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SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of a *Camellia oleifera* C.Abel seed extract for use in all animal species except fin fish (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 a *Camellia oleifera* C.Abel seed extract (Cosap®) as technological feed additive for all animal species except fin fish. In the absence of adequate tolerance studies in the target species or toxicological studies with the additive under assessment, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not conclude on the safety of *C. oleifera* extract for the target species. The use of the additive in animal nutrition is not expected to cause concern for consumer safety. The additive is considered as irritant to the eyes and mucous membranes. No conclusions can be reached on the potential of the additive to be irritant to the skin or to be a dermal sensitiser. The use of the additive under the proposed conditions of use is considered safe for the terrestrial compartment. However, no conclusion can be reached on the safety of the additive for the environment when used in feed of aquatic animals other than fin fish. The Panel concluded that the additive has the potential to be efficacious as an emulsifier when used according to proposed conditions of use.

KEYWORDS

Camellia oleifera C.Abel, *Camellia oleifera* extract, cosap, efficacy, emulsifiers, flavonol glycosides, pentacyclic triterpenoid saponins, safety, technological additives

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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 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 SAS[2] for the authorisation of the additive consisting of *Camellia oleifera* extract, when used as a feed additive for all animal species except fin fish (category: technological additives; functional group: emulsifiers).

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 *C. oleifera* extract, when used under the proposed conditions of use (see Section 3.1.4).

1.2 | Additional information

The additive has not been previously authorised as a feed additive in the European Union (EU).

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 *C. oleifera* extract as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 21 January 2022 to 21 April 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 additive.[4]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *C. oleifera* extract is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: 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), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019), Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023), Statement on the genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019).

[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, 49070 Beaucouze, 3 rue Amedeo Avogadro, France.

[3]FEED dossier reference: FAD-2021-0071.

[4]The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2021-0071_en.

[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.

3 | ASSESSMENT

The additive under assessment, *C. oleifera* extract (COE), is a liquid extract derived from the seed meal[6] of *C. oleifera* C.Abel. It is intended to be used as a technological additive (functional group: emulsifiers) in feed for all animal species except fin fish.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The additive COE is obtained from seeds of *C. oleifera* used by the edible-oil industry. The extraction of the seed meal, left after cold pressing to remove the oil, is made with aqueous alcohols [redacted]

The additive is specified to contain a minimum of 10.5% of saponins. Compliance with the proposed specification was shown by analysis of five batches,[7] which showed an average content of 11.6% (range: 11.3%–11.8%) of total saponins, when analysed by an in-house spectrophotometric method for quantification of total saponins in the additive.[8]

The applicant provided a proximate analysis of the same five batches of the additive.[9] [redacted]

The dry matter fraction was further characterised[10] by high performance liquid chromatography (HPLC) with diode array detection (DAD), evaporative light scattering detection (ELSD) or mass spectrometry (MS) detection. The HPLC with DAD detection identified three groups of components: carbohydrates and the secondary metabolites flavonoids and saponins, whose identity was confirmed by HPLC–MS^n and quantified by HPLC-ELSD. When analysed by using HPLC–ELSD the carbohydrate content in five batches[11] was on average 20.3% (range: 19.8%–20.8%), flavonoids were 12.8% (12.7%–13.1%) and saponins were 10.8% (10%–11.6%). The results are summarised in Table 1.

All the constituents (average content of carbohydrates, flavonoids, saponins, protein, fat, ash and fibre) sum up to approximately 50.9% which is above the analytically determined DM content (mean DM is 43.9%). The FEEDAP Panel notes that this difference may be attributed to the variety of analytical methods used to quantify different constituents of the additive, which all have different measurement uncertainties.

TABLE 1 Characterisation of the additive *Camellia oleifera* extract based on the analysis of five batches. The results are expressed as % (w/w) of the extract.

[redacted]

Further analyses by HPLC coupled with electrospray ionisation mass spectrometry (HPLC–ESI-MS^n) allowed the identification of most of the individual components of the three fractions.

[redacted] The identified saponins already described

[6]Seed meal or seed cake that remained after the extraction of the oil.

[7]Technical dossier/Section II/Annex_II_9 to Annex_II_13.

[8]Saponins were determined by a patented spectrophotometric method which includes derivatisation and reading absorption at 600 nm, with aescin as standard; Technical dossier/Section II/Annex II_8, Annex II_66, Annex II_70 and Annex II_71.

[9]Technical dossier/Section II/Annexes_II_2 to II_6.

[10]Technical dossier/Section II/Annex II_7.

[11]Technical dossier/Section II/Annex_II_7.

in literature [redacted] are pentacyclic oleanane-type triterpenoid saponins. Based on the molecular formula and the MS fragmentation patterns, the other saponins without a fully resolved structure, are also assumed to be pentacyclic oleanane-type triterpenoid saponins.

The results of the characterisation of the individual components of the flavonoid and saponin fractions (secondary metabolites) by HPLC–ELSD in five batches of the additive are summarised in Table 2.

TABLE 2 Characterisation of the individual components of the flavonoid and saponin fractions (secondary metabolites) of the additive *Camellia oleifera* extract based on the analysis of five batches by high performance liquid chromatography with evaporative light scattering detector, expressed as % (w/w) of the extract.

[redacted]

Based on the available data on the characterisation, the additive is considered a fully defined mixture (EFSA Scientific Committee, 2019).

3.1.1.1 | *Impurities*

Three batches of the additive were analysed for impurities.[12] The concentrations of cadmium, lead, mercury and arsenic were below the limit of quantification (LOQ). The analysis of aflatoxins (B1, B2, G1, G2) and ochratoxin A resulted in values

[12]Technical dossier/Section II/Annex II_14; Annex II_15 and Annex II_16.

below the LOQ, except for aflatoxin B1, which was detectable in two batches at 0.42 and 0.48 μg/kg. In the same batches, the results of a multiresidue pesticide analysis showed that all pesticide residues were below their respective LOQ.[13]

Ethanol ([redacted])[14] and methanol ([redacted])[15] quantified in four batches were below the corresponding ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) thresholds for residual solvents (5000 mg/kg for ethanol and 3000 mg/kg for methanol; EMA, 2024).

Polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated dibenzofuran (PCDF) and dioxin-like polychlorinated biphenyls (PCBs) were analysed in three batches.[16] The calculated upper bond concentrations for the sum of dioxins ranged between 0.0156 and 0.0272 ng WHO-PCCD/F TEQ/kg, and between 0.239 and 0.416 ng WHO-PCCD/F + PCB TEQ/kg for the sum of dioxins and dioxin-like PCBs. The upper bond sum of non-dioxin-like PCBs ranged between 0.0049 and 0.0216 μg/kg (all expressed on a 88% dry matter basis).

Microbiological contamination[17] was assessed by the determination of *Escherichia coli* and *Salmonella* spp. with values of < 1 colony forming units (CFU)/g and no detection in 25 g, respectively. Counts of aerobic bacteria were < 100 CFU/g; filamentous fungi and yeasts were < 10 CFU/g, and coagulase-positive staphylococci were not detected in 1 g. In three additional batches, Enterobacteriaceae were < 10 CFU/g.[18]

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

3.1.2 | Physical properties of the additive

COE is a brown liquid with an average density[19] of 1040 kg/m^3. The pH of the additive is specified to be in a range from 5 to 7 at 20°C, with a reported average from five batches[20] of pH 5.5.

3.1.3 | Stability and homogeneity

3.1.3.1 | *Shelf life*

The proposed shelf life of COE is 12 months. No losses in the total saponin content were observed after storage at room temperature for 16 or 17 months.[21]

Three batches of COE were stored at 40°C/80% of relative humidity for 6 months. There were no losses in total saponin content after the storage period.[22]

3.1.3.2 | *Stability in premixtures and feed*

The stability of the additive incorporated at 3% in a premixture (containing calcium carbonate as carrier and trace elements) was assessed by measuring camelliaside A in one batch after 6-month storage (in aluminium bags at room temperature).[23] The loss of camelliaside A was 18.6%.[24]

The stability of the additive in feed at the inclusion level of 0.015%, was studied by measuring camelliaside A in three different feeds: a mash finisher feed for chickens, a mash starter feed for piglets and a pelleted feed for dairy cows.[25] After 3-month storage (in aluminium bags at room temperature) the losses of camelliaside A were 3.5%, 10.7% and 14%, respectively.

[13]Technical dossier/Section II/Annex II_14; Annex II_15 and Annex II_16. LOQ(Pb) = 0.01 mg/kg; LOQ(Cd) = 0.005 mg/kg; LOQ(As) = 0.05 mg/kg and LOQ(Hg) = 0.005 mg/kg; LOQ (aflatoxins B1, B2 and G1) = 0.2 μg/kg; LOQ (aflatoxin G2) = 0.4 μg/kg; LOQ(ochratoxin A) = 2 μg/kg; LOQs (multiresidue pesticide analysis) = 0.005–0.05 mg/kg.

[14]Technical dossier/Section II/Annex II_22; Annex II_23, Annex II_24, Annex II_25.

[15]Technical dossier/Section II/ Annex II_25 and Supplementary information June 2023/Annexes 1–3.

[16]Technical dossier/Supplementary information June 2023/Annexes 4–6. Upper bound concentrations are calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification. TEQ = toxic equivalency factors for dioxins, furans and dioxin-like PCBs established by WHO in 2005 (van den Berg et al., 2006).

[17]Technical dossier/Section II/Annex II_15, Annex II_17 and Annex II_18.

[18]Technical dossier/Supplementary information June 2023/Annexes 7–9.

[19]Technical dossier/Section II/Annex II_33.

[20]Technical dossier/Section II/Annex II_34.

[21]Technical dossier/Section II/Annex_II_36 and Annex_II_37.

[22]Technical dossier/Annex II_39, 41, 43, 45, 47, 48.

[23]Technical dossier/ Annex II_62–1 and Supplementary information June 2023.

[24]Technical dossier/Section II/Annex II_53, Annex II_54, Annex II_55, Annex II_55–1 and Annex II_55–2.

[25]Technical dossier/Section II/Annex II_61, Annex II_62 and Annex II_62–1.

3.1.3.3 | *Homogeneity*

The capacity for homogeneous distribution of the additive in feed was assessed by measuring camelliaside A content in 10 sub-samples of a feed supplemented with 150 mg COE/kg. The average content of camelliaside A was 3.47 mg/kg and the corresponding coefficient of variation was 8.4%.[26]

3.1.4 | Conditions of use

The additive COE is intended to be used in feed for all animal species except fin fish at a minimum use level of 30 mg/kg complete feed. The maximum proposed use levels are 200 mg COE/kg feed for non-ruminant species, 500 mg COE/kg feed for dairy cows and 750 mg COE/kg feed for other ruminants.

These inclusion levels would correspond to the following minimum and maximum levels[27] of saponins in feed: 3.15–3.54 to 21–23.6 mg saponins/kg feed for non-ruminant species; 3.15–3.54 to 52.5–59 mg saponins/kg feed for dairy cows and 3.15–3.54 to 78.8–88.5 mg saponins/kg feed for other ruminants.

3.2 | Safety

The safety assessment of the additive is based on the maximum proposed use levels in feed, i.e. 200 mg/kg for all non-ruminant species (except fin fish), 500 mg/kg for dairy cows and 750 mg/kg for other ruminants.

No studies to support the safety for the target species, the consumers and the users were provided for the additive under assessment.

The applicant carried out an extensive literature search (ELS) to support the safety evaluation of the additive for the target species, the consumer, user and the environment.[28] The search strategy was described in detail and search terms were provided (substance descriptors, effects and target species). The search was carried out in SciFinder® database covering the period until 2023, without a start date. After applying the inclusion and exclusion criteria, 15 publications were considered relevant.

In addition to the ELS, the applicant carried out a manual search on PubMed, Google scholar and ResearchGate. The search syntax was provided. After removing the duplicates, the manual search resulted in additional 24 publications considered by the applicant as relevant.

The main components of the additive are carbohydrates, flavonoids and saponins (Table 1). Mineral components (expressed as ash), proteins, lipids, fibre and carbohydrates do not raise concern and are not further considered in the assessment. Among the identified secondary plant metabolites of the extract, pentacyclic oleanane-type triterpenoid saponins represent up to 11.6% (w/w) and flavonoids up to 13.1% (w/w) (Table 2).

Pentacyclic oleanane-type triterpenoid saponins

Pentacyclic oleanane-type triterpenoid saponins are common in all *Camellia* species. This type of structure is also present in many other plants including some edible legumes cultivated in Europe (e.g. soybean, peas, broad bean or chickpeas). Saponins in these edible legumes occur in a range from 2.5% to 5.6% dry weight (dw, w/w) (Shi et al., 2004; Vincken et al., 2007).

The FEEDAP Panel notes that the EFSA Panel on Food Additives and Flavourings (EFSA FAF Panel) evaluated similar compounds in the scientific opinion on the re-evaluation of Quillaia extract as a food additive (EFSA FAF Panel, 2019). Considering the limited data available on the absorption, distribution, metabolism and excretion (ADME) of Quillaia saponins, the FAF Panel applied read-across from structurally similar saponins, assuming that Quillaia saponins would share a similar fate. The FEEDAP Panel considers that an approach based on read-across from pentacyclic triterpenoid saponins would be appropriate for the assessment of the ADME and the genotoxicity of saponins from COE.

When considering toxicity, the structural differences in the side chains of the pentacyclic triterpenoid saponins, e.g. in the number of sugar units attached at different positions to the sapogenin, are expected to influence the interaction of saponins with cell membranes. Therefore, differences in the size and the substitution pattern of the saponins would argue against the read-across of toxicological data among saponins sharing the same pentacyclic triterpenoid structure, but with a different glycosylation/side chain pattern.

Saponins can exert deleterious effects on cell membranes (Kawaguchi et al., 1994; Shen et al., 2008). This is partly due to the formation of complexes with cholesterol, which is an important component of the lipid barrier of cells (Böttger & Melzig, 2013; Navarro del Hierro et al., 2018). As a result, the permeability of the membranes is enhanced which may lead to a collapse of cell integrity. This is the underlying reason for the haemolytic property of saponins and the irritation of

[26]Technical dossier/Section II/Annex II_63.

[27]Range is calculated by using the minimum of 10.5% of saponins in the COE and a maximum of 11.8% saponins (maximum analytical value reported in the certificates of analyses).

[28]Technical dossier/Supplementary information June 2023/Annex 10.

mucous membranes of the oral cavity, gastrointestinal tract, eyes and respiratory tract. Damage to the intestinal mucosa may enhance the absorption of saponins and facilitate their systemic toxicity (Guo et al., 2018).

Flavonoids

The flavonoids identified in COE are flavonol-3-O-glycosides, whose aglycone is kaempferol. Kaempferol glycosides are present in several edible vegetables such as cabbage, parsley, radish, turnip, Welsh onion and broccoli (Sakakibara et al., 2003).

The following sections focus on pentacyclic triterpenoid saponins and flavonol glycosides based on the evidence provided by the applicant in the form of literature searches described above. The publications considered relevant by the FEEDAP Panel are described in the following sections.

3.2.1 | Absorption, distribution, metabolism and excretion

3.2.1.1 | *Pentacyclic triterpenoid saponins*

In the absence of ADME data on saponins from *C. oleifera*, the FEEDAP Panel considered that read-across from other saponins characterised by chemical similarity (pentacyclic triterpenoid saponins with sugar moieties attached at the same position) could be applied for ADME for saponins from *C. oleifera*. ADME data for pentacyclic triterpenoid saponins (e.g. glycyrrhizin, β-aescin, anhuienoside C) have been reported in the FAF Panel opinion on Quillaia extract (EFSA FAF Panel, 2019).

The FAF Panel reviewed in vitro studies performed to evaluate the stability/biotransformation of some pentacyclic triterpenoid saponins (glycyrrhizin, aescin, anhuienoside C) incubated in different media: (i) isolated bacteria from the rumen of yearling steers, (ii) fresh faeces from male Wistar rats, (iii) microbial glucuronidases from various intestinal bacteria and (iv) human intestinal bacteria isolated from faeces. The results consistently indicated that the saponins were hydrolysed, releasing the sugar moieties and the respective sapogenins. Additionally, some metabolites of the released aglycones were identified. When sections of small intestine, cecum and colon isolated from rats, mice or chicks were incubated (at physiological pH conditions) with a soybean saponin extract, only saponins were found in the small intestine digests, while both, saponins and sapogenins, were present in the cecum and colon digests, demonstrating the action of the microbiota present in cecum and colon.

Some in vivo studies are also described in the FAF Panel opinion which justify the read-across to other oleanane-type triterpenoid saponins. Soybean saponins crossed the gastrointestinal tract intact, being hydrolysed only in the colon and caecum of mice, rats and chicks (Gestetner et al., 1968, as referenced in EFSA FAF Panel, 2019); no saponins or sapogenins were detected (limit of detection, LOD, 40 μg) in blood of the animals after feeding 10 days a diet containing 20% heated soybean flour. Other examples are studies carried out in rats given orally soybean saponins, aescin, glycyrrhizin, pulsatilla saponin D or DS-1 from *Dianthus superbus*. The bioavailability was very low for all compounds ranging from 0.16% to 4.0%, depending on the saponins tested and whether given as extracts or individually (EFSA FAF Panel, 2019).

The applicant provided a review paper on the gastrointestinal behaviour of several saponins, including pentacyclic triterpenoid saponins, in relation to their bioavailability and bioactivity (Navarro del Hierro et al., 2018). The bioavailability of saponins is expected to vary depending on the chemical structure of saponins and sapogenins and on the different animal species. Saponins are usually poorly absorbed in the gastrointestinal tract, and sapogenins released from glycosides in the colon by bacterial enzymes are better absorbed than the parent glycosides.

Overall, the pentacyclic triterpenoid saponins from *C. oleifera*, as known for saponins in general, are expected to be poorly absorbed in the gut of experimental animals and in target species. Following hydrolysis by the microbiota in colon and caecum, the respective sapogenins may be absorbed, although to a limited extent. The sapogenins absorbed, with hydroxyl groups in their structures, are prone to be glucuronidated and excreted in urine.

3.2.1.2 | *Flavonol glycosides*

The flavonoids identified in COE are flavonol-3-O-glycosides, whose aglycone is kaempferol.

The literature search provided by the applicant identified two publications with relevant data on the ADME of kaempferol and its hydroxylated derivative quercetin: a pharmacokinetics study of flavonols in rats (Chen et al., 2013) and a review on the bioavailability and metabolism of flavonoids (Viskupičová et al., 2008).

The pharmacokinetics parameters of the flavonols kaempferol and quercetin (which is formed by enzymatic oxidation of the B-ring of kaempferol via the cytochrome P450 system) have been described by Chen et al. (2013). Following oral administration of a standardised ginkgo biloba extract (GBE50 containing kaempferol 12.2% and quercetin 16.7%) at 10, 30 and 90 mg/kg, the total flavonols showed a biphasic plasma concentration.[29] A first peak (corresponding to glucuronides and glycosides) was observed after 15 min followed by a rapid decrease in plasma concentrations, suggesting a fast absorption of the aglycones after hydrolysis of the glycosides in the small intestine, which are then promptly conjugated and

[29]Measured after hydrolysis of rat plasma samples with hydrochloric acid (HCl; 4 M) to release the flavonol aglycones from the glycosides and conjugated metabolites. The measured flavonol levels are expressed as concentrations of total kaempferol (t-kaempferol) and total quercetin (t-quercetin).

excreted. The second peak (corresponding to glucuronides) appeared between 4 and 6 h after administration and was followed by a slow decrease in plasma concentrations, probably reflecting biliary excretion followed by the hydrolysis of non-absorbed glycosides by colonic bacteria and absorption of the aglycones with subsequent enterohepatic circulation and excretion. Plasma C_{max} of flavonols (free and conjugated) increased linearly with the dose.

After the oral application of 10 mg GBE50/kg BW (equivalent to 68.1 µg kaempferol), kaempferol plasma concentration was 14.1 ng/mL and the absorption rate was 6.58%. For quercetin, present in GBE50 in similar concentration, the absorption rate was 10-fold lower (0.48%) than for kaempferol. The flavonol aglycones kaempferol and quercetin were not detected in plasma (limit of quantification 0.32 pg, Zhao et al., 2008, as referenced in Chen et al., 2013), where the compounds were present mainly as their glucuronides. After the oral application of 90 mg GBE50/kg BW, flavonol concentrations in tissues were highest in kidney, followed by liver, lung and heart. Several flavonol glycosides and flavonol aglycone conjugates (glucuronides) as well as the sulfated kaempferol glycoside were recovered in rat bile. The recovery of flavonols in feaces was considerably lower than in the bile. Flavonol glycosides, together with low levels of glucuronides were recovered in urine.

The bioavailability and metabolism of flavonoid glycosides, including glycosides of kaempferol and quercetin, was reviewed by Viskupičová et al. (2008). After ingestion, these compounds are hydrolysed by β-glucosidases of the intestinal cells generating the respective aglycones which are then absorbed. The remaining intact glycosides may be hydrolysed in the colon by microbiota enzymes and the aglycones partially absorbed or excreted in faeces. After absorption, the aglycones are conjugated mainly in liver, giving rise to glucuronides and sulfates, although methylated derivatives can also be formed. These conjugates can be excreted in urine, but bile seems to be the principal via of excretion.

Studies on the metabolism of flavonoids in the target species were not available. However, equivalent metabolic pathways exist in all species routinely exposed to flavonoids and related compounds found in diets. Therefore, it can be assumed that food-producing animals have the ability to metabolise and excrete the flavonoids present in the additive, and they are not expected to accumulate in tissues and products of animal origin.

Overall, the available data indicate that flavonoids present in the additive are poorly absorbed in the glycosidic form. However, after intestinal metabolism the aglycones can be absorbed and extensively metabolised, mainly by conjugation, giving rise to glucuronides, sulfates and methylated derivatives. Both the flavonoids and their metabolites are widely distributed in animal tissues and excretion occurs in urine, and through bile in faeces. Thus, it is not expected that accumulation of such compounds and their metabolites in tissues and products of target species occurs.

3.2.2 | Toxicological studies

3.2.2.1 | *Genotoxicity*

For fully defined mixtures, the EFSA Scientific Committee recommends applying a component-based approach, i.e. assessing all components individually for their genotoxic potential using all available information, including read-across and quantitative structure–activity relationship (QSAR) considerations about their genotoxic potential (EFSA Scientific Committee, 2019).

Therefore, the potential genotoxicity of the identified constituents of the additive was first considered and evaluated through the analysis of the studies retrieved in a literature search[30] provided by the applicant. Then, genotoxicity studies performed with the additive under assessment or with *C. oleifera* extracts similar to the additive under assessment were taken into account. The studies deemed relevant are discussed below.

Pentacyclic triterpenoid saponins

The saponins identified in the additive under assessment are pentacyclic triterpenoid saponins structurally similar to saponins detected in Quillaia extract. Triterpenoid saponins in Quillaia extract consist of glycosides of quillaic acid, which is a major pentacyclic triterpenoid aglycone of quillaia saponins. Based on this structural similarity of the aglycones, the FEEDAP Panel considers that applying read-across from pentacyclic triterpenoid saponins from Quillaia extract would be appropriate for the assessment of genotoxicity of saponins from COE. No concern for genotoxicity of saponins was identified by the FAF Panel in the scientific opinion on the re-evaluation of Quillaia extract as food additive (EFSA FAF Panel, 2019). Therefore, the FEEDAP Panel concluded that saponins from COE do not raise concern for genotoxicity.

Flavonol glycosides

The flavonoids present in the additive under assessment are mainly camelliaside A and B (average 5.6% and 7% of the extract, respectively). These compounds are flavonol triglycosides, consisting of the aglycone kaempferol and a carbohydrate chain.

The genotoxic potential of the aglycone kaempferol was evaluated in vitro in studies which showed the induction of gene mutations in bacterial and mammalian cells in the presence of metabolic activation (Brown & Dietrich, 1979; Carver et al., 1983; Hardigree & Epler, 1978; Nagao et al., 1981; Silva et al., 1997). It was demonstrated that these mutagenic effects

[30]Technical dossier/Supplementary information 15 March 2024.

were mainly related to quercetin formed by enzymatic oxidation of the B-ring of kaempferol via the cytochrome P450 system (Nagao et al., 1981; Silva et al., 1996, 1997). For a kaempferol-rich food produced from enzyme-treated horseradish leaves (16.8% kaempferol as aglycone), positive results were reported for the induction of gene mutations by the Ames test in the presence or absence of metabolic activation (Kimoto et al., 2022).

An increase of chromosomal aberrations in CHO and V79 cell induced by the aglycone kaempferol was observed in the presence and absence of metabolic activation (Carver et al., 1983; Silva et al., 1996, 1997). The effects observed in the absence of metabolic activation were attributed to inhibition of the enzyme topoisomerase II triggering the induction of DNA double strand breaks (Zhang et al., 2015).

No follow-up in vivo genotoxicity studies on kaempferol were retrieved by the applicant. The only in vivo micronucleus test identified by the literature search was performed in rats with a kaempferol-rich food produced from enzyme-treated horseradish leaves (16.8% kaempferol as aglycone). No cytotoxic effects and no increase in the frequency of micronuclei in bone marrow erythrocytes were observed (Kimoto et al., 2022). The FEEDAP Panel notes that the negative results were obtained with no evidence of target tissue exposure; in addition, the test item had a low purity, even with a content of kaempferol in the form of the aglycone comparable to the additive under assessment (i.e. 12.8%). Thus, the FEEDAP Panel considered the study of low relevance for the assessment.

Overall, after ingestion flavonol glycosides are hydrolysed by intestinal enzymes and colonic bacteria to release the aglycone which is absorbed and then conjugated in the liver with glucuronic acid or sulfate to facilitate excretion (see Section 3.2.1.2). In general, when conjugated, flavonols do not pass through the cell membranes and would not reach and damage DNA.

On the other hand, the aglycone kaempferol has a genotoxic potential observed in vitro in the absence of metabolic activation, that could be expressed (i) in vivo systemically and (ii) at the site of contact. In this respect, the FEEDAP Panel considered that:

(i) Systemic effects: a pharmacokinetic study performed in rats, showed that free aglycones were not detected in plasma following oral administration of ginkgo extracts rich in glycosides of kaempferol and quercetin (Chen et al., 2013). On this basis, no systemic exposure to the aglycones was observed and potential systemic genotoxic effects following oral exposure are not expected.

(ii) Effects on the site of contact: the intestine, where the aglycones are released and absorbed, and the liver, where the aglycones are metabolised, are identified as the sites of contact. A carcinogenicity study showed that kaempferol did not induce malignant tumours in ACI rats after chronic application of 0.04% kaempferol in the diet (Takanashi et al., 1983). Since the potential genotoxicity of the aglycone kaempferol at the level of intestine and liver may result in carcinogenicity, these negative results support the conclusion that the potential genotoxicity of kaempferol is not expressed in vivo at the sites of contact.

The genotoxic effects of kaempferol observed in vitro in the presence of metabolic activation are related to the formation of quercetin by enzymatic oxidation of kaempferol (Nagao et al., 1981; Silva et al., 1996, 1997). The FEEDAP Panel noted that quercetin was not detected in plasma of rats following oral administration of an extract rich in quercetin glycosides (Chen et al., 2013), and that quercetin did not show carcinogenic activity in a chronic feeding experiment in rats (Takanashi et al., 1983). On this basis, the FEEDAP Panel concluded that quercetin does not raise concern for systemic and local genotoxic effects.

Overall, the FEEDAP Panel concludes that the potential genotoxic activity of kaempferol observed in vitro is not expressed in vivo. This is based on considerations on the metabolism of flavonol glycosides and on the absence of carcinogenicity in feeding studies with kaempferol and quercetin.

Camellia oleifera extracts

The applicant did not provide genotoxicity studies with the additive under assessment.

An Ames test and an in vivo micronucleus test in bone marrow erythrocytes were performed with a water extract of the fruit hull of *C. oleifera*, prepared using hot-reflux method, containing 9.8% triterpenoid saponins and 25.1% polyphenols (Zhang et al., 2011). The FEEDAP Panel noted that the test item was derived from a different plant part and obtained by a different extraction process compared to the additive under assessment, without a complete characterisation and quantification of the saponin and flavonoid fractions. Therefore, the study was not further considered for the evaluation of the additive under assessment.

3.2.2.2 | *Repeated-dose toxicity studies*

Pentacyclic triterpenoid saponins

Toxicological studies with the pentacyclic triterpenoid saponins identified in COE were not submitted.

In the absence of toxicological data with saponins from *C. oleifera*, the FEEDAP Panel considered that read-across from other saponins, i.e. pentacyclic triterpenoid saponins from *Quillaia saponaria*, could not be applied. The high number of sugar moieties present in Quillaia glycosides, resulting in high molecular weight, reduced lipophilicity and steric hindrance,

is expected to play a role in the toxicological properties of the molecule, reducing the contact and the effects on the gastrointestinal tract. For the pentacyclic triterpenoid saponins from *C. oleifera* with a lower substitution, an interaction with the intestinal mucosa is more likely to occur.

Flavonol glycosides

Toxicological data for the kaempferol glycosides identified in COE were not submitted by the applicant.

Camellia oleifera extracts

The literature search performed by the applicant identified 90-day oral toxicity studies in mice (Ahmed et al., 2020) and in rats (Kawaguchi et al., 1994). However, the test items used in both studies are not comparable with the additive under assessment. In the study by Ahmed et al., 2020, the saponin mixture was obtained by solid phase adsorption extraction from *C. oleifera* and was administered to experimental animals in oil. The presence of oil is expected to influence the toxicokinetic and consequently the toxicodynamic of the components. Therefore, the results cannot be extrapolated to the additive under assessment. In the study by Kawaguchi et al., 1994, the test item containing 48% saponins from the seeds of *C. sinensis* was not fully characterised. Due to the differences in the plant species, the extraction process and the polarity of the solvents used,[31] the constituents present in the test item may differ from those present in the additive under assessment. Therefore, the FEEDAP Panel concludes that neither studies could be used to derive a NOAEL for the additive under assessment.

The applicant also provided studies of shorter duration in mice (Ahmed et al., 2021; Li et al., 2010; Shen et al., 2008). One study was not further considered because the saponin mixture tested was administered in oil (Ahmed et al., 2021). In the other two studies, a saponin extract, obtained from the remainder of the seeds of *C. oleifera*[32] after oil extraction using 75% ethanol as solvent, was orally administered to mice at 100, 200 and 400 mg/kg BW per day for 42 days (Li et al., 2010; Shen et al., 2008). In particular, the study by Shen et al. (2008) showed that the highest dose (400 mg/kg BW per day) was lethal to the mice. Effects on the gastrointestinal integrity and motility were seen at all doses. Significant increases of the plasma level of aspartate transaminase (GOT) were seen at 100 and 200 mg/kg BW. In addition, there was a dose-dependent, although non-significant, drop in the blood concentration of glucose, cholesterol and triglycerides, which might be associated with changes in the intestinal absorption. This hypothesis is supported by a slight dose-dependent reduction in body weight. Histological investigation confirmed the damage by the test substance on the gastrointestinal mucosa at 400 mg/kg BW per day, the highest dose tested.

Considering the nature of the effects seen in the study by Shen et al. (2008) and the potential species differences with respect to saponin toxicity (George, 1965), the FEEDAP Panel considered that there is high uncertainty when extrapolating conclusions from experimental studies made with single animal species to other animal species and categories.

3.2.2.3 | *Conclusions on toxicology*

The FEEDAP Panel did not identify a concern for genotoxicity for the individual components of the additive under assessment. Based on the toxicological data provided, a NOAEL for the individual components of the additive, pentacyclic triterpenoid saponins and flavonol glycosides, or for the additive under assessment could not be identified.

3.2.3 | Safety for the target species

The applicant did not provide tolerance trials to support the safety of the additive for the target species. From the literature search described in Section 3.2, the applicant identified a number of studies in target species considered relevant to the safety assessment of the additive. Most of the identified studies were designed to investigate the effects of saponins on the zootechnical performance and immune function of the target species, or on the reduction in methane production in ruminants. In the majority of cases, the studies showed relevant shortcomings that prevented to consider them as evidence of the safety of the different target species (e.g. no monitoring of relevant safety endpoints, lack of overdose levels tested). In addition, the source of the saponin-containing material in many of the studies was *Camellia sinensis* or was of uncertain origin. The FEEDAP Panel considered that results obtained with extracts which could not be established as coming from *C. oleifera* seeds could not be extrapolated to the additive under assessment, and therefore, these publications were not further considered.

Out of the studies retrieved from the literature search, one study evaluated the effect of an extract of *C. oleifera* seeds (levels up to 5000 mg/kg feed) with a composition approximating that of the additive (Table 3), on the zootechnical performance, blood haematology and biochemistry, gross pathology and histopathology evaluation of a series of organs and tissues of weaned piglets (Wang et al., 2020). The extract used in the study was described as 'saccharicterpenin', a dry commercial feed additive manufactured in China by extraction of the seed meal after oil removal. The applicant provided

[31]Fat removal with hexane, extraction with ethanol, addition of ether, the resulting precipitate filtered and dried.

[32]Described by the applicant using the synonym as *Camellia sasanqua*.

analytical evidence[33] to support that 'saccharicterpenin' used in the study is similar in composition of the dry matter to the additive under assessment. Some minor differences were identified in the polysaccharide profile of the test item used in the study which may be due to the enzymatic processing that was carried out during the test item preparation.

TABLE 3 A comparison of the composition between the additive under assessment and the *Camellia oleifera* extracts used as test items in the identified target animal studies (expressed as a percentage of dry matter[34]).

	Feed additive (COE) under assessment	Piglet study* ('Saccharicterpenin')
Saponins	24.4	31.6
Carbohydrates	46.2	34.4
Proteins	3.4	–
Ash	6.4	–

*Wang et al. (2020).

In the study of Wang et al. (2020), 150 crossbred[35] piglets (initial BW 7.3 kg, 50%♀: ♂) were distributed in 30 pens of five animals each, which were randomly allocated to five groups (six replicates per group). Two basal diets (pre-starter, from day 1 to 35; starter, from day 36 to 70) based on maize and soybean meal were either not supplemented (control) or supplemented with 500, 1000, 2500 or 5000 mg of 'saccharicterpenin'/kg feed. Based on the analytical composition of the extract, these supplementation levels would correspond to 0 (control), 158, 316, 790 and 1580 mg saponins/kg feed. The content of saponins in the feed was not confirmed analytically. The experimental diets were offered ad libitum for 70 days. The Panel noted that limited information was available about the husbandry conditions in which the animals were kept during the trial. The piglets were weighed on days 1, 36 and 71, and the daily feed intake was recorded. The average daily weight gain, average daily feed intake and gain-to-feed ratio were calculated for the pre-starter (1–35) and starter (36–70) periods. One piglet per pen (close to the pen's average weight) was blood sampled on days 1, 35 and 70, and different haematology[36] and biochemistry[37] parameters were analysed. On days 35 and 70, the selected animals were killed; the heart, liver, kidney, spleen and pancreas were weighed, and a histopathology evaluation was performed on them. The experimental data were analysed with a generalised linear model, including the diet as a fixed effect and the pen as an experimental unit. Group means were compared with Duncan's multiple comparison test when differences were observed. Linear, quadratic and cubic contrasts were also performed to evaluate dose-dependent relationships. The significance level was set at 0.05.

No deaths were declared during the trial. However, little information was reported regarding the health status of the animals during the trial, including the administration of veterinary treatments. Both during the pre-starter and starter phases, a significant linear dose-dependent reduction of the average daily feed intake and daily weight gain was observed, which became significant between the groups supplemented at 2500 (pre-starter phase) and 5000 (both phases) mg/kg compared to the control. A linear effect was also observed in the gain-to-feed ratio during the pre-starter phase; however, only a significant difference between the 500 and 5000 mg/kg groups was found when group means were compared. During the starter phase, a significant linear increase in the rate of diarrhoea was seen with increasing levels of the test item, which resulted in a significant difference between the 5000 mg/kg group and the control.

Regarding the blood haematology, the results on day 35 showed a linear reduction in the red blood cell counts, haemoglobin and haematocrit values with increasing additive levels, becoming significantly lower from 1000, 2500 and at 5000 mg/kg feed, respectively, compared to the control. There were no differences in the red blood cell counts and haematocrit at day 70, while the haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin levels were lower at the highest level than the control. No other differences in the haematology parameters evaluated were found. On day 35, the effects in the blood biochemistry were limited to a linear reduction of the total protein and albumin content, significantly different from the control with levels higher than 500 and 2500 mg/kg, respectively, and a linear increase in the glucose concentration, which became significant only at the highest level tested compared to the control. On day 70, no differences in the total protein content and glucose concentration were observed, whereas the difference in the albumin content was only found between the 5000 mg/kg group and the control. Instead, a significant linear reduction of the blood urea nitrogen and creatinine concentrations was observed with increasing levels of the test item, significantly different from the control at 2500 and 5000 mg/kg, respectively. The Panel noted that some of the blood haematology

[33]Technical dossier/Supplementary information 15 March 2024.

[34]Total saponins here are reported in reference to the dry matter fraction. [redacted].

[35][(Yorkshire×Landrace)×Duroc].

[36]White blood cell count (WBC), neutrophil count (NEUT#), eosinophil count (EO#), basophil count (BASO#), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), red cell distribution width-SD/CV (RDW-SD/CV), mean haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), standard deviation in red cell distribution width (RDW-SD), platelet (PLT) and thrombocytocrit (PCT).

[37]Blood urea nitrogen (BUN), total protein (TP), glucose (GLU), albumin (ALB), alkaline phosphatase (ALP), creatinine (CREA), alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol determination (TC) and bilirubin total (TBIL).

(prothrombin time, fibrinogen) and biochemistry (sodium, chlorine, calcium, phosphate, magnesium, amylase, lactate dehydrogenase, gamma-glutamyl transferase, creatine kinase, acute phase proteins) parameters usually requested for tolerance trials, according to the Guidance on the assessment of the safety for the target species of feed additives (EFSA FEEDAP Panel, 2017b) were not evaluated. Instead, the effect of the test item on serum antioxidant parameters[38] was reported. The inclusion of the test item in the feed of the piglets resulted in a reduction of some of the antioxidant parameters measured (CAT, GSH-PX, GSH-S, SOD), with a particular relevance of the significantly lower CAT and GSH-PX observed at day 35 from 1000 mg/kg feed compared to the control.

The relative weight of the pancreas (day 35) and the liver (day 70) of the animals fed with the test item at 5000 mg/kg was significantly higher compared to the control group. In both cases, a linear dose-dependent increase was observed. Based on the histopathological evaluation, the supplementation of the test item at the highest levels led to different degrees of degeneration in the hepatic (2500 and 5000 mg/kg) and splenic (5000 mg/kg) cells.

The study's results showed that the inclusion of 'saccharicterpenin' in the feed of weaned piglets at levels above 2500 mg/kg feed led to poor growth performance, haematological abnormalities and organ damage in piglets. Adverse dose-dependent effects were also observed from 1000 mg/kg feed on blood haematology and antioxidant parameters.

The FEEDAP Panel noted that the maximum use level of 200 mg COE/kg feed was not tested and that at 500 mg/kg, the lowest level included in the design, limited significant differences were observed in any of the recorded parameters compared to the control group. However, the Panel considered that, (i) based on the dose-dependent linear impact observed in the zootechnical performance and other blood parameters (especially in younger animals), (ii) the lack of essential safety information (health status of the piglets during the trial, relevant blood haematology/biochemistry parameters) and (iii) the absence of the reporting of the cumulative data for the whole experimental period, it is not possible to establish a precise safety level based on this study. Therefore, no conclusion can be made on the safety of the proposed maximum level of 200 mg COE/kg feed for piglets.

3.2.3.1 | *Conclusions on safety for the target species*

In the absence of studies with the additive under assessment, the FEEDAP Panel cannot conclude on the safety of the additive for the target species.

In general, considering the nature of the effects seen in some studies and the potential species differences in sensitivity with respect to saponin toxicity, the FEEDAP Panel considers that there would be high uncertainty when extrapolating conclusions from studies made with single animal species to other animal species.

3.2.4 | Safety for the consumer

No data on residues in products of animal origin were made available for any of the constituents of the extract.

However, the FEEDAP Panel recognises that the individual constituents of the additive COE are expected to be either poorly absorbed (pentacyclic triterpenoid saponins) or poorly absorbed and extensively metabolised and excreted in the target species (flavonol glycosides) (see Section 3.2.1). In addition, the components of the additive are already present in food of vegetable origin (see Section 3.2). Potential exposure to saponins from their use as food additive, is higher than from the consumption of products of animals fed with the additive under assessment. Therefore, a relevant increase in the uptake of these compounds originating from the normal diet by humans consuming products of animals exposed to the feed additive is not expected.

No safety concern is expected for the consumer from the use of COE at the proposed use level in feed for the target animals.

3.2.5 | Safety for the user

No specific studies on user safety with the additive under assessment have been submitted by the applicant.[39]

The applicant made a literature search aimed at retrieving studies related to the safety of preparations obtained from *C. oleifera* for the user.[40] None of the references retrieved were considered relevant to the safety assessment.

Due to the presence of saponins (see Section 3.2), the additive should be considered as irritant to mucous membranes of the oral cavity, eyes and the respiratory tract.

In the absence of data, the FEEDAP Panel cannot conclude on the skin irritation and skin sensitisation potential of the additive.

[38]Catalase (CAT), glutathione peroxidase (GSH-PX), glutathione S-transferase (GSH-S), malondialdehyde (MDA), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC).

[39]Technical dossier/Annex III 7; Annex III 9 and Supplementary information June 2023.

[40]Technical dossier/Supplementary information June 2023.

3.2.6 | Safety for the environment

The applicant carried out a literature search[41] to demonstrate that the compounds present in the additive (see Table 2) are similar to those also present in other European plant species. The search resulted in five papers, three of which were considered relevant by the FEEDAP Panel to the current assessment (Sakakibara et al., 2003; Shi et al., 2004; Vincken et al., 2007).

Since the saponins identified in COE belong to the oleanane-type sapogenins and the flavonoids identified in *C. oleifera* are structurally related to kaempferol-3-O-glycosides, these two classes of compounds (oleanane saponins and kaempferol glycosides) were used to confirm the presence and occurrence of these specific structures in plants naturally occurring in Europe.

3.2.6.1 | *Saponins*

The saponins present in the additive are oleanane-type triterpenoid saponins, which are common in all *Camellia* genus. This type of structure is present in many other botanical species including some edible legumes cultivated in Europe (e.g. soybean, peas, broad bean or chickpeas). Saponins in these edible legumes are all oleanane-type triterpenoid saponins whose content range from 2.5% to 5.6% dry weight (dw, w/w) (Shi et al., 2004; Vincken et al., 2007).

The level of saponins is not expected to be above 12% in the additive. The saponins concentration in feed resulting from the use of the additive under the proposed conditions of use (up to 750 mg/kg) will be up to 0.009%. This value is below the natural occurrence range for oleanane-type triterpenoid saponins in edible legumes.

The FEEDAP Panel notes that the use of the additive in sea farming/aquaculture (for crustaceans and molluscs) may have an adverse effect on the non-target organisms sensitive to saponins (e.g. fin fish). Saponins have been reported to be highly toxic to fish because of their damaging effect on the respiratory epithelial (Francis et al., 2002). However, the extent of this effect could not be assessed based on available data in the dossier.

3.2.6.2 | *Flavonoids*

The additive COE contains two major flavonoid compounds: camelliaside A and camelliaside B. These compounds, whose aglycone is kaempferol, belong to the class of organic compounds known as flavonol-3-O-glycosides.

According to Sakakibara et al. (2003) kaempferol glycosides are present in edible vegetables such as cabbage, parsley, radish, turnip, Welsh onion and broccoli with concentrations that range from 1.6 to 95 µmol/100 g of the fresh edible plant part. Assuming a kaempferol-3-O-glucoside, with a molecular weight of 448.4 g/mol, this occurrence level would correspond up to 0.04% (w/w) in the fresh vegetables, which is higher than the expected content in feed of 0.00042% of camelliaside A (when the additive is added at 750 mg/kg feed).

3.2.6.3 | *Conclusions on safety for the environment*

The components of the additive are naturally present in edible vegetables and other plants occurring in the EU, therefore the FEEDAP Panel concludes that the use of the additive at the proposed conditions of use is not expected to pose a risk to the terrestrial compartment. However, the data available in the dossier does not allow to conclude on the safety of the additive for the environment when used in feed for aquatic animals other than fin fish.

3.3 | Efficacy

Four studies aimed to evaluate the effects of the addition of the additive on the stability of water/oil emulsions were provided. Four oils (sunflower oil, extra virgin soybean oil, canola oil and salmon oil) were mixed with water ([redacted]), in which the additive was previously dissolved. The additive was added to water at two different inclusion levels, corresponding to [redacted] mg/kg oil. These doses were selected to represent the minimum inclusion level of 30 mg/kg complete feed in feeds with fat concentrations of [redacted]% and [redacted]%, respectively. [redacted] The results are summarised in Table 4

[41]Technical dossier/Supplementary information June 2023 and corresponding clarification (2023-08-25).

In the four oil/water emulsions tested, the results of [redacted] analysis showed a higher stability ($p < 0.01$) of the emulsions containing COE at [redacted] mg/kg oil [redacted] oil compared to the respective controls.

3.3.1 | Conclusions on efficacy

The FEEDAP Panel concludes that the additive has the potential to be efficacious as an emulsifier in feed at the proposed conditions of use.

4 | CONCLUSIONS

In the absence of adequate studies, the FEEDAP Panel cannot conclude on the safety of COE for the target species.

The use of COE in animal nutrition at proposed use levels is not expected to cause concern for the consumer.

The additive is considered as irritant to the eyes and mucous membranes. No conclusions can be reached on the potential of the additive to be a dermal irritant or a dermal sensitiser.

The use of COE as a feed additive is not expected to pose a risk to the terrestrial compartment. However, no conclusions can be reached on the safety of the additive for the environment when used in feed for aquatic animals other than fin fish.

The additive has the potential to be efficacious as an emulsifier when used according to the proposed conditions of use.

5 | RECOMMENDATIONS

The Panel recommends that the maximum level of saponins in the additive should not exceed 11.8%, the highest analysed concentration.

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
BW	body weight
CAS	Chemical Abstracts Service
CAT	catalase
CFU	colony forming unit
COE	*Camellia oleifera* extract
CV	coefficient of variation
DAD	diode array detection
DM	dry matter
ELSD	evaporative light scattering detection
ELS	extensive literature search ELS
EMA	European Medicines Agency
ESI	electrospray ionisation
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GSH-PX	glutathione peroxidase
GSH-S	glutathione S-transferase
HPLC	high performance liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MS	mass spectrometry
NOAEL	no observed adverse effect level

PCBs	polychlorinated biphenyls
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
QSAR	quantitative structure–activity relationship
RH	relative humidity
SOD	superoxide dismutase
WHO	World Health Organization

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CONFLICT 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.

REQUESTOR
European Commission

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