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SCIENTIFIC OPINION

Assessment of the feed additive consisting of folic acid for all animal species for the renewal of its authorisation (Chr. Olesen A/S and DSM Nutritional Products Ltd)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the assessment of the application for the renewal of the authorisation of folic acid as a nutritional feed additive. The additive is authorised for use in feed and water for drinking for all animal species. The applicant provided evidence that the additive currently in the market complies with the existing conditions of authorisation and the production process has not been modified. The FEEDAP Panel considers that there is no evidence to revise the conclusions reached in the previous assessment for the safety for the terrestrial species, consumers and for the environment. The use of folic acid in aquatic animal species to cover their nutritional needs is considered safe. However, the Panel is not in a position to set a maximum safe level for all fish and crustacean species. Considering the narrow margin between the requirement and the tolerated levels seen in some aquatic animal species, the FEEDAP Panel considers that supplementation should not exceed the requirements of the different aquatic animal species. The additive is neither a skin irritant nor a dermal sensitiser. The exposure through inhalation is likely. Due to the lack of data, the FEEDAP Panel is not in the position to conclude on the potential of folic acid to be harmful to the respiratory system and irritant to eyes. The Panel retains that the previously made conclusion on the efficacy remains valid.

KEYWORDS

all animal species, efficacy, nutritional additives, safety, vitamin

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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 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from Chr. Olesen A/S and DSM Nutritional Products Sp.z.o.o.[2] for the renewal of the authorisation of the additive consisting of folic acid when used as a feed additive for all animal species (category: nutritional additives; functional group: vitamins, pro-vitamins and chemically well-defined substances having similar effect).

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 14(1) (renewal of the authorisation). 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 24 January 2023.

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 folic acid, when used under the proposed conditions of use (see **Section 3.1.5**).

1.2 | Additional information

In 2000, the Scientific Committee for Food established the upper tolerable level (UL) for folic acid (SCF, 2000). The EFSA Scientific Panel on Nutrition, Novel Foods and Food Allergens (NDA) has recently set ULs for folates for supplemental uses, i.e. when added to foods (fortified foods) and used in food supplements (EFSA NDA Panel, 2023).

EFSA issued an opinion on the safety and efficacy of folic acid when used in feed for all animal species (EFSA FEEDAP Panel, 2012).

Folic acid is currently authorised for use in feed and via water for drinking for all animal species (3a316)[3] and is described in a monograph of the European Pharmacopeia (PhEur, 2022).

Folic acid is used as a medicinal product for human[4] and veterinary use[5] and cosmetic product/ingredient.[6]

It is authorised as a vitamin in foods,[7] it is permitted as a food supplement[8] and in infant formula and follow-on formula, processed cereal-based foods and baby foods, foods for special medical purposes and total diet replacement for weight control.[9]

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[10] in support of the authorisation request for the use of folic acid as a feed additive. The dossier was received on 13/9/2023 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00555.

[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]Chr. Olesen A/S, Jaegersborg Allé 164, 2820 Gentofte, Denmark and DSM Nutritional Products Sp.z.o.o. representing DSM Nutritional Products Ltd., Tarczynska 11, 96–320 Mszczonów, Poland.

[3]Commission Implementing Regulation (EU) No 803/2013 of 22 August 2013 concerning the authorisation of folic acid as a feed additive for all animal species. OJ 225/17, 23.8.2013, p. 3.

[4]EMA/144279/2018, List of nationally authorised medicinal products. Active substance: folic acid. Procedure no.: PSUSA/00001459/201706.

[5]Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ 15/1, 20.1.2010, p. 72.

[6]Commission Implementing Decision (EU) 2022/677 of 31 March 2022 laying down rules for the application of Regulation (EC) No 1223/2009 of the European Parliament and of the Council as regards the glossary of common ingredient names for use in the labelling of cosmetic products. OJ L 127, 29.4.2022, p. 442.

[7]Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ 404/26, 31.12.2006, p. 13.

[8]Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ 183/51, 12.7.2022, p. 7.

[9]Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ 181/35, 29.6.2013, p. 22.

[10]Dossier reference: FEED-2022-8610.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 24 January 2023 to 24 April 2023 for which the received comments were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[11] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[12] a non-confidential version of the dossier has been published on Open.EFSA at https://open.efsa.europa.eu/questions/FEED-2022-00555.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[13] EFSA carried out a public consultation on the non-confidential version of the technical dossier from 27 April to 18 May 2023. One entry was registered but the comment submitted dimmed as not relevant to the scope of the public consultation, and therefore, were not considered further.

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.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the folic acid in animal feed are valid and applicable for the current application.[14]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of folic acid is in line with the principles laid down in Regulation (EC) No 429/2008[15] and the relevant guidance documents: Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021).

3 | ASSESSMENT

Folic acid is authorised as a nutritional additive (functional group: vitamins, pro-vitamins and chemically well-defined substances having similar effect) for use in all animal species. This assessment regards the renewal of the authorisation of folic acid for all animal species.

3.1 | Characterisation

The authorised form of folic acid is its pure form at 96% on anhydrous basis (hereinafter called additive). Folic acid can be used in animal nutrition in its pure form and as preparations. The applicants provided an example of a formulated additive preparation with a concentration of 80% of folic acid obtained using maltodextrin (20%) as a carrier (hereinafter called formulated additive).

3.1.1 | Characterisation of the additive

Folic acid (anhydrous substance, CAS No. 59-30-3) is currently authorised with a purity of 96% on anhydrous basis.

Analytical data to confirm the existing specifications were provided from the two applicants for a total of 23 batches of the additive, showing the following average values: 97.8% folic acid on anhydrous basis (range: 96.5%–98.7%).[16] The same batches were also tested for water content (≤ 8.5%) which ranged from 7.5% to 8.4%.

The FEEDAP Panel noted that these values are in compliance with those described in the European Pharmacopoeia (PhEur, 2022) for 'folic acid hydrate' for which the PhEur defines a 'variable quantity of water' and describes a content of 5.0%–8.5% of water. Folic acid dihydrate (CAS No. 75709-92-8) has a water content of 7.6%.

[11]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[12]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[13]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

[14]Evaluation report 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

[15]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.

[16]Annex_II_01; Annex_II_02; Annex_II_04.

According to the applicant, no changes have been introduced in the manufacturing process, composition, purity or activity of the additive since the original authorisation (EFSA FEEDAP Panel, 2012).

The additive is currently authorised to comply with the purity criteria laid down by the European Pharmacopeia 6th edition 01/2008/0067. The two applicants provided a total of 13 certificates of analysis (10 and 3 batches of the additive from each producer, respectively), to demonstrate that the levels of substance-related impurities complied with the requirements described in the PhEur (2008).[17] However, the 10 certificates from one producer reported only statements of compliance with the substance-related impurities. The other three certificates reported the following results: impurity A ranged from 0.15% to 0.19%, impurity B was < 0.025% (LOD) in all batches, impurity C ranged from 0.31% to 0.34%, impurity D ranged from 0.15% to 0.19%, impurity E ranged < 0.025% (LOD) to 0.06% and impurity F was < 0.025% (LOD) in all batches. Sulphated ash was measured on three batches and was on average 0.08% (range: 0.06%–0.1%).[18] These values demonstrated compliance with the requirements as in the PhEur (2008).

The FEEDAP Panel noted that, since the previous assessment, a new version of the PhEur (2022)[19] was made available, reporting different impurities and related limits.

The two applicants sent additional analysis from a total of eight batches (five and three from each producer, respectively) to demonstrate compliance with the new specifications as set in the PhEur (2022): impurity A ranged from 0.09% to 0.33%), impurity C ranged from 0.08% to 0.29%), impurity D ranged from 0.04% to 0.3%), impurities E and G were both in the range < 0.05%–0.29% and impurities H and I were both in the range < LOD to 0.1%; any other unspecified impurities ranged from < LOD and 0.06%; the total ranged from 0.24% to 1.03%). Water was on average 7.86% (range: 7.8%–7.9%) and the sulfated ask was 0.046% (range: 0.03%–0.06%).[20] These values demonstrated compliance with the requirements as in the PhEur (2022).

The applicants demonstrated the compliance of the additive with the more recent version of the PhEur (2022), in which the specifications for impurities are more conservative than the ones in the previous version (PhEur, 2008), included in the current authorisation. Therefore, the FEEDAP Panel considers that the lack of demonstration of compliance with the PhEur (2008) in a sufficient number of batches of the additive has no impact on the characterisation and on the safety assessment of the additive.

Six batches (three batches each applicant) of the active substance/additive were analysed for arsenic (< 0.5 mg/kg and 0.013 mg/kg, for the first and second applicant, respectively); cadmium (< 0.1 mg/kg and 0.01 mg/kg, for the first and second applicant, respectively) and mercury (< 0.1 mg/kg and 0.04 mg/kg, for the first and second applicant, respectively).

Chromium and lead were tested on three batches only and levels were on average 0.17 mg/kg and < 0.05 mg/kg, respectively.[21]

Analyses performed on three batches of the additive demonstrated that methanol was found below the LOD (20 mg/kg).[22]

The detected amounts of the above described impurities do not raise safety concerns.

3.1.2 | Physical properties of the additive

Folic acid is a synthetic folate compound belonging to the vitamin B family and becomes biologically active after reduction.

The active substance folic acid is a yellowish to orange, crystalline powder with almost no odour or taste.

[17] Limits:

– impurity A (2S)-2-[(4-aminobenzoyl)amino]pentanedioic acid (N-(4-aminobenzoyl)-l-glutamic acid): not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (0.5%)
– impurity D 4-[[(a-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amoni]benzoic acid (pteroic acid): not more than the area of the principal peak in the chromatogram obtained with reference solution (e) (0.6%)
– any other impurities: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5%)
– total of other impurities: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (1.0%)
– disregard limit: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05%)
– water: 5.0%–8.5%, determined on 0.150 g
– sulphated ash: maximum 0.2%, determined on 1.0 g.

[18] Annex_II_02; Annex_II_04.

[19] Limits:

– impurity A (2S)-2-(4-aminobenzamido)pentanedioic acid (N-(4-aminobenzoyl)-ʟ-glutamic acid): maximum 0.5%
– impurity D 4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)-methyl]amino]benzoic acid (pteroic acid): maximum 0.4%
– impurities C (2S)-2-[4-[[(2-amino-4-oxo-1,4-dihydropteridin-7-yl)-methyl]amino]benzamido]pentanedioic acid (isofolic
– acid), E (2S)-2-[4-[bis[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)-methyl]amino]benzamido]pentanedioic acid (6-pterinylfolic acid), G (2S)-[4-[(2-amino-7-methyl-4-oxo-1,4-dihydropteridin-6-yl)amino]benzamido]pentanedioic acid: for each impurity, maximum 0.3%
– impurities H (2S)-2-[4-[(4S)-4-[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino]benzamido]-4-carboxybutanamido]benzamido]pentanedioic acid, I (unknown structure): for each impurity, maximum 0.15%
– unspecified impurities: for each impurity, maximum 0.10%
– total: maximum 1.2%
– reporting threshold: 0.05%
– water: 5.0%–8.5%, determined on 0.150 g
– sulfated ash: maximum 0.2%, determined on 1.0 g

[20] Annex_II_05; Reply_RFI_24Apr23_Annex_01_CONF.pdf.

[21] Annex_II_4 and Annex_II_5.

[22] Annex_II_1.

The bulk density was measured on three batches and was on average 340 g/m^3.[23]

The dusting potential of three batches of the additive was determined using the Stauber-Heubach method and showed values on average of 253 mg/m^3 (range 220–285 mg/m^3) (mg airborne dust per m^3 of air).[24]

Particle size of the additive was measured by laser diffraction on a total of six batches (three batches each producer). For the first producer the results showed that 10% of the particles had an average diameter of 110 μm (range: 105–115 μm), 50% 150 μm (range: 145–155 μm) and 90% 173 μm (range: 170–180 μm).[25] For the second producer the results showed that 10% of the particles had an average diameter of 2.36 μm (range: 2.3–2.46 μm), 50% 15.46 μm (range: 14.1–17.3 μm) and 90% 54.23 μm (range: 51.3–56.1 μm).[26]

Folic acid is practically insoluble in water (1.6 mg/L at 25 °C, up to about 1% in boiling water (PubChem, NCBI)). However, folic acid recrystallised four times showed a solubility of 561 mg/L at pH 6 (Wu et al., 2010). It dissolves in diluted acids and in alkaline solutions.

When considering that the additive is an acid soluble substance and its expected use levels (up to 20 mg/kg complete feed, see Section 3.1.5), the Panel concluded that any potential folic acid nanoparticles present in the additive would be fully solubilised in the gastrointestinal tract of all animal species and, therefore, a conventional risk assessment should be sufficient.

3.1.3 | Characterisation of the formulated additive

The additive can be placed in the market as a preparation. The applicant described one of such preparations with a concentration of 80% of folic acid and 20% of maltodextrin used as a carrier.

Analyses on six batches of the formulated product showed that the concentration of folic acid was on average 82.6% (range: 80.8%–84.7%).[27]

The analysis conducted of the same three batches of the formulated additive showed a dusting potential of 3220 mg/m^3 (range: 3085–3445 mg/m^3).[28]

Particle size of the formulated additive was measured by laser diffraction on a total of six batches (three batches each producer). For the first producer the results showed that 10% of the particles had an average diameter of 27.5 μm (range: 28.1–26.9 μm), 50% 81.8 μm (range: 80.3–84.5 μm) and 90% 167.33 μm (range: 160–179 μm).[29] For the second producer the results showed that 10% of the particles had an average diameter of 89.33 μm (range: 54–112 μm), 50% 157.33 μm (range: 136–174 μm) and 90% 253.66 μm (range: 242–266 μm).[30]

3.1.4 | Stability and homogeneity

3.1.4.1 | *Shelf life*

The shelf life of the additive (four batches, from one applicant) was investigated when stored at 25°C and 60% relative humidity (RH) (packaging not specified) for 48 months. Losses at the end of the storage period ranged from 0.5% to 0.98%.[31]

Further, the stability of one batch of the additive was also tested under accelerated conditions (40°C and 75 RH) over 6 months. At the end of the experiment, the recovery of folic acid content was 99.7%.[32]

The stability of a formulated additive containing 80% folic acid was measured on three batches stored at 25°C and 60% RH. After 3 years of storage, the recovery of folic acid was on average 99.9%.[33]

The stability of the active substance/additive and formulated additive in premixtures, feed and water for drinking was evaluated in a previous assessment (EFSA FEEDAP Panel, 2012).

3.1.4.2 | *Homogeneity*

The capacity for homogeneous distribution of the additive in mash feed for pigs was studied in 10 subsamples of one batch of folic acid. The coefficient of variation (CV) was 6.75%.[34]

The capacity for homogeneous distribution of the three batches of the formulated additive (containing 80% folic acid) in a corn-wheat based broiler feed was studied in 10 subsamples each. The CV was on average 5.56% (range: 5.3%–5.7%).[35]

[23]Annex_II_05.
[24]Annex_II_18.
[25]Annex_II_05.
[26]Annex_II_06.
[27]Annex_II_03.
[28]Annex_II_19.
[29]Annex_II_07.
[30]Annex_II_08.
[31]Annex_II_11.
[32]Annex_II_11.
[33]Annex_II_11.
[34]Annex_II_20.
[35]Annex_II_13.

3.1.5 | Conditions of use

The additive is currently authorised for use in feed for all animal species without a specified minimum/maximum use level.

Under other provisions of the authorisation, it is specified that:

1. If the preparation contains a technological additive or feed materials for which a maximum content is set or which is subject to other restrictions, the feed additive manufacturer shall provide this information to the customers.
2. In the directions for use of the additive and premixture, indicate the storage and stability conditions.
3. Folic acid may be used also via water for drinking.
4. For safety: breathing, eye and skin protection shall be used during handling.

The applicant has requested to maintain the same conditions of use.

The applicant reported typical use levels below 20 mg/kg complete feed.

3.2 | Safety

The safety of the additive was evaluated in a previous EFSA opinion (EFSA FEEDAP Panel, 2012). The FEEDAP Panel concluded that folic acid was safe for the target animals, the consumers and the environment. In the absence of any data, the FEEDAP Panel considered the additive as an irritant to skin, eyes and to the respiratory tract and as a skin and respiratory sensitiser.

For the current assessment, the applicants provided the outcomes of the complaint reporting systems stating that no adverse effect notifications were received as concerns the safety for the target species, consumers, users/workers and the environment since the authorisation of the additive.[36]

The applicants have sent results from a comprehensive automatic literature search[37] conducted to identify new data related to the safety of the additive which were made available since the previous authorisation.

Four cumulative databases (LIVIVO, NCBI, Ovid and Toxinfo), 13 single databases and 12 publisher databases were used. The search covered the period from the previous assessment (January 2012) to May 2022. No other limits were set. The keywords used covered different aspects of safety and the inclusion and exclusion criteria were provided by the applicants.

In addition to the automatic literature search, a manual search was conducted using LIVIVO database and Google Scholar. The search was restricted to the years 2012 to current. Patents were excluded, while citations in Google Scholars were included. The same inclusion and exclusion criteria as for the automatic search were applied.

In total, 43 publications were identified as relevant for the safety of the additive: 34 for the safety for the target species, 8 for the safety for the consumers and 1 for the safety for the users. No relevant papers were identified in support of the safety for the environment.

Considering the above, the FEEDAP Panel concluded that the additive remains safe for the environment.

3.2.1 | Safety for the target species

For the functional group vitamins, pro-vitamins and chemically well-defined substances having similar effect, maximum contents are only provided for vitamin A and vitamin D. Vitamins from the so-called group B are generally considered to be not toxic even at higher dietary levels (NRC, 1987; EMA, 1997). For this reason, in its previous opinion, the FEEDAP Panel concluded that folic acid is safe for all animal species and there is no need to define a maximum content in feed (EFSA FEEDAP Panel, 2012). It was also noted that the amount of information on the tolerance and toxicity levels of folic acid in domestic animals was limited.

Requirements for folic acid are in the range of 0.25–1.0 mg/kg feed for poultry (NRC, 1994), 0.3–1.3 mg/kg for pigs (NRC, 1998), 1–10 mg/kg for fish (NRC, 2011) and 0.2–0.8 mg/kg for pets (NRC, 2006). Ruminants are assumed to cover their requirement by microbial synthesis in the rumen. However, in cases of insufficient or disturbed rumen fermentation, or particular metabolic challenge, an oral supply may become necessary.

Out of the 34 publications retrieved in the literature search, six were considered relevant for the assessment of the safety of poultry species (two in chickens for fattening and four in laying hens).[38] No relevant adverse effects were observed in any of the health and performance parameters evaluated. The results suggest that levels of folic acid up to 13.5 mg/kg complete feed are well tolerated in chickens for fattening and up to 100 mg/kg complete feed in laying hens.

[36] Annex_III_01 and Annex_III_02.

[37] Annex_III_03.

[38] Li et al 2021.pdf; Gouda et al., 2020.pdf; Bagheri_et_al_2019.pdf; Bai_et_al_2021.pdf; Jing_et_al_2014.pdf; TactacanGB-etal_2012.pdf

Regarding swine, one study in sows[39] and one in piglets[40] were considered relevant; none of the studies described adverse effects of the dietary supplementation with folic acid. The results suggest that folic acid is well tolerated at levels up to 20 and 12.5 mg/kg complete feed in sows and weaned piglets, respectively.

Six studies in ruminants (three in calves[41], two in cattle for fattening[42] and one in dairy cows[43]) were considered relevant for assessing the safety of folic acid. The results of the studies showed no adverse effect of the supplementation of folic acid in the diet of calves, cattle for fattening and dairy cows at levels up to 7–10 mg/kg complete feed.

Considering the requirements of folic acid established and the studies with poultry, pigs and ruminants identified in the literature search, the FEEDAP Panel concludes that, in light of the current knowledge, there is no new evidence to reconsider the previous conclusions that folic acid is safe for terrestrial species with no need for maximum content.

With regards to fish and crustacean species, four of the studies retrieved were considered relevant for the assessment of the safety. Two of them (one in gilthead seabream[44] and one in grass carp[45]) showed no negative effects on those species when folic acid was supplemented at levels up to 2–3 mg/kg complete feed. Two other studies were instead identified (Asaikkutti et al., 2016; Sesay et al., 2016) in which the additive showed some adverse effects at levels relatively close to the requirements.

Sesay et al. (2016) carried out a 10-week study to investigate the effects of dietary folic acid on growth, digestive enzyme activity, immune response and antioxidant enzyme activity of blunt snout bream (*Megalobrama amblycephala*) fingerlings. Six semi-purified diets formulated to contain graded levels of folic acid (0, 0.5, 1, 2, 5 and 10 mg/kg diet) were fed to near satiation four times daily. Group size was three replicates (tanks) with 20 fish each (initial body weight (bw) 27 g). Survival rate was 90% in the control group and increased to 95, 95 and 98% for folic acid supplements of 0.5, 1 and 2 mg/kg feed, respectively; at dietary folic acid levels of 5 and 10 mg/kg mortality increased to values comparable to those of the unsupplemented control. The final body weight (69.45 and 63.94 g), specific growth rate (1.34% and 1.22%) and feed to gain ratio (0.79 and 0.76) were significantly better in the groups fed 1 and 2 mg/kg feed compared to the unsupplemented control group (57.2 g, 1.07%/day and 0.64). At higher levels (5 and 10 mg folic acid/kg feed), the performance showed values numerically higher to those of the unsupplemented group. Haematological parameters showed numerical differences with no dose related effects. Malondialdehyde (MDA) significantly decreased and superoxide dismutase (SOD) significantly increased in the groups receiving 1, 2 and 5 mg folic acid/kg feed, returning to the levels of the unsupplemented control group at 10 mg folic acid/kg feed. Muscle crude protein content, serum total protein and immunoglobulin M contents, and intestinal amylase, lipase, trypsin and chymotrypsin activities were significantly enhanced as the dietary folic acid level increased up to 1 mg/kg but showed values comparable to those of the unsupplemented controls at 2 mg/kg feed (trypsin and immunoglobulin M), 5 mg/kg feed (intestinal amylase and lipase) and 10 mg/kg feed (serum total protein and chymotrypsin). Based on the broken line-regression analysis of weight gain rate, specific growth rate and hepatosomatic index, the optimum dietary folic acid for blunt snout fingerlings was estimated to be 0.68, 0.68 and 0.82 mg/kg, respectively. It could be concluded from the data that about 2 mg folic acid/kg feed would be well tolerated in *M. amblycephala* fingerlings.

Asaikkutti et al. (2016) conducted a 12-week feeding trial on freshwater prawn (*Macrobrachium rosenbergii* – start length = 1.46 cm and bw = 0.25 g) to determine the effects of dietary folic acid on growth performance, digestive enzyme activity, muscle composition, immune response and antioxidant ability. Six diets supplemented with 0 (control; background folic acid concentration: 0.04 mg/kg), 0.5, 1, 2, 4 or 8 mg folic acid/kg feed dry matter (DM) were given. Group size was three tanks with 50 prawns each. Survival was 70.2% in the control group and increased to 78.2%, 83.2%, 90.7% and 70.9% in the groups of animals treated with for folic acid at 0.5, 1, 2 and 4 mg/kg feed DM, respectively. The survival rate was significantly lower (67.7%) when folic acid was supplemented at the highest dose (8 mg/kg). The growth performance (final bw, specific growth rate, feed to gain ratio) of prawns showed a significant improvement at all levels of folic acid supplementation compared with the control, best values found at 2 mg/kg. Similarly, intestinal enzymes (protease, amylase, lipase), crude protein content of the prawn and haemocyte counts (total and differential) reached the highest values at 2 mg folic acid/kg and decreased at higher folic acid supplementation rates. After 90 days of supplementation, biomarkers of antioxidant status (superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA)) and liver enzymes (glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GPT)) in muscle and hepatopancreas were about control level until 2 mg folic acid/kg feed DM but reached significantly higher values at 4–8 mg folic acid/kg feed DM. Based on the results, the FEEDAP Panel concludes that the addition of folic acid up to 2 mg/kg feed DM for prawns has beneficial effects on health and performance. However, higher levels of folic acid (4–8 mg/kg feed DM) seem not to be well tolerated by the prawns.

Based on the data provided by the applicant, the FEEDAP Panel notes that the margin of safety of folic acid in feed for fish and crustaceans at levels meeting the requirement is small. The FEEDAP Panel further notes that the requirement for Fleshy Prawn (*Fenneropenaeus chinensis*) according to NRC (2011) is 5 mg folic acid/kg feed DM, while similar levels in freshwater prawn (*Macrobrachium rosenbergii)* do not seem to be well tolerated (Asaikkutti et al., 2016). The wide range of

[39] vanWettereWHEJ-etal_2012.pdf
[40] Wang et al., 2020.pdf
[41] Liu_et_al_2020.pdf; Li_et_al_2021.pdf; WangC-et al_2019.pdf
[42] Wu et al., 2019.pdf; Zhang et al., 2020a.pdf
[43] Zhang et al., 2020b.pdf
[44] Amri_et_al_2020.pdf
[45] ShiL-et al_2015.pdf

aquatic species, as well as the age, breed and environmental factors (e.g. temperature, water salinity) might influence the requirements and possibly tolerance to folic acid.

3.2.1.1 | *Conclusion on the safety for the target species*

The FEEDAP Panel concludes that: (1) there is no new evidence to lead it to reconsider the previous conclusions that folic acid is safe for terrestrial species under the authorised conditions of use and (2) the use of folic acid in aquatic animal species to cover their nutritional needs is considered safe. However, the Panel is not in a position to set a maximum safe level for all fish and crustacean species. Considering the narrow margin between the requirement and the tolerated levels seen in some aquatic animal species, the FEEDAP Panel considers that supplementation should not exceed the requirements of the different aquatic animal species.

3.2.2 | Safety for the consumers

Some studies were identified in the literature search reporting deposition of folates in food of animal origin. The FEEDAP Panel noted that new data derived from publications by Tactacan et al. (2012), Altic et al. (2016) and Bagheri et al. (2019), all concerning deposition in the eggs, give very similar values to those retained in the former EFSA assessment (EFSA FEEDAP Panel, 2012). The study by Czarnowska-Kujawska et al. (2020) concerning the deposition of folates in livers of chickens, turkeys, pigs and beef collected in retail/butchers, shows high deposition values however, no information was provided in the publication whether the samples were obtained from animals fed with folic acid-supplemented diets.

The FEEDAP Panel noted that currently there is not an UL for natural folates. Recently the EFSA NDA Panel issued an opinion establishing UL for supplemental folates (EFSA NDA Panel, 2023), however this is not considered relevant for the current assessment.

In the current assessment, the FEEDAP Panel considered the following: (1) no new evidence was made available that would change the conclusions previously reached on the low deposition rate of folates in animal products (EFSA FEEDAP Panel, 2012); (2) folates levels in liver and milk are not influenced by dietary folic acid (EFSA FEEDAP Panel, 2012); (3) folates occur naturally in several sources of human and animal food, particularly in fresh vegetables, mushrooms and yeast, as well as green leaves, grasses and citrus fruit.

Considering the above, a significant increase of the exposure to folates due to the consumption of products of animal origin given folic acid in feed is not expected and therefore the FEEDAP Panel concluded that the feed additive is safe for the consumers.

3.2.3 | Safety for the user

From the literature search conducted by the applicant (see Section 3.2), a single paper was identified but was not considered relevant by the FEEDAP Panel. Consequently, the applicant has provided new data in order to assess the safety of the additive for the users.

3.2.3.1 | *Effect on respiratory system*

The highest dusting potential of the additive is up to 285 mg/m^3, consequently the FEEDAP Panel considered that the exposure through inhalation is likely. No studies have been submitted to evaluate the potential effects of the additive on the respiratory system.

3.2.3.2 | *Effects on skin*

A skin irritation study performed according to OECD Testing Guideline (TG) 439 (which was in the validation phase and not adopted at that time) (Spielmann et al., 2007), was submitted.[46] The results of the study showed that folic acid is not irritant to skin.

A local lymph node assay was performed to assess the skin sensitisation potential of folic acid. The study was performed following OECD TG 429.[47] The results of the study showed that folic acid is not a dermal sensitiser.

3.2.3.3 | *Conclusions on safety for the user*

The FEEDAP Panel concluded that the exposure through inhalation is likely, however, no data are available on the potential of the additive to be harmful for the respiratory system. Folic acid is not a dermal irritant nor a dermal sensitiser. Due to lack of data, no conclusions can be drawn on the potential of the additive to be an eye irritant.

[46]Reply_RFI_05Apr23_Annex_01_CONF.pdf.
[47]Reply_RFI_05Apr23_Annex_02_CONF.pdf.

3.3 | Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the conditions of the original authorisation that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

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[48] and Good Manufacturing Practice.

4 | CONCLUSIONS

The applicant has provided evidence that the additive currently on the market complies with the existing conditions of authorisation.

The Panel concludes that folic acid remains safe for all terrestrial species, consumers and the environment under the authorised conditions of use. The FEEDAP Panel concludes that the use of folic acid in aquatic species to cover their nutritional needs is considered safe. However, the Panel is not in a position to set a maximum safe level for all fish and crustacean species. Considering the narrow margin between the requirement and the tolerated levels seen in some species, the FEEDAP Panel considers that supplementation should not exceed the requirements of the different aquatic species.

Regarding user safety, the additive is neither a skin irritant nor a dermal sensitiser. In the absence of data, no conclusions can be drawn on the eye irritation and on the potential of the additive to be harmful for the respiratory system. The exposure through inhalation is likely.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

5 | RECOMMENDATIONS

The FEEDAP Panel recommends that the description of the additive and its specifications for impurities follow those set in the updated version of the PhEur (2022).

The FEEDAP Panel recommends that further research is conducted to allow setting a maximum safe level of folic acid in aquatic animal species.

ABBREVIATIONS

bw	body weight
CAT	catalase
CAS	Chemical Abstracts Service
CV	coefficient of variation
PhEur	European Pharmacopeia
EURL	European Union Reference Laboratory
FEEDAP Panel	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
HRP	maximum highest reliable percentile
LOD	limit of detection
MDA	Malondialdehyde
NDA Panel	EFSA Scientific Panel on Nutrition, Novel Foods and Food Allergens
OECD	Organisation for Economic Co-operation and Development
SC	Scientific Committee
SOD	superoxide dismutase
TG	Testing Guideline
UL	upper level

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.

[48]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.

REQUESTOR
European Commission

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