studies should be removed from section 3.4.2.	The Panel considers that for effects on methane production, long-term efficacy studies are required to measure the possible adaptive response to the additive, which would compromise the overall efficacy.
			Line 446-449: Next to phosphorus and nitrogen, there may be other nutrients in the manure that are (or may become) of environmental concern, like trace elements. The option to prove efficacy by allowing the use of diets with lower nitrogen or phosphorus content while maintaining a physiologically adequate level of absorption may be extended to those other effluents.	The text was modified to address the comment.
			Additionally, we suggest deleting requests for balance trials due to the conflict with ethical requirements (balance crates). Please consider the following amendment: ?For additives aimed at reducing the output to the environment of nitrogen, phosphorus or other nutrients to the of environmental concern (e.g., by allowing the use of diets with lower nitrogen or phosphorus content) short-term studies can substitute for long-term studies, provided that adequately defined and specific methods are applied.?	The Panel considers that balance trials are the most accurate way to assess the retention of nutrients in the animals. Deviations from this requirement should be duly justified and will be considered on a case-by-case basis.
			Line 450-454: The guidance does not indicate how the CH4 data shall be presented/measured: methane output or methane intensity: g/day, g/kg DMI,	The Panel considers all these ways of expressing the methane data relevant.

			g/kg milk/meat/? Is it at the discretion of the applicants or does the panel could provide recommendations?	
			Could EFSA also clarify the meaning of "internationally recognised methods"? In this context the wording remains ambiguous. We would welcome the inclusion of a definition within this section for clarity and better understanding.	Internationally recognised methods refer to methods validated according to international procedures to ensure that the data are of high quality and reliable.
			In addition, we understand that the sentence "and consider the possibility of an adaptive response to the additive" has been extracted from Regulation 429/2008. To enhance clarity, could further details be provided on the specific data or analyses needed to address this aspect? Additionally, clarification on the types of acceptable adaptive responses, if identified, would be beneficial.	Long-term studies are needed to assess the adaptive response and show that the effects are maintained (persistent) during the whole experimental period. At present, it is not possible to define acceptable adaptive responses, and the assessment will be done on a case-by-case basis.
61	3.4.2 Additives favourably affecting the environmental consequences of animal production	FEFAC	Line 443: "For additives which favorably affect the environment by direct or indirect means." FEFAC representing manufacturers of compound feed and premixtures for food producing animals, considers that any favourable effect on the environment (especially indirect effect) of a feed additive used for another, principal function for which it has been authorized or an application for authorisation has been made should not require an efficacy assessment by EFSA. Many feed additives may be considered as having a positive effect on the environment, e.g. when reducing resources wastage or improving the feed conversion ratio and it would make little sense and would be disproportionate to require that such benefits for the environment require a specific authorization under the functional group of ?substances that favourably affect the environment?. This would add unnecessary costs and administrative constraints that would undermine the much needed transition towards more environmentally friendly livestock production practices. We therefore propose rewriting the sentence as follows: "For additives whose principal function is to favorably affect the environment".	See reply to #60.
62	3.4.2 Additives favourably affecti	Adisseo	Lines 446-491, page 12: "For additives aimed at reducing the output of nitrogen or phosphorus to the environment (e.g., by allowing the use of diets with lower nitrogen or phosphorus content), short-term (balance) studies can substitute for long-term studies, provided that adequately defined and specific methods are applied"	Comment not related to risk assessment.

	ng the environmental consequences of animal production		The example of additives cited by EFSA are additives already authorized in others categories such as nutritional additive for amino acids and zootechnical additive/digestibility enhancer for enzymes (phytase, protease, carbohydrase,?), so these additives are not aimed at reducing the output of N or P to the environment but aimed to satisfy the nutritional needs of animals (for amino acids) and to increase the digestibility of the diet through action on target feed materials. For those additives (amino acids and enzymes), it is already well known and scientifically proven that, through their mode of action, they indirectly decrease the excretion of N and P. Their mode of action has already been evaluated by EFSA in their respective feed additive categories and they are considered positive and efficient because of authorizations delivered by EFSA. As their positive impacts on environment is a consequence of this same mode of action scientifically recognized and well available in the public domain, it would be a waste of resources at EFSA, Industry and EU commission to go through an authorization process as suggested, especially when the consecutive application would not target an innovative use, but instead cover a indirect effect. So, we would suggest another approach, where EFSA and /or the EU commission (e.g. via an EFSA mandate) could alternatively work on all additive categories and their functions, listing all additives with a scientifically proven mode of action known to have indirect benefits on environment as a result of their primary intended use. Call of data could as well be launched by EFSA, as it regards many additives categories (independently whether it is generic or holder specific categories).	
63	3.4.2 Additives favourably affecting the environmental consequences of animal production	AFCA-CIAL	p. 12 Line 441 : Compared to the other paragraphs, here the exact name of the functional group is used. For consistency (and also in line with the other categories of Feed Additives) we would very much appreciate to have the name of the functional groups clearly stated (line 425, line 465)	Editorial comment. The Panel notes that, in this case, the name of the functional group and the expected effect of the additive coincide.

			p. 12 Line 450-454 : Regulation 429/2008 (4.2.4 (2)) indicates that evidence of efficacy can be given by three short term efficacy studies. Respiration chambers are also recognized methods to assess this end-point.	See reply to #60. The reference to specific methods was removed from the text. The Panel considers that any internationally recognised method can be used if appropriate.
			Should it be possible to Allow one in vitro study on the 3 required (1 in vitro or preclinical model, and 2 in vivo) when the mode of action is determined?	The Panel considers that for additives affecting the environmental consequences of animal production, three *in vivo* studies are required to demonstrate the efficacy.
			It is not indicated how the CH_4 data have to be presented/measured: methane output or methane intensity: g/day, g/kg DMI, g/kg milk/meat/? Is it at the discretion of the applicants or does the panel could provide recommendations?	See reply to #60.
			Text should be amended to reflect the requirements established in the guidelines: « For additives aimed at reducing the production of methane and other greenhouse gases, the reduction in gas production should be measured by internationally recognised methods (e.g., GreenFeed, SF6 tracer) short-term studies. The studies should assess the potential effects of the additive on zootechnical parameters, in vitro model, preclinical model, and consider the possibility of an adaptive response to the additive. »	See the reply to the comment above.
64	3.4.2 Additives favourably affecting the environmental consequences of animal production	Chr. Hansen A/S	Line 443, page 13 "For additives which favourably affect the environment by direct or indirect means,…" Comment: What is meant by "..by direct or indirect means.."?. By mentioning indirect means, would it be acceptable to show an indirect environmental impact by for example improved feed efficiency, thereby reducing emission of greenhouse gases?	See reply to #60.

			Lines 450-452, page 13 For additives aimed at reducing the production of methane and other greenhouse gases, the reduction in gas production should be measured by internationally recognised methods (e.g., GreenFeed, SF6 tracer) in long-term studies. Comment: "… in long-term studies." We expect the mentioned long-term studies are still in accordance with the table 6 "Minimum duration of long-term efficacy studies" of the present EFSA guidance on efficacy. Proposal: Please include reference to this table.	The reference to the duration of trials has been included at the beginning of the section.
			Lines 453-454 + 458-459, page 13 "… consider the possibility of an adaptive response to the additive." Comment: Please clarify which kind of data/analyses are required for addressing this point. Which adaptive response would be acceptable, if found? Proposal: Additionally, clarification on the types of acceptable adaptive responses, if identified, would be beneficial.	See reply to #60.
65	3.4.2 Additives favourably affecting the environmental consequences of animal production	Oy Medfiles Ltd	Thank you for providing more guidance now.	Comment not related to risk assessment.
66	3.4.4 Additives affecting animal	FEFANA asbl	Lines 465ff: Reference shall be made to the actual functional group as mentioned in Annex I of Reg. 1831/2003. In addition, in the current EU regulatory framework, the term welfare refers to other aspects than those that are addressed with feed (e.g. stocking density, animal husbandry conditions). Rather feed additives and their benefits targeted here are related to the functional group of "physiological condition stabilisers": substances or, when applicable microorganisms, which, when fed to animals in good health, favourably affect	There is no specific functional group defined in Regulation (EC) No 1831/2003 restricted to animal welfare.

	welfare		their physiological condition, including their resilience to stress factors. The link with the definition of the functional group would better help applicants to prepare applications targeting this functional group.	
			Line 465ff: The chapter does not provide guidance for the applicant with regard to endpoints, parameters and mode of actions. This clear guidance is required to enable targeted applications for this functional group. For instance, could end-points/parameters such as blood or milk markers or behavioural measurements/observations be acceptable to demonstrate efficacy? In order to avoid confusion and study rejections, also examples of stress factor endpoints (e.g. IgA, faecal score, etc.) that are expected to be measured by the applicants should be indicated within the section. Please add a non-exhaustive list of endpoints, parameters and stress factors.	The text was updated to develop the endpoints that can be provided to demonstrate the claimed effect of the additive further.
			Line 469-471: For additives affecting stress resilience, to demonstrate efficacy, it might be possible to apply stressing factors representing realistic situations of the life/productive cycle of the animals and challenging their optimum physiological status (e.g., heat stress, transport). When stressing factors are mentioned ?(e.g., heat stress, transport)?, we understand them as examples (e.g.,) but the sentence could be rephrased or clarified to avoid the understanding that it is only limited to these two specific stressors	The Panel does not propose or limit the stressors/challenges: "A clear description of the rationale for selecting the stressor(s) and the endpoints monitored should be provided." However, the Panel considers that feed additives are not intended to compensate for poor hygiene conditions or poor animal health status.
67	3.4.4 Additives affecting animal welfare	FEDIAF European Pet Food Association	Lines 471 - 473 (page 15) FEDIAF believes that it would be helpful to consider including a specific paragraph or examples for pets and non-food-producing species. As they are not intended for food, unlike other species mentioned in the guidance, the purpose of feed additives in these cases is aimed to optimize the health of pets through nutrition. The possibility of applying physiological stress-type factors to prove efficacy of zootechnical additives would be useful.	The paragraph, as it is now, applies both to food-producing and non-food-producing animals. The possibility of applying stressors is already foreseen.
68	3.4.4 Additives affecting animal welfare	Association of Veterinary Consultants (AVC)	Page 15, Line 467 to Line 485 "Additves affecting animal welfare" & Other additives. EFSA should allow similar challenge/stress models as permitted for coccidiostats, to demonstrate potential welfare benefits of various types of feed addtives, such as probiotics (live micro-organisms) bacteriophages, enzymes, essential oils, & otherr botanical extracts/derivatives.	See reply to #66.

69	3.4.4 Additives affecting animal welfare	Pen & Tec Consulting, SLU trading as Argenta ®	465-468 3.4.4 Additives affecting animal welfare For additives favourably affecting welfare, the choice of long-term or short-term studies to demonstrate efficacy will depend on the nature of the substance and intended purpose. The selection of the endpoints should be adequately justified. Comment: More information in relation to the studies affecting welfare and the endpoints and/or the addition of some examples could be useful for applicants.	The text was updated to develop the endpoints that can be provided to demonstrate the claimed effect of the additive further. It is the responsibility of the applicant to design and conduct the studies in a way that is considered better to support the efficacy in relation to the intended effect and the conditions of use.
			469-471 For additives affecting stress resilience, to demonstrate efficacy, it might be possible to apply stressing factors representing realistic situations of the life/productive cycle of the animals and challenging their optimum physiological status (e.g., heat stress, transport). Comments: Can EFSA confirm that studies with stressing factors can also be used in applications intended for other functional groups? E.g., improvement of zootechnical performance. Studies that use pathogenic microorganisms as a source of stress are useful to represent challenging conditions that farmers can face in commercial farms. Would EFSA accept these studies for this and/or other functional groups? E.g., introducing microbial contamination using trays disseminated on top of clean/new bedding, or via oral gavage. We believe that it would be helpful to consider a specific paragraph or examples for pets and non-food-producing species. As they are not intended for food, unlike other species mentioned in the guidance, the purpose of feed additives in these cases is mostly aimed to optimize the health of pets through nutrition. The possibility of applying specific physiological stress-type factors (in addition to environmental factors) to prove efficacy of zootechnical additives would be useful.	See reply to #66.
70	3.4.4 Additives affecting animal welfare	Nor-Feed SAS	Lines 469-471: Another stressor can be the presence of a microorganism (infectious pressure), representing realistic situations of animal production, and at level sufficiently low not to induce disease, and thus be in line with the definition of healthy animals (as defined in lines 690-691). We suggest to review lines 469-471 as "For additives affecting stress resilience, to demonstrate efficacy, it might be possible to apply stressing factors representing realistic situations of the life/productive cycle of the animals and challenging their	See reply to #66.

			optimum physiological status (e.g., heat stress, transport, infectious pressure that does not induce clinical disease)."	
			Lines 471-473: Should this a priori evidence be part of the dossier Section IV, or be validated prior to submission during a pre submission meeting with EFSA ?	This should be part of the study design and submitted with the technical dossier.
71	3.4.4 Additives affecting animal welfare	ADISSEO	Lines 469-471, page 13: "For additives affecting stress resilience, to demonstrate efficacy, it might be possible to apply stressing factors representing realistic situations of the life/productive cycle of the animals and challenging their optimum physiological status (e.g., heat stress, transport)." Next to the physical challenges cited by EFSA (heat stress, transport), could also consider the epidemiological stress referring to the infectious challenge with parasite or pathogenic organisms. Indeed, the exposure of animals to low doses of those infectious agents will generate a discomfort without causing diseases in animals. This sub-optimal status will be sufficient to demonstrate the efficacy of the nutritional solution to improve the animal's health and prevent them from disease.	See reply to #66.
72	3.4.4 Additives affecting animal welfare	AFCA-CIAL	p. 13 Line 465: For clarity, reference has to be made with the actual functional group as mentioned in Annex I of 1831/2003. In addition, in the current EU regulatory framework, the term welfare refers to other aspects than those that are addressed with feed (e.g stocking density, animal husbandry conditions). Rather feed additives and their benefits targeted here are related to the functional group of "physiological condition stabilisers": substances or, when applicable microorganisms, which, when fed to animals in good health, favourably affect their physiological condition, including their resilience to stress factors. The link with the definition of the functional group would better help applicants to prepare applications targeting this functional group.	See reply to #66.
			p. 13 Line 466-468 : The definition of the functional group is more or less disconnected from "welfare" and there is a need to reconcile the two as it creates confusion for applicants regarding what is acceptable in terms of studies/end-points. In terms of the acceptable end-points to measure animal welfare/stress, what kind of end-points could be listed here as a guidance? Please include a non-exhaustive list of relevant Physiological responses, stress biomarkers that could be measured to demonstrate efficacy as feed additives under this functional group. Alternatively, a technical note could be prepared jointly with stakeholders in order to provide further assistance to applicants. As it was done in the past for example for Feed hygiene enhancers.	See reply to #66.

			p. 13 Line 469-471 : In addition to heat stress or transport; it would be beneficial to include other types of acceptable challenges to provide further assistance to applicants. A proposal is made hereafter: "For additives affecting stress resilience, to demonstrate efficacy, it might be possible to apply stressing factors representing realistic situations of the life/productive cycle of the animals and challenging their optimum physiological status (e.g., heat stress, transport, level of microorganism that does not include clinical disease)."	See reply to #66.
			p. 13 Line 471-473 : To avoid that efficacy studies under this functional group are not considered acceptable by the FEEDAP, industry is claiming for meaningful pre-submission advices where scientific and technical advices would be provided by the EFSA. Pre Submission Advice as provided until now have shown not to provide the expected level of commitment that is needed for industry. Pre submission advices could help to reduce the use of experimental animals.	Comment not related to risk assessment.
			p. 13 Line 477: Could end-points/parameters such as blood or milk markers or behavioural measurements/observations be acceptable to demonstrate efficacy ?	The text was updated to develop the endpoints that can be provided to demonstrate the claimed effect of the additive further. The endpoints monitored should be provided a priori based on the stressor applied, the claimed effect of the additive and the conditions of use.
73	3.4.4 Additives affecting animal welfare	Oy Medfiles Ltd	Thank you for providing more guidance now.	Comment noted. Comment not related to risk assessment.
74	3.4.5 Other additives	Federal Office of Consumer Protection and	Page 13 L 479 Please add "zootechnical": 3.4.5 Other zootechnical additives	The proposed modification is not considered appropriate to avoid confusion with the functional group "other zootechnical additives". These provisions are for any zootechnical additives with intended effects not included in the previous sub-sections.

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75	3.4.5 Other additives	FEFANA asbl	Line 479: As in other paragraphs of the guidance, there is a need to connect this with the actual functional groups? names of Regulation (EC) No 1831/2003. Without this, it is creating a lot of confusion for applicants. Here the guidance would better refer to: "Other zootechnical additives".	The proposed modification is not considered appropriate to avoid confusion with the functional group "other zootechnical additives". These provisions are for zootechnical additives with intended effects not included in the previous sub-sections. The particularity of zootechnical additives has been described at the beginning of the section.
			Line 481 "term studies to demonstrate the efficacy of other additives under this category will depend on the": The term "category" is not correctly used here. The legal term is "functional group". Unless the goal is to refer to zootechnical additives as a whole. Hence, the sentence would benefit by being revised in order to better connect with the regulation (1831/2003 and 429/2008) that should remain the basis for applicants to prepare technical dossiers.	The word refers to other additives under the category of zootechnical additives for which the intended effects are not included in the previous section.
76	3.4.5 Other additives	AFCA-CIAL	p. 13 Line 479 : As in other paragraphs of the guidance, there is a need to connect this with the actual functional groups' names of 1831/2003. Without this, it is creating a lot of confusion for applicants. Here the guidance would better refer to: the functional group of "Other zootechnical additives". It would also be beneficial to clarify if for this functional group the same recommendations (as for the other zootechnical additives and the link with Art 5) are established in terms of claimed effects or if it is, as suggested in the draft guidance, under the responsibility of applicants to provide justification for the end-points. The guidance would gain in clarity if lines 421-424 would make reference the functional groups and animal categories to which the recommendation aims to apply.	See reply to #74.
			p. 13 Line 481 : The term "category" is not correctly used here. The legal term is "functional group". Unless the goal is to refer to zootechnical additives as a whole - Hence, the sentence would benefit by being revised in order to better connect with the regulation (1831/2003 and 429/2008) that should remain the basis for applicants to prepare technical dossiers.	See reply to #74.

77	3.5 Coccidiostats and histomonostats	Pen & Tec Consulting, SLU trading as Argenta ®	494 * artificial infection to simulate use conditions (e.g., floor pen studies with poultry, battery... Comment: EFSA should allow similar challenge/stress models to demonstrate potential welfare benefits of other feed additives, such as probiotics, bacteriophages, enzymes, essential oils, & other botanical extracts	See reply to #66.
78	3.5 Coccidiostats and histomonostats	FEFANA asbl	Line 485-486 "These additives protect animals from the consequences of an invasion of Eimeria spp. or Histomonas meleagridis": The regulatory definition (Regulation 1831/2003) does not mention the protection of animals from the consequences of an invasion of Eimeria spp. or Histomonas meleagridis. The sentence should be revised to be in line with the EU Regulation (art. 2.2 "coccidiostats" and "histomonostats" means substances intended to kill or inhibit protozoa & art. 5.3 (g) have a coccidiostatic or histomonostatic effect.) Please consider removing: These additives protect animals from the consequences of an invasion of Eimeria spp. or Histomonas meleagridis.	The text was modified to address the comment.
			Line 487 "coccidiostats in poultry and rabbits": We propose adding other relevant animal species affected by coccidiosis, such as calves	The possibility of considering other species is already foreseen in the first paragraph of section 3.5: "The requirements below should be adapted and justified for applications covering other animal species or histomonostats."
79	3.5 Coccidiostats and histomonostats	AFCA-CIAL	p. 13 Line 485-486 : The regulatory definition (Regulation 1831/2003) doesn't mention the protection of animals from the consequences of an invasion of *Eimeria* spp. or *Histomonas melegridis*. The sentence should be revised to be in line with the EU Regulation (art. 2.2 "coccidiostats" and "histomonostats" means substances intended to kill or inhibit protozoa & art. 5.3 (g) have a coccidiostatic or histomonostatic effect.) It is also proposed adding other relevant animal species affected by coccidiosis such as calves. These additives are intended to kill or inhibit protozoa.	The text was modified to address the comment. See reply to #78.
			p. 13 Line 492-496 : Regulation 429/2008 allows three types of experiments for the demonstration of the efficacy: - artificial single and mixed infections - natural/artificial infection to simulate use conditions - actual use conditions in field trials	The Panel does not consider field trials adequate to demonstrate the efficacy of the coccidiostats since they do not allow for proper measurement of the additive's coccidiostatic effect. However, they can be submitted as supportive evidence.

			Rewording proposed : Efficacy data, testing the minimum inclusion levels, should derive from different types of target animal experiments, notably: field trials with natural infection from qualified litter contaminated and validation of infestation by different of end point by a not contaminated litter batch, contaminated litter batch not treated, contaminated and treated litter batch or artificial infection to simulate use conditions (e.g., floor pen studies with poultry, battery cage studies with rabbits) ? Warning: not representative of reality, given the mode of contamination/propagation between individuals. or anticoccidial sensitivity tests (AST)	Moreover, even though artificial infection does not reproduce the natural way of disseminating Eimeria in commercial farming systems, the Panel considers that natural infection methods often do not allow the assessor to evaluate the additive's coccidiostatic effect.
80	3.5.1 Floor pen studies with poultry /battery cage studies with rabbits	Federal Office of Consumer Protection and Food Safety	Page 14 L508 Please replace "(see Section 4.2.2.1)" by "(see Section 5.2.2.1)".	The text was modified to address the comment.
81	3.5.1 Floor pen studies with poultry /battery cage studies with rabbits	Pen & Tec Consulting, SLU trading as Argenta ®	Lines Guidance Text Comments 508 Link states section 4.2.2.1 Link directs to section 5.2.2.1	The text was modified to address the comment.
82	3.5.1 Floor pen studies	AFCA-CIAL	p. 13 Line 508 : "see Section 4.2.2.1" replace by "see Section 5.2.2.1"	The text was modified to address the comment.

	with poultry /battery cage studies with rabbits			
83	3.5.1 Floor pen studies with poultry /battery cage studies with rabbits	FEFANA asbl	Line 498-499: For floor pen studies with poultry/battery cage studies with rabbits, "three studies with inocula from different country regions within the EU are required." Previous guidance: "Three studies with different inocula from different geographical locations within the EU are required." This means same inocula from different geographical locations can be applied? Please clarify	The text was modified to address the comment.
			Line 499 : Might be a typo "it should probably be either country or region"	The text was modified to address the comment.
			Line 509-510 "The different endpoints should be measured at least 6-7 and 14 days after inoculation and at the end of the study.": Allow for flexibility of when scoring lesions and oocyst excretion depending on the Eimeria species present in the inoculum. - Poultry: Include D5 post-inoculum to not miss *E. acervulina* lesions when relevant. - Rabbits: need to start at d4 as prepatent period of Eimeria media, one of the most prevalent species in rabbits, is 4.5 days.	The text was modified to address the comment.
			There is no scientific value in evaluating the stated endpoints at d14 post inoculation as intestinal lesions will be minimal by then and there will not be much oocyst excretion both in poultry and in rabbits. This leads to an unnecessary waste of animals. Please consider the following amendment: The different endpoints should be measured for poultry between 5 to 7 days after inoculation and for rabbits between 4 to 7 days after inoculation (depending on the prepatent period of Eimeria species present in the inoculum) as well as at the end of the study.	The Panel considers that the monitoring of the intestinal lesions on day 14 after inoculation and at the end of the trial is a necessary endpoint to assess the efficacy of this type of additive.

84	3.5.2 Anticoccidial sensitivity tests	FEFANA asbl	Line 513-514: Does the text refer to "different countries within the EU" OR "different regions of EU countries within the country"? We suggest providing a clarification or reformulating the text.	The text was modified to address the comment.
			Line 522 : Would it be possible to include a non-exhaustive list of the most relevant endpoints to measure	The list of most relevant endpoints is already included at the beginning of section 3.5. "The capacity of anticoccidial substances to control coccidiosis should be demonstrated by targeting specific endpoints (e.g., lesion/faecal score, oocyst excretion, morbidity, coccidiosis-related mortality). Data on body weight and feed intake should be provided as supportive information."
			Line 522-523 "Examination of endpoints should generally be done 6 to 7 days after inoculation.": Allow for flexibility of when scoring lesions and oocyst excretion depending on the Eimeria species present in the inoculum. Please consider the following amendment: Examination of endpoints should generally be done for poultry between 5 to 7 days after inoculation and for rabbits between 4 to 7 days after inoculation, depending on the prepatent period of Eimeria species present in the inoculum	See reply to #83.
85	3.5.2 Anticoccidial sensitivity tests	AFCA-CIAL	p. 14 Line 513-514 : "different countries within the EU" OR "different regions of EU countries within the country" The criteria of 3 regions is not relevant. The three populations of coccidia have to be different whatever where they are coming from. Rewording proposed: "Done with different inocula" instead of "done with inocula from different country regions"	The text was modified to address the comment.
			p. 14 Line 522 : Could you please provide a non-exhaustive list of the most relevant end-points to measure	See reply to #84.
86	3.5.3 Inocula	FEFANA asbl	Line 531"Molecular characterisation of the strains should be provided.": Please consider providing method name or reference for the molecular characterisation of the strains	It is up to the applicant to provide the method considered more appropriate and to justify the selection.

			Line 536 "turkeys: E. meleagrimitis and E. adenoeides": Some important turkey Eimeria species are missing. Please consider the following addition: E. meleagrimitis, E. adenoeides, E. meleagridis, E. gallopavonis	The text was modified to address the comment.
			Lines 544-547: The use of artificial infection/ inoculation studies makes it unlikely to be able to replace current coccidiostats (ionophores and chemicals) with biological solutions. As challenges studies are very harsh and somewhat very different from natural production circumstances where the solutions should be applied and work. If the requirements for depression in BW and lesion scoring were reduced, it would be more likely for biological solutions in this space and closer to natural production conditions.	The virulence test aims to assess the effect of the inocula on the animal, not the effect of the coccidiostat product on the protozoa.
			There is also a lesion scoring system available in turkeys Gadde et al. 2020: Pathology caused by three species of Eimeria that infect the turkey with a description of a scoring system for intestinal lesions Avian Pathology. 2020 2020 Feb;49(1):80-86	It is up to the applicant to provide the method considered more appropriate and to justify the selection.
			Please consider the following amendment taking into consideration the provided information: Virulence is assumed when weigh gain is depressed in the experimental period by 15 % in Chickens and 15% in turkeys and/or intestinal lesion score increased significantly in chickens or turkeys.	The Panel considers that the current thresholds are adequate to ensure that the inocula's virulence is sufficient to assess the coccidiostat's effect properly.
87	3.5.3 Inocula	AFCA-CIAL	p. 14 Line 531 : Could you please provide method name or reference for the molecular characterisation of the strains p. 15	See reply to #86.
			Line 544-546 : percentages indicated are not requested bu regulation 429/2008. These percentages are an obstacle to the authorisation of new solutions which could nevertheless demonstrate efficacy against coccidiosis. WE propose to suppress:	The indications provided relative to the impairment in performance refer to the virulence test and aim to demonstrate that the Eimeria spp contained in the inoculum has deleterious effects on the performance of the target animal.
			Virulence is assumed when weight gain is depressed in the experimental period by 25% in chickens and 15% in turkeys and/or intestinal lesion score increased by a minimum of two units on a five-point scale7 in chickens or a comparable increase in faecal score for turkeys. In addition, mortality/morbidity should be reported	The FEEDAP Panel considers that the combination of both ASTs and floor pen studies should provide evidence of the pure coccidiostatic effect of the additive and the inhibition of the negative effects of Eimeria on performance.

88	4 Number of in vivo efficacy studies required	Chr. Hansen A/S	Lines 586-587, page 18 // Table 5, in general Comment: We are of the opinion that the number of studies should not be reduced compared to present table 5 of the efficacy guidance. We find it important that feed additives brought to the market are shown to be efficient in several studies and we find the present numbers of studies to be relevant. Proposal: Please consider keeping Table 5 as it is.	In the review of the efficacy Guidance, the minimum number of studies required to conclude on the efficacy of feed additive has been re-checked. The intention was to reduce the number of studies needed in compliance with the 3Rs policy while ensuring that sufficient and good quality and scientifically based data is provided to demonstrate the efficacy of the additive under assessment.
			Lines 586-587, page 18; Table 5 Comment: Why is "Pets" not included in Table 5 (dogs and cats studies)	Table 5 covers only food-producing animals.
			All poultry: Why not "2 chickens OR 1 in chickens & 1 in turkey" in line with the Poultry for fattening.	The Panel considers that for all poultry, 2 trials with chickens for fattening and 2 in laying hens are necessary.
89	4.1 Single animal category	Federal Office of Consumer Protection and Food Safety	Page 15 L 556 Please replace "Section 2" by "Section 3".	The text was modified to address the comment.
90	4.1 Single animal category	Pen & Tec Consulting, SLU trading as Argenta ®	556-557 If the application covers only one animal category, the studies required in Section 2 should be performed in that animal category Comment: If the application covers only one animal category, the studies required in Section 3 (should say) should be performed in that animal category.	The text was modified to address the comment.
91	4.1 Single	FEFANA asbl	Line 556: Link to Section 2 should probably be a link to Section 3. Section 2 does not give indications about the number and nature of studies (line 143-145 says	The text was modified to address the comment.

	animal category		"the number of studies depends [?]"), but Section 3 generally gives the nature of studies to be performed. Please adapt the link accordingly.	
92	4.2 Multiple categories of the same species of food-producing animals	FEFANA asbl	Line 559-561 "In principle, conclusions from studies in fattening animals are extended to include animals of the same species that are reared for reproduction, e.g., from chickens for fattening to chickens reared for laying/breeding, from turkeys for fattening to turkeys reared for breeding.": The text would probably benefit by extending the example to other types of animals (at least ruminants and pigs). Or by making reference to the table 4 related to extrapolations.	The Guidance includes a non-exhaustive list of examples.
			There is also a lack of clarity regarding the specie extension rules between growing and rearing for reproduction animal (swine and poultry). Does "rearing for reproduction" stand for pullet and future sow?	Comment unclear. The current text reflects the potential extension within categories of the same species, and Table 4 clearly states the potential extrapolations between different physiologically related species.
93	4.2 Multiple categories of the same species of food-producing animals	AFCA-CIAL	p. 15 Line 559-561 : The text would probably benefit by extending the example to other types of animals (at least ruminants and pigs). Or by making reference to the table 4 related to extrapolations.	See reply to #92.

94	4.2 Multiple categories of the same species of food-producing animals	Oy Medfiles Ltd	p. 15 Line 564-565 : Could it be envisaged to extend the possibility to extrapolate data from one animal species to another species when the mode of action of the feed additive is documented and known to be the same across the species even if they do not share the same gastrointestinal function. In this case, similarities in terms of metabolism and mode of action would have to be provided. In addition, while it is understood that extrapolation between categories of the same species at different production stages is not possible (eg layers vs broilers) when zootechnical performance are targeted; it should be possible to seek extrapolation between categories in case for examples of "physiological condition stabilisers" when the claimed effect/benefit is common to the different categories/. Actually, for additive affecting stress resilience, data can be extrapolated between categories of the same species at different production stage.	The text was modified to address the comment. The possibility of extrapolating the efficacy results between categories of the same species at different production stages is considered appropriate if the claimed effect can be reasonably presumed to be the same between categories. In these cases, when efficacy has been demonstrated in one category, one additional study in the category it is intended to be extrapolated should be provided to show the minimum efficacious level.
			Should section 4.2.2.1 be actually section 5.2.2.1 and section 4.2.2.2 section 5.2.2.2 ?	The text was modified to address the comment.
95	4.3 Multiple species of food-producing animals	FEFANA asbl	Line 568 "unrealistic to expect studies in all potential target species for which the application is made.": For clarity we would appreciate the following addition: ?And/or categories?	The term "categories" has been removed from the text to avoid confusion.
			Line 586 in Table 5 "All terrestrial species": - All terrestrial species: Including pets or only livestock species? Including insects or only vertebrates?	Table 5 refers to food-producing animals. Specific provisions for "all insects" have been provided in Table 5.
			- All in fish: Would this cover as well "ornamental fish"? If this is the case, the guidance would be clearer if making reference to "all fish"	According to Regulation (EC) 429/2008, the extrapolation from other physiologically related species is only considered from major species. The table was modified to avoid confusion.

			- 1 in crustacean/mollusc: 1 study in shrimps (instead of molluscs, unless studies in oysters are anticipated) would make more sense in terms of market representativeness	Shrimps are included within "crustaceans".
			- 3 covering both weaned piglets/pigs for fattening for All Suidae for fattening and reared for reproduction: does it mean 2 piglets + 1 pig trial or 1 piglet + 2 pigs trials?	The text was modified to address the comment.
			- Please include an application covering all pigs, all poultry and fin fish. Not all feed additives (.f.ex. certain enzymes or probiotics) will have a use in ruminants: 3 covering both chickens for fattening and laying hens + 3 covering both weaned piglets/pigs for fattening and sows+ 3 covering both salmonid and non-salmonid species	The number of studies covering porcine species, poultry, and fin fish has already been reduced from the previous requirements. The required number of studies considered by the Panel is reflected in Table 5.
			- Why are pets not included in Table 5 (dogs and cats studies)	See reply to #88.
			- All poultry: Why not "2 chickens OR 1 in chickens & 1 in turkey" in line with the Poultry for fattening.	See reply to #88.
			- We would appreciate a reformulation or clarifications on footnote (1).	The text was modified to address the comment.
			Line 596: Section link should go to section 3. Please adapt accordingly.	The text was modified to address the comment.
			Lines 602-611 "For enzymes (as zootechnical additives), if in vitro validated systems are available (?)": It is appreciated to reduce the requirements for enzymes (as zootechnical additives) by implementation of in vitro studies. This fulfils the increasing ethical resource constraints. However, we do not understand why the possibility to use in vitro models for zootechnical additives is restricted to enzymes. Actually, it should be possible to use in vitro models for any type of additive as long as the model corresponds to a "validated system" and is suitable for the demonstration of the intended effect in the animal. Of particular importance is to apply a similar approach for substances which favourably affect the environment and physiological condition stabilisers.	For the time being, the Panel considers that this approach should be limited to enzymes used as zootechnical additives.
			Lines 602-611: We would appreciate clarity on the following aspects:	The suggestion proposed to modify the text is unclear.

			- The effect is usually in the digestibility and not on the animal, so the wording is not clear.	
			- The meaning of "battery" of in vitro studies is unclear. Suggestion is to define the "battery" of in vitro studies (in a relevant range of compound feeds)? in order to clarify to applicants how many in vitro studies are needed to define a battery. A suggestion would be (at least) three in vitro studies with three different compound feeds.	The text was modified to address the comment.
			- Suggestion to define the "in vitro validated system". The description in this paragraph is too generic and very difficult to understand and bears the risk of misinterpretations. To the best of our knowledge, there are no recognized, authorized nor validated in vitro systems for demonstrating the efficacy of feed enzymes in vivo. Different university and company research groups use different models. Please consider providing examples for "in vitro validated systems". A suggestion could be "in vitro models/systems published in scientific peer-reviewed research journals".	*In vitro* methods used to support the efficacy of enzymes as zootechnical additives should be validated *in vivo* within the same animal species, taking into account the feed characteristics (e.g. particle size; composition/substrate) and the animal species/category-specific digestive conditions mimicking the physical, chemical and microbiological characteristics of the *in vivo* gut fluid (e.g. pH, Eh (redox potential), temperature, DM content, endogenous enzyme activities, bile salt content and profile, MRT (Mean Retention Time), gut microbiota/inoculum, animal physiological state), preferably based on knowledge of mode of action. The aim of these tests is not to assess the enzyme "activity", which is usually carried out in simple, standardized laboratory conditions (e.g. optimum pH, temperature, synthetic substrate) without guaranteeing the same efficacy in *in vivo* gut conditions. Several validated in vitro methods (model: static, dynamic, ex vivo; GIT segment: stomach, fore-gut, hindgut) to predict the in vivo response are described for different animal species in the literature

				in peer-reviewed papers. EFSA is not a position to recommend any specific one.
			- What is the requirement if you want to request all poultry and all suidae? For instance, for All poultry for fattening and reared for laying, would 1 in-vivo study in chickens for fattening + 2 in vitro studies in two different feeds- be acceptable?	According to the proposed text, for "all poultry and all porcine species", in vitro studies covering a representative range of feeds to which the additive will be used under different in vitro conditions covering both species, plus one vivo in poultry and one in vivo study in porcine species.
			- Could EFSA consider an additional table as table 5 for in vivo and implement a replacement equivalence list?	The Panel considers that the text proposed in the current document is clear enough and no table is necessary.
			Lines 602-606 and 900-923: Could you please share your recommendations about acceptable/scientifically recognized in vitro tests? Could the clarification on in vitro studies provided in paragraph 6 (L900-923) also be considered for enzymes?	The recommendations included in section 6 would apply to all in vitro studies referred to within the Guidance.
			Line 611 : It would be relevant to add another animal group "crustaceans"	The applicant can provide studies in additional species to those indicated in the Guidance.
96	4.3 Multiple species of food-producing animals	Erawan Consulting SARL	Line 578 Table 4 / Comment: At chickens for fattening category, the ostrich species is gone. I suggest adding it.	The text includes a non-exhaustive list of examples.
			Line 578 Table 4 / Comment: In the last line "Salmon and trout", I suggest completing the physiologically related species "ornamental fish" by the following: "other fish (carnivore / herbivore)".	The number of studies requested for the extrapolation to other fin fish is included in Table 5.

			Line 578 Table 4 / Comment: I suggest adding a line for "crustaceans" with "other mollusc" as physiologically related species	The number of studies requested for the extrapolation to crustaceans and aquatic species is included in Table 5
			Line 587 Table 5 / Comment: We have 3 groups for bovine: one for fattening and reared for reproduction second for milk production and a third one for which production? I suggest clarifying.	The group named "all bovines, ovines, caprines, cervids and camelids" aims to cover all categories of these species.
			Line 587 Table 5 / Comment: In this table there is no information for pets and NCA (New Companion Animals). I suggest adding these groups.	See reply to #88.
97	4.3 Multiple species of food-producing animals	Federal Office of Consumer Protection and Food Safety	Page 15 LL 570-577 , including Table 4 Please note, that with regard to extrapolation, it is pointed out that the species mentioned differ in terms of the physiology of their gastrointestinal tract and that extrapolation is not scientifically sound for certain additives, e.g. zootechnical additives. In particular, it is considered that the extrapolation of weaned piglets to suckling piglets and the extrapolation of chickens for fattening to turkeys for fattening is not sufficiently justifiable (Everaert et al., 2017, Pieper et al., 2009; Mach et al., 2015, Wei et al., 2013; Arsenault et al., 2014).	The point raised in the comment was subject of discussion with the Member States, and the FEEDAP Panel decided that the cited extrapolations were appropriate.
			Page 16 L 577 Table 4: - line Chickens for fattening, column To physiologically related species : Please delete turkeys. The extrapolation of chickens for fattening to turkeys for fattening is not sufficiently justifiable (Everaert et al., 2017, Pieper et al., 2009; Mach et al., 2015, Wei et al., 2013; Arsenault et al., 2014). - line Laying hens, column To physiologically related species : Please delete turkeys. The extrapolation of chickens for fattening to turkeys for fattening is not sufficiently justifiable (Everaert et al., 2017, Pieper et al., 2009; Mach et al., 2015, Wei et al., 2013; Arsenault et al., 2014). - Please add a new line below the line for laying hens. In the new cell column "From" please add "Turkeys for fattening", in the new cell column "To physiologically related species" please add Turkeys reared for breeding.	See the reply to the comment above.
			Page 17 LL 586-587 General comments on Table 5: It is noted, that in this guidance draft, it could not be supported that the required numbers of efficacy studies are reduced.	See reply to #88.

			Column "Number of studies required and species": The "/" symbol is ambiguous, because it can be interpreted as either "and" or "or". The literal formulation instead of symbols is desirable.	The text was modified to address the comment.
			Line "All Suidae" and line "All terrestrial species" It is noted that to ensure that extrapolation to all pigs is permissible, the studies on weaned piglets should also include suckling piglets from the time when solid feed is administered	See the reply to the comment above.
			For consistency, please add "dairy" before "cows"	The text was modified to address the comment.
			Line "all terrestrial species", column "Number of studies required and species": Please add in cell additionally "+1 in insects (honey bees)"	The table was modified to address the comment.
			Line "all fin fish", column "Number of studies required and species": Please add in cell additionally "1 in ornamental fish"	Table 5 covers only food-producing animals.
			The German proposals for correction in Table 5 are provided in a separate document (Annex German Proposal for Table 5).	The proposed Table has been carefully screened, and Table 5 has been modified for those suggestions that were considered appropriate by the FEEDAP Panel.
			Page 18 L 596 Please replace "Section 2" by "Section 3".	The text was modified to address the comment.
98	4.3 Multiple species of food-producing animals	Pen & Tec Consulting, SLU trading as Argenta ®	586 Table 5: All terrestrial species – Comment: Does this include pets?	Table 5 covers only food-producing animals.
			Application for: All bovines, ovines, caprines, cervids and camelids - Number of studies required and species: 1 in calves + 1 in cattle for fattening + 2 in cows. Comments: Might there be flexibility in delivery of the 4 studies (e.g., could a dairy cow study be replaced with an additional study in calves)?	The Panel considered a minimum of two studies on growing animals and another two studies on milking cows in order to draw a conclusion.

			There is growing interest in additives which favourably affect the environment; reducing methane and other greenhouse gases. For this additive category (and other categories intended to be fed to animals with a functional rumen), the study group "calves" (defined in Table 6 as "<6 weeks of age at study start") can lack biological relevance owing to an undeveloped rumen. In consideration of the above, please include a clarification in the post-script (line 588-589) indicating how the study in calves might be substituted/adapted (e.g. allowing calves <12 weeks of age / indicating weaned calves in full rumination).	The applicant has the possibility of deviating from the Guidance provided that it is adequately justified. The Panel will assess the deviations in a case-by-case basis.
			595-596 binders and nutritional additives, the number of studies and the target species are given in Section 2. Comment: binders and nutritional additives, the number of studies and the target species are given in Section 3 (should say).	The text was modified to address the comment.
99	4.3 Multiple species of food-producing animals	AFCA-CIAL	p. 15 Line 568 : For clarity: please add "And/or categories"	The term "categories" was removed from the text to avoid confusion.
			p. 16 Line 577 : table 4 - Insects : What about extrapolations between insects types: from bees to all insects as it is done from shrimps to "all crustaceans"?	See reply to #95.
			- Fish : Would extrapolation be accepted also from other farmed fish species to ornamental fish	See reply to #95.
			- Horses : In previous opinions on the efficacy of feed additives in horses, the FEEDAP considered that the effect of feed additive in a hindgut fermenter, such as the horse, is likely to be similar to that seen in ruminants. When it was considered that evidence has been provided to show that the effect of the additive in horses is similar to that observed in ruminants, only one study in ruminant was considered necessary When an additive is already authorised in a major ruminant species/category, it should therefore be sufficient to provide one single in vivo study in horses to support the efficacy of the additive in that species. And according to Table 5; to demonstrate the efficacy in "all bovines,	As a general principle, three in vivo studies are required to support the efficacy of the additives in horses. Deviations should be appropriately justified and will be considered by the Panel on a case-by-case basis.

			ovines, caprines, cervids and camelids, and all horses/Equidae", it should be acceptable to provide the following number of studies: 1 in calves + 1 in cattle for fattening + 2 in cows + 1 in horses	
			p. 17 Table 5 : We would like to thank EFSA for the global reduction of number of in vivo efficacy studies to reach an authorisation for main animal species/categies (eg all ruminants). However, some clarifications related to this table 5 are required to support the applicants. - "all terrestrial species": Including pets or only livestock species? Including insects or only vertebrates?	See reply to #95.
			- Would "All fin fish" cover as well "ornamental fish" ? If this is the case, the guidance would be clearer if making reference to "all fish"	See reply to #95.
			- "all aquatic species" : 1 study in shrimps (instead of mollusc, unless studies in oysters are anticipated)	See reply to #95.
			- Would make more sense in terms of market representativeness Instead of « 1 in crustacean/mollus?, we suggest : ?1 in crustacean/shrimp/mollusc?	See reply to #95.
			p. 18 Line 596 : replace "Section 2" by "Section 3"?	The text was modified to address the comment.
			p. 18 Line 602-611 : We would like to thank EFSA for the possibility to use in vitro models for enzymes. However, it is restricted to only one functional group (i.e. enzymes). According to Guidelines Annex III - 4.2.4. (1) related to Additives favourably affecting animal production, performance or welfare and for the functional group "other zootechnical additives": "Evidence of the mode of action can be provided by short term efficacy studies or laboratory studies measuring relevant end-point." It should be possible to open the use of in vitro models for other zootechnical additives as long as the model corresponds to a "validated system" and is suitable for the demonstration of the intended effect/mode of action in the animal.	The comment mentioned in Regulation (EC) 429/2008 refers to the demonstration of the mode of action. The proposed text in the Guidance allows the use of in vitro systems to replace in vivo animal studies as a demonstration of efficacy, not only to support the mode of action of the additive.
			Instead of "for enzymes", we suggest : "-For additives favourably affecting animal production, performance or welfare and for the functional group "other zootechnical additives" (as zootechnical additives), if in vitro [?]	See reply to #95.

100	4.3 Multiple species of food-producing animals	FPS Public Health, Food Chain Safety and Environment	Table 4: Extrapolation of efficacy data from certain species to other physiologically related species. In the EU, camelids are generally not kept for fattening or milk production purposes. How to obtain authorization for camelids kept for wool production? Or simply for pleasure? Legally, authorizations must explicitly exclude dairy or fattening camelids, as the case may be, which does not make much sense.	Comment not related to risk assessment.
101	4.3 Multiple species of food-producing animals	Oy Medfiles Ltd	Tbl 5: Thank for reducing the number of studies needed and hence animals.	Comment noted. Comment not related to risk assessment.
102	4.4 Pets and other non-food-producing animals	FEFANA asbl	Line 619-622 "three in vivo studies in one pet/non-food-producing species are required. If the application is for more than one pet/non-food-producing species, a single additional study would be needed for each additional target species with a maximum of three species in total." In line with the adaptations of the number of studies when using multiple species of food producing animals, it would make sense to adapt the number of studies in non-food producing species too. The current text assumes that there is 1 main species in which 3 studies need to be conducted + 1 (to a max of 3) studies in addition for multiple species. In line with extrapolation possibilities given for zootechnical enzymes or substances for the reduction of the contamination by mycotoxins, it would make sense that there needs to be a minimum of 3 studies in relevant different species (1 study per species) for all terrestrial non-food-producing animals (+1 aquatic if for all non-food-producing animals). - This would reduce studies to a maximum of 4 (rather than 6) which is also the current suggestion for other larger categories (e.g. all suidae, all poultry, all fin fish or bovines etc.). - It would also enhance flexibility when only 2 species are involved. Please consider adapting the text based on the information provided.	The text was modified to address the comment. New provisions for applications intended for “all pets/non-food-producing animals” have been included in the Guidance.

103	4.4 Pets and other non-food-producing animals	AFCA-CIAL	p. 18 Line 617-622 : Instead of the 3 - 1 - 1 combination as proposed, could it be envisaged to submit a 2 - 2 ? 1 combination for an application in all pets ? For example: 2 studies in dogs, 2 studies in cats, 1 study in another pet species instead of 3 in dogs or cats, 1 in cats or dogs, 1 in another species ?	See reply to #102.
104	4.4 Pets and other non-food-producing animals	Pen & Tec Consulting, SLU trading as Argenta ®	614-616 For additives for which efficacy has been demonstrated in a food-producing animal species and the intended effect is the same, one in vivo study is required for each target pet/non-food producing species with a maximum of three species in total. Comment: Will applications for all terrestrial species cover also pets? This is not clear.	Table 5 refers to food-producing animals. New requirements for applications covering pets have been included in Section 4.4.
			Comment: Certain feed additives exert the same effect in food-producing animals and pets, while the endpoints used to measure the effect is different, e.g. gut flora stabilisers are "micro-organisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora". In food producing animals, the endpoint used is "improved zootechnical performance" (e.g. body weight gain); in pets, other endpoints are used. We suggest that the text reflects that the same effect can be measured by different endpoints and include the above example.	For zootechnical additives such as the one in the example provided, the efficacy assessment will be performed exclusively based on the effect(s) of the additive expected. It is up to the applicant to provide evidence that the effects seen in the food-producing animals and pets are the same.
			617-620 Where the intended effect in the pet/non-food-producing species is different from that described for the food-producing animal species or when efficacy has not been demonstrated in food- producing animal species, three in vivo studies in one pet/non-food-producing species are required. Comment: For microorganisms, the effect in the gut is the same for food-producing animals and for pets. However, the endpoints are different. EFSA should consider that only 1 pet study is required when efficacy has been established in food-producing animals.	The number of studies required for applications covering pets and other non-food-producing animals are included in Section 4.4.

105	5 In vivo efficacy studies	FEFANA asbl	Line 628-629 "parameters (e.g., absorption, digestibility, excretion, retention). The choice of short- or long-term studies or a combination of both will depend on the effect and/or mode of action of the additive.": What are the recommendations in terms of number of studies? 2 long term and 1 short term or is it sufficient to provided 2 short term and 1 long term? Please clarify.	The number of long- or short-term studies will depend on the category of the additive and the claimed effect, as described in Section 3.
			Line 640 "Evidence should be provided that the work was done by qualified personnel": Could you please provide further information on the "evidence" requested? Which kind of documents?	For example, a detailed list of the personnel involved in each of the aspects of the trial (i.e., animal management, preparation of feed, sampling, laboratory analysis), including their competence and responsibility.
			Line 638-641 "Trials should follow the criteria established by recognised externally-audited quality assurance schemes (e.g., good laboratory practice, good clinical practice). Evidence should be provided that the work was done by qualified personnel using appropriate facilities and equipment, with a named study director responsible of the research.": This does not seem to be in line with Regulation 429/2008 which says: "trials shall ideally be compliant with the criteria established by a recognised, externally-audited, quality assurance scheme. In the absence of such a scheme, evidence shall be provided to show that the work was done by qualified personnel using appropriate facilities and equipment and responsible to a named study director." Comment: In order to align with the guidelines, we would appreciate if the text could be modified to avoid that the EFSA guidance would be more stringent than the regulation.	EFSA considers that the requirements indicated in the Guidance and the Regulation (EC) 429/2008 are essentially the same.
			Lines 644-647 "For that purpose, the approval by a competent authority or independent ethical committee, clearly declaring compliance with the animal welfare requirements under Directive 63/2010, should be documented (also for studies conducted outside the EU)." AND Lines 943-946 "Ethical statement: Certificate of approval of the study protocol by a competent authority or independent animal welfare committee (including number/code of authorization) clearly declaring compliance with the animal welfare requirements, according to EU legislation.": The introduction of written approval for in vivo studies is a new requirement which is highly likely to cause practical problems. On one hand, under applicable welfare legislation, an animal study is defined as a study where animals may experience a certain amount of suffering exceeding that of commercial practice. This is not the case for all animal studies executed for	The compliance of the studies with the European Union (EU) legislation, particularly those listed in Directive 63/2010/EU, should be documented. It is acknowledged that the certification of the approval, e.g., by a competent authority or independent ethical committee, may vary depending on the nature of the study and the EU country in which the study is done (if relevant).

			efficacy, e.g., when only feed intake is measured or for pure performance studies. When a study does not qualify as "animal study" such independent approval may not be granted. It is recommended, therefore, to restrict the obligation for approval by an independent body to those study designs that really qualify as animal studies in accordance with Directive 63/2010. On the other hand, the availability of a written approval from a competent authority is dependent on national laws and study type. Studies subject to approval by local authorities differ across EU countries and in many cases such certification cannot be issued. Not all trial facilities will feature an ethical committee to compensate for this since they may have a general approval for certain types of trial. In such cases all they can do is provide an internal (signed) statement which indicates the laws and guidelines they followed. Another aspect to be consider concerns trials done outside the EU: what would then be the requirement? Competent authorities in third countries would be unable to declare compliance with EU legislation. Additionally requiring certifications which are not automatically part of a trial usually comes at extra costs. Please adapt the requirement so that it remains feasible by accepting the currently required "compliance statement" and by recognising that Directive 63/2010 is not applicable to all kind of efficacy studies. The possibility of accepting a statement from a veterinarian not directly involved in the study should be also considered.	
			Line 648-650 "aim of the project requires that the animals are kept under conditions similar to those commonly observed in commercial farms, the husbandry conditions should meet the requirements of Directive 98/58/EC as well as, where relevant, the species-specific legislation." Comment: What about studies in pets that may be performed in breeding facilities or with privately owned dogs or cats? For some species, animals have to be kept in group and for such animals, e.g., cats (kept by two for welfare reasons), it is not possible to record individual feed intake. How could this be taken into consideration for the assessment, and would such studies be accepted when it is not possible to have enough collective pens for statistical purpose: in short, would a study with cats kept by pair be acceptable when feed intake is recorded at the pen level and not per cat?	The experimental unit is the smallest entity to which a given treatment is applied. If animals are housed in groups (e.g., pen, cage) and all the animals in the pen share the same feed source (and feed intake is not measured individually), then the experimental unit for all parameters is the pen, not the individual animal.
106	5 In vivo efficacy studies	FEDIAF European Pet Food	FEDIAF believes that a clear definition of what will be understood by "independent" ethical committee is needed for clarity, in terms of dependency and relationship with the entity conducting the trial. For example, is an "animal-welfare body" (as defined by Directive 63/2010/EU), or an "Institutional Animal Care and Use Committee" (IACUC - as recommended by AAALAC International),	The concept of independence refers to the lack of conflict of interest between the persons involved in the ethical revision of the study protocol and the personnel involved in its design and

		Association	convened by a research organisation with the specific purpose of ensuring compliance with animal welfare requirements considered within the definition of "independent"? We would suggest mentioning them as an acceptable entity issuing certificates of approval for study protocols.	conduct (e.g., if the study director is also a member of the Ethics Committee of the research centre, a conflict of interest should be declared to avoid her/him participating in the decision).
107	5 In vivo efficacy studies	Association of Veterinary Consultants (AVC)	Page 21, Lines 624 to 643. In vivo efficacy studies. Challenge studies cannot be obliged to adhere fully to EU health, welfare & hygiene legislation, but must be accepted as models that are used to demonstrate potential benefits of feed additives, especially when animals may be subjected, under commercial EU farming conditions, to adverse physiological or environmental conditions (e.g. dysbiosis, wet litter, power failures leading to excess heat or cold, or automatic feed delivery failures). A challenge study is a model that uses small scale simulations of certain adverse conditions that may be experienced by animals, at certain times under commercial production of during routine lefe events (e.g., live vaccine stress in poultry, pets stressed by separation, fireworks, kenneling during holiday periods, etc).	The approval from the ethical committee is considered a mandatory requirement for any animal trial submitted. The relevance of the study design to support the efficacy of the additive is defined in the current Guidance and is not linked to the above approval.
			Page 21, Lines 644 to 651. Ethical committees & local authorities can decide where challenge studies are acceptable, & EFSA should accept such derogations from full compliance with EU animal welfare legislation.	In principle, if the study design is considered acceptable by a relevant ethical committee, the studies will be assessed by the FEEDAP Panel. However, the Panel will assess each application on a case-by-case basis, considering the additive function, conditions of use, animal species and category.
108	5 In vivo efficacy studies	Pen & Tec Consulting, SLU trading as Argenta ®	633-634 No single design is recommended; flexibility is provided to allow for scientific discretion in the design and conduct of the studies. Comment: Little flexibility has been observed from EFSA when applicants are proposing their study designs. In the case of microorganisms and enzymes, the effects can vary from each CRO, therefore, the use of mild stressors can simulate the real farming conditions without affecting the animal welfare.	Comment noted.
			636-637 The trials should be conducted such that their health and husbandry conditions do not adversely affect the interpretation of the results.	See reply to #107.

			Comments: Challenge studies cannot be obliged to adhere fully to EU health, welfare & hygiene legislation, but must be accepted as models that are used to demonstrate potential benefits of feed additives, especially when animals may be subjected, under commercial EU farming conditions, to adverse physiological or environmental conditions (e.g. wet litter, power failures leading to excess heat or cold, or automatic feed delivery failures.) A challenge study is a model that uses small scale simulations of certain adverse conditions that may be experienced by animals, at certain times under commercial production (e.g. live vaccine stress, pets stressed by separation/fireworks/kennelling during holiday periods, etc).	
			643-650 "Studies involving animals should respect the rules on animal welfare laid down by the European Union (EU) legislation, particularly those listed in Directive 63/2010/EU. For that purpose, the approval by a competent authority or independent ethical committee, clearly declaring compliance with the animal welfare requirements under Directive 63/2010, should be documented (also for studies conducted outside the EU). It is noted that, according to Directive 63/2010/EC, when the aim of the project requires that the animals are kept under conditions similar to those commonly observed in commercial farms, the husbandry conditions should meet the requirements of Directive 98/58/EC as well as, where relevant, the species-specific legislation". Comments: We believe that a clear definition of what will be understood by "independent" ethical committee is needed for clarity, in terms of dependency and relationship with the entity conducting the trial. For example, is an "animal-welfare body" (as defined by Directive 63/2010/EU), or an "Institutional Animal Care and Use Committee? (IACUC – as recommended by AAALAC International), convened by a research organisation with the specific purpose of ensuring compliance with animal welfare requirements considered within the definition of "independent"? We would suggest mentioning them as an acceptable entity issuing certificates of approval for study protocols.	See reply to #106.
			645-647 Approval by a competent authority or independent ethical committee, clearly declaring compliance with the animal welfare requirements under Directive 63/2010, should be documented (also for studies conducted outside the EU). Comments: Ethical committees & local authorities can decide where challenge studies are acceptable, & EFSA should accept such derogations from full compliance with EU animal welfare legislation.	See reply to #107.

109	5 In vivo efficacy studies	Not Applicable (Submission on Personal Capacity)	426 For additives affecting animal production or the performance of animals, long-term efficacy studies 427 should be provided unless the use of the additive/active substance is restricted to specific short 428 term periods for which particular provisions apply (see Section 4.2.2.1) Comment: The reference is erroneous, it should be 5.2.2.1	The text was modified to address the comment.
			785 Short-term studies are defined as studies with duration shorter than the minimum duration given 786 in Section 4.2.2.1. Comment: The reference is erroneous, it should be 5.2.2.1	The text was modified to address the comment.
			710 Specific endpoints will depend on the nature of the additive and its intended effects. More 711 information can be found in Section 2 and Section 4.2.2.2 below. Comment: The reference is erroneous, it should be Section 3 and Section 5.2.2.2.	The text was modified to address the comment.
			In general, the hyperlinks have to be checked throughout the whole document.	The hyperlinks have been reviewed throughout the document and updated or removed accordingly.
			2) Question on the Table 6. What was the basis for the choice of the minimum daily milk production of cows in the efficacy trials (>30 kg/day)? This limitation is not mentioned in the Regulation 429/2008. Average milk production in the EU is around 26 kg/day (Eurostat). Isn't it too restrictive based on the EU stats?	The minimum daily milk production, as reflected in Table 6, refers to the production at the start of the trial, not to the average of the whole experiment. This minimum requirement aims to ensure that the trial covers the most productive period of the milk cycle. If the additive is not intended to be effective during the whole cycle, the experimental design would need to be justified according to the additive function and its conditions of use.
110	5 In vivo efficac	AFCA-CIAL	p. 19 Line 636-641 : This does not seem to be in line with Regulation 429/2008 which says: "trials shall ideally be compliant with the criteria established by a recognised, externally-audited, quality assurance scheme. In the absence of such a scheme, evidence shall be provided to show that the work was done by	See reply to #105.

	y studies		qualified personnel using appropriate facilities and equipment and responsible to a named study director. - In order to align with the guidelines, we would appreciate if the text could be modified to avoid that the EFSA guidance would be more stringent than the regulation.	
			- Could it be possible to provide examples of the evidence that are expected by the EFSA to demonstrate that the study has been supervised by qualified personnel? In addition to the name of the study director	See reply to #105.
			p. 19 Line 644-646 : What would be considered as an independent ethical committee? Would any ethical committee nominated in a research facility or in a private company/industry qualify as being independent ?	See reply to #106.
			p. 19 Line 648-650 : What about studies in pets that may be performed in breeding facilities or with privately owned dogs or cats ? For some species, animals have to be kept in group and for such animals, eg cats (kept by two for welfare reasons), it is not possible to record individual feed intake. How could this be taken into consideration for the assessment and would such studies be accepted when it is not possible to have enough collective pens for statistical purpose: in short, would a study with cats kept by pair be acceptable when feed intake is recorded at the pen level and not per cat ?	See reply to #105.
111	5 In vivo efficacy studies	Chr. Hansen A/S	Lines 653-654, page 20 - Efficacy studies should be based on the additive(s) for which the application is made. Comments: Suggest to keep the following sentence from the present version of the guidance: "Any deviations because of practical or other considerations should be justified"	The text was modified to address the comment.
			Lines 667-671, page 20-21 - In ruminants…based on the daily ration. Comment: From the text it is not clear how to calculate. It is important that we also in ruminants can extrapolate from feed to water/milk. Measuring water intake is not easy per cow.	Considering the high variability in the water consumption of ruminants, it is not possible to provide a fixed feed-to-water ratio. The applicant can propose a ratio based on the nature and the conditions of use of the additive.
			Lines 691-692, page 21 - In production animals, this includes the state allowing optimal productivity. Comment: How is optimal productivity defined? Productivity in line with commercial levels and/or breeders' recommendations?	Performance standards of breeder companies are considered a good indicator of optimal productivity.

			Lines 693-694, page 21 The health and welfare status of the animals should be monitored by a veterinarian at the beginning and throughout the whole duration of the experiment. Comment: "…animals should be monitored by a veterinarian…" Not needed to have a veterinarian to evaluate health and welfare throughout (daily or how often?) the whole duration of the study. Proposal: Please consider changing to "…. Animals should be monitored by a trained animal caretaker, and a vet should be involved if any disease/sick animals as relevant."	The Panel considered that animal health and welfare can only be certified by a veterinarian, who is responsible for ensuring that proper monitoring of the animals during the trial is in place.
			Lines 699-701, page 21 - "..including the number of animals treated, duration of the treatment, distribution between experimental groups and severity of the disease." Comment: Important also to take the full study duration into account, see suggested update of the text. Proposal: Please consider these additions: "..including the number of animals treated, duration of the treatment, distribution between experimental groups, the study duration and severity of the disease. Treatment days out of days at risk will be part of the assessment of the acceptability of the studies."	The duration of the veterinary treatments applied in relation to the study length is already considered during the assessment.
			5.2 Typology of in vivo studies Line 786, page 23 Comment: Typo in the link: Section 4.2.2.1. Proposal: Change to Section 5.2.2.1.	The text was modified to address the comment.
112	5.1.1 Test item	FEFANA asbl	Line 653 "Efficacy studies should be based on the additive(s) for which the application is made.": We notice that a sentence is missing compared to current guidance and we consider it should be also included in this revised version. Please consider adding the sentence: Any deviations because of practical or other considerations should be justified.	See reply to #111.
113	5.1.2 Route of delivery	FEFANA asbl	Line 661 "use in feed and water. Therefore, studies can be made in either feed, water, or a mixture of both,": Please add Water for drinking.	The editorial change was not considered appropriate.
			Lines 667-671 "In ruminants and horses, concentrations of an additive cannot be consistently extrapolated from feed to water using a fixed ratio of feed-to-water	See reply to #111.

			intake. However, these concentrations can be converted to daily amounts and equally administered via feed or water. Consequently, the conversion of feed concentration to water concentration should be done based on the daily ration": The text lacks clarity on the calculation method. It is important that we can extrapolate from feed to water/milk in ruminants. Measuring water intake per cow is a challenging task. We propose providing additional explanations to enhance comprehension.	
			Line 672-673 "The concentration of the active substance(s)/agent(s) in the feedingstuffs/water should be confirmed by analysis.": For clarity, please consider using the term "feed or compound/complete feed, as relevant".	The text was modified to address the comment.
			Line 686-687 "Additional groups with the additive supplemented at different levels or a positive control may be included, as appropriate.": We suggest adding after line 687 the following sentence to facilitate novel research approaches that go beyond the classical comparison control-treatment group: When duly substantiated, the study design may replace the normal control with a positive control that contains a higher level of the nutrient that the additive intends to (partially) replace or which absorption it intends to increase. Efficacy of the additive is then defined by maintaining the body reserves of the nutrient despite a lower level in the feed.	The study design should be appropriately justified in relation to the intended effect of the additive. The Panel will evaluate each application on a case-by-case basis.
114	5.1.2 Route of delivery	Federal Office of Consumer Protection and Food Safety	Page 19 L 657 Please add "of administration": Use of the additive in efficacy studies should respect the proposed conditions of use (e.g. use level, route of administration, number of administrations, duration of administration). Page 20 L 671 Please add "or the daily water intake": Consequently, the conversion of feed concentration to water concentration should be done on the basis of the daily ration and the daily water intake. Page 20 LL 671-673 Please note that by administration of the additive via water for drinking and to make these calculations, you need clear knowledge of the water intake!	The text was modified to address the comment. The text was modified to address the comment. The text was modified to address the comment.
115	5.1.2 Route of delivery	AFCA-CIAL	p. 19 Line 661 : replace "water" by "Water FOR DRINKING"	See reply to #113.

			p. 20 Line 672-673 : For clarity, the term "feed or compound/complete feed, as relevant" should be used.	The text was modified to address the comment.
116	5.1.3 Experimental groups	Federal Office of Consumer Protection and Food Safety	Page 20 after L 687 Please add the following sentences: A helpful publication on setting up bioavailability studies can be found here: Brugger, Daniel, et al. "Bioavailability of trace elements in farm animals: Definition and practical considerations for improved assessment of efficacy and safety." animal 16.8 (2022): 100598. Link: https://www.sciencedirect.com/science/article/pii/S1751731122001513	The Panel acknowledges the relevance of the publication. However, it was not considered necessary to add any reference in this context to the current document.
117	5.1.3 Experimental groups	Pen & Tec Consulting, SLU trading as Argenta ®	679-680 Feed of the control group should contain the nutrient at concentrations marginally below the animals? requirements. Comment: "marginally below": could you please indicate an acceptable range and/or percentage?	The concept of "marginally below" intends to allow the basal diet to be formulated so that a physiological effect of the supplementation with the additive can be expected. Severely deficient diets which would compromise animal health status/welfare should be avoided. The text was modified to improve the clarity.
118	5.1.4 Animals	FEFANA asbl	Line 689-692 "Animals used should be healthy and preferably from a homogeneous group. For the purpose of this Guidance, health is considered the absence of disease, which allows normal functioning and behaviour of the animal. In production animals, this includes the state allowing optimal productivity.": Could it be clarified if/that the healthy status/absence of disease should be confirmed "at enrolment/start of the studies"? Meaning that a study would remain acceptable as long as mortality/morbidity observed during the study allows for statistical evaluation? For example, if an animal shows a gut disorder leading to a nutritional diarrhoea in calves or linked to SARA signals in cows, would that be acceptable to show that at the time of the study, the animals were performing on average compared to the farm performance indicators, which would demonstrate a "normal functioning"/healthy status? OR could the study be considered as not acceptable?	The animals involved in the trial should be healthy at the start of the trial. The relevance of any morbidity/mortality recorded during the trial would be assessed on a case-by-case basis according to standard farming practices. The health and husbandry conditions of the animals involved should not adversely affect the interpretation of the results.
			Lines 691-692: It is understood that health is considered the absence of disease which allows normal functioning and behaviour of the animal. However, the formulation "in production animals this includes the state allowing optimal productivity" is problematic since "optimal" can be interpreted to imply "maximum" productivity. An animal maximum productivity is a) difficult to obtain	The text was modified to address the comment.

			under practical circumstances and b) unlikely to see any improvement of productivity by an additive. It would therefore be recommended to delete the sentence or to further elucidate its meaning. Please delete "In production animals this includes the state allowing optimal productivity". Alternatively, please clarify how this sentence has to be interpreted and applied in practice, especially when considering that certain additives that particularly show a good effect under practical/commercial situations would possibly not make it to an authorisation due to inconclusive efficacy data (e.g., physiological condition stabilisers would prevent a challenge from occurring in the treated group, but not in the control group, thus implying that animals were not able to reach optimal productivity and creating reasons to reject the trial) A possibility would be: "this includes the state allowing a productivity level in line with accepted commercial standards".	
			Line 693-694 "The health and welfare status of the animals should be monitored by a veterinarian at the beginning and throughout the whole duration of the experiment.": It is unclear exactly how a veterinarian should monitor the animals "throughout the whole duration of the experiment". The trial should still simulate normal production circumstances in the EU where animals are not monitored by a veterinarian at all times. It should be clarified whether the regular monitoring can be handled by the daily caretaker. Please consider the following proposal: The health and welfare status of the animals should be monitored and documented by the caretaker throughout the study. With signs of illness a veterinarian should be consulted.	See reply to #111.
			Line 695-696 "Routine vaccinations across all groups are acceptable, but preventive treatments with antibiotics/antimicrobials before the start of the trial should be avoided.": The sentence says: "should be avoided". For clarity and better assistance to applicants, revising the sentence may help as it does not make clear if this can be accepted if properly justified, and in exceptional cases, to make preventive treatments with antibiotics/antimicrobials? It would be very much appreciated if EFSA could clarify if a study where such treatments have been made can be accepted or not. Or it should be specified that such preventive treatments would render the study not acceptable. Actually, the term "avoided" may create confusion.	The applicants may provide justifications for the need to apply preventive treatments with antibiotics/antimicrobials to the animals before the start of the trial; these situations will be assessed on a case-by-case. However, as a general rule, these treatments are not considered acceptable and will likely imply the rejection of the study.

			Lines 699-701 "including the number of animals treated, duration of the treatment, distribution between experimental groups and severity of the disease.": It is also important to take the study duration into account. Please consider the following additions: including the number of animals treated, duration of the treatment, distribution between experimental groups, the study duration and severity of the disease. Treatment days out of days at risk will be part of the assessment of the acceptability of the studies.	See reply to #111.
			Lines 702-703 "an abnormally high mortality rate (i.e., above current European commercial production standards) will not be accepted.": Article 7(6) of Regulation (EC) No 1831/2003 states that "The Authority shall publish detailed guidance to assist the applicant in the preparation and the presentation of its application" and we consider that the guidance is not responding to the regulatory principles regarding the following aspects: 1. For the sake of clarity and transparency, it is essential to provide information on the referenced "European commercial production standards." The current mention lacks any kind of additional guidance, leaving applicants without clear indications for assessing the acceptability of a study. The guidance should explicitly specify the standards used as a reference by FEEDAP during the efficacy assessment.	It is the responsibility of the applicant to provide evidence that the performance and mortality reached by the animals are in line with current European commercial production standards. These situations will be assessed on a case-by-case.
			2. Mortality rate is a parameter that may be affected by certain husbandry practices that cannot be used in studies. E.g., a slight feed restriction, or a selection for early slaughter of 20% of the broilers may help prevent part of the broiler mortality. Or a group antibiotic treatment may be done at an early stage of an infection in a flock, while in research this can only be done on individual animals. It is therefore recommended to apply a certain upper tolerance to commercial mortality standards per species. This could be introduced in the form of range values and not as a strict threshold, allowing applicants to deviate of duly justified.	See the reply to the comment above.
			3. Despite the lack of acceptable ranges, it is understood that abnormally high mortality rates will not be accepted. However, we consider that additional aspects should be mentioned. Experience teaches, that even studies in which chickens performed lower than expected (e.g. final body weight of 70% of breeding standard's maximum) will not be considered as adequate to conclude on the efficacy of a FA. This needs to be mentioned in this guidance, together with a clear and scientifically justified cut-off, since this may rule out some trial facilities for certain trial setups. In line with the 3R principle, it is necessary for	It is challenging to define a specific threshold that applies to all species and categories. The acceptability of studies that substantially deviate from European commercial standards will be assessed on a case-by-case basis. It is the responsibility of the applicant to provide evidence that the performance and

			applicants to compare the expected performance in a trial facility under given circumstances to an acceptance criterium since trials will otherwise be run in vain and animals' lives, as well as lots of effort, time and money will be wasted. We would appreciate the addition of clear and justified rejection criteria for performance characteristics such as final body weight or any other rejection criteria that might internally already be handled.	mortality reached by the animals are in line with current European commercial production standards.
			Line 705: Link should refer to section 5.2.2.1 instead of 4.2.2.1- the link works but numbering still as in current guidance. Please adapt link.	The text was modified to address the comment.
119	5.1.4 Animals	Association of Veterinary Consultants (AVC)	Page 22, Line 693 to 694 Challenge studies must be excepted. from full compliance with animal welfare regulations since the study design may involve exposure to undesirable micro-organisms or to pathogens.	See reply to #107.
			Page 23, Lines 702 to 704. Challenge studies must be excepted, since the experimental models may involve higher mortality due to the challenge conditions.	See reply to #107.
120	5.1.4 Animals	Federal Office of Consumer Protection and Food Safety	Page 20 L 705 Please replace "Section 4.2.2.1" by "Section 5.2.2.1".	The text was modified to address the comment.
121	5.1.4 Animals	Pen & Tec Consulting, SLU trading as Argenta ®	691-694 In production animals, this includes the state allowing optimal productivity. Housing and husbandry conditions should be adequate for the study design and conform to animal welfare regulations. The health and welfare status of the animals should be monitored by a veterinarian at the beginning and throughout the whole duration of the experiment. Comments: In several opinions, EFSA have referred to the "standards of the breed", rejecting in some cases studies on the basis that the performance was below these standards. The breed standards data are produced by commercial companies and generated in optimal conditions as a marketing tool to show the	The breed standards are reference values considered by the FEEDAP Panel to ensure that the rearing conditions applied in the trial are similar and comparable to common farming practices within the EU and that animals are in adequate conditions of health and welfare. It is acknowledged that the maximum values cannot always be reached; for that reason, each study is

			genetic potential of the breed (reported as "performance targets"). These conditions do not represent all the management conditions and factors in the field/farms, and the actual animal performance will depend on a variety of factors, including among others: country, housing, diet, environmental factors and animals. Most of the standards supplements acknowledge this; some even recommend to "contact the local technical representative to help develop a program designed specifically to suit the local conditions". Furthermore, these standards may not be generated in compliance with EFSA efficacy guidance (conditions, replicates, reporting, availability of raw data, transparency etc.). Hence, we believe that EFSA should not use these data to assess the validity of studies. Studies performed in compliance with EFSA guidance (e.g. housing, husbandry conditions etc.) were all animals (including the controls) are healthy should be accepted.	considered on a case-by-case, and certain flexibility is applied depending on the overall outcome. The applicant may provide alternative evidence that the performance reached by the animals is in line with current European commercial production standards.
			705- detailed in Section 4.2.2.1. Comment: The link directs to Section 5.2.2.1	The text was modified to address the comment.
122	5.1.4 Animals	AFCA-CIAL	p. 20 Line 689-692 : Does "absence of disease" mean that microbial challenge at level not inducing disease and a higher significative mortality in the exposed group(s) will be considered as non-acceptable by EFSA? Could it be clarified if/that the healthy status/absence of disease should be confirmed "at enrolment/start of the studies"? Meaning that a study would remain acceptable as long as mortality/morbidity observed during the study allows for statistical evaluation? For example, if an animal shows a gut disorder leading to a nutritional diarrhoea in calves or linked to SARA signals in cows, would that be acceptable to show that at the time of the study, the animals were performing on average compared to the farm performance indicators, which would demonstrate a "normal functioning"/healthy status? OR could the study be considered as not acceptable ?	See reply to #118.
			p. 20 Line 695-696 : The sentence says: "should be avoided". For clarity and better assistance to applicants, revising the sentence may help as it does not make clear if this can be accepted if properly justified, and in exceptional cases, to make preventive treatments with antibiotics/antimicrobials? It would be very much appreciated if FEEDAP could clarify if a study where such treatments have been made can be accepted or not. Or it should be specified that such preventive treatments would render the study not acceptable. Actually the term "avoided" may create confusion.	See reply to #118.

			p. 20 Line 702-703 : For clarity and transparency, it is of prime importance to have information about those European Commercial Standards: what are the standards used as reference by the FEEDAP ? Without this information, it is rather difficult to assess if a study is acceptable or not and making reference in the guidance to "European commercial production standards" (without any other indication) does not provide the assistance to applicants as required by Regulation 1831/2003	See reply to #118.
			p. 20 Line 705 : replace section 4.2.2.1 by Section 5.2.2.1	The text was modified to address the comment.
123	5.1.5 General endpoints	Federal Office of Consumer Protection and Food Safety	Page 21 L 711 Please replace "Section 2" by "Section 3" and "Section 4.2.2.2" by "Section 5.2.2.2".	The text was modified to address the comment.
124	5.1.5 General endpoints	Pen & Tec Consulting, SLU trading as Argenta ®	Line 711. information can be found in Section 2 and Section 4.2.2.2 below. Comment: Links direct to sections 3 and 5.2.2.2	The text was modified to address the comment.
125	5.1.5 General endpoints	FEFANA asbl	Line 707-709 "For all in vivo studies, the following parameters should be reported: clinical observations including general health status, morbidity/mortality, feed intake and water intake for those additives administered via water, initial and final body weight, and milk/egg production (as appropriate).": We believe that some of the required parameters are not applicable to all in vivo studies. For example, it is not possible to record body weight in bees. In this case, the weight of the hive may be an indicator but would suffer significant bias. What about other production endpoints such as honey?	The text was modified to address the comment. Regarding the body weight of bees, the Guidance aims to encompass the most usual situations within animal production; the deviation from the requirements, if scientifically justified, will be duly evaluated by the Panel.
			Line 711 : Link should refer to section 5.2.2.2 instead of 4.2.2.2- the link works but numbering still as in current guidance. Also Section 2 should be replaced by Section 3. Please adapt link	The text was modified to address the comment.

126	5.1.5 General endpoints	AFCA-CIAL	p. 20 Line 707-709 : What about other production end-points such as honey ? We believe that some of the required parameters are not applicable to all in vivo studies. For example, it is not possible to record body weight in bees. In this case, the weight of the hive may be an indicator but would suffer significant bias.	See reply to #125.
			p. 21 Line 711 : Replace section 4.2.2.2 by Section 5.2.2.2	The text was modified to address the comment.
127	5.1.6 Statistical considerations	FEFANA asbl	Lines 714-718 "The experimental unit is the smallest entity to which a given treatment is applied. If animals are housed in groups (e.g., pen, cage) and all the animals in the pen share the same feed source (and feed intake is not measured individually), then the experimental unit for all parameters is the pen, not the individual animal. The experimental unit would be the individual animal when individual feed intake is registered.": For design of experiments, it could be argued to use animal as statistical unit if the main readout is bodyweight even though feed intake is recorded on pen level. This could contribute to minimizing the number of animals used in the studies and increase power through individual body weight registration. Please consider the following proposal: The experimental unit is the smallest entity to which a given treatment is applied. If animals are housed in groups (e.g., pen, cage) and all the animals in the pen share the same feed source (and feed intake is not measured individually), then the experimental unit for all parameters is the pen, not individual animal. The experimental unit would be the individual animal when individual feed intake is registered. If body weight is the main read-out then animal can be used as statistical unit if all animals are measured individually by individually identification.	The experimental unit is the smallest entity to which a given treatment is applied. If animals are housed in groups (e.g., pen, cage) and all the animals in the pen share the same feed source (and feed intake is not measured individually), then the experimental unit for all parameters is the pen, not the individual animal.
			Line 722 "should be the same for the various groups, including housing, husbandry, and diet/water": As elsewhere, it is considered appropriate to refer to feed/compound feed, as appropriate, instead of Diet.	The text was modified to address the comment and harmonised throughout the document.
			Line 730-731 "These factors might include initial body weight, sex, age, stage of lactation, milk yield, parity, and egg production.": What about honey production? Could this be included?	These factors represent a non-exhaustive list that might be considered for stratification.
			Line 750 : There is a numbering error. It should read v) instead of iv)	The text was modified to address the comment.
			Lines 754-757 "As a guide, a power greater than or equal to 80% (75% for ruminants, minor species, pets and non-food-producing animals) should be ensured. Generally, when testing differences, a confidence level of 90% is	One of the aims of this Guidance is to move further within the 3Rs approach. For that purpose, when animal studies

			adopted for ruminants, minor species, pets, and non-food-producing animals and 95% for all other animal species and categories.": In the light of stronger enforcement of animal welfare rules and the 3R policy; and considering that the statistical power of 80% (75% for ruminants etc.) is a guide rather than a hard limit, does this mean that a statistical power lower than 80% (75% for ruminants etc.) could be accepted in cases where animal welfare would otherwise be compromised because of large numbers of animals used? If this is the case, please add this information to line 755.	are considered necessary, "refinement" and "reduction" are aspects to be considered in the experimental design. For instance, good welfare increases uniformity among experimental subjects, leading to the need for smaller sample sizes; for ensuring "reduction", the optimisation of the sample size is essential, meaning to clearly defining the objectives of the study, reducing the variability and ensuring sufficient power/statistical significance to detect the differences considered relevant.
			Line 765-767 "Under certain conditions, a log or other transformations can be needed to linearise the relationship with the explanatory factors": Please provide examples of parameters where such log or other transformations may be needed	For example, the oocysts count in studies assessing the efficacy of coccidiostats.
			Line 775-776 "factor means. Independently from the outcome of normality tests, non-parametric tests should be used when only a small sample size is available and/or there is evidence of outliers and/or": Please indicate what is meant by small sample size.	The Panel considers that testing the normality assumptions with small sample sizes lacks sufficient power to reject normality even in case of substantial deviation from the normal distribution. Therefore, in those situations or in case of doubt, the use of non-parametric testing is always a valid alternative.
			Line 781-782 "Pooling data from different studies of comparable design may substitute for a single efficacy study provided that the interaction treatment x study is not significant": Please provide minimum number of studies for pooling data to provide a single efficacy study. Furthermore, we observe a lack of specification regarding the recommended statistical tests for assessing significance and the criteria to be applied in the evaluation process. It seems that EFSA is requesting the use of the same significance criteria outlined in Section 5.1.6.2 'Sample size.' To promote clarity for applicants and prevent complications during the assessment process, we propose an inclusion in this section of the suggested statistical tests and criteria	It may be possible to pool data from different independent studies of comparable design with or without significant differences to substitute for a single efficacy study. It is up to the applicant to propose the number of studies to be pooled, as far as the interaction diet × study is not significant (the significance level considered will be that referred to in 5.1.6.2)."

			for determining the statistical significance of the interaction between treatment and study in pooled data.	
128	5.1.6 Statistical considerations	Association of Veterinary Consultants (AVC)	Page 23 Line 714 to 718. Design of the experiment. Regardless of not having individual feed consumption data, for many parameters related to the efficacy of an additive, the statistical analyses of individual efficacy data can provide relevant data on desired efficacy. Examples: · Pigmentation of skin, feathers, egg yolk, (chickens, hens, fish, etc?): the study of the coefficient of variation of controls versus an added pigment · Individual serum vaccinal titres / antibody development: effect of an additive vs controls on the immune responses · Acute phase proteins in blood: effect of an additive versus controls on stress factors · Individual blood concentrations of antioxidants, effects of an additive versus controls	See reply to #127.
129	5.1.6 Statistical considerations	Pen & Tec Consulting, SLU trading as Argenta ®	714 - 718 The experimental unit is the smallest entity to which a given treatment is applied. If animals are housed in groups (e.g., pen, cage) and all the animals in the pen share the same feed source (and feed intake is not measured individually), then the experimental unit for all parameters is the pen, not the individual animal. The experimental unit would be the individual animal when individual feed intake is registered. Comments: Based on the definition "The experimental unit is the smallest entity to which a given treatment is applied", even if animals are grouped in pens, the smallest entity to which the feed (“treatment”) is supplied is the individual animal. In line with this definition, peer reviewed journals accept that the experimental unit is the individual animal when the endpoints (e.g. zootechnical performance or other endpoints as relevant) are measured in the individual animals. Significant differences in feed intake would be directly reflected in differences in weight and/or performance of the animals. It would benefit animal welfare (the 3Rs principle aiming to reduce the use of animals in research) if EFSA consider adopting the interpretation of the peer reviewed journals.	See reply to #127.
130	5.1.6 Statistical considerations	Not Applicable (Submission on Personal	Lines 781-782. The description of the pooling of data is too vague. A more detailed approach would be suitable, including which statistical tests are required/recommended for pooling data from different studies, as well as the criteria to be used when testing for the significance of the interaction treatment x study. If applicable, also include clear indications on which of the statistical considerations included in this section are applicable to the pooling of data. Discussion of other specific situations such as: if EU and third country studies are	See reply to #127.

		Capacity)	pooled, EFSA will consider that the pooled data is from a (new?) site located in the EU/a thrid country/both? This clarification could be critical to meet the requirements set out in section 3.4. Thanks!	
131	5.1.6 Statistical considerations	AFCA-CIAL	p. 21 Line 722 : What about honey production – As elsewhere, it is considered appropriate to refer to feed/compound feed, as appropriate, instead of Diet.	See reply to #127.
			p. 21 Line 730 : What about honey production ?	See reply to #127.
			p. 22 Line 766 : Provide examples of parameters where such log or other transformations may be needed	See reply to #127.
			p. 22 Line 775-776 : What is meant by small sample size ?	See reply to #127.
			p. 22 Line 781 : Could EFSA provide recommendations in terms of the minimum number of studies for pooling data (ie meta-analysis) to provide a single efficacy study	See reply to #127.
132	5.2.1 Short term efficacy studies	FEDIAF European Pet Food Association	Lines 828-829 (page 23) FEDIAF would like to emphasize that the two diets should be essentially equal in composition, with the only difference being the presence of the additive in the test diet at the proposed inclusion rate (analytically confirmed). Therefore, FEDIAF would like to request that inclusion rate be confirmed analytically or qualitatively (i.e., by good manufacturing practice (GMP)). There can be significant technical challenges to the development of analytical methods for certain feed additives. For example, if the additive is coming from a natural source other ingredients may provide similar molecules meaning a specific method of analysis cannot be developed. Regulation 429-2008 has a requirement to provide "Methods of analysis for the active substance". It states "Detailed characterisation of the qualitative and, where applicable, quantitative analytical method(s) for determining compliance with maximum or minimum proposed levels of the active substance(s)/agent(s) in the additive, premixtures, feedingstuffs and, when appropriate, water, shall be provided". Based on Regulation 429-2008 qualitative confirmation of the inclusion of the additive (i.e. through GMP) should be an acceptable alternative to an analytical confirmation where justified.	In general, the concentration of the active substance(s) or agent(s) in the feeds to which the additive is added should be periodically analysed and reported to confirm the inclusion rate. Deviations to this approach should be duly justified.
133	5.2.1 Short	FEFANA asbl	Line 786 : Reference to Section 4.2.2.1 should be replaced to section 5.2.2.1.	The was modified to address the comment.

	term efficacy studies			
			Lines 803-804: As mentioned for section 3.4.1, balance studies request to keep animals in balance crates which - even though assure scientifically more accurate sampling - is constantly criticised by ethical committees. Balance studies even though scientifically preferred can be replaced by true or apparent ileal or total tract digestibility studies. We would appreciate if this could be considered and the text modified accordingly.	The Panel considers that balance trials are the most accurate way to assess the retention of nutrients in the animals. Deviations from this requirement should be duly justified and considered on a case by case.
			Line 816-817: In many cases an increased nutrient availability affects the output in depleted diets but is otherwise excreted. Therefore, in some cases analysis of the output should not be necessary (except for nutritional supplements) and balance studies again to be questioned. To avoid that efficiency demonstrated by improved digestibility is not discredited just because e.g. eggs were not analysed. We propose the following modification: For additives where the output of layers, sows or cows can be influenced (e.g. eggs, milk, litter, products of conception) additional analyses of those outputs can be used as evidence for the efficacy.	The analysis of the output in this type of studies is considered relevant by the Panel. The text was modified to improve the clarity.
			Line 817-818: For applications in gestating and lactating sows, digestibility studies should be performed in both gestating and lactating sows. From the text it is unclear if the same sows can be used in gestation and lactation. We suggest the following addition: For applications in gestating and lactating sows, digestibility studies should be performed in both gestating and lactating sows (the same or different sows can be used).	The requirement refers to the fact that digestibility should be assessed both during the gestation and the lactation phase, which could be performed with the same or different sows.
			Line 819 : Please consider adding crustaceans into this paragraph. Additionally, we request detailed clarifications on the study design for both fish and crustaceans, including the minimum pre-period and collection period for retention studies.	The text was modified to address the comment.
134	5.2.1 Short term efficac	Federal Office of Consumer Protectio	Page 22 L 786 Please replace "Section 4.2.2.1" by "Section 5.2.2.1"	The was modified to address the comment.

	y studies	n and Food Safety		
			Page 22 LL 791-792 It is noted that the definition of bioavailability should be reconsidered., see Germany´s proposal for addition of literature on page 20 after L 687	See reply to #116.
			Page 23 LL 803-805 It is noted, that it is not clear why fistulization is generally rejected on the basis of animal welfare considerations. In our experience, the use of fistulated animals is much gentler on the animals than long or repeated periods in metabolic cages.	The text has been modified to address the comment. However, EFSA does not encourage the use of invasive procedures in animals when alternative methods are available.
			Page 23 LL807- 808 Please replace : "3 days" by "7-10 days": The minimum duration of this pre-period depends on the species: 7-10 days for poultry, pigs, dogs and cats, 14 days for ruminants and 7 days for Equidae. Germany wants to point out, that the minimum duration of the pre-period for adaptation to the diet (and experimental conditions) is too short for poultry, pigs, dogs and cats. In publications, the usual duration of such a period is 7 to 10 days. In addition to "emptying" the gastrointestinal tract of the previous feed, the digestive enzymes and intestinal microbiome also need sufficient time to adapt.	The text was modified to address the comment.
			Page 23 L 808-810 Please change the duration for poultry, dogs and cats into at least 5 days, for ruminants 7-10 days: For studies using the total collection method of faeces/urine/excreta, the duration of the collection should be 5 days in poultry, dogs and cats, 4-6 days in pigs and horses, 7-10 days in ruminants.	The text was modified to address the comment.
135	5.2.1 Short term efficacy studies	Pen & Tec Consulting, SLU trading as Argenta ®	786- in Section 4.2.2.1. Comment: Again links to section 5.2.2.1	The text was modified to address the comment.
			813 - 815 The use of a marker in the diet would avoid the need for quantitative collection of faeces; the same time given above for the pre-period and collection period should be retained. Comment: The authorisation of titanium dioxide as a feed additive for all animal species has been denied (e.g. Regulation 2021/2090). Please confirm if efficacy	The Panel raised safety concerns about the use of titanium dioxide in animal feed. Therefore, the Panel considers it should not be used in animal studies.

			studies using titanium dioxide are accepted by EFSA for the assessment of efficacy.	
136	5.2.1 Short term efficacy studies	AFCA-CIAL	p. 22 Line 786 : replace section 4.2.2.1 by Section 5.2.2.1	The text was modified to address the comment.
137	5.2.2 Long term efficacy studies	FEFANA asbl	Line 838 in Table 6: Changes in the minimum age for some of the categories is appreciated but could be kept a bit wider as this sometimes helps to improve welfare if animals need to be adapted to new environments before the trial start. For instance, consider piglets: < 10 days after weaning. In addition, we see some of the information provided as unnecessary and suggest the following: - Pigs for fattening: delete until slaughter (the text not less than 70 days is fine enough). - Sows: for effects on lactation - until the end of the weaning period	The Panel considers there are no grounds to change the current requirements.
			Line 838, Table 6: "22-25 weeks of age" is <30 weeks of age. Thus "22-25 weeks of age" can be deleted	The text was modified to address the comment.
			Line 838 in Table 6: Could you please clarify if the minimum duration is meant to be the total duration of the study? The period during which the additive is fed? Or the period during which the end points are recorded?	The duration of the study is considered the period during which the additive is fed to the animals. This time is generally aligned with the recording of the endpoints.
			Line 838 in Table 6 dairy cow - Milk yield = 30 kg/d": Together with laying hens, dairy cows are the only species for which such threshold/minimum production level is defined. 30kg milk per day is not realistic for some high productive cows of certain breed (e.g., Jersey cows) or under common feeding practices in the EU. It is rather difficult for FEFANA to understand such requirements that are not in line with EU average milk production. It is also important to mention that the Regulation 429/2008 does not prescribe for a minimum performance level only for the demonstration of efficacy between control and treated group. And what about dairy ewes and goats, should a minimum milk yield be also achieved for a study to be accepted? The same applies to laying hens. For fairness across animal species/categories, no minimum performance threshold should be	This requirement aims to assess efficacy studies in high-producing animals; it is considered that demonstrating efficacy under these productive conditions would better allow extrapolation to other situations in which animals are in less demand. For other dairy animals (e.g., sheep and goats), there are no specific milk production requirements due to greater differences between individual breeds.

			established. We understand that in case of safety studies, working with high yielding animals may be relevant as they may be more sensitive but not in case of performance/efficacy studies.	
			Line 838 in Table 6 "other insects - Whole production cycle": It may not be realistic to perform studies during the whole production cycle as some insect species have a cycle longer than a year. We suggest an amendment of the text in order to consider this aspect.	Deviations from the generally established minimum duration of the studies should be justified.
			Line 838 in Table 6, page 26 "honey bees - 28 days": Similarly, as for the other insect species, to establish de facto a study duration of 28 days minimum is not appropriate and given the lack of expertise for such dossiers (only one application since 2003), it would be more appropriate to let applicants define the study duration depending on the targeted benefits. A duration of 21 days seems more appropriate.	Deviations from the generally established minimum duration of the studies should be justified.
			Line 838 in Table 6: We suggest adding crustaceans to Table 6	The text was modified to address the comment.
			Line 861 "dairy animals: milk production (including fat/energy corrected milk), feed efficiency, milk": Should ECM be calculated according to a specific formula as several formulas are proposed in the literature or as in the case of DM for silage, is it up to applicants to decide on the formula? We would appreciate clarifications on this aspect.	The methodology used for the calculation should be appropriate and duly referenced.
			Line 866 : Crustaceans should be added to the list including specific parameters.	The text was modified to address the comment.
			Line 882-886 "Endpoints for Environmental effects": In line with our comment on Line 443 (Section 3.4.2), the reference to indirect/direct effects goes beyond the scope of the regulatory framework for feed additives. Please refer to our previous comments and consider an amendment of this section too.	See answer to comment #60.
138	5.2.2 Long term efficacy studies	Biochem Zusatzstoffe Handels- und Produkti	Thanks a lot for providing the possibility to comment on the draft. I would like to leave a comment on one topic: Start of the study for laying hens and dairy cows. In both cases the new guidance foresees a certain performance level at the start of the study in order to ensure that high-performing animals are used in the study. Requesting the high performance level already at start of the study will make planning of the study start more complicated as animals may not reach the requested performance level within the expected time. So studies may have to	The Panel considers that the current requirements allow enough margin for appropriate planning of the studies.

		onsges. mbH	start with delay and thus will also end with delay. So it will become difficult or impossible to keep the planned and notified study dates. To avoid those problems around study planning and notification the guidance could ask for a certain performance level (min. 90% laying rate or min 30 kg/day milk yield) that has to be reached during the study. That would ensure that high-performing animals are used in the study, but would also allow to have a fix study start. Furthermore this approach would allow to obtain further information on potential effects of the additive (effect on time to reach peak production, duration of peak production).	
139	5.2.2 Long term efficacy studies	FEDIAF European Pet Food Association	Lines 887-889 (page 26) FEDIAF would like to request verified faecal consistency scoring by a standalone parameter for a demonstration of efficacy. Faecal consistency is a sign of pet food quality for pet owners. Small changes in dry matter content may not be relevant for them. There are faecal consistency testing protocols available which are widely used by the pet food industry.	The Panel recommends using objective measurements such as dry matter content of faeces. Subjective observations of faecal consistency alone are discouraged.
140	5.2.2 Long term efficacy studies	Erawan Consulting SARL	Line 838 Table 6 / Comment: I suggest adding "crustaceans" category	See reply to #137
			Line 838 Table 6 / Comment: In EU we have several types of breeds and the milk production at the pic vary from 16 Kg (Jersey breed) to 33 Kg (Holstein breed). We also have different feeding systems such as concentrate feed and pasture. In EU we have ca 20 million of cows with an average at the pic of 7300 Kg (305 days). With a such requirement we discard less intensive system based on forage or pasture. In addition, when performing trial <16 weeks, the genetics of the animals will be tested, not the feed additive. The restriction should be skip. The feeding system (intensive or extensive) and the breed should be taken into consideration. The best is to carry out efficacy trial in the 2nd part of lactation.	See reply to #109.
141	5.2.2 Long term efficacy studies	Association of Veterinary Consultants (AVC)	Page 26, Lines 838 to 839 Table 6: Minimum duration of long-term efficacy studies. In the case of laying hens, 84 days would not be necessary for some studies, taking into account the length of the ovarian cycle. Example: for a pigmentation test, 28 days would be enough to see the effect (14 days to stabilize the tissue reserves of the pigment additive & 14 more days to see the colouring effect in egg yolk). In the case of botanical additives with pathology control effects, & in efficacy tests with challenge infections or infestations, so	The possibility of performing short- or long-term studies would depend on the category of the additive as described in section 3. The experimental design and methodology used should be appropriate to the intended effects of the additive and must be justified according to the

			many days of study would not be necessary in all animal species. The effect/effectiveness can be measured in much shorter time frames.	additive function, conditions of use, animal species and category.
142	5.2.2 Long term efficacy studies	Federal Office of Consumer Protection and Food Safety	Page L838 Comments on Table 6: - Please keep the minimum duration of long-term efficacy studies according to Regulation EC No 429/2008 Annex IV – for laying hens 168 days, cattle for fattening 168 days, salmon and trout 90 days	Thank you for the comment. The current requirements established in Table 6 reflect the adequate minimum duration of the studies based on the experience.
			- Please decide to use in table 6 column "category" either for singular or plural and change it in all rows- we prefer the animals in plural.	The text was modified to address the comment.
			- Please add additional rows for o Chickens reared for laying 1 day of age 112 days (if the efficacy data are not available for chickens for fattening)	Thank you for the comment. The current requirements established in Table 6 reflect the adequate minimum duration of the studies based on the experience.
			- Calves for fattening <6 months 84 days	Thank you for the comment. The current requirements established in Table 6 reflect the adequate minimum duration of the studies based on the experience.
			- Row "Laying hens", column "Start of the Study": Please delete "22-30 weeks of age"	The text was modified to address the comment.
			- Row "Sows" column "Start of the study" please correct "no later" to "not later"	The text was modified to address the comment.
			- Please correct in column "Category" : Calves into Calves for rearing; Dairy ewe to Dairy sheep; and Rabbits (growing) to Rabbits	Thank you for the comment. The current requirements established in Table 6 reflect the adequate minimum duration of the studies based on the experience.
			- The German proposals for correction of Table 6 are provided in a separate document (Annex German Proposal for Table 6).	Thank you for the comment. The current requirements established in Table 6 reflect the adequate minimum duration of the studies based on the experience.

			Page 25 L 852 Please replace "(see Section 2)" by "(see Section 3)"	The text was modified to address the comment.
143	5.2.2 Long term efficacy studies	AFCA-CIAL	p. 24 table 6 -For laying hens, there are two different information that can be confusing regarding the age of animals: "<30 weeks of age [?] 22-25 weeks of age" proposal to suppress : 22-25 weeks of age	The text was modified to address the comment.
			-sow - For effects on reproduction: two full reproduction cycles : It is considered hardly realistic to perform such studies in existing CRO, one full cycle would be more realistic and in line with other major specie (it is understood that this requirement comes from 429/2008. Hence, a modification may require to be agreed by the EC/the member states.	The requirement for studying the effects in two full reproduction cycles is established to consider the potential compensation effects between litters.
			-dairy cow - Milk yield - 30 kg/d : Together with laying hens, dairy cows are the only species for which such threshold/minimum production level is defined. 30kg milk per day is not realistic for some high productive cows of certain breed (eg Jersey cows) or under common feeding practices in the EU. It is rather difficult for industry to understand such requirements that are not in line with EU average milk production. It is also important to mention that the Regulation 429/2008 does not prescribe for a minimum performance level only for the demonstration of efficacy between control and treated group. And what about dairy ewes and goats, should a minimum milk yield be also achieved for a study to be accepted ? The same applies to laying hens. For fairness across animal species/categories, no minimum performance threshold should be established. We understand that in case of safety studies, working with high yielding animals may be relevant as they may be more sensitive but not in case of performance/efficacy studies	See reply to #137.
			-other insects - Whole production cycle: This may not be realistic to perform studies during the whole production cycle as some insect species have a cycle longer than a year. Based on the specificity of those species and the lack of track records and applications, it should be up to the applicant to propose a study duration and justify it	See reply to #137.
			-honey bees - 28 days : Similarly as for the other insects species, to establish de facto a study duration of 28 days minimum is not appropriate and given the lack of expertise for such dossiers (only one application since 2003), it would be more	See reply to #137.

			appropriate to let applicants define the study duration depending on the targeted benefits. A duration of 21 days seems more appropriate.	
			Could EFSA provide recommendations in terms of minimum study duration for crustaceans to Table 6? Would 28 days be sufficient ?	See reply to #137.
			p. 25 Line 851 : replace “Section 2” by "section 3"	The text was modified address the comment.
			p. 25 Line 861 : Should ECM be calculated according to a specific formula as several formulas are proposed in the literature or as in the case of DM for silage, is it up to applicants to decide on the formula ?	See reply to #137.
			p. 25 Line 865 : Add crustaceans next to the fish for clarity.	See reply to #137.
			p. 26 Line 881-885 : The differentiation of direct and indirect effects may be not pertinent for this functional group as it is considered that feed additives applications and their assessment should focus on the primary function(s) of the additives and that indirect/secondary effects benefits of feed additives that are a consequence of the primary functions should not require pre-market authorisation. Proposal to remove : Indirect effects on the environment may result from increased nutrient utilization and reduced excretion of, e.g., nitrogen, phosphorus and sulphur, if appropriate dietary adjustments are made.	See reply to #60.
			p. 26 Line 891-892 : Not enough precise: could you please clarify by adding example (not a limitative list of examples) of endpoints.	The text was modified to address the comment.
144	5.2.2 Long term efficacy studies	FPS Public Health, Food Chain Safety and Environment	Table 6: Minimum duration of long-term efficacy studies The table of test durations is problematic because it disagrees with the table which appears in R429/2008. This regulation was voted as a compromise between the MS and must be considered representative of current practices in the different countries. For example, for dairy cows, Milk yield = 30 kg/d is not representative of milk production in all regions of the EU.	The current requirements established in Table 6 reflect the adequate minimum duration of the studies based on the experience of the Panel. The minimum milk yield indicated is at start and not the average of the whole production cycle.
145	5.3 Studies on the	Federal Office of Consumer	Page 26 L 899 Please replace “Section 4.2.2.2” by “Section 5.2.2.2”.	The text was modified to address the comment.

	quality of products when this is not the effect claimed	Protection and Food Safety		
146	5.3 Studies on the quality of products when this is not the effect claimed	Pen & Tec Consulting, SLU trading as Argenta ®	898-899 Appropriate end points may be found under Section 4.2.2.2. Again links to section 5.2.2.2	The text was modified to address the comment.
			898 "Omission of these studies should be adequately justified" - Text is absent Comment: 2018 guidance indicated that this justification was required. No equivalent statement in the new guidance. Is this intentional or an oversight?	The text was modified to address the comment.
147	5.3 Studies on the quality of products when this is not the effect	AFCA-CIAL	p. 26 Line 899 : replace "section 4.2.2.2" by "section 5.2.2.2"	The text was modified to address the comment.

	claimed			
148	5.3 Studies on the quality of products when this is not the effect claimed	FEFANA asbl	Line 899 : Link should refer to section 5.2.2.2 instead of 4.2.2.2- the link works but numbering still as in current guidance.	The text was modified to address the comment.
149	6 In vitro studies	FEDIAF European Pet Food Association	Lines 903-904 (page 26): FEDIAF would like to point out that the concentration of the active substance(s) or agent(s) in the feedingstuffs/water should be confirmed by analysis. FEDIAF requests for the inclusion rate to be confirmed analytically or qualitatively (i.e., by good manufacturing practice (GMP)). There can be significant technical challenges to the development of analytical methods for certain feed additives. For example, if the additive is coming from a natural source other ingredients may provide similar molecules meaning a specific method of analysis cannot be developed. Regulation 429-2008 has a requirement to provide "Methods of analysis for the active substance". It states "Detailed characterisation of the qualitative and, where applicable, quantitative analytical method(s) for determining compliance with maximum or minimum proposed levels of the active substance(s)/agent(s) in the additive, premixtures, feedingstuffs and, when appropriate, water, shall be provided?. Based on Regulation 429-2008 qualitative confirmation of the inclusion of the additive (i.e. through GMP) should be an acceptable alternative to an analytical confirmation where justified.	See reply to #132.
150	6 In vitro studies	FEFANA asbl	Line 900ff: Please could you share your recommendations about acceptable/scientifically recognized in vitro tests? Shouldn't the recommendations in this section be also applicable for other categories of additives?	The methodology used should be appropriate to the intended effects of the additive. The recommendations included in section 6 apply to all categories of additives requiring in vitro studies to demonstrate efficacy.

			Lines 906-908 "Studies should be designed to demonstrate the efficacy of the minimum use level(s) of the additive by targeting sensitive parameters compared to a control group.": We repeat our comment to line 258-261 (section 3.1.1.2) that multiple levels of preservatives or hygiene condition enhancers can be tested to be evaluated by regression analysis. Please consider the following amendment: Studies should be designed to demonstrate the efficacy of the minimum use level(s) of the additive by targeting sensitive parameters compared to a control group. For preservatives and hygiene condition enhancers, multiple use levels may be tested, and data can be analysed by regression techniques to arrive at a general conclusion on effectiveness.	The Guidance foresees that at least the minimum inclusion level proposed is tested. Apart from this, no single design is recommended, and flexibility being provided to allow for scientific discretion in the design and conduct of the studies.
			Lines 908-910 "The study should be designed to cover a representative range of materials to which the additive will be applied (feed materials, complete or complementary feed or water, depending on 908 the intended use).": If the recommendations for in vitro studies given in section 6 are also applied for enzymes, we suggest enlarging the list of the materials by adding purified substrates which can clearly demonstrate the mode of action of enzymes. Please consider the following addition: The study should be designed to cover a representative range of materials to which the additive will be applied (purified substrate, feed materials, complete or complementary feed or water, depending on the intended use).	For enzymes, the efficacy by in vitro methods should be demonstrated in the intended feed in which the additive is intended to be added, not in the purified substrate. See reply to #95.
			Line 921 "Evidence should be provided that?": What documents can be used as evidence? For clarity, please add examples of documents.	See reply to #105.
151	7 Reporting of efficacy studies	FEDIAF European Pet Food Association	Lines 943 - 945 (page 30) FEDIAF believes that a clear definition of what will be understood by "independent" ethical committee is needed for clarity, in terms of dependency and relationship with the entity conducting the trial. For example, is an "animal-welfare body" (as defined by Directive 63/2010/EU), or an "Institutional Animal Care and Use Committee? (IACUC - as recommended by AAALAC International), convened by a research organisation with the specific purpose of ensuring compliance with animal welfare requirements considered within the definition of "independent"? We would suggest mentioning them as an acceptable entity issuing certificates of approval for study protocols.	See reply to #106.
152	7 Reporting of efficac	FEFANA asbl	Lines 935-936 "Title: The title should provide a concise and precise description of the study, including the type of study, the product under assessment and the animal species/category.": Please consider that EFSA/EC platform restricts titles to 80 digits which is in conflict with the guidance request. Please delete:	The Panel considers the current wording appropriate.

	y studies		including the type of study, the product under assessment and the animal species/category.	
			Lines 943-946 "Ethical statement": Please refer to our comment to lines 644-647	See reply to #105.
			Line 951 "procedure, physiological stage and general health certified by a veterinarian" AND Lines 693-694 (sub-section 5.1.4): There is a discrepancy in the requirements here. In chapter 5.1.4 it is stated that the health and welfare status should be "monitored" by a veterinarian at the beginning and throughout the trial. In chapter 7, however, an additional certificate seems to be required ("general health *certified* by a veterinarian") It would be helpful if the two chapters were harmonised to each other: Either to cut out the health certificate requirement in chapter 7 or to add that this will be necessary in chapter 5.1.4. In the latter case, if a certificate is needed, it should be clarified whether a take-in certificate of the animals' health at trial start will suffice. Please clarify the requirement	Animal health and welfare should be monitored by a veterinarian, as requested in section 5.1.4. A certificate released by the veterinarian should be included in the dossier to report the monitoring of the health status of the animals at the start and during the study (including any adverse situation that may require veterinary interventions).
			Line 958-960 "In addition, for studies with enzymes, the diets should be analysed for the enzyme-specific substrate (e.g., non-starch polysaccharides, phytate-P).": The requests for substrate analyses should be deleted. It is already a request to use diets representative for EU feed compositions and all diets contain such substrates and can roughly be calculated. Please delete: In addition, for studies with enzymes, the diets should be analysed for the enzyme-specific substrate (e.g., non-starch polysaccharides, phytate-P)	The Panel considers the current requirement relevant to the assessment.
			Lines 993-996 "For all endpoints measured on individual animals in a pen, a summary parameter of the endpoint in the experimental unit should be used (e.g., mean for continuous measures such as body weight, median and counts for quantal measurements such as severity of an outcome or mortality)": The meaning of "Summary parameter of the endpoint" is unclear. Please provide the necessary clarification.	For example, when the body weight of chickens raised in the same pen is measured individually, but the feed intake is recorded by pen, the experimental unit is considered the pen, so that the mean of the body weight should be calculated for the pen.
			Lines 1001-1003 "The likely cause of death and/or reason for culling should be established by a veterinarian and reported (including the necropsy report, where relevant)." AND Line 1030 "reports of the veterinary observations": "The likely cause should be established by a veterinarian and reported"? A requirement for a vet. report for each case is in our view not relevant. A trained animal caretaker can handle the daily management of the animals, supervised by the veterinarian. Please refer to our comment on line 694.	The Panel considered that animal health and welfare can only be certified by a veterinarian, who is responsible for ensuring proper monitoring of the animals during the trial.

			Lines 1007-1009 "The concentration of the active substance(s) or agent(s) in the feedingstuffs to which the additive is added should be periodically analysed and reported. A certificate of analysis of the test item used in the study should be provided.": Periodical measurement of the active substance in the test feeds is not needed when the additive is stable in compound feed and a composite sample of sufficient size is taken at the start of the study to test the initial level. A stability test is already foreseen as part of the requirements in the Identity section. In case an additive is not stable this would already come out in that study. Only in cases where multiple test feeds are produced consecutively due to multistage feeding test and/or execution of the study with consecutive batches of animals, every batch of feed needs to be measured. Please consider the following amendment: *The concentration of the active substance(s) or agent(s) in the feedingstuffs to which the additive is added should be analysed and reported for each tested batch. A certificate of analysis of the test item used in the study should be provided*.	The text was modified to address the comment.
			Line 1013 "analysis performed for all measured endpoints and each time-point." We suggest deleting "and each time-point." Or to clarify the meaning in this sentence, which now is unclear.	The text was modified to address the comment.
			Line 1016-1017 "26) The interpretation of the results, considering the study objectives and hypotheses and other relevant studies in the literature.": To avoid interpreting that literature must be included, please add: "and studies in the literature if relevant".	The text was modified to address the comment.
			Line 1018-1019 "27) Comments on the study limitations, including any potential sources of bias, any limitations of the animal model and the imprecision associated with the results.": Please consider the following modification: "If relevant, comments on the study limitations, including any potential sources of bias, any limitations of the animal model and the imprecision associated with the results."	The text was modified to address the comment.
			Line 1027-1028 "30) All codes, logs and complete outputs for the final statistical analysis (i.e., the results and analysis reported) should be provided in an electronic and readable format": Applicants already submit the statistical output where the structure of the codes used can be checked. We suggest referring to it only to avoid the risk of mistakes due to the impossibility of checking the coding in many different software. Please consider the following change: *Complete*	The current text is considered adequate.

			outputs for the final statistical analysis (i.e., the results and analysis reported) should be provided in a readable format.	
153	7 Reporting of efficacy studies	Pen & Tec Consulting, SLU trading as Argenta ®	943-946 Ethical statement Comments: What about studies carried out outside of the EU? A certificate from local authority stating a study is compliant with EU regulations is not realistic. Will this be a requirement only for studies carried out in EU?	See reply to #105.
			944-946 1) Certificate of approval of the study protocol by a competent authority or independent animal welfare committee (including number/code of authorization) clearly declaring compliance with the animal welfare requirements, according to EU legislation. Comments: We believe that a clear definition of what will be understood by "independent" ethical committee is needed for clarity, in terms of dependency and relationship with the entity conducting the trial. For example, is an "animal-welfare body" (as defined by Directive 63/2010/EU), or an "Institutional Animal Care and Use Committee" (IACUC - as recommended by AAALAC International), convened by a research organisation with the specific purpose of ensuring compliance with animal welfare requirements considered within the definition of "independent"? We would suggest mentioning them as an acceptable entity issuing certificates of approval for study protocols.	See reply to #106.
			951- general health certified by a veterinarian. Comments: What are the criteria for health certificate? What should it contain?	The Panel considers that a veterinarian is qualified to certify the animal health and welfare status of the animals involved in the trial.
154	7 Reporting of efficacy studies	Nor-Feed SAS	Lines 956-961 : Should the COA be provided for all type of feed additive category, or only for "studies with enzymes" ?	The text was modified to address the comment.
155	7 Reporting of	AFCA-CIAL	p. 27 Line 956-961 : If the requirement to provide Certificate Of Analysis is for all feed additives, the sentence should be indicated before the paragraph related to enzymes only, such as : - Diets: description of manufacture and quantitative	The text was modified to address the comment.

	efficacy studies		composition of the diet(s) in terms of ingredients used (including premixes), relevant nutrients (calculated and analysed values) and energy (digestible, metabolisable or net). The certificates of analysis of the proximate composition of the diets should be provided. In addition, for studies with enzymes, the diets should be analysed for the enzyme-specific substrate (e.g., non-starch polysaccharides, phytate-P).?	
156	7 Reporting of efficacy studies	Chr. Hansen A/S	Lines 993-996, page 29 For all endpoints measured on individual animals in a pen, a summary parameter of the endpoint in the experimental unit should be used (e.g., mean for continuous measures such as body weight, median and counts for quantal measurements such as severity of an outcome or mortality) Comment: Unclear section. "Summary parameter of the endpoint" – what does it mean?	See reply to #152.
			Lines 1001-1002, page 29-30, plus 1029-1031, page 30 "The likely cause…should be established by a veterinarian and reported…" and "31) The report should include the certificates for the different analyses performed and the reports of the veterinary observations (including, gross pathology and histopathology, haematology, and clinical chemistry, when appropriate)." Comment: A requirement for a Vet. Report for each case is not relevant. A trained animal caretaker can handle the daily management of the animals, supervised by the Vet. Also ref. to comments for lines 693-4	See reply to #152.
			Line 1013, page 30 "24) …and each time-point." Comment: Unclear what it means. Proposal: suggest to delete "and each time point"	See reply to #152.
			Lines 1016-17, page 30 "26) …and other relevant studies in the literature." Comment: The text can be read as if we must include literature. Proposal: Change to "…and studies in the literature if relevant. "	See reply to #152.
			Lines 1018-19, page 30 "27) Comments on the study limitations, including any potential sources of bias, any limitations of the animal model and the imprecision associated with the results."	See reply to #152.

			Proposal: Change for "27) If relevant, comments on the study limitations, including any potential sources of bias, any limitations of the animal model and the imprecision associated with the results."	
			Lines 1027-28, page 30 30) "All codes, logs and complete outputs for the final statistical analysis…" Comment: We do already submit the output, and here the reviewer can see the structure of the codes used. Risk to submit something wrong as we can't check the coding in many different software's. Proposal: Please delete "All codes, logs and "	See reply to #152.
157	9 References	Oy Medfiles Ltd	As a general note, it would be helpful if you/EFSA made the revisions in different color similarly to ECHA when it revises its guidance documents. Thank you for considering.	Comment not related to safety assessment.

#: Comment no. ; EFSA: European Food Safety Authority; AFCA-CIAL: L'Association des Fabricants de Compléments pour l'Alimentation Animale; FEEDAP: Panel on Additives and Products or Substances used in Animal Feed; FEFANA: Fédération Européenne des Fabricants d'Adjuvants pour la Nutrition Animal; EC: European Commission; MS: Member State(s); SLU: Sociedad Limitada Unipersonal; GD: Guidance; EU: European Union; FEDIAF: European Pet Food Industry Federation; SARL: Società A Responsabilità Limitata; SAS: Société par Actions Simplifiée; FM: Feed Material; DLG: Deutsche Landwirtschafts-Gesellschaft; DM: Dry MAtter; HCE: Hygiene Condition Enhancers; MO: Micro-organism(s); FA: Feed Additives; ACIAC: Acids Authorisation Consortium; CFU: colony forming unit; FPS: Federal Public Service of Belgium; ZEA: Zeralenone; SRCM: Substances to Reduce the Contamination by Mycotoxins; FLAVIS: Flavour Information System database; COE: Council of Europe; IU: International Units; ATTD: apparent total tract digestibility; NSPases: Non-Starch Polysaccharidases; AFD: Apparent Feed Digestibility; ME: Metabolisable Energy; DMI: Dry Matter Intake; FEFAC: European Feed Manufacturers' Federation; AVC: Association of Veterinary Consultants; AST: anticoccidial sensitivity test; BW: Body Weight; NCA: New Companion Animals; IACUC: Institutional Animal Care and Use Committee; AAALAC: Association for Assessment and Accreditation of Laboratory Animal Care; CRO: Contract Research Organization; SARA: Sub Acute Ruminal Acidosis; GMP: good manufacturing practice; ECM: Energy-corrected Milk Yield; ECHA: European Chemicals Agency

Abbreviations

FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
FEFANA	Fédération Européenne des Fabricants d'Adjuvants pour la Nutrition Animal
EC	European Commission
MS	Member State(s)
SLU	Sociedad Limitada Unipersonal
GD	Guidance
FEDIAF	European Pet Food Industry Federation
SARL	Società A Responsabilità Limitata
SAS	Société par Actions Simplifiée
FM	Feed Material
DLG	Deutsche Landwirtschafts-Gesellschaft
DM	Dry Matter
HCE	Hygiene Condition Enhancers
MO	Micro-organism(s)
FA	Feed Additive(s)
ACIAC	Acids Authorisation Consortium
CFU	colony forming unit
FPS	Federal Public Service of Belgium
ZEA	Zeralenone
SRCM	Substances to Reduce the Contamination by Mycotoxins
FLAVIS	Flavour Information System database
COE	Council of Europe
IU	International Units
ATTD	apparent total tract digestibility
NSPases	Non-Starch Polysaccharidases
AFD	Apparent Feed Digestibility
ME	Metabolisable Energy
DMI	Dry Matter Intake
FEFAC	European Feed Manufacturers' Federation
AVC	Association of Veterinary Consultants
AST	anticoccidial sensitivity test
BW	Body Weight
NCA	New Companion Animals
IACUC	Institutional Animal Care and Use Committee
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
CRO	Contract Research Organization
SARA	Sub-Acute Ruminal Acidosis
GMP	good manufacturing practice
ECM	Energy-corrected Milk Yield
ECHA	European Chemicals Agency