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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of *Quillaja saponaria* Molina and *Yucca schidigera* Roezl ex Ortgies (Magni-PHI®) for all poultry species (to slaughter age/weight, or to the point of lay) and ornamental birds (Phibro Animal Health Corporation)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of a feed additive consisting of *Quillaja saponaria* powder and *Yucca schidigera* powder (Magni-Phi®) for all avian species (to slaughter age/weight, or to the point of lay) and ornamental birds, as a zootechnical additive (digestibility enhancer and other zootechnical additives). The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive is safe for chickens for fattening at the level of 250 mg/kg complete feed with a margin of safety of 20 assuming that the additive contains 3.58% of saponins. This conclusion was extrapolated to all growing poultry species and ornamental birds. The Panel concluded that the use of the feed additive in animal nutrition at 250 mg/kg complete feed is of no concern for the safety for the consumer and the environment. The Panel also concluded that the additive is not irritant to skin, but irritant to the eyes and to the respiratory system. Due to the lack of data, the FEEDAP Panel could not conclude on the skin sensitisation potential of the additive. The FEEDAP Panel was not in the position to conclude on the efficacy of the additive for all poultry species and ornamental birds.

KEYWORDS

all avian species, digestibility enhancers, efficacy, Quillaja, safety, Yucca, zootechnical 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 (Phibro Animal Health Corporation)[2] for the authorisation of the additive consisting of Quillaja saponins (Magni-PHI®) when used as a feed additive for all avian species to slaughter age/weigh, or to the point of lay (category: zootechnical additive; functional groups: digestibility enhancer and other zootechnical additives).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1). 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 8 October 2021.

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 *Quillaja saponaria* and *Yucca schidigera* (Magni-PHI®), when used under the proposed conditions of use (see Section 3.1.5).

1.2 | Additional information

The feed additive consisting of a preparation of powdered *Quillaja saponaria* Molina (85% w/w) (wood including bark) and *Yucca schidigera* Roezl ex Ortgies (15% w/w) (stem) is not currently authorised for use in feed in the EU.

Yucca schidigera is currently authorised as a feed additive (functional group: flavouring compounds) in all animal species, subject to the re-evaluation.

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 *Quillaja saponaria* Molina and *Yucca schidigera* Roezl ex Ortgies (Magni-PHI®) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' (elicitation) 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 saponins in the feed additive and in the animal feed.[4]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Quillaja saponaria* Molina and *Yucca schidigera* Roezl ex Ortgies (Magni-PHI®) is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019) and the Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023).

[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]Phibro Animal Health S.A. Chemin du Stocquoy, 3.1300, Wavre, Belgium.

[3]Dossier reference: FAD-2021-0046.

[4]Evaluation report received on 11/11/2022 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_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

This opinion assesses the safety and efficacy of the product that contains powdered *Quillaja saponaria* Molina (85% w/w) (wood including bark) and *Yucca schidigera* Roezl ex Ortgies (15% w/w) (stem), intended for use as a zootechnical feed additive (functional groups: digestibility enhancer and other zootechnical additive) for all poultry species (to slaughter age/weight, or to the point of lay), and ornamental birds. The additive under assessment will be hereafter referred to as Magni-Phi®.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

The additive is a mixture consisting of dry and powdered *Quillaja saponaria* Molina (family: Quillajaceae) (wood including bark) (85%) and *Yucca schidigera* Roezl ex Ortgies (family: Asparagaceae) (stem) (15%). Quillaja saponins are the main active substances and marker compounds.

The applicant proposed the following specifications for the additive: Quillaja saponins > 3.5% w/w; moisture < 7% w/w; extractable solids[6] > 15% w/w; granulometry > 90% w/w (passing 250 μm) and an ammonium binding capacity or B50 (g) < 6.

Data to demonstrate compliance with the proposed specifications were provided on five batches of the additive[7]: moisture 1.7% (range 1.5%–2.1%); granulometry 93.5% (range 92.2%–94.0%); extractable solids 16.7% (range 15.3%–17.4%); Quillaja saponins 4.5% (range 4.0%–5.1%). The ammonium binding capacity or B50 (g) was 4.7 (range 3.6–5.7).

The applicant provided the analytical data on the content of phenolic acids and flavonoids, resveratrol[8,9] and saponins[10] in *Quillaja saponaria* wood, including bark, each of them measured in five different recent batches using ultra-high-performance liquid chromatography with ultraviolet detection mass spectrometry (UHPLC–UV-MS) (Table 1). In addition, the moisture content was evaluated in the same batches showing the average value of 4.68% (range 2.86%–6.67%). The Quillaja saponins structure are reported in Annex A.

TABLE 1 Average content and range values of compounds identified in batches of *Quillaja saponaria* (wood, including bark).

Compound (CAS)	Average content (range) mg/kg	Calculated average content in the final additive (mg/kg) (85% of *Quillaja saponaria*)
Phenolic acids and flavonoids (5 batches)		
Piscidic acid (469–65-8)	12,683.6 (11,499–13,489)	10781.1
Syringic acid (530–57-4)	3168.8 (2977–3440)	2693.5
(+)-Catechin (154–23-4)	1522.2 (147–2474)	1293.9
Vanillic acid (121–34-6)	1932.4 (1715–2031)	1642.5
Rutin (153–18-4)	2702.8 (1698–2976)	2297.4
Ferulic acid (1135-24-6)	2420.8 (2265–2528)	2057.7
Coumaric acid derivatives	2192.8 (1859–2551)	1863.9
Total phenolic acids and flavonoids	26620.6 (23439–28,901)	22627.5
Resveratrol (5 batches)		
Resveratrol (501–36-0)	61.4 (54–66)	52.2
Saponin content (5 batches)		
QS-7	1400 (1200–1600)	1190
QS-17	5000 (3300–6600)	4250
QS-18	5500 (3400–6900)	4675
QS-21	1700 (800–2900)	1445
Non-identified saponins[11]	32,800 (42000–50,000)	27,880
Total saponins in Quillaja saponaria	46,400 (50700–68,000)	39,440

[6]The density (absolute and solid density) is determined by means of a pycnometer according to DIN 66137–2 (determination of solid state density – Part 2: Gaspycnometry).

[7]Technical dossier/Section II/Annexes II_1_4_1_1, II_1_4_1_2, II_1_4_1_3 (part 5) and Annex II_1_3.

[8]Technical dossier/ Supplementary information August 2022/ Annex_Q1_11445.

[9]The terms resveratrol or *trans*-resveratrol are used through the opinion depending on which one is indicated in the underlying documents/literature.

[10]Technical dossier/ Supplementary information August 2022/ Annex_Q1_2.

[11]Generated by calculation.

The identification of phenols and saponins was also provided for five batches of *Yucca schidigera* stems.[12] The phenolic fraction included resveratrol and other stilbenoids. Other phenolic compounds are present but have not been identified and quantified. A total of 12 stilbenoids and a range of 73–108 saponins with an average content for the latter of 58 mg/kg (range 47.8–69.7) were identified. The average and range values of the stilbenoids are presented in Table 2. The Yucca saponins contribute only minimally to the total concentration of saponins in the additive and are not detailed here (~0.022% of the total saponins present in the final additive).

TABLE 2 Average content and range values of stilbenoids identified in five recent batches of *Yucca schidigera* (stems).

Compound	Average content (range) mg/kg	Calculated average content in the final additive (mg/kg) (15% of *Y. Schidigera*)
***trans*-3,3′,5,5′-Tetrahydroxy-4′-methoxystilbene**	806 (580–1020)	121
Resveratrol	302 (180–470)	45
Yuccaol E	248 (170–340)	37
Yuccaol C	831 (560–1100)	125
Yuccaol B	406 (270–550)	61
Yuccalide A	140 (90–180)	21
Yuccaol D	753 (500–1070)	113
Yuccaol A	286 (200–380)	43
Gloriosaol E	138 (80–200)	21
Gloriosaol D	135 (60–220)	20
Gloriosaol A	165 (120–220)	25
Gloriosaol C	161 (80–250)	24
Sum of 1–12 stilbenoids in Yucca schidigera	4370 (3070–5150)	656

Saponin[13] and calcium oxalate[14] content was measured in five batches of the final additive. The average total saponin content originating from *Quillaja saponaria* powder and *Yucca schidigera* powder was 4.10% w/w (range 3.69–4.81); 0.19% w/w QS-7 (range 0.16–0.22); 0.31% w/w QS-17 (range 0.28–0.30); 0.57% w/w QS-18 (0.51–0.65) and 0.12% w/w QS-21 (range 0.10–0.15). The concentration of calcium oxalate was below the limit of detection (LOD 21.34 mg/kg) in all batches.

No analytical data on the other individual compounds identified for *Quillaja saponaria* powder and *Yucca schidigera* powder (Tables 1, 2) was provided in the final additive.

Three batches of the additive were analysed for the possible presence of impurities/contaminants. Cadmium was detected at a mean value of 0.03 mg/kg (0.02–0.05 µg/kg), lead at 0.26 mg/kg (0.17–0.33 mg/kg), and arsenic at 0.043 mg/kg (0.03–0.06 mg/kg). Mercury was below the LOD.[15,16]

The analysis of aflatoxins B1, G1, B2, G2, fumonisin (total), HT-2 toxin, T-2 toxin, ochratoxin A, vomitoxin and zearalenone[17] and pesticides showed values below the respective limits of quantification.[18]

Analysis of three batches of the additive showed that the aerobic plate count (< 10 colony forming unit (CFU)/g)), the mean probable number of Enterobacteriaceae (< 3 CFU/g), the numbers of yeasts and filamentous fungi (both < 10 CFU/g), and the numbers of *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* (each absent in 10 g) met the specifications set by the applicant for microbial contamination.[19]

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (coplanar PCBs) were analysed and found on average 0.098 ng/kg (0.094–0.101 ng/kg).

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

[12]Technical dossier/Supplementary information August 2022/ Annex_Q1_3 and Annex_Q4_1.

[13]Technical dossier/ Supplementary information August 2022/ Annex_Q1_2.

[14]Technical dossier/ Supplementary information August 2022/ Annex_Q1_5.

[15]LOD: 7.23 µg/kg.

[16]Technical dossier/Section II/Annexes II_1_4_1_1, II_1_4_1_2, II_1_4_1_3 (part 3).

[17]Technical dossier/Section II/Annexes II_1_4_1_1- II_1_4_1_3 (part 1).

[18]Aflatoxins B1, B2, G1, G2: < 5 µg/kg; Fumonisin: < 30µg/kg, HT-2 toxin: < 10 µg/kg, T-2 toxin, ochratoxin A: < 5 µg/kg, Vomitoxin: < 10 µg/kg and zearalenone < 25 µg/kg); pesticides 0.01 mg/kg.

[19]Technical dossier/Section II/Annexes II_1_4_1_1- II_1_4_1_3 (part 4).

3.1.2 | Physical properties of the additive

Bulk density was on average 348 kg/m^3 (344–354 kg/m^3) and true density was on average 1462 kg/m^3 (1461–1464 kg/m^3) in three batches of the products.[20]

The dusting potential[21] of the same three batches of the additive was determined using the Stauber–Heubach method and showed values on average of 2248.3 mg/m^3 (2110–2430 mg/m^3).

The particle size distribution of the additive was measured on the same batches by laser-diffraction. A 10% of the particles had a diameter below 8.39 μm, 50% were below 95.3 μm and 90% were below 368.70 μm.

The applicant also analysed the particle size of the dust by laser-diffraction;[22] the results showed that on average 10% of the particles were below 3.21 μm, 50% below 13.24 μm, and 90% below 30.11 μm.

3.1.3 | Manufacturing process

[redacted]

3.1.4 | Stability and homogeneity

The shelf life of the additive was studied in three batches when stored at 25°C for 42 (two batches) and 45 months (one batch). The initial estimated level of Quillaja saponins was > 3.5% and at the end of the experiment was on average 4.3% (3.8%–4.7%).[23] In the absence of precise information on the initial content of the compound, the FEEDAP Panel is not in the position to properly assess the shelf-life of the additive.

The stability of the additive was evaluated in three feeds for chickens for fattening and in one for turkeys (each feed in mash and pelleted form), when supplemented with 250 mg/kg of the additive and stored at 25°C and 60% relative humidity. Saponin levels were measured at 0, 4, 8 and 12 weeks.[24] There were no losses at the end of the storage periods except in one batch of mashed pelleted feed for chickens, where losses accounted for 6.5% of saponins.

The homogeneous distribution of the additive in feed was studied in 10 subsamples of the same previous four feeds (each feed in mash and pelleted form).[25] The average coefficient of variation (CV) was 12.3% and 10.3% in mash and pelleted feed for chickens, respectively, and 10% and 9% in mash and pelleted feed for turkeys, respectively.

3.1.5 | Conditions of use

The additive is intended for use in feed for all avian species (to slaughter age/weight, to point of lay) and ornamental birds (with no maximum age) at a proposed minimum concentration level of 250 mg/kg complete feed. No maximum content is proposed by the applicant.

3.2 | Safety

In support of the safety of the additive, the applicant submitted one tolerance trial, genotoxicity studies and some papers retrieved in the public literature.

3.2.1 | Existing assessments

The EFSA Food Additives and Flavourings (FAF) Panel evaluated the toxicological profile of Quillaja extract and an acceptable daily intake (ADI) was established at 3 mg Quillaja saponins/kg body weight (bw) per day, based on the no observed

[20]Technical dossier/Section II/Annex_II_1_5_.
[21]Technical dossier/Section II/Annex_II_1_5_1.
[22]Technical dossier/Section II/Annex_II_1_5_5.
[23]Technical dossier/Section II/ Annex II_1_3.
[24]Technical dossier/Section II/ Annex II_4_1.
[25]Technical dossier/Section II/Annex II_4_1.

adverse effect level (NOAEL) of 1500 mg Quillaja extract/kg bw per day, the highest dose tested, from a 2-year study in rats (EFSA FAF Panel, 2019).

The European Medicines Agency (EMA) Committee for veterinary medicinal products (CVMP) concluded in 1996 that Quillaja saponins had low toxicity and poor absorption after oral administration, and that Quillaja saponins are a natural component of the diet in all food producing species (EMA CVMP, 1996). Therefore, the CVMP considered that there was no need to establish a maximum residue limit for Quillaja saponins in foodstuffs of animal origin from all animal species.

In 2016, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) evaluated the safety of synthetic trans-resveratrol as a novel food and concluded that it is safe under the proposed conditions of use (as a food supplement in capsule or tablet form at daily doses up to 150 mg/day) (EFSA NDA Panel, 2016).

3.2.2 | Absorption, distribution, metabolism and excretion (ADME)

Studies on bioavailability and ADME of saponins of *Quillaja saponaria* powder and of *Yucca schidigera* powder present in the additive under assessment were not made available.

EFSA delivered an opinion on 2019 on the re-evaluation of the safety of Quillaja extract as a food additive and considered that the data available on ADME of saponins of Quillaja extract were not adequate to make a conclusion (EFSA FAF Panel, 2019). Due to this limitation, the Panel considered the available data on ADME for other saponins structurally similar to those of Quillaja, namely soyabean saponins. Based on read across from these data the FAF Panel concluded that Quillaja saponins follow the same fate in organism as those structurally similar: are poorly absorbed, hydrolysed in the colon, and the formed sapogenins are absorbed to a limited extent (EFSA FAF Panel, 2019). In the context of the current assessment, the FEEDAP Panel shares the same conclusions.

In addition, the EMA-CVMP concluded that saponins are poorly absorbed in the gut in rats, mice and chickens based on the results of a study performed by Gestetner et al. (1968), in which soybean flour was given orally to these animal species (EMA CVMP, 1996). Male mice (10 per group), male rats (3 per group) and male chickens (3 per group) were fed for 10 days standard diets containing 20% heated soybean flour. After this period the animals were killed, blood collected, and their digestive tracts were removed and divided into three parts: (i) small intestine including duodenum, (ii) cecum and (iii) colon. Saponins and sapogenins were extracted from these separated intestine parts and analysed by paper chromatography. In the small intestine of rats, mice and chickens, only saponins were detected while in cecum and colon both saponins and sapogenins were present, indicating microbial hydrolysis of saponins. Moreover, neither soybean saponins nor sapogenins were found in the blood of the rats, mice and chickens at levels above the respective LOD.

The bioavailability of soyasaponins was also evaluated in humans (Hu et al., 2004). Eight women ingested 4 g of a commercial product consisting in a concentrated soy extract containing 434 μmol of group B soyasaponins. Urine was collected during the following 24 h and faeces during the following 5 days. Soyasaponins or their metabolites were not detected in urine. In the faeces, only soyasapogenol B, a major metabolite of group B soyasaponins, was found. Five days after dosing, soyasapogenol B in faeces was about 8% of the corresponding ingested amount of soyasaponins, these being not detected (LOD not given). In the same study, the in vitro absorption was evaluated by mean of the Caco-2 cell model. The transfer of soyasaponin I and soyasapogenol B from the apical to the basal chamber after 4 h incubation was 0.5%–2.9% and 0.2%–0.8%, respectively, corroborating the low absorption observed in vivo.

The FEEDAP Panel considered that the same considerations above would apply also to saponins from Yucca which represent a minor portion of saponins in the additive under assessment.

Following hydrolysis by the microbiota in colon and caecum, the respective sapogenins may be absorbed, although to a limited extent and residues, if present, should be negligible. Overall, saponins from *Quillaja saponaria* and *Yucca schidigera*, as known for saponins in general, are expected to be poorly absorbed in the gut of animals. The sapogenins absorbed, having hydroxyl groups in their structures, are prone to be glucuronidated and excreted in urine.

The other identified components (phenolic acids, flavonoids and stilbenoids) are readily metabolised in the intestine and liver and excreted and are not expected to accumulate in animal tissues and products.

3.2.3 | Toxicological studies

Genotoxicity studies, including mutagenicity

The applicant provided genotoxicity studies conducted with the main components of the additive. The studies are described below.

Quillaja saponaria

In order to investigate the potential of *Quillaja saponaria* powder [redacted] to induce gene mutations in bacteria, an Ames test was performed [redacted]

[redacted] The FEEDAP Panel concludes that the test item did not induce gene mutations in bacteria under the experimental conditions employed in this study.

Quillaja saponaria powder [redacted] was tested for the potential to induce chromosomal damage with the in vitro micronucleus test performed [redacted] The FEEDAP Panel concludes that the test item did not induce chromosome damage in vitro in mammalian cells under the experimental conditions employed in this study.

Yucca schidigera

Yucca schidigera powder [redacted] was tested for the potential to induce gene mutations in bacterial cells in a study conducted [redacted] The FEEDAP Panel concludes that the test item did not induce gene mutations in bacteria under the experimental conditions employed in this study.

Yucca schidigera powder [redacted] was tested for the potential to induce chromosomal damage with the in vitro micronucleus test performed [redacted] The FEEDAP Panel concludes that the test item did not induce chromosome damage in vitro in mammalian cells under the experimental conditions employed in this study.

Conclusions on genotoxicity

Based on the studies available, the FEEDAP Panel concludes that the main components of the additive (*Quillaja saponaria* powder and *Yucca schidigera* powder) did not induce gene mutations and did not cause numerical and structural chromosomal damage.

3.2.4 | Safety for the target species

The applicant provided a tolerance study with the additive under assessment in chickens for fattening to support the safety for the target species.[26]

[26]Technical dossier/Section III/ Annex III.1.1.1.

A total of 2112 one-day-old male chickens for fattening (Ross 308) were distributed in 96 pens in groups of 22 animals and allocated to six dietary treatments (16 replicates per treatment). Two basal diets (starter, from days 1 to 21; grower, from days 22 to 42) based on maize and soybean meal were either not supplemented (control) or supplemented with 250 (1× minimum recommended level), 500 (2×), 1000 (4×), 2500 (10×) or 5000 (20×) mg additive/kg complete feed. The analysed saponin contents were (for starter/grower diets) 14.7/11.3, 32.1/21.8, 59.4/48.0, 148.2/130.7 and 255.0/255.0 mg of saponins/kg complete feed, respectively.[27] The experimental diets were offered ad libitum in mash form for 42 days.

Mortality and health status were checked daily, dead animals were necropsied, and the most probable cause of death reported. Birds were weighed at the start of the trial (day 1). Thereafter, body weight and feed intake (pen basis) were registered at days 21 and 42. The average daily feed intake, average daily weight gain and feed to gain ratio were calculated (corrected for mortality) for the starter, grower, and whole feeding period. Blood samples were obtained from one randomly selected bird per pen on day 42 for haematology and biochemistry analysis.[28]

The experimental data were analysed with a Generalised Linear Model including the diet and the block (location of the pen) as fixed effects. When significant effects were observed, group means were compared with Tukey test. Significance level was set at 0.05.

Mortality (including culling) was on average 3.6% and not affected by treatment. No significant differences were observed between the control and any treated group in the performance parameters (values of the control group: average final body weight = 2860 g; average daily feed intake = 110 g; and feed to gain ratio = 1.64) or for the haematological parameters. Regarding blood biochemistry, higher levels of amylase in the 4x group (810 vs 630 IU/L) and globulin in the 2x group (2.4 vs 2.1 g/dL) were observed in comparison with the control. However, these differences were considered of no relevance.

Based on this study, the FEEDAP Panel concludes that the additive Magni-PHI® is safe for chickens for fattening at the level of 250 mg/kg complete feed. Considering the values of saponins analysed in the feeds in this study and the highest content of saponins analysed in the additive (5.1%, see Section 3.1.1) the margin of safety would correspond to 20. This conclusion can be extrapolated to all poultry for fattening and reared for laying/breeding growing and ornamental birds.

The applicant also provided some published studies to support the safety of Quillaja and Yucca saponins[29] The FEEDAP Panel notes that the studies reported in the papers refer to Quillaja and Yucca, however the relationship of the test item used in those studies and the additive under assessment is not fully clarified. In addition, few adverse effects were reported for Quillaja powder only, and at concentrations higher than those present in the additive under assessment (0.1%–1.2% of the diet).

Conclusions on safety for the target species

The Panel concludes that the additive Magni-Phi® is safe for all poultry species (to slaughter age/weight, or to the point of lay) and ornamental birds at the level of 250 mg/kg complete feed with a margin of safety of 20.

3.2.5 | Safety for the consumer

No data on residues in products of animal origin were made available for the additive under assessment.

However, the FEEDAP Panel recognises that the individual constituents of *Quillaja saponaria* powder and *Yucca schidigera* powder are expected to be either poorly absorbed (saponins) or absorbed and extensively metabolised and excreted in the target species (see Section 3.2.2).

In addition, the FEEDAP Panel notes that an extract of *Quillaja saponaria* is authorised as additive in food (E999), and resveratrol was assessed by the NDA Panel who considered it safe up to 150 mg/day (EFSA NDA Panel, 2016). The FEEDAP Panel considers that this exposure is higher than that expected 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 would be expected for the consumer from the use of the additive at the proposed use level of 250 mg/kg complete feed.

3.2.6 | Safety for the user

Considering the dusting potential of the additive (highest value measured: 2430 mg/m^3), exposure to the additive through inhalation is likely.

[27]Considering the content of saponin in the certificate of analysis of 3.58%, the intended concentration of saponins in feed was 8.95, 17.9, 35.8, 89.5 and 179 mg of saponins/kg complete feed, respectively.

[28]Total RBC counts, packed cell volume, haemoglobin, MCV, MCVH, MCHC; total & differential counts of leukocytes.
platelet counts, prothrombin time, fibrinogen; Na, K, Cl, Ca, P, Mg, total protein, albumin, globulin, glucose, urea/uric acid, cholesterol, creatinine, bilirubin, acute phase proteins, amylase, ALT, AST, LDH, GGT, AP, CK and haptoglobin.

[29]Annex_III_3_2_3_3_1a_Literature_search.

Effect on eyes and skin

The skin corrosion potential of the additive was tested in a valid study performed according to OECD TG 431, which showed that it is not corrosive to skin.[30]

The skin irritation potential of the additive was tested in a valid study performed according to OECD TG 439, which showed that it is not irritant to skin.[31]

The eye irritation potential of the additive was tested in a valid study performed according to OECD TG 437, which showed that it is an eye irritant.[32]

No experimental data on the potential skin sensitisation of the additive were submitted.[33] Due to the lack of data, the FEEDAP Panel is not in the position to conclude on the potential skin sensitisation of the additive.

Conclusions on safety for the user

The additive Magni-PHI® is not a skin irritant. The additive should be considered an eye and respiratory irritant. Due to the lack of data, the FEEDAP Panel cannot conclude on the skin sensitisation potential of the additive. The FEEDAP Panel concludes that the exposure to the additive through inhalation is likely.

3.2.7 | Safety for the environment

The applicant provided data on the occurrence of some of the main compounds present in the additive (saponins, resveratrol) which showed that their occurrence is similar to those naturally present in European plant species.[34]

Considering the above, the FEEDAP Panel concludes that no further environmental risk assessment is considered necessary and that the use of the additive under the proposed conditions of use in animal feed is not expected to pose a risk to the environment.

3.3 | Efficacy

The applicant submitted four long-term trials and one publication (Bafundo et al., 2021) to support the efficacy of the additive in chickens for fattening. The Panel notes that the husbandry conditions applied in three of the trials submitted,[35,36,37] and in the publication from Bafundo et al. (2021) did not reflect those in which the birds are raised in the EU and were not in line with Directive 2007/43/EC[38] (regarding the bedding used, the spiking with avian pathogens and/or the lighting program). Therefore, these trials were not further considered as evidence for the assessment of the efficacy.

In trial 1,[39] a total of 480 one-day-old male chickens for fattening (Cobb 500) were distributed in 24 pens in groups of 20 animals and allocated to three dietary treatments (8 replicates per treatment). Two basal diets (starter, from days 1 to 14; grower from days 15 to 42) based on maize and soybean meal were either not supplemented (control) or supplemented with Magni-Phi® to provide 250 or 500 mg per kg feed, which would correspond to 8.95 and 17.9 mg of saponins/kg complete feed, considering the content of saponin in the certificate of analysis of 3.58%. The inclusion level of the additive in the feed was determined based on the content of saponins, showing analytical values about 60% higher than the intended ones.[40] The experimental diets were offered ad libitum in mash form for 42 days. The grower diet contained an external marker for digestibility analysis.

Mortality and health status were checked daily, animals found dead were necropsied, and the most probable cause of death was reported. The birds were weighed at the start of the trial (day 1). Thereafter, body weight and the feed intake were measured weekly (pen basis). The average daily feed intake, average daily weight gain and the feed-to-gain ratio were calculated and corrected for mortality for whole feeding period.

From days 21 to 25, two birds per pen were moved to metabolic cages (eight replicates per treatment), and samples of excreta were collected and pooled per replicate. Feed and excreta samples were analysed for dry matter, crude ash, crude protein, ether extract, calcium, phosphorus and the external marker, and nutrient retention was calculated.

The experimental data were analysed by one-way analysis of variance (ANOVA) with diet as a fixed effect. When significant effects were observed, group means were compared with the Tukey test. The significance level applied was 0.05.

[30]Technical dossier/Section III/ Annex III.3.3.1.1.

[31]Technical dossier/Section III/ Annex III.3.3.1.3.

[32]Technical dossier/Section III/ Annex III.3.3.1.2 (a-b-c).

[33]Technical dossier/Section III/ Annex III.3.3.1.4.

[34]Technical dossier/Supplementary information August 2022/0_MagniPhi_Cover_Letter.

[35]Technical dossier/Section IV/Annex_IV_3_2.

[36]Technical dossier/Section IV/Annex_IV_3_3.

[37]Technical dossier/Section IV/Annex_IV_3_4.

[38]Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production, OJ L 182 12.7.2007, p. 19.

[39]Technical dossier/Section IV/Annex_IV_3_1.

[40]Annex IV_3_5; analytical content of saponins in feed (mg/kg): not detected, 15.4/13.8 and 30.6/25.5 for the control, 1× and 2× groups, respectively.

The results of the study are given in Table 3. No differences in mortality were observed among treatments. Birds supplemented with the additive at 250 and 500 mg/kg feed had a significantly higher final body weight and body weight gain in comparison with the control diet. No difference in the average daily feed intake was observed. The feed-to-gain ratio showed an improvement at 500 mg/kg feed only.

TABLE 3 Effects of the additive on the performance of chickens for fattening.

Trial	Groups (mg additive/kg feed)	Daily feed intake (g)	Final body weight (g)	Average daily weight gain (g)	Feed to gain ratio	Mortality and culling (%)
1	0	113	3019^{b}	70.8^{b}	1.59^{a}	6.3
	250	113	3077^{a}	72.2^{a}	1.56^{ab}	5.0
	500	112	3092^{a}	72.6^{a}	1.55^{b}	4.4

Mean values with a different superscript are significantly different $p < 0.05$.

The retention (as % of intake) of crude ash (17.1, 20.9 and 21.5%, for the control, 250 and 500 mg/kg groups, respectively), protein (51.5, 60.9 and 65.1%) and fat (85.7, 88.5 and 87.9%) was higher in both supplemented diets compared to the control, and that of calcium (40.4, 43.0 and 43.5%) at 500 mg/kg feed.

Conclusions on efficacy

The efficacy of Magni-Phi® as a zootechnical feed additive was demonstrated in one study with chickens for fattening at 250 mg/kg complete feed. According to the EFSA FEEDAP Panel Guidance on efficacy (EFSA FEEDAP Panel, 2018), two additional studies demonstrating efficacy of the additive are needed to positively conclude on the efficacy of the additive. Therefore, the FEEDAP Panel is not in the position to conclude on the efficacy of Magni-Phi® for poultry species for fattening and reared for laying, and ornamental birds.

3.4 | Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[41] and Good Manufacturing Practice.

4 | CONCLUSIONS

Magni-Phi® is safe for chickens for fattening at the level of 250 mg/kg complete feed with a margin of safety of 20. This conclusion can be extrapolated to all growing poultry species and ornamental birds.

The use of the feed additive in animal nutrition at 250 mg/kg complete feed is of no concern for the safety for the consumer and the environment.

The additive is not irritant to skin, but irritant to the eyes and respiratory system. Due to the lack of data, the FEEDAP Panel cannot conclude on the skin sensitisation potential of the additive.

The FEEDAP Panel is not in the position to conclude on the efficacy of the additive for all poultry species and ornamental birds.

5 | RECOMMENDATIONS

The FEEDAP Panel recommends setting a maximum content of 5.1% Quillaja saponins in the specifications of the additive.

ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
ANOVA	analysis of variance
BW	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CV	coefficient of variation

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

CVMP	Committee for Veterinary Medicinal Products
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FAF	EFSA Panel on Food Additives and Flavourings
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
IU	International Unit
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCDDs	polychlorinated dibenzodioxins
PCDFs	polychlorinated dibenzofurans
TDQS	total dissolved Quillaja solids
TDYS	total dissolved Yucca solids
UHPLC-UV-MS	ultra-high-performance liquid chromatography with ultraviolet detection mass spectrometry

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.

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European Commission

QUESTION NUMBER

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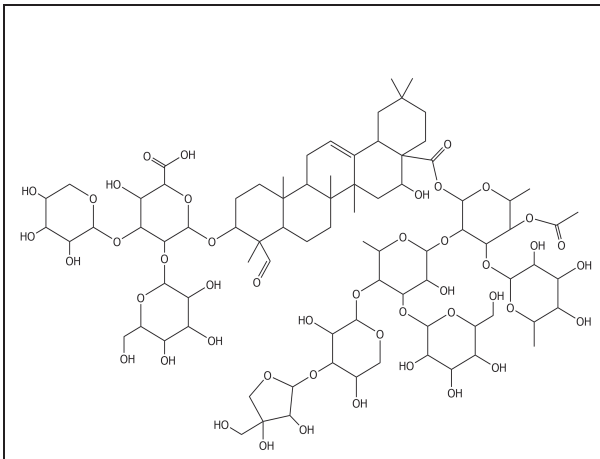
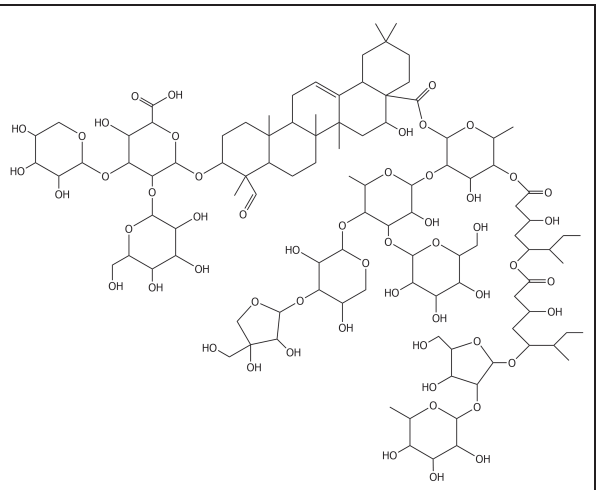
ANNEX A

Chemical structure of the major saponin components in Quillaja extracts, as proposed by the applicant and according to Kensil et al. (1991).



QS-7



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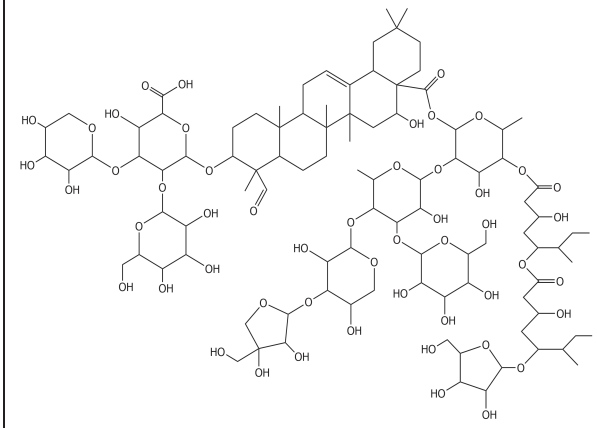
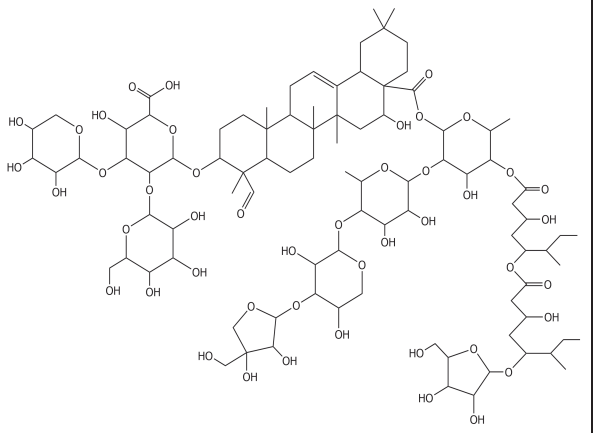


QS-18



QS-21