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Safety and efficacy of a dried aqueous ethanol extract of leaves from *Olea europaea* L. when used as a sensory additive in feed for all animal species

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of a dried aqueous ethanol extract of leaves from *Olea europaea* L., when used as a sensory feed additive for all animal species. The extract is specified to contain ≥ 20% oleuropein. As a full analysis of the extract was not provided, about 70% of the extract remained uncharacterised. In view of the inadequate chemical and toxicological characterisation of the additive, the FEEDAP Panel is unable to conclude on the safety for the target species, the consumers and the users. *O. europaea* L. is a native species to Europe where it is widely grown for commercial purposes. Use of the extract from the plant in animal production is not expected to pose a risk for the environment. In the absence of data showing the sensory properties of the additive under assessment, the Panel could not conclude on the efficacy of olive leaf extract from *O. europaea* L. when used as sensory additive.

Keywords: *Olea Europaea* L., leaf extract, oleuropein, consumer safety, target animal safety, environment, efficacy

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of 7 years after the entry into force of this Regulation.

The European Commission received a request from Company NOR-FEED A/S[2] for the re-evaluation of the product olive leaf extract from *Olea europaea* L., for all animal species (category: sensory additives; functional group: flavourings).

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). The particulars and documents in support of the application were considered valid by EFSA as of 11 September 2018.

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 product olive leaf extract from *O. Europaea* L., when used under the proposed conditions of use (see Section 3.2.3).

1.2. Additional information

The preparation under assessment, olive leaf extract from *O. europaea* L. is currently authorised as 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). It has not been assessed as feed additive in the EU.

'Olive Leaf Dry Extract' is described in a monograph of the European Pharmacopoeia 9.0 (PhEur, 2017). It is defined as a dry extract which is produced from the dried leaves from *O. europaea* L. and which has a minimum content of 16.0% of oleuropein. The extract is produced by using ethanol (65–96% v/v).

For *O. europaea* L. folium, the European Medicines Agency (EMA) issued an assessment report (EMA, 2017a) and a monograph for human medicinal use (EMA, 2017b).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of olive leaf extract from *O. europaea* L. as a feed additive.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) used the data provided by the applicant together with data from other sources, such as peer-reviewed scientific papers 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 olive leaf extract in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of a dried aqueous ethanol extract of leaves from *O. europaea* L. is in line with the principles laid down in

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] NOR-FEED A/S, Kanalholmen 2, 2650 Hvidovre, Denmark.
[3] FEED dossier reference: FAD-2010-0368.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0368-olive-leaf-extract.docx_.pdf

Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Guidance on the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2017b), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance document on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019a), Genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019b).

3. Assessment

This opinion deals with the assessment of a dried aqueous ethanol extract of leaves from *Olea europaea* L. when used as sensory additive (functional group: flavouring compounds) in feed for all animal species.

3.1. Origin and extraction

O. europaea L. belongs to the Oleaceae, is native of the Mediterranean region but is now extensively cultivated worldwide.

The applicant provided a description and a flow chart of the manufacturing process of the additive. Freshly picked olive leaves are washed and then macerated eight times in ethanol (70%, v/v) at 75–85°C for 3 h. The extract is filtered and recycled at lower temperature (65–70°C) and partial vacuum until a concentrated cream occurs, which is evaporated to dryness. The resulting dry mass is crushed, sieved and the oleuropein content is determined. Maltodextrin is added to adjust the oleuropein content to 20%.

3.2. Characterisation

3.2.1. Characterisation of the additive

Olive leaf dry extract is identified by the Chemical Abstract Service (CAS) No 8001-25-0, the European Inventory of Existing Commercial Chemical Substances (EINECS) No 232-277-0 and the Council of Europe (CoE) No 309.

The additive contains by specification ≥ 20% oleuropein (which the applicant identifies as the phytochemical marker). Analysis of five batches showed an average content of [redacted] in compliance with specification and with the description of the PhEur.[6] The loss on drying is [redacted] and ash [redacted]

Oleuropein (International Union of Pure and Applied Chemistry (IUPAC) name: (4S,5E,6S)-4-[2-[2-(3,4-dihydroxy-phenyl)ethoxy]-2-oxoethyl]-5-ethylidene-6-[[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-tetrahydropyranyl]oxy]-4H-pyran-3-carboxylic acid, methyl ester) is identified with the CAS No 32619-42-4. It has a molecular mass of 540.514 g/mol. Its molecular formula is C25H32O13. The structural formula of oleuropein is given in Figure 1.

[5] 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.

[redacted]

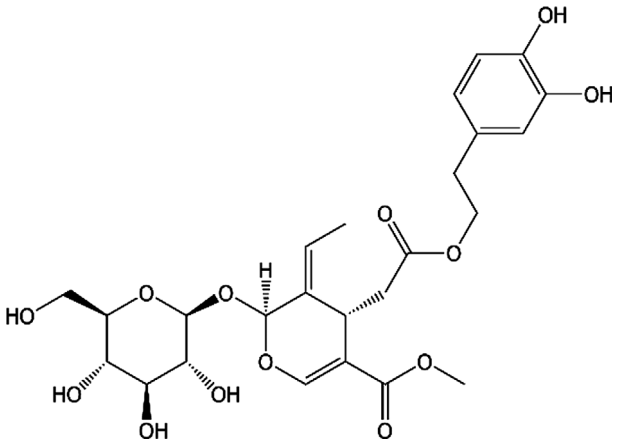

Figure 1: Molecular formula of oleuropein

The applicant provided the characterisation of additional six batches where, in addition to oleuropein, other phenolic compounds were detected and quantified by high-performance liquid chromatography (HPLC) analysis: verbascoside, demethyloleouropein, hydroxytyrosol, tyrosol, elenoic-7-O-glucoside, luteolin-7-O-glucoside, luteolin, apigenin-7-O-glucoside, diosmetin-7-O-glucoside, diosmetin, caffeic acid, vanillic acid and vanillin.[7] The analytical results are summarised in Table 1. Taken together, these compounds account on average for [redacted]

Table 1: Major constituents of a dried aqueous ethanol extract of leaves from *Olea Europaea* L. based on the analysis of six batches (mean and range)

Constituent	CAS no	FLAVIS no	Percentage in the extract	
			Mean	Range
Oleuropein	32619-42-4	–	[redacted]	[redacted]
Verbascoside	61276-17-3	–	[redacted]	[redacted]
Demethyloleuropein	52077-55-1	–	[redacted]	[redacted]
Hydroxytyrosol	10597-60-1	–	[redacted]	[redacted]
Tyrosol	501-94-0	02.166	[redacted]	[redacted]
Elenolic-7-O-glucoside acid	60539-23-3	–	[redacted]	[redacted]
Luteolin-7-O-glucoside	5373-11-5	–	[redacted]	[redacted]
Luteolin	491-70-3	–	[redacted]	[redacted]
Apigenin-7-O-glucoside	578-74-5	–	[redacted]	[redacted]
Diosmetin-7-O-glucoside	20126-59-4	–	[redacted]	[redacted]
Diosmetin	520-34-3	–	[redacted]	[redacted]
Caffeic acid	331-39-5	–	[redacted]	[redacted]
Vanillic acid	121-34-6	08.043	[redacted]	[redacted]
Vanillin	121-33-5	05.018	[redacted]	[redacted]
Total			24.3	23.2–24.7

CAS No: Chemical Abstracts Service number; FLAVIS No: EU Flavour Information System number.

The FEEDAP Panel notes that the characterisation of the additive is incomplete. A full analysis of the extract was not provided, more than 70% of the extract remains uncharacterised. The literature data (Sangodele et al., 2014) provided on an extract described as an olive leaf extract, which clearly contained other components because of the high fat content,[8] was not relevant for the assessment of the additive.

[redacted]

[8] Technical dossier/Supplementary information June 2019/Clarification.

3.2.1.1. Impurities

Multiresidue analysis for pesticides of two batches of the additive showed that [redacted]

Mycotoxins (ochratoxin A, aflatoxin B1 and the sum of aflatoxins (B1+B2+G1+G2)) [redacted] in three batches.[10] Residual solvents [redacted] in additional three batches.[11] Yeast and moulds were [redacted] in five batches of olive extract.[6] The levels of dioxins and dioxin-like PCBs were not determined, but according to the risk analysis provided by the applicant the risk for contamination is low.[12]

In two batches, heavy metals ([redacted]) and arsenic ([redacted]) were low and are considered of no concern.[13] In additional three batches, heavy metals (as lead) were [redacted], lead and arsenic was [redacted] each.[14]

3.2.1.2. Physicochemical properties

The extract under assessment is a green to brown powder with a characteristic odour and taste. It has a density of 200–500 kg/m^3 and is partially soluble in water.[15]

3.2.2. Stability

The stability of the additive was demonstrated for at least 24 months at 25°C (60% relative humidity, RH) and for 6 months at 40°C (75% RH) in three batches of the additive.[16] [redacted].

No data to support the stability of the additive in water for drinking was provided.

3.2.3. Conditions of use

The additive is intended for use in feed for all animal species. The maximum recommended level of inclusion in feed is 70 mg/kg feed for chickens and laying hens, 120 mg/kg for turkeys, 80 mg/kg for piglets, 135 mg/kg for pigs for fattening and sows, 135 mg/kg for lambs, 125 mg/kg for dairy sheep, ewes for reproduction, and goats, 195 mg/kg for dairy cows and 300 mg/kg for calves, cattle, cows for reproduction, 300 mg/kg feed for salmon and trout, 100 mg/kg for rabbits and does and 200 mg/kg for horses. Assuming that the additive contains at least 20% of oleuropein, the maximum recommended levels in feed correspond to maximum levels ranging from at least 14 to 60 mg oleuropein/kg complete feed.

The recommended level of inclusion in water is 50 mg additive/kg water for drinking for all animal species (corresponding to at least 10 mg oleuropein/kg water). The inclusion level of the additive in milk replacer for calves ranges from 5 to 30 mg/L or 50 to 300 mg/animal and day. A minimum or a maximum content is not proposed.

The Panel notes that the list of inclusion levels in feed considers only food producing animal species. No use levels are proposed for non-food producing animals.

3.3. Safety

The safety assessment is based on the maximum recommended use levels proposed by the applicant for the different target species.

3.3.1. Absorption, distribution, metabolism and excretion

No absorption, distribution, metabolism and excretion (ADME) studies of the active substances contained in olive leaf extract in target species or laboratory animals were submitted by the applicant.

[redacted]

[12] Technical dossier/Section II/page 8.

[redacted]

[15] Technical dossier/Section II/Annex_II_11.

[redacted]

Kendall et al., 2009 reviewed the ADME of phenols present in olive products (mostly olive oil) and concluded based on the limited experimental data, that the bioavailability of oleuropein from the gut is low. Oleuropein is the glucosidic ester of elenolic acid with 3,4-dihydroxyphenylethanol (hydroxytyrosol). The possibility of hydrolysis of the glycosidic bond at gastrointestinal level with the formation of the aglycone is hypothesised, that is further hydrolysed to hydroxytyrosol, which are subsequently absorbed, and extensively conjugated in the organism as glucurono-, sulfate- and methylconjugates being rapidly excreted in urine (Kendall et al., 2009). These principal phase II conjugation reactions were confirmed in the rat after intravenous administration of hydroxytyrosol (D'Angelo et al., 2001) and in human volunteers after oral administration of olive leaf extract, encapsulated or in the liquid form (de Bock et al., 2013). The other identified phenolic compounds are each present in the additive at low concentrations (0.01–1.6%). These compounds are present in many food (e.g. olive oil) and feed plants and all are absorbed from the intestinal lumen and transformed to innocuous metabolites (Vissers et al., 2004; Serra et al., 2012).

Although ADME studies in target animals are not available, it can be assumed that the same metabolic pathways occur in the target animals.

3.3.2. Genotoxicity

For mixtures containing a substantial fraction of substances that have not been chemically identified, the EFSA Scientific Committee recommends that the chemically defined substances should first be assessed individually for their potential genotoxicity (EFSA Scientific Committee, 2019b). Therefore, the potential genotoxicity of oleuropein and other identified components is considered first.

The applicant provided *in silico* analysis performed with OECD QSAR ToolBox (version not specified) for all the identified components listed in Table 1.[17] For all the identified components, no alerts were identified for *in vitro* mutagenicity (Ames test), for genotoxic and non-genotoxic carcinogenicity and for other endpoints.

Although the identified components did not raise a concern for genotoxicity, considering that there is uncertainty on the composition of the additive, as 70% remains uncharacterised, the FEEDAP Panel could not conclude on the genotoxicity of the additive under assessment.

3.3.3. Toxicological studies

No repeated-dose toxicity studies with the additive under evaluation were submitted. The applicant submitted two studies, one in laboratory animals and one in pigs (see safety for target species). Although the test materials used in the studies, a water extract and leaf powder, are different compared to the ethanolic extract under application, the Panel considers these studies relevant for the present assessment. Concerning the extracts, water and ethanol are expected to extract a similar spectrum of polar compounds giving rise to comparable qualitative composition, although differences might occur in the quantitative composition. In the study in pigs, the content of oleuropein in dried olive leaf powder (2.2%) was determined after extraction with ethanol allowing a comparison with the additive under assessment.

In a 6-week toxicity study, groups of six male Wistar rats each were fed 2,000, 4,000, 7,000 or 9,000 mg/kg of a water extract of olive leaves via feed (Omer et al., 2012). The extract was obtained by extracting 100 g of dried leaves with 500 mL water for 24 h, then the extract was air evaporated for 3 days. No information was available on the content of oleuropein. A control group received normal diet without the extract. At the end of the study, blood samples were taken for the determination of haematological and biochemical parameters. The animals were necropsied and samples taken for histopathology of liver, kidney and spleen. A significant reduction of packed cell volume and haemoglobin was observed in the groups receiving 2,000 and 9,000 mg/kg of olive leaf extract, but not in the intermediate groups. A significant but not dose-related reduction in white blood cells was observed in all treatment groups. Red blood cell counts and mean corpuscular haemoglobin concentration significantly decreased in the animals that received the highest dose (9,000 mg/kg). There was a significant and dose-related increase in the serum levels of alkaline phosphatase in all treated groups. Total bilirubin was significantly increased in groups receiving 4,000 mg/kg olive leaf extract and higher compared to the control group. Microscopically both liver and kidneys showed histological alterations characterised by fatty cytoplasmic vacuolation and necrosis of hepatocytes and haemorrhages and congestion in the cortex of the kidneys. The slight haemorrhage recorded in the

[17] Technical dossier/Supplementary information/June 2019.

kidneys of the experimental animals was most pronounced in those fed 9,000 mg/kg olive leaf extract. Because adverse effects were observed in all treatment groups, and considering the short duration of the study, a no observed adverse effect level (NOAEL) cannot be established. Although the test item was an aqueous extract from olive leaves, it can be expected that an aqueous ethanolic extract would show the same adverse effects.

The FEEDAP Panel identified a 14-week study aimed at investigating the toxicity of a commercial olive leaf extract (containing 18–23% oleuropein) on the liver function in mice (Arantes-Rodrigues et al., 2011). Four groups of 10 female ICR mice each received 0, 2,500, 5,000 and 7,500 mg extract/kg feed. Serum biochemical determinations included alanine aminotransferase, alkaline phosphatase, total bilirubin and albumin. At the end of the study, the animals were killed and samples taken for histopathology of the liver. Liver mitochondrial bioenergetics were also assessed. The serum enzyme activities of alanine aminotransferase and alkaline phosphatase increased significantly in the groups fed with 5,000 and 7,500 mg/kg olive leaf extract. Histopathologically, all the groups including the lower dose tested (2,500 mg/kg) exhibited hyperplasia of the bile ducts, cholestasis, hepatocyte necrosis and inflammatory infiltrates. Hepatic fibrosis was observed in the groups receiving 5,000 and 7,500 mg/kg olive leaf extract. The mitochondrial membrane potential, respiratory control ratio and the efficiency of ATP synthesis of samples from animals fed the higher concentration of olive leaf extract was significantly decreased when compared to the control group.

The studies with olive leaf extracts in laboratory animals did not allow deriving an NOAEL as they showed adverse effects already at the lowest concentration tested. Moreover, the validity of the studies was very limited, and the studies were not performed under GLP and not according to OECD guidelines.

3.3.4. Safety for target species

Different concentrations of a dried olive leaf powder were administered to pigs for 8 weeks to investigate the effect on haematology and lipid profile of blood and on the oxidative stability of red blood cells (Paiva-Martins et al., 2014). Thirty male pigs (Large White $\times$ Landrace $\times$ Pietrain), 12 week old (67.4 $\pm$ 4.7 kg), were assigned to three experimental groups: a control group fed unsupplemented diet and two groups fed diet supplemented with 50 and 100 g/kg dried olive leaf powder, containing 2.2% oleuropein. These dose levels are comparable with 1,100 and 2,200 mg oleuropein/kg feed. Pigs were individually housed. Blood was collected for haematological and biochemical analysis, and haemostatic investigations. The main zootechnical parameters were significantly adversely affected by olive leaf supplementation at all doses tested (50 and 100 g/kg compared to the control group final body weight 113.9 and 111.7 kg vs. 130.5 kg in controls, daily weight gain 0.84 and 0.79 kg vs. 1.10 kg, daily feed intake 2.44 and 2.44 vs. 2.90 kg and feed to gain ratio, 2.95 and 3.11 vs. 2.64), indicating that the test item is not well tolerated by the animals. Olive leaf powder supplementation resulted in a significant decrease in plasmatic triacylglycerols concentration, lower body mass and lower fat storage, but no significant reductions were found for low-density lipoprotein cholesterol (LDLc) and oxidised low-density lipoprotein (oxLDL) levels. No significant differences in haematological parameters were observed by the treatment with dried olive leaf powder; however, a trend towards lower values of red blood cells, haemoglobin concentration and haematocrit were observed. Liver enzymes were not tested. The Panel notes that a safe dose could not be identified.

The applicant submitted four published studies as evidence of the safety for poultry of olive leaf powder or extract.[18] Three of these could not be considered as the doses described (based on the oleuropein content) were either considerably lower than the recommended dose for the extract under application (Cayan and Erener, 2015) or could not be established (Jabri et al., 2017; Nafea and Hussein, 2018).

In the remaining study, the effect of an olive leaf extract on the performance of chickens raised under tropical conditions (annual mean temperature 34°C and relative humidity 82%) was investigated in an experiment of 8-week duration (Oke et al., 2017). In this study, the olive leaf extract was added to water for drinking at 5, 10 or 15 mL/L, corresponding to 22, 44 or 66 mg oleuropein/L. Based on the oleuropein content, the highest level tested was approximately sixfold higher than the recommended level for the olive extract under application when used in water for drinking. Although no adverse effects were observed on performance characteristics, haematological parameters or the measured stress markers, the growth conditions employed and the limited overdosing (6 $\times$) precludes

[18] Technical dossier/Supplementary information June 2019/Efficacy.

any conclusion on safety that can be drawn with any confidence for chickens for fattening raised under typical European conditions.

The applicant provided evidence that olive leaves and twigs are commonly used as forage for ruminants and that feeding animals olive vegetation is a common practice.[19] Taking into consideration oleuropein content in olive leaves (0.75%, Council of Europe), as well as the intake of dried olive leaves (23–43 g dry matter (DM)/kg body weight (bw)$^{0.75}$ in sheep, 71 g DM/kg bw$^{0.75}$ in goats, FAO, 1985), the intake of oleuropein was calculated to be 4.4 g/day in sheep, 10.8 g/day in goats. These intakes of oleuropein are 150- to 360-fold higher than the oleuropein intake (30 mg/day) from the high use level of the additive in feed (125 mg/kg) proposed by the applicant as feed flavouring.

However, considering

i) the inadequate characterisation of the additive, which does not allow a comparison of the preparation with feeding materials,
ii) the differences in matrix effects leading to different absorption,
iii) the uncertainty in relation to possible genotoxicity of the extract,
iv) the fact that severe effects have been observed in all animal studies provided, without identifying an NOAEL, substances of concern and underlying mechanisms of toxicity,
v) the lack of studies testing the additive or other comparable olive leaf extracts in ruminants,

the FEEDAP Panel cannot conclude on the safety for ruminants.

3.3.4.1. Conclusions on safety for the target species

In view of the inadequate chemical and toxicological characterisation of the additive, the FEEDAP Panel is unable to conclude on the safety for the target species.

3.3.5. Safety for the consumer

In view of the inadequate chemical and toxicological characterisation of the additive, the FEEDAP Panel is unable to assess the safety for the consumers following the use of olive leaf extract as a flavouring in animal feed.

3.3.6. Safety for the user

No specific data were provided by the applicant regarding the safety of the additive for users. In the absence of these data and considering that the chemical and toxicological characterisation of the additive was inadequate, no conclusions can be drawn on the additive's potential to be a dermal/eye irritant or a skin sensitiser nor on the effects on the respiratory system.

3.3.7. Safety for the environment

O. europaea L. is a native species to Europe where it is widely grown for commercial purposes. Use of the extract from the plant in animal production is not expected to pose a risk for the environment.

3.4. Efficacy

The applicant submitted an efficacy study in weaned piglets and four studies in broilers, which showed an increase in feed intake after supplementation of olive leaf powder via feed or olive leaf extract in water for drinking.[20] However, these studies are designed to show an improvement of performance parameters and therefore are inappropriate for demonstrating the sensory properties (to influence feed smell and/or increase feed palatability).

No conclusion can be drawn on the sensory properties of the additive under assessment.

4. Conclusions

Approximately 70% of the extract under assessment remained uncharacterised.

In view of the inadequate chemical and toxicological characterisation of the additive, the FEEDAP Panel is unable to conclude on the safety for the target species, the consumers and the user.

[19] Technical dossier/Section II.
[20] Technical dossier/Supplementary information/June 2019/9.Efficacy.

O. europaea L. is a native species to Europe where it is widely grown for commercial purposes. Use of the extract from the plant in animal production is not expected to pose a risk for the environment.

In the absence of data showing the sensory properties of the additive under assessment, the Panel could not conclude on the efficacy of olive leaf extract from *O. europaea* L. when used as sensory additive.

Documentation provided to EFSA/Chronology

Date	Event
08/11/2010	Dossier received by EFSA. Olive extract, olive leaf extract (*Olea europaea* L.) for all animal species. Submitted by Nor-Feed A/S
07/08/2018	Reception mandate from the European Commission
11/09/2018	Application validated by EFSA – Start of the scientific assessment
27/09/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the consumer, safety for the user and efficacy*
12/12/2018	Comments received from Member States
17/06/2019	Reception of supplementary information from the applicant – Assessment remains suspended
08/08/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment re-started
28/01/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
bw	body weight
CAS	Chemical Abstracts Service
CoE	Council of Europe

DM	dry matter
EINECS	Existing Commercial Chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
HPLC	high-performance liquid chromatography
LDLc	low-density lipoprotein cholesterol
LOD	limit of detection
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OLE	Olive leaf extract
oxLDL	oxidised low density lipoprotein
QSAR	quantitative structure–activity relationship
RH	Relative humidity
SC	EFSA Scientific Committee
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Olive leaf extract

In the current application, authorisation is sought under Article 10(2) for the botanically defined *olive leaf extract* under the category/functional group (2 b) 'sensory additives'/'flavouring compounds', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the *feed additive* is sought to be used for all animal species and categories.

The *feed additive* is a powder extract derived from the leaves of olive trees containing a minimum of 20% (w/w) of *oleuropein* which is considered by the Applicant as a phytochemical marker. The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* at the levels of *olive leaf extract* ranging from 10 to 300 mg/kg *feedingstuffs*.

For the quantification of *oleuropein* (phytochemical marker) in the *feed additive,* the Applicant submitted a method based on reversed phase high-performance liquid chromatography (HPLC) coupled to ultraviolet (UV) detection. The method is based on the HPLC-UV protocol described in the European Pharmacopoeia monograph 01/2008:1878 dedicated for the determination of *oleuropein* in olive leaves.

The Applicant analysed five batches of the *feed additive* and a relative standard deviation for *repeatability* (RSDr) of 1.2% was reported for an average content of *oleuropein* of 20.5% (w/w).

Based on the acceptable performance characteristics presented the EURL recommends for official control the reversed phase HPLC-UV method based on the European Pharmacopoeia monograph 01/2008:1878 for the quantification of *oleuropein* (phytochemical marker) in the *feed additive*.

For an additional identification/characterisation of the *feed additive*, the EURL considers a method based on spectrophotometry after derivatisation with Folin–Ciocalteau reagent suitable for the determination of total polyphenols in the *feed additive*.

The Applicant did not provide experimental data or analytical methods for the determination of *olive leaf 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 *olive leaf extract* in *premixture*s 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.