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Safety and efficacy of a feed additive consisting of an aqueous extract of *Citrus limon* (L.) Osbeck (Citrozest®) for weaned piglets and all growing poultry species (Nor-Feed SAS)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of an aqueous extract of *Citrus limon* (L.) Osbeck (Citrozest®) when used as a zootechnical additive in feed for weaned piglets and all growing poultry species. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive under assessment is safe for the target species up to the maximum proposed use level of 1,000 mg/kg complete feed. No concerns for consumers were identified following the use of additive up to the highest safe level in feed. The additive should be considered a skin and eye irritant, and a potential corrosive. The use of Citrozest® as a feed additive is considered safe for the environment. In the absence of adequate data, the FEEDAP Panel could not conclude on the efficacy of Citrozest® as a zootechnical additive for weaned piglets and all growing poultry species under the proposed conditions of use.

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Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference.....	4
1.2. Additional information.....	4
2. Data and methodologies.....	4
2.1. Data.....	4
2.2. Methodologies.....	4
3. Assessment.....	5
3.1. Characterisation.....	5
3.1.1. Characterisation of the additive.....	5
3.1.2. Stability and homogeneity.....	5
3.1.2.1. Shelf life.....	5
3.1.2.2. Stability.....	5
3.1.2.3. Homogeneity.....	6
3.1.3. Conditions of use.....	6
3.2. Safety.....	6
3.3. Efficacy.....	6
3.4. Post-market monitoring.....	7
4. Conclusions.....	7
5. Documentation provided to EFSA/Chronology.....	7
References.....	7
Abbreviations.....	8
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for Lemon extract (<i>Citrus limon</i> Burm).....	9

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Nor-Feed SAS² for the authorisation of the additive consisting of an aqueous extract of *Citrus limon* (L.) Burm³ (Citrozest®), when used as a feed additive for weaned piglets and all growing poultry species (category: zootechnical additives; functional group: other zootechnical additives).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 21 January 2022.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of an aqueous extract of *Citrus limon* (L.) Osbeck (Citrozest®), when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The additive Citrozest® consists of lemon extract, an aqueous extract of *C. limon* (L.) Osbeck.

EFSA issued an opinion on the safety and efficacy of the additive when used as a flavouring in feed for all animal species (EFSA FEEDAP Panel, 2021). Lemon extract from *C. limon* (L.) Burm f. is currently authorised as a feed additive according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁴ in support of the authorisation request for the use of an aqueous extract of *C. limon* (L.) Osbeck (Citrozest®), as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the total polyphenols/eriodictin in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁵

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of an aqueous extract of *Citrus limon* (L.) Osbeck (Citrozest®) is in line with the principles laid down in Regulation (EC) No 429/2008⁶ and the relevant guidance documents: Guidance on studies concerning the safety

¹ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

² Nor-Feed SAS, 3 rue Amedeo Avogadro, 49,070 Beaucouzé, France.

³ Accepted name: *Citrus limon* (L.) Osbeck, synonym: *Citrus limon* (L.) Burm. f.

⁴ FEED dossier reference: FAD-2021-0070.

⁵ The full report is available on the EURL website: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>.

⁶ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The additive under assessment, lemon extract (Citrozest®), is an aqueous extract derived from the fruit of *C. limon* (L.) Osbeck (isonym *C. limon* (L.) Burm. f.; originating from the South of Spain) and is intended for use as a zootechnical additive (functional group: other zootechnical additives) in feed for weaned piglets and all growing poultry species.

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive under assessment is an aqueous extract of the material remaining after the extraction of juice from the fruit.

The additive Citrozest® is a dark orange to yellow liquid. It is standardised in the content of total polyphenols (selected as a phytochemical marker), eriocitrin (selected as a phytochemical marker) and hesperidin, in order to meet the following specification: total polyphenols $\geq 1\%$ (determined by spectrophotometry at 760 nm, expressed as pyrogallol equivalents), eriocitrin $\geq 4,000$ mg/kg and hesperidin $\geq 2,000$ mg/kg, (both determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection). The additive is specified to contain 4–8% citric acid. The additive also contains 1% propionic acid (E 280) as a preservative.

The same product has been recently evaluated in a previous opinion for use as a sensory additive (functional group: flavouring compounds) in feed and water for drinking for all animal species (EFSA FEEDAP Panel, 2021). In that opinion, the additive was fully characterised, therefore, all data pertaining to composition, purity, physicochemical properties described thereof are considered valid also for this application. For the current dossier, the applicant submitted some new data that are described below.

The applicant provided a nutrient analysis for six batches⁷ and data on additional three batches showed that all pesticides were below the corresponding limit of quantification (LOQ), except a few of them,⁸ which were below the EU maximum residue levels (MRLs) for lemon defined by relevant regulations.⁹

3.1.2. Stability and homogeneity

3.1.2.1. Shelf life

The shelf life of the additive (five batches) was studied when stored at room temperature in plastic bottles for 12 months. The content of total polyphenols and eriocitrin was determined at the end of the storage period. No losses in the content of total polyphenols were detected. For eriocitrin, losses at the end of the storage period ranged 1.7–13%.¹⁰

3.1.2.2. Stability

The stability of the additive (one batch) in a premixture containing trace elements was studied when supplemented at 30,000 mg/kg premixture and stored at room temperature in aluminium bags for 6 months. Only the content of eriocitrin was measured at the end of the storage period. The loss in the content of eriocitrin at the end of the storage period was 3%.¹¹

⁷ Technical dossier/Section II/Annexes_21, 22, 23, and 26 and Annexes_24 and 25_conf: crude protein 3.1% (2.4–3.7%), crude fat 0.3% (0.2–0.4%), crude ash 2.7% (1.7–3.8%), crude fibre 0.1%, water 54.9% (49.2–58.2%).

⁸ Technical dossier/Section II/Annex 36_Conf, Annex_II_38_Conf and Annex_II_40_Conf.

⁹ Technical dossier/Section II/Annex 35.

¹⁰ Technical dossier/Section II/Annex_II_58_conf.

¹¹ Technical dossier/Section II/Annexes_II_62 to 64, Annex_II_64-1 and Annex_II_64-2.

The stability of the additive in mash feed and pelleted feed for chickens for fattening and in mash feed for piglets was studied when supplemented at 150 mg/kg complete feed. Samples were stored at room temperature in aluminium bags for 3 months. No losses in the content of eriocitrin were observed at the end of the storage period.¹² The analysis of feed samples prior and after pelleting showed a loss of about 26% in the eriocitrin content due to pelleting.¹³

3.1.2.3. Homogeneity

The capacity for homogeneous distribution of the additive in feed was studied in 10 subsamples of a mash feed for piglets supplemented with Citrozest® at 150 mg/kg feed. The coefficient of variation (CV) for the content of eriocitrin was 6.9%.¹⁴

3.1.3. Conditions of use

Citrozest® is intended for use in feed for weaned piglets and all growing poultry species at a minimum proposed use level of 120 mg/kg complete feed and a maximum proposed use level of 1,000 mg/kg complete feed.

3.2. Safety

The safety of the additive Citrozest® when used at the maximum proposed use levels in feed for weaned piglets and growing poultry species has been already established in a previous opinion (EFSA FEEDAP Panel, 2021). The Panel concluded that the additive is safe for weaned piglets and all growing poultry up to 1,000 mg/kg complete feed. The Panel also concluded that the use of the product as a feed additive raises no concern for consumer safety or for the environment. Concerning the safety for the user, the Panel concluded that the additive should be considered a skin and eye irritant, and potential corrosive, owing to low pH of the additive (< 4). No conclusion could be drawn on the skin sensitisation potential.

Since the new use as a zootechnical additive would not introduce concerns to those already assessed in the former opinion, the previous conclusions on safety apply to the current application.

3.3. Efficacy

Citrozest® is proposed for classification as a zootechnical additive – functional group: other zootechnical additives (improvement of zootechnical parameters) for weaned piglets and all growing poultry species.

The applicant submitted two studies and a meta-analysis in weaned piglets¹⁵ and three in chickens for fattening¹⁶ to support the efficacy of the additive.

The Panel evaluated the studies and noted that the test item used in all efficacy studies was a premixture [REDACTED]

[REDACTED]. The control diet in all the studies submitted did not include the components of the Nor-Spice AB premixture given to the treated animals without Citrozest®. Therefore, the Panel considers that the studies do not allow for adequately assessing the effects of the additive under assessment.

In addition to this limitation, some studies showed major flaws that would prevent considering them for the efficacy assessment. In the trials in chickens for fattening, one of them¹⁷ showed high mortality, while another¹⁸ lacked experimental replicates. In weaned piglets, one meta-analysis was submitted, encompassing 10 individual animal trials performed with Nor-Spice AB.¹⁹ However, the experimental design of most of the individual trials included in the meta-analysis showed substantial shortcomings (short duration, high use levels, lack of adequate control), which limited the acceptability of the overall study.

¹² Technical dossier/Section II/Annex_II_68 and Annex_II_69.

¹³ Technical dossier/Supplementary Information/June 2022.

¹⁴ Technical dossier/Section II/Annex_II_70.

¹⁵ Technical dossier/Section IV/Annexes_IV_1-IV_6, Annexes IV_7-IV_9, Annexes_IV_10-IV_13.

¹⁶ Technical dossier/Section IV/Annexes_IV_14-IV_19, Annexes IV_21-IV_24, Annexes_IV_25-IV_30.

¹⁷ Technical dossier/Section IV/Annexes_IV_14-IV_19.

¹⁸ Technical dossier/Section IV/Annexes_IV_21-IV_24.

¹⁹ Technical dossier/Section IV/Annexes_IV_7-IV_9.

Therefore, considering the above limitations and in the absence of adequate data, the Panel is not in the position to conclude on the efficacy of Citrozest® as a zootechnical additive for all growing poultry and weaned piglets.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation²⁰ and Good Manufacturing Practice.

4. Conclusions

The additive under assessment is safe for weaned piglets and all growing poultry species at the maximum proposed use levels of 1,000 mg/kg complete feed.

The use of the feed additive Citrozest® in animal nutrition under the conditions of use proposed is of no concern for consumer safety.

The additive should be considered a skin and eye irritant, and a potential corrosive. No conclusion can be drawn on the potential for skin sensitisation.

The use of Citrozest® as a feed additive is considered safe for the environment.

In the absence of adequate data, the FEEDAP Panel could not to conclude on the efficacy of Citrozest® as a zootechnical additive for weaned piglets and all growing poultry species under the proposed conditions of use.

5. Documentation provided to EFSA/Chronology

Date	Event
10/05/2021	Dossier received by EFSA. Citrozest (Lemon extract (<i>Citrus limon</i> Burm)) for piglets (weaned) and all growing poultry species. Submitted by Nor-Feed S.A.S.
10/05/2021	Reception mandate from the European Commission
21/01/2022	Application validated by EFSA – Start of the scientific assessment
23/03/2022	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation/safety for the user</i>
22/04/2022	Comments received from Member States
20/05/2022	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
24/06/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
28/09/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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²⁰ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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Abbreviations

CV	coefficient of variation
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
HPLC	high-performance liquid chromatography
LOQ	limit of quantification
MRL	maximum residue level
UV	ultraviolet

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for Lemon extract (*Citrus limon* Burm)

In the current application an authorisation is sought under Article 4 for *lemon extract* (*Citrus limon* Burm) under the category / functional group (4 d) “zootechnical additives”/“other zootechnical additives”, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the *feed additive* to be used for weaned piglets and all growing poultry species.

The Applicant proposed as phytochemical markers for the characterisation of the *feed additive* (i) *total polyphenols* and (ii) *eriocitrin*. The product contains respectively a minimum content of 1% of *total polyphenols* and 4,000 mg/kg product of *eriocitrin*. The *feed additive* (Citrozest®) is a liquid extract from the peel and the seeds of *Citrus limon* (L.) Burm. fruit. The product is to be used through *premixtures* or directly into *feedingstuffs* at proposed inclusion levels ranging from a minimum of 120 to a maximum 1,000 mg/kg *feedingstuffs*.

For the determination of the *total polyphenols* in the *feed additive* the Applicant proposed a colourimetric method described in the European Pharmacopoeia monograph 2.8.14. Upon request of the EURL, in the frame of the similar “FAD-2010-0130 – Lemon extract” dossier, the Applicant provided experimental data for the analysis of the *total polyphenols* content in two different batches of *lemon extract*. Based on the acceptable performance characteristics presented the EURL recommends for official control the colourimetric method described in the European Pharmacopoeia 2.8.14 monograph for the determination of the *total polyphenols* content in the *feed additive*.

For the determination of the *eriocitrin* content in the *feed additive* the Applicant presented an extension of scope of the ring-trial validated method from the “International Fruit and Vegetable Association” (IFU 580) dedicated to the determination of hesperidin and naringin in juices. In the frame of the similar “FAD-2010-0130 – Lemon extract” dossier, the Applicant provided experimental data for the analysis of the *eriocitrin* content in two different batches of *lemon extract*. According to the results provided, the EURL calculated a relative standard deviation for repeatability (RSDr) of 0.1% and 1.8%, and a relative standard deviation for intermediate precision (RSDip) of 8.1% and 8.5%. Based on the experimental evidences provided, the EURL considers the above mentioned HPLC-UV method fit-for-purpose for the determination of the *eriocitrin* content in the *feed additive*.

For the determination of the *eriocitrin* content in *premixtures* the Applicant submitted a single-laboratory validated and further verified method based on HPLC-UV. For the determination of the *eriocitrin* content in *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified method based on ultra-high performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS).

Based on the experimental evidences provided, the EURL considers the proposed HPLC-UV and UPLC-ESI-MS/MS methods fit-for-purpose for the potential official control of *eriocitrin* content respectively in *premixtures* and *feedingstuffs*.

Furthermore, the Applicant did not provide experimental data or analytical methods for the determination of *lemon extract* in *premixtures* and *feedingstuffs*, as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control for the determination of *lemon extract* in *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.