

ADOPTED: 22 March 2023

doi: 10.2903/j.efsa.2023.7964

Safety and efficacy of a feed additive consisting of a dry grape extract (Nor-Grape[®] α) for all avian species (Nor-Feed S.A.S.)

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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 a feed additive consisting of a dry grape extract (Nor-Grape[®] α) as a zootechnical feed additive, functional group physiological condition stabilisers - increase antioxidant defences, for all avian species. The additive is already authorised for use as a feed flavouring for all animal species, except dogs. The FEEDAP Panel concluded that the additive is safe for all avian species. The use of the additive in animal nutrition is of no concern for consumer safety. Based on the data submitted, the FEEDAP Panel could not conclude on the potential of the additive to be a skin or eye irritant or a dermal or respiratory sensitiser. However, the Panel considered that exposure through inhalation is likely. The use of the feed additive is considered safe for the environment. The Panel was unable to conclude on the potential of the additive to be efficacious under the proposed conditions of use.

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Keywords: zootechnical additives, dry grape extract, polyphenols, proanthocyanidins, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2021-00467

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Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output Daniel Pagès Plaza.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Brantom P, Chesson A, Martelli G, Westendorf J, Ortuño J, Manini P and Pizzo F, 2023. Scientific Opinion on the safety and efficacy of a feed additive consisting of a dry grape extract (Nor-Grape® α) for all avian species (Nor-Feed S.A.S.). *EFSA Journal* 2023;21(4):7964, 15 pp. <https://doi.org/10.2903/j.efsa.2023.7964>

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



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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 S.A.S.² for the authorisation of the additive consisting of dry grape extract (Nor-Grape[®] α), when used as a feed additive for all avian species (category: zootechnical additive; functional group: (e) physiological condition stabilisers).

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 7/4/2022.

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 dry grape extract (Nor-Grape[®] α), when used under the proposed conditions of use (see Section 3.1.6).

1.2. Additional information

The additive is a preparation containing a dry grape extract of *Vitis vinifera* ssp. *vinifera*, a mixture of dry grape extracts from seed and skins.

EFSA issued an opinion on the safety and efficacy of the same product when used as a sensory additive (functional group: (b) flavouring) in feed for all animal species and categories (EFSA FEEDAP Panel, 2016a) and another opinion when used as flavouring in water for drinking for all animal species and categories (EFSA FEEDAP Panel, 2016b).

The additive under evaluation is currently authorised as a feed flavouring for all animal species except dogs (2b485).³

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 dry grape extract (Nor-Grape[®] α) as a feed additive. The dossier was received on 27/5/2021 and the general information and supporting documentation is available at <https://open.efsa.europa.eu/questions/EFSA-Q-2021-00467>.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 8 April 2022 to 8 July 2022 for which the received comments were considered for the assessment.

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 active substance in animal feed.⁵

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, 49070. Beaucoz , France.

³ Commission Implementing Regulation (EU) 2017/307 of 21 February 2017, concerning the authorisation of dry grape extract of *Vitis vinifera* spp. *vinifera* as a feed additive for all animal species except for dogs. OJ L 44/1, 22.2.2017, p. 5.

⁴ FEED dossier reference: FAD-2021-0069.

⁵ The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of dry grape extract (Nor Grape^{®-α}) 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 of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (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

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive consists of a mixture of dry grape extracts from seed and skins.

For the additive under assessment, the applicant proposed the same specifications as those for the additive already authorised (2b485) as a feed flavouring and evaluated by the FEEDAP Panel in 2016 (EFSA FEEDAP Panel, 2016a). These were polyphenols $\geq 80\%$ expressed as catechin content (including proanthocyanidins $\geq 60\%$, anthocyanins and anthocyanidins $\geq 0.75\%$) and water content $\leq 10\%$.

The applicant characterised the polyphenol fraction analysing three batches of the additive.⁷ For these batches polyphenols represented on average 86.2% (range: 83.1–89.9%) of the additive, proanthocyanidins were 69% (range: 66.6–70.6%), anthocyanins and anthocyanidins were 1.5% (range: 1.1–1.7%), malvidin 3-*O*-glucoside was 0.22% (range: 0.22–0.23%),

Analytical data for the water content were not provided.

Nine additional batches of the additive were analysed and mean values were 82.8% (range 80.1–88.3%) for polyphenols and 0.18% (range 0.09–0.26%) for malvidin-3-*O*-glucoside.

Analytical data provided demonstrated compliance with the proposed specifications as described above.

Seven batches of the additive were analysed for impurities.⁹ The lead content ranged between 0.04 and 0.08 mg/kg, cadmium between < 0.005 and 0.009 mg/kg, arsenic between 0.09 and 0.23 mg/kg, and mercury between 0.005 and 0.044 mg/kg. In addition, the content of copper (average: 3.3 mg/kg, range: 2.87–3.62 mg/kg) was analysed in three batches of the additive.¹⁰

Mean values for dioxins and the sum of dioxins plus dioxin like PCBs concentrations in three batches of the additive were 0.11 ng WHO-PCDD/F-TEQ/kg and 0.17 ng WHO-PCDD/F-PCB-TEQ/kg, respectively, non-dioxin-like PCBs levels ranged from 0.10 to 0.8 $\mu\text{g}/\text{kg}$ additive.¹¹ The dioxin content was also analysed in three additional batches. The sum of dioxins plus dioxin like PCBs concentrations

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.

⁷ Technical dossier/Supplementary Information (December 2022)/Section II/Annex_II_23 to Annex_II_25 and Annex_II_26 to Annex_II_28.

⁸ Technical dossier/Supplementary Information (December 2022)/Section II/Annex_II_1 to Annex_II_22.

⁹ Technical dossier/Section II/Annex_II_14 to Annex_II_20.

¹⁰ Technical dossier/Supplementary Information (December 2022)/Section II/Annex_II_30 to Annex_II_32.

¹¹ Technical dossier/Section II/Annex_II_16, Annex_II_17 and Annex_II_29.

were 0.06 ng WHO-PCDD/F-TEQ/kg and 0.1 ng WHO-PCDD/F-PCB-TEQ/kg. Non-dioxin-like PCBs levels ranged from 0.04 to 0.1 µg/kg additive.¹²

Four batches of the additive were analysed for the possible presence of pesticide residues.¹³ All of the pesticides tested were below the respective limit of quantification (LOQ) of the analytical method. The same batches were also analysed for the possible presence of aflatoxins (B1, G1, B2, G2) and ochratoxin A. The results for aflatoxins were below their respective LOQ¹¹ of the analytical methods, while ochratoxin A ranged from < 2 to 2.6 µg/kg.

The applicant also provided an analysis of three batches of the additive for residual amounts of ethanol used in the manufacturing process (average: 546.6 mg/kg; range: 120–800 mg/kg).¹⁰

Microbiological contamination was assessed by the determination of *Escherichia coli* in six batches of the additive (not detected in 1 g), coagulase-positive staphylococci in three batches (not detected in 1 g), *Salmonella* spp. in seven batches (not detected in 25 g), total aerobic plate counts in seven batches (< 1 colony forming units (CFU)/g), yeasts and filamentous fungi in two and five batches, respectively (< 10 CFU/g), yeasts and filamentous fungi in a single batch (< 40 and < 10 CFU/g), respectively and *E. coli* β-glucuronidase positive in a single batch (< 1 CFU/g).⁹

The Panel considered that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

3.1.2. Manufacturing process

[REDACTED]

3.1.3. Characterisation of the active substances

The polyphenol fraction, which represents the bulk of the additive, consists of proanthocyanidin oligomers with minor amounts of catechin, epicatechin, epicatechin gallate, gallic acid and minor amounts of anthocyanins and anthocyanidins (Figure 1) (see Section 3.1.1). Proanthocyanidins, also known as condensed tannins, are dimers, oligomers and polymers of flavan-3-ols (catechins) covalently linked between C4 and C8 (or C6). Analytical data on the characterisation of the polyphenol fraction of the additive are reported in Section 3.1.1.

Procyanidins are a subclass of proanthocyanidins, consisting of oligomers of (+)-catechin and (–)-epicatechin molecules exclusively. According to the analysis provided by the applicant, the most important procyanidins in grape are procyanidins B1, B2, B3 and B4.

The different anthocyanidins ($R_6 = \text{OH}$) or anthocyanins ($R_6 = \text{O-glycosyl}$) resulting for substitution on R1–R5, are shown in Figure 2.

The phytochemical proposed by the applicant is malvidin-3-O-glucoside. The IUPAC name of malvidin is 5,7-dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-3-chromeniumyl β-D-glucopyranoside, the molecular formula is $\text{C}_{23}\text{H}_{25}\text{O}_{12}$, the molecular weight (g/mol) 493.43 and the CAS number 7228-78-6.

¹² Technical dossier/Supplementary Information (December 2022)/Section II/Annex_II_29 to Annex_II_31.

¹³ Technical dossier/Section II/Annex_II_14 to Annex_II_17.

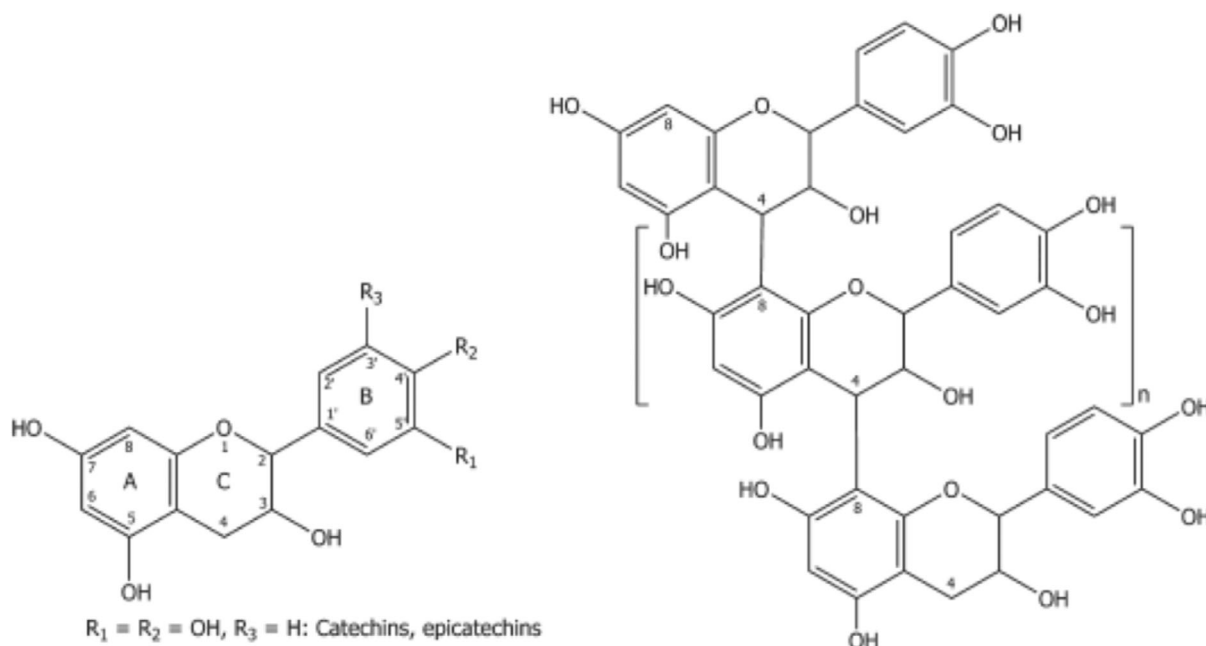


Figure 1: General structure of flavan-3-ols, the building blocks for proanthocyanidins ($n = 0$ proanthocyanidins dimers, $n = 1$ trimers, etc.)

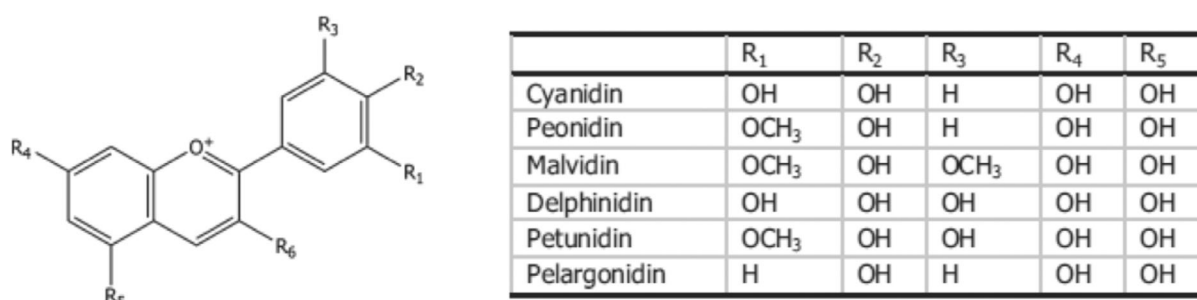


Figure 2: General structure of anthocyanidins ($R_6 = \text{OH}$) or anthocyanins ($R_6 = \text{O-glycosyl}$)

3.1.4. Physical properties of the additive

The additive is a purple to brown free-flowing powder. The bulk density ranges from 0.3 to 0.6 g/mL and the solubility of the additive in water (at 50 g/L) was measured on three batches and was on average 91.5% (89.4–92.8%).

The dusting potential was determined in two replicates of one batch of the additive using the Stauber–Heubach method and showed values of 79 mg/m³. The dusting potential of the additive was further measured with three batches using the type II (DIN 55992) Heubach Dust meter. The dusting potential showed a mean of 6.2 g/m³ (range: 5.3–6.8 g/m³).¹⁴ Approximately 85–95% of the additive consists of particles between 100 and 315 μm with less than 0.15% measuring < 100 μm.¹⁵

3.1.5. Stability and homogeneity

3.1.5.1. Shelf life

The shelf life of five batches of the additive was determined when stored at 15–25 °C in sealed plastic bags in sealed drums for up to 30 months. Losses, in terms of total polyphenol content,¹⁶ at the end of the storage period ranged between 2% and 6.4%.¹⁷

¹⁴ Technical dossier/Supplementary Information (December 2022)/Section II/Annex_II_34 to Annex_II_36.

¹⁵ Technical dossier/Section II/Annex_II_30.

¹⁶ Expressed as catechin equivalent.

¹⁷ Technical dossier/Section II/Annex_II_35.1–35.5.

3.1.5.2. Stability in premixtures

The stability of the additive was determined [REDACTED] of the malvidin-3-*O*-glucoside content. In a first trial, a mineral premixture was supplemented with the additive [REDACTED] and its malvidin-3-*O*-glucoside content analysed at 0, 2, 3, 4 and 6 months.¹⁸ In the second and third trial, the vitamin–mineral premixtures were supplemented with 60.7 and 71.4 mg additive/kg, respectively.¹⁹ The expected content of malvidin-3-*O*-glucoside was calculated from the amount of the additive used, at the start of the trial (121 µg/kg and 143 µg/kg, respectively) and then analysed after 3 and 6 months.

After 6 months, the losses ranged from 0% to 20.5%.

3.1.5.3. Stability in feeds

The stability of the additive was analysed in feeds for chickens for fattening (two pelleted finisher feeds and one mash finisher feed).²⁰ One batch of feed was supplemented with 30 mg additive/kg and then pelleted (pelleting conditions not reported). A second batch of feed was also supplemented with 30 mg/kg of the additive and divided into two aliquots; one was analysed as pellets and the other one as mash feed. The malvidin-3-*O*-glucoside content was analysed after supplementation and after 3 months of storage in a dry and cool place and protected from light. Losses at the end of the storage period ranged from 0% to 8.4%.²¹

3.1.5.4. Homogeneity

The capacity of the additive to homogeneously distribute in feed was studied in 10 subsamples of pelleted finisher feed for chickens for fattening. Each subsample (15 g) was supplemented with 30 mg/kg of the additive. The coefficient of variation was 7%.²²

3.1.6. Conditions of use

The additive is intended for use in feed for all avian species with a recommended use level from 30 to 100 mg/kg complete feed.

3.2. Safety

The assessment of safety is based on the highest use level proposed by the applicant (100 mg/kg complete feed).

In the current assessment, the applicant provided results of a literature search carried out to update knowledge on the safety of the additive and its components. Seven databases (the EFSA Register, TOXLINE, PubMed, Google Scholar, Researchers Gate, Science Direct, SciFinder) were used. The literature search covered the period from 2015 up to December 2020. Main keywords used were: "chicken" OR "poultry" AND "grape" OR "vitis" AND "safety", "toxicity" "adverse effect", "negative effect", "NOAEL", "ADI". A detailed description of the iterations used, and the inclusion/exclusion criteria applied for the selection were provided.²³

The outcome of the literature search is described in the sections below.

3.2.1. Absorption, distribution, metabolism and excretion

The absorption, distribution, metabolism and excretion (ADME) of the constituents of the additive when intended to be used as flavouring for all animal species, was described in the previous EFSA assessment (EFSA FEEDAP Panel, 2016a), based on studies conducted in rats and pigs. It was shown that polyphenols, depending on the degree of polymerisation, were partly absorbed, as such or after degradation in the gut, extensively metabolised and excreted in urine and faeces in both species.

In the current application, the applicant submitted two published papers reporting studies carried out in chickens.

¹⁸ Technical dossier/Section II/Annex II_38 to Annex II_40.3.

¹⁹ Technical dossier/Section II/Annex II_45 and 45–1.

²⁰ Technical dossier/Section II/Annex II_46–47.

²¹ Technical dossier/Section II/Annex II_49 to Annex II_51.

²² Technical dossier/Section II/Annex II_54.

²³ Technical dossier/Section III/Annex III_1_1.

In the study by Chamorro et al. (2019),²⁴ chickens for fattening were fed for 21 days with control diets or diets supplemented with the additive under assessment (see Section 3.1.1) at 2.5 or 5 g/kg complete feed (representing 25 or 50 times the highest proposed use level, respectively). Excreta from birds were collected during the last 24 h of the experiment and the ileal contents from some birds on day 21. The polyphenolic compounds, present in the additive as well as in the ileal contents and excreta, were evaluated by High-performance liquid chromatography–mass spectrometry (HPLC-MS); the metabolites in the intestine and excreta were analysed by UPLC-DAD-ESI-TQ-MS. There was a significant increase in the content of all compounds analysed in the ileal contents of birds fed the diet with the additive at 5 g/kg complete feed as compared with 2.5 g/kg. Catechin (C), epicatechin (EC), procyanidin dimers PB1 and PB2 in ileal contents increased ($p < 0.001$) by 65, 83, 35 and 63%, respectively. The same trend, although to a lower extent, was observed for C (24%, $p < 0.001$), EC (34%, $p < 0.01$) and PB1 (5%, $p < 0.05$) in excreta concentrations. The calculated ileal digestibility of the monomeric compounds (C and EC) was in the range of 84–87% in birds fed the diets with the additive, while for PB1, PB2 and ECG, it ranged from 50% to 69%. These data show that the degree of digestibility is determined by the polymerisation and galloylation (esterification with gallic acid) of the compounds. Twenty-two phenolic derivatives of benzoic acids, phenylacetic acids, phenylpropionic acids, cinnamic acids and phenyl- γ -valerolactones were identified in the excreta samples of the chickens given the additive at both levels, indicating degradation by microbiota. Almost all these metabolites were also present in excreta from the control group resulting from components of the diet, except for 3,4,5-trihydroxybenzoic acid, 5-(3',4'-dihydroxyphenyl)- γ -valerolactone and m-hydroxycinnamic acid which were only present in excreta of birds fed with the additive.

In the study by Muñoz-González et al. (2019)²⁵, chickens for fattening were fed for 35 days with a diet containing grape seed extract with a composition close to that of the additive under assessment, at 1,000 mg/kg complete feed (representing more than 10 times the highest proposed use level). Phenolic compounds from the grape seed extract and their metabolites were evaluated by HPLC-QTOF-MS in plasma and thigh meat of broilers at day 35 of the experiment. Several phenolic derivatives were identified in plasma of animals fed the grape seed extract, with seven at significantly higher concentrations as compared with the control group. Some were conjugated catechin/epicatechin metabolites only identified in chickens fed a diet with the extract. In thigh meat, 14 phenolic metabolites were identified, at concentrations lower than those in plasma with no differences between control and grape seed extract diet groups, except for 3-(3-hydroxyphenyl)-propionic acid, which was at significantly higher concentrations in the grape seed extract group (0.96 ± 0.66 ng/g meat, $p = 0.03$) compared to the control group (not detected; limit of detection (LOD) not available). The FEEDAP Panel noted that a dose of GSE more than 10x times higher than that resulting from the recommended highest use level did not lead to differences in concentrations of metabolites in thigh meat between control and grape seed extract groups, except for 3-(3-hydroxyphenyl)-propionic acid, a known metabolite from dietary polyphenols intake.

These two studies, carried out with the additive under assessment or with a grape seed extract with a composition close to that of the additive under assessment at high concentrations in diet, demonstrate the ability of chickens for fattening to metabolise at different extents the phenolic components of the additive in the intestine, as part of the formed metabolites is absorbed and residually distributed in meat and part is directly excreted.

Thus, the use of the additive at the highest proposed use level in the present application, is not expected to result in a significant increase of the phenolic content of meat of the exposed target species above that present in birds not fed with the additive.

3.2.2. Toxicological studies

The toxicity and genotoxic potential of the additive, when intended to be used as a flavouring for all animal species was evaluated in the previous opinion issued by the FEEDAP Panel (EFSA FEEDAP Panel, 2016a), based on evidence found in the literature.

3.2.2.1. Genotoxicity

In 2016, the FEEDAP Panel concluded that the additive is not mutagenic and does not cause chromosomal damage. These conclusions were based on evidence found in the literature and

²⁴ Technical dossier/Section III/Annex_III_4.

²⁵ Technical dossier/Section III/Annex_Muñoz-González et al. - 2019.pdf.

assuming that the test items used in the published studies evaluated, although not exactly the same of the additive under assessment, were considered representative enough.

No new studies were found in the literature search provided by the applicant on the genotoxic potential of grape extracts.

In addition, the applicant provided an Ames test performed with the GSKE used in the formulation of the additive.²⁶ The study was conducted in accordance with the OECD Testing Guideline (TG) 471

No increase in the number of revertant colonies was observed at any concentration and with any tested strain. Therefore, the test item did not induce gene mutations in bacteria under the experimental conditions employed in this study.

3.2.2.2. Subchronic oral toxicity studies

In 2016, the FEEDAP Panel identified a no observed adverse effect level (NOAEL) of 1,780 mg/kg bw per day for both extracts (GSE and GSKE) from a 90-day study in rats (Bentivegna and Whitney, 2002). In this study, groups of CD[®] (Sprague–Dawley) CrI:CD-1[®]IGS BR rats (20 animals per group and sex) were fed diets containing GSE at concentrations of 0%, 0.63%, 1.245% or 2.5% (w/w) or GSKE at 2.5% (w/w) for 90 days. No mortality or signs of toxicity were observed. No significant dose-related effects on body weight, feed consumption, haematological parameters, clinical chemistry, organ weight and histopathology were observed. For both extracts, the highest dose tested (2.5%, corresponding to ~ 1,780 mg/kg bw per day in male rats and 2,150 mg/kg bw per day in female rats) was considered the NOAEL.

No new studies were found in the literature search provided by the applicant on the toxicity of grape extracts, therefore, the NOAEL considered in the previous evaluation is retained for the current assessment.

3.2.2.3. Conclusions on toxicological studies

Based on the data available, the additive under assessment is considered to be not genotoxic. The FEEDAP Panel confirmed the previously established NOAEL of 1,780 mg/kg bw per day.

3.2.3. Safety for the target species

No tolerance trials conducted in the target species were provided by the applicant.

The safety of the additive dry grape extract, when used as a feed flavouring, was already evaluated by EFSA (EFSA FEEDAP Panel, 2016a). At that time, the FEEDAP Panel identified a NOAEL, for GSE and GSKE, of 1,780 mg/kg bw per day from the study by Bentivegna and Whitney (2002) and concluded that the additive was safe at the maximum proposed use level (100 mg/kg complete feed) for all animal species, except dogs, for which no safe dietary concentration could be established.

In the current application, the same approach applies for the use of the product as a zootechnical additive. The highest recommended use level is the same as that proposed in the past for the use as a flavouring (100 mg/kg complete feed); therefore, the FEEDAP Panel concludes that the additive is safe for chickens for fattening, laying hens and turkeys for fattening at the proposed conditions of use. These conclusions are extended to all the other avian species.

From the literature search performed (see details in Section 3.2.2), the applicant retrieved three papers reporting studies conducted with the additive under assessment in chickens for fattening (Viveros et al., 2011; Chamorro et al., 2013; Chamorro et al., 2019) and other six papers using different grape extracts with similar composition to the additive under assessment and conducted in poultry species (Hajati et al., 2015; Farahat et al., 2017; Muñoz-González et al., 2019; Rajput et al., 2019; Ao and Kim, 2020; Barbe et al., 2020).

None of these papers indicated a safety concern for the target species and therefore the Panel retains the NOAEL previously identified to conclude on the safety for the target species.

3.2.4. Safety for the consumers

The safety for the consumer has been evaluated in a previous assessment (EFSA FEEDAP Panel, 2016a). The FEEDAP Panel considered that the consumers are exposed to the compounds under assessment (polyphenols consisting of proanthocyanidin oligomers with minor amounts of

²⁶ Technical dossier/Supplementary Information (December 2022)/Section III/Annex_III_38.

catechin, epicatechin, epicatechin-3-O-gallate, gallic acid and minor amounts of anthocyanins) due to their natural occurrence in food and their occurrence as a component of food additives from botanical origin. The additional polyphenol exposure of consumers via food derived from animals given the additive could not be calculated because residue data in food-producing animals were not available. However, considering the natural exposure, data on absorption, metabolism, distribution and excretion submitted in the present application (see Section 3.2.1) and the low toxicity of the extracts under consideration, the FEEDAP Panel concludes that supplementation of animals' diets with the additive under assessment up to the highest safe level in feeds would not raise concerns for consumer safety.

3.2.5. Safety for the user

3.2.5.1. Effect on respiratory system

The dusting potential of the additive measured on three batches of the additive was up to 6.8 g/m³. The FEEDAP Panel considered that the exposure through inhalation is likely.

No studies to investigate the potential toxicity by inhalation of the additive have been provided.

3.2.5.2. Effect on eyes and skin

The applicant did not provide any data on sensitisation or skin or eye irritancy on the additive under assessment. Reports of eye and skin irritancy studies conducted on rabbits were provided for two related products which showed divergent results.^{27,28} These studies were not considered relevant due to the test item used and, consequently, were not further considered for the risk assessment.

3.2.5.3. Conclusions on safety for the user

Based on the data submitted, the FEEDAP Panel cannot conclude on the potential of the additive to be a skin or eye irritant.

In the absence of data, it is not possible to conclude on the potential of the additive to be a dermal or respiratory sensitiser. However, the FEEDAP Panel considered that the exposure through inhalation is very likely.

3.2.6. Safety for the environment

The additive is composed of natural substances and its use will not result in a measurable increase of their concentration in the environment. The FEEDAP Panel concluded that the use of the additive does not raise any safety concern for the environment.

3.3. Efficacy

The additive is proposed as a zootechnical additive (functional group: physiological condition stabiliser – increase antioxidant defences) for use in feed for all avian species.

The applicant submitted three *in vitro* trials, three *in vivo* studies in chickens for fattening and one *in vivo* study in laying hens. The three *in vitro* trials evaluated the antioxidant capacity of the additive.

[REDACTED].²⁹ The other two were described in publications focused on the evaluation of the antioxidant capacity of the additive before and after heat (Chamorro et al., 2012a) or enzymatic (Chamorro et al., 2012b) treatment, measured by different methods (ABTS, DPPH, photochemiluminescence).

The FEEDAP Panel concluded that these *in vitro* studies do not provide evidence of the efficacy of the additive in the animals and therefore, were not further considered for the risk assessment.

3.3.1. Efficacy in chickens for fattening

The applicant submitted three short-term trials to support the efficacy of the additive in chickens for fattening. However, one of the trials included an artificial infection with coccidia and was not considered further.³⁰

²⁷ Technical dossier/Section III/Annex_III_10-1.

²⁸ Technical dossier/Supplementary Information (December 2022)/Section III/Annex_III_39.

²⁹ Technical dossier/Section IV/Annex_IV 3-1 Conf.

³⁰ Technical dossier/Section IV/Annex_IV 10.

Trials 1³¹ and 2³² shared a similar design, which is summarised in Table 1.

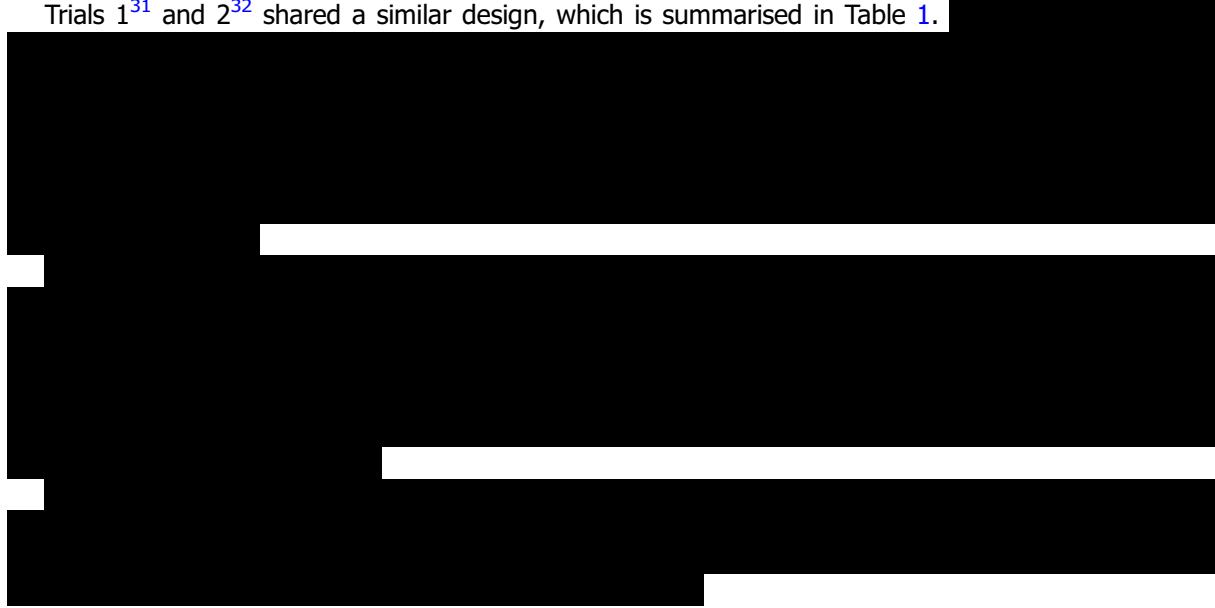


Table 1: Trial design and supplementation levels in the efficacy trials in chickens for fattening

Trial	Supplementation level	Duration	Parameters measured	Outcome		
				Significance	Effect size	Direction
1	0.1%	12 weeks	Antioxidant capacity	Significant	Increased	Positive
2	0.1%	12 weeks	Antioxidant capacity	Not significant	Increased	Positive



The Panel notes that, in each study, only one oxidative status related parameter was influenced by the additive. In Trial 1, [redacted]; however, no details on the ratio of the oxidised and reduced forms were available. In Trial 2, [redacted]; however, no effects were observed on the same parameter in the first study and the biological relevance of the increase of such parameter alone is questionable. Therefore, the Panel concludes that there is not enough evidence to demonstrate the improvement of the antioxidant defence in chickens for fattening.

3.3.2. Efficacy in laying hens

The applicant submitted one short-term trial to support the efficacy of the additive in laying hens. For that purpose, [redacted]

³¹ Technical dossier/Section IV/Sect_IV_Efficacy_4.2.3 and Annex_IV_21.

³² Technical dossier/Section IV/ Sect_IV_Efficacy_4.2.4 Annex_IV_30.

³³ Technical dossier/Section IV/Sect IV_4.2.2 and Annex_IV 14.



No hen died during the experimental period. No differences were observed in the laying performance or the plasma antioxidant capacity parameters measured at the end of the supplementation period between the two groups.

3.3.2.1. Conclusions on efficacy

Due to the lack of sufficient data, the FEEDAP Panel cannot conclude on the efficacy of the additive to improve the antioxidant defence status of the chickens for fattening and laying hens, and consequently, to any other avian species.

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 Regulation³⁴ and Good Manufacturing Practice.

4. Conclusions

The additive is considered safe for the target species, the consumers and for the environment at the proposed conditions of use.

Based on the data submitted, the FEEDAP Panel cannot conclude on the potential of the additive to be a skin or eye irritant. In the absence of data, it is not possible to conclude on the potential of the additive to be a skin or respiratory sensitizer.

The FEEDAP Panel cannot conclude on the efficacy of the additive.

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³⁴ Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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Abbreviations

BW	body weight
CAS	Chemical Abstracts Service
CATH	Total hydrophilic antioxidant capacity
CATL	Total lipophilic antioxidant capacity
CFU	colony forming unit
CV	coefficient of variation
DM	dry matter

EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GSE	grape seed extract
GSKE	grape skin extract
GPx	glutathione peroxidase activity
GSH	total glutathione
HPLC-MS	high performance liquid chromatography-mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LOQ	limit of quantification
MW	molecular weight
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
TBAR	thiobarbituric acid reactive substance
UPLC-DAD-ESI-TQ-MS	ultra-performance liquid chromatography-triple quadruple mass spectrometry
UPLC-ESI-MS/MS	ultra-performance liquid chromatography coupled to electrospray ionisation tandem mass spectrometry