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Safety and efficacy of Biomin® DC-P as a zootechnical feed additive for chickens for fattening, chickens reared for laying and minor avian species to the point of lay

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Abstract

The additive (trade name Biomin®DC-P) is a blend of five individual compounds (carvacrol, thymol, D-carvone, methyl salicylate and L-menthol) encapsulated with a hydrogenated vegetable oil. The additive is intended for use in feed for various poultry species at a minimum concentration of 65 mg/kg complete feed and a recommended maximum level of 105 mg/kg complete feed. The results of a tolerance study show that Biomin® DC-P is safe for chickens for fattening at the maximum recommended application rate of 105 mg/kg complete feed; this conclusion is extended to include chickens reared for laying and extrapolated to minor poultry species The active components of a previously evaluated additive (Biomin®DC-C) were shown to be not genotoxic; owing to the similarity on composition, this conclusion can be also applied to Biomin®DC-P. Notwithstanding the uncertainties identified in the residue study, after applying a worst-case scenario to calculate potential exposure of consumers to menthol and carvone, and since that the components of the additive are considered safe for their use as food and feed flavourings, the FEEDAP Panel concludes that the use of the additive in animal nutrition is considered safe for consumers. The FEEDAP Panel considered that exposure of users by inhalation is unlikely, but cannot conclude on the effects of Biomin®DC-P on skin and eyes. The use of Biomin®DC-P is not expected to pose a risk for the environment. Biomin®DC-P has a potential to increase the growth performance of chickens for fattening when incorporated into feed at a minimum application rate of 65 mg/kg complete feed; the conclusion can be extended to chickens reared for laying and extrapolated to minor poultry species reared up to the point of lay.

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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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Biomin GmbH[2] for authorisation of the product Biomin® DC-P, when used as a feed additive for chickens for fattening, chickens reared for laying, minor avian species to the point of lay (category: zootechnical additives; functional group: 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) (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 2 July 2018.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product Biomin® DC-P, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

Biomin® DC-P has not previously been assessed in the European Union.

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 Biomin® DC-P 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.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the Biomin® DC-P as a zootechnical feed additive for chickens for fattening, chickens reared for laying, minor avian species to the point of lay. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Biomin® DC-P is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical Guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Biomin GmbH, Erber Campus 1, 3131 Getzersdorf, Austria.
[3] FEED dossier reference: FAD-2018-0023.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0023-biomin-dc-p.pdf
[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

Biomin® DC-P is composed of a mixture of chemical compounds intended to be used as a zootechnical feed additive for chickens for fattening, chickens reared for laying and minor avian species to the point of lay. It has not been previously assessed in the European Union (EU).

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive is a blend of five individual compounds (carvacrol, thymol, D-carvone, methyl salicylate and L-menthol).[6] Amorphous silica is added as a carrier and the mixture then encapsulated with a hydrogenated vegetable oil. The approximate quantitative composition of the resulting additive is shown in Table 1.[7]

Table 1: Typical composition of Biomin® DC-P

Ingredient (CAS number)	Content (mg/g additive)
Carvacrol (499–75–2)	120–160
Thymol (89–83–8)	1–3
D-Carvone (2244–16–8)	3–6
Methyl salicylate (119–36–8)	10–35
L-Menthol (2216–51–8)	30–55
Amorphous silica (68611–44–9)	Maximum 100
Hydrogenated vegetable oil	Maximum 700

The applicant is responsible for the manufacture of the additive itself, but raw materials are sourced from third party suppliers. In each case, raw materials are purchased to specifications established by the manufacturer, which set a purity of ≥ 99% for each of the active compounds of the additive;[8] certificates of analysis were provided confirming that the specification for each individual compound was met. The sources and specifications of pure silica[9] and hydrogenated vegetable oil[10] are described in the technical dossier.

Analysis of five batches of the additive for the five active compounds showed compliance with the specified content;[11] there was little variation between batches confirming the consistency in composition. Mean values (and range) in mg/g reported were: carvacrol 150.5 (142.5−156.6), thymol 2.3 (2.1−2.5), D-carvone 4.9 (4.5−5.4), methyl salicylate 31.3 (26.7−36.3) and L-menthol 49.3 (35.3−54.9).

Analysis of three batches of the additive for heavy metals (lead (Pb), cadmium (Cd) and mercury (Hg)) and arsenic (As) was provided.[12] Data showed that in each case the content was below the respective limits of quantification (LOQ)[13] and complied with the specifications (in mg/kg, for As: < 2, for Pb: < 10, for Cd: < 0.5, for Hg: < 0.1).

A further set of three batches of the product was analysed for the presence of mycotoxins,[14] pesticide residues[15] and dioxins (polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo

[6] According to the information provided by the applicant all components are manufactured from a pure chemical synthesis, except L-Menthol which is manufactured from extraction of a botanical source.
[7] Technical Dossier/Section II and Technical Dossier/Supplementary Information.
[8] Technical Dossier/Section II/Annex II_45 to II-49.
[9] Technical Dossier/Section II/Annex II_57 and Annex II_58.
[10] Technical Dossier/Section II/Annex II_56.
[11] Technical Dossier/Section II/Annex II_02 to II_06.
[12] Technical Dossier/Section II/Annex II_07 to II_09.
[13] LOQ (in mg/kg) for As: < 0.5, for Pb: < 0.5, for Cd: < 0.1, for Hg: < 0.01.
[14] Technical Dossier/Section II/Annex II_10.
[15] Technical Dossier/Section II/Annex II_17 to II_19. The battery of pesticides analysed included the following: aldrin, dieldrin, chlordane, DDT, endosulfan, endrin, heptachlor, hexachlorobenzene, hexachlorohexane, methoxychlor, nitrofen, quintozene, tecnazene and tetradifon.

(p)dioxins (PCDD)) and dioxin-like polychlorinated biphenyls (PCBs).[16] All of the mycotoxins tested (deoxynivalenol, zearalenone, aflatoxins (B1, B2, G1, G2), ochratoxin A, fumonisin B1 and B2, HT-2 toxin and T-2 toxin) were below their respective limits of detection (LOD).[17] The analysis of pesticide residues resulted in all cases either not detected or below the LOQ.[18] Dioxins and the sum of dioxins and dioxin-like PCBs amounted to 0.06–0.09 ng WHO-PCDD/F-TEQ/kg and 0.11–0.14 ng WHO-PCDD/F-PCB-TEQ/kg, respectively.

Another set of three batches was analysed for the presence of residual solvents (methanol, acetone, 2-propanol and *n*-hexane) used during the manufacture of the additive or its components.[19] Results showed only residual amounts of methanol at a mean concentration of 92 μg/g additive; this is substantially below the maximum value of 3,000 μg/g additive recommended in the Guidance document on the International Cooperation on Harmonisation of Technological Requirements for Registration of Medicinal Products (VICH, 2011).

Limits are set for microbial contamination, which are specified not to exceed 30 CFU/g additive for total coliforms, 100 CFU/g additive for yeasts and 100 CFU/g additive for filamentous fungi; in addition, no *Escherichia coli* or *Salmonella* should be detected in 25 g additive. The analysis of three batches of additive showed compliance with these specifications.[20]

The product Biomin®DC-P is an off-white powder with flowing properties. Three batches of the additive were examined for particle size distribution by laser diffraction[21] and for dusting potential using the Stauber–Heubach method.[22] Only 0.11% of particles with diameter < 100 μm could be detected and 0.01% with diameter < 10 μm. The measurement of dusting potential gave a mean value of 0.03 g/m^3. In the same three batches, the bulk density and pH were analysed resulting in an average of 530.2 kg/m^3 and 5.86, respectively.[23]

3.1.2. Stability and homogeneity

3.1.2.1. Shelf-life

The shelf-life of the additive was assessed using three batches of the additive stored in sealed air-tight packaging at either 22±2 °C or 37±0.5 °C for up to 18 months.[24] Stability was assessed by following the concentration of the five active compounds listed in Table 1 after 6, 12 and 18 months of storage. Recoveries of all of the individual compounds were > 87% and > 83% at the lower and higher storage temperature, respectively.

3.1.2.2. Stability in premixtures and feed

Biomin® DC-P was incorporated into a commercial 'mineral feed' containing minerals and phytase and stored in sealed packaging for six months at 22 ± 2°C; the additive was incorporated into the mineral feed at a ratio 1:150.[25] After 3 months, the concentrations of thymol, carvacrol and methyl salicylate substantially remained unchanged, but those of D-carvone and L-menthol were reduced by about 20%. After 6 months, the recoveries of carvacrol and methyl salicylate were > 80%, but the concentration of the remaining three compounds had fallen to approximately 60% of their initial values.

In a similar study, a single batch of the additive was incorporated into a mash feed for chickens based on maize and soybean at 65 mg/kg complete feed.[26] The feed was stored in a sealed packaging at 22 ± 2°C for 5 months then analysed for the five active compounds. All five of the compounds showed recoveries of at least 85% of their respective initial values.

In a separate study to investigate the effects of pelleting on the stability of the additive, Biomin® DC-P was incorporated into a mash feed (wheat, maize and soybean based) at the minimum recommended inclusion level and the mash feed then pelleted at temperatures of 85 and 95°C

[16] Technical Dossier/Section II/Annex II_14 to II_16.
[17] LOD (in μg/kg) for deoxynivalenol: 20 , for zearalenone: 4, for aflatoxin B1: 0.2, aflatoxin B2: 0.2, aflatoxin G1: 0.2, aflatoxin G2: 0.2, for ochratoxin A: 0.2, for fumonisin B1: 20, for fumonisin B2: 20, for HT-2 Toxin: 2 and for T-2 Toxin: 2.
[18] 0.010 mg/kg, for each of the pesticides tested.
[19] Technical Dossier/Section II/ Annex II_20. According to the information provided by the applicant, all components of Biomin®DC-P originate from chemical synthesis, with the exception of L-menthol which derives from extraction from herbs.
[20] Technical Dossier/Section II/ Annex II_11 to II_13.
[21] Technical Dossier/Section II/ Annex II_21.
[22] Technical Dossier/Section II/ Annex II_22 to II_24.
[23] Technical Dossier/Section II/ Annex II_25 to II_27.
[24] Technical Dossier/Section II/ Annex II_62.
[25] Technical Dossier/Section II/ Annex II_63.
[26] Technical Dossier/Section II/ Annex II_64.

(conditioning time of 60 s);[27] the pellets were then stored in sealed packaging at 21°C for 3 months and the concentration of the five active compounds were measured in the mash feed prior to pelleting, immediately after pelleting and after the 3-month storage period. Recoveries immediately after pelleting were > 90% at 85°C and > 80% at 95°C. Subsequent storage of the pellets resulted in further losses of around 10% independent of pelleting temperature.

3.1.2.3. Homogeneity

One tonne of a mash feed for chickens was prepared incorporating Biomin® DC-P at a target inclusion level of 65 mg/kg complete feed.[28] The feed was distributed in 20 kg bags and samples taken from 10 randomly selected bags. Samples were then analysed for the concentrations of the five active compounds. The resulting coefficients of variation (CV) for four of the five individual compounds were in the region of 10 %; the result for methyl salicylate was 23.7 %.

3.1.3. Conditions of use

Biomin® DC-P is intended for use as a zootechnical additive (functional group: other zootechnical additives) in feed for chickens for fattening, chickens reared for laying and minor avian species to the point of lay at a minimum concentration of 65 mg/kg complete feed and a recommended maximum level of 105 mg/kg complete feed. No withdrawal period is foreseen.

3.2. Safety

3.2.1. Safety for the target species

3.2.1.1. Tolerance study

A tolerance test was made with 1520 one-day-old male Ross 308 chickens for fattening using a randomised complete block design with four treatments with 19 replicate pens each holding 20 birds per treatment.[29] The duration of the study was 35 days. The four treatments were a group given only the basal diet based on wheat, maize and soybean and provided in mash form and three groups in which the basal diet was supplemented with 105 (×1), 525 (×5) or 1050 (×10) mg additive/kg complete feed. Analysis of feed for the five active compounds suggested that recoveries were approximately half of the intended values; this is likely due to a limitation of the analytical method, which has been also highlighted by the EURL. The analysis of the premixture used to deliver the additive in the study indicates that the overdose supplementation was achieved. The basal diet also contained coccidiostats and enzyme supplements.

Birds were monitored throughout the study and were weighed on pen basis at the start and on days 21 and 35. Feed intake per pen was measured for the periods 0–21 days and 21–35 days. From these data, the average daily gain and feed to gain ratio were calculated. One bird per pen from 12 randomly selected pens per treatment was killed and samples of blood and tissues were collected on day 36. Blood samples were analysed for haematology[30] and clinical chemical[31] parameters. Tissue samples (breast muscle, liver, kidneys and skin/fat from the breast area) were obtained to perform the residue study (see Section 3.2.4). Caecal contents and samples from the distal jejunum were taken for microbial profiling. A section of the mid-jejunum was also taken for histomorphological examination (villus length, crypt depth, villus to crypt ratio, goblet cell number per villus).

Data were analysed with analysis of variance (ANOVA) with treatment means compared with Fisher's least significant differences after significant effects of the treatment were identified in the ANOVA. In all cases, the pen was the experimental unit.

Overall mortality was 2.6% with no evidence of a treatment-related effect. There were no significant differences in final body weight (range of values across treatments: 1.99–2.01 kg), feed intake per bird (range of values across treatments: 2.97–2.99 kg) or feed to gain (range of values across treatments: 1.51–1.53) between treatment groups. Other than serum glucose and potassium

[27] Technical Dossier/Section II/ Annex II_65.
[28] Technical Dossier/Section II/ Annex II_66.
[29] Technical Dossier/Section III/ Annex III_01.
[30] Red blood cells, packed cell volume, haemoglobin, white blood cells, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume.
[31] Albumin, albumin:globulin, total protein, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, creatine phosphokinase, lactate dehydrogenase, creatinine, glutamate dehydrogenase, bilirubin, sodium, potassium, chloride, calcium, phosphate, triglycerides, urea and glucose.

values, none of the clinical chemistry or haematological parameters showed significant differences. Both glucose and potassium showed elevated levels in the ×10 treatment group compared to the control values (glucose 17.7 vs 21.2 mmol/L, potassium 13.9 *vs* 18.5 mmol/L). The FEEDAP Panel notes that glucose values fall within the normal ranges in chicken, and that the high potassium mean value for ×10 treatment came from an outlier. The additive did not affect any of the histomorphological measurements of the jejunal tissue.

3.2.1.2. Gut microbiota

The potential effect of Biomin® DC-P on the intestinal microbiota of digesta taken from the chickens selected for gross pathology in the tolerance study was studied using denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified 16S rRNA gene fragments.[32] This provided a fingerprint of the dominant bacteria present. Computational analysis of DGGE patterns showed 25–27 prominent bands. The number of dominant bands (species richness) did not differentiate the samples according to treatment. Cluster analysis also showed no effect of the additive on the bacterial diversity of dominant members of the gut microbiota.

3.2.1.3. Conclusions on safety for the target species

The results of the tolerance study show that the additive is safe for chickens for fattening at the maximum recommended application rate of 105 mg/kg complete feed; this conclusion is extended to chickens reared for laying and extrapolated to minor poultry species to the point of lay.

3.2.2. Safety for the consumer

3.2.2.1. Toxicological studies

The applicant made reference to the toxicological studies submitted in another dossier with a preparation of the 'active' components of a similar additive (Biomin® DC-C) intended for use with pigs, and argued that the same active compounds are present in both additives at approximately the same concentrations (see Table 2). The safety of this additive has been recently evaluated by the FEEDAP Panel (EFSA FEEDAP Panel, 2019). In both additives, Biomin® DC-C and Biomin® DC-P, the active components represent one quarter of the product, the remainder being silica and hydrogenated vegetable fat.

Although thymol was not routinely measured in Biomin® DC-C, it is expected to be present in approximately the same concentration as that of Biomin® DC-P arising from the use of the essential oil of oregano known to contain thymol.

Table 2: A comparison of the specified concentration (mg/g) of the five active compounds in Biomin® DC-P with those in Biomin® DC-C used for the toxicological studies

Compound	Biomin® DC-P	Biomin® DC-C
Carvacrol	120–160	120–131
Thymol	1–3	Not measured
D-carvone	3–6	4
Methyl salicylate	10–35	23–30
L-menthol	30–55	45–48

The toxicological studies submitted with the dossier Biomin® DC-C, two *in vitro* genotoxicity studies (a bacterial reverse mutation test and a mammalian chromosome aberration test) and a repeated dose oral toxicity study were assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2019). Based on the outcome of the two *in vitro* studies and considering that all the identified components of the additive (linalool, carvone, carvacrol, thymol, methyl salicylate and menthol) have been assessed and considered safe for use as flavourings, and are currently authorised for food[33] and feed[34] uses, the

[32] Technical Dossier/Section III/ Annex III_04.

[33] Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1.

[34] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-eu-reg-comm_register_feed_additives_1831-03.pdf

Panel concluded that Biomin® DC-C was non-genotoxic. As the repeated dose oral toxicity did not comply with OECD 408, it was not considered in the assessment of Biomin® DC-C.

The FEEDAP Panel considers that the studies already assessed for Biomin® DC-C are relevant for the assessment of the safety of the additive currently under assessment (Biomin® DC-P) and the conclusions reported previously can be retained for the current assessment.

3.2.2.2. Residue study

The applicant provided analysis of residues of the components of the additive in samples of tissues (muscle and skin + fat) taken from the tolerance study (see Section 3.2.1.1) performed with chickens for fattening (Table 3).[35]

Table 3: Concentration (μg/kg) of the five active compounds in Biomin® DC-P in muscle and skin + fat. Samples taken from the chickens used in the tolerance study.

Compound	Breast muscle		Skin + Fat	
	Control	Biomin® DC-P 105 mg/kg feed	Control	Biomin® DC-P 105 mg/kg feed
L-menthol	10.57[a]	16.69[b]	24.08	24.47
D-carvone	77.13	74.46	159.74	170.25
Methyl salicylate	34.73	40.51	13.95	24.67
Thymol	5.28[a]	8.60[b]	--(1)	--(1)
Carvacrol	8.35[a]	11.28[b]	--(2)	--(2)

a,b: For a given compound and tissue, means with different superscripts are significantly different ($p < 0.05$).
(1): All measurements below LOD. LOD (μg/kg) L-menthol: 1.15, methyl salicylate: 1.32, D-carvone: 0.88, thymol: 0.81 and carvacrol: 1.45.
(2): All measurements below LOQ. LOQ (μg/kg) L-menthol: 3.46, methyl salicylate: 3.95, D-carvone: 2.63, thymol: 2.42 and carvacrol: 4.36. For those analytes in which some results gave < LOQ the applicant chose the value LOQ/2 for statistics.

The FEEDAP Panel has serious doubts on the reliability of the residue data since (i) the levels of residues in control animals were unexpectedly high, (ii) the control and the supplemented diets led to a similar deposition levels in tissues for carvone and methyl salicylate in muscle and for three compounds (L-menthol, carvone and methyl salicylate) in skin + fat, and (iii) the levels of carvacrol were 100-fold higher than the levels of thymol and carvone in the additive, but in the same order of magnitude (thymol) or even 10-fold lower (carvacrol) in muscle. Overall, the data indicate that it is likely that sample contamination occurred, or analytical artefacts were generated during the extraction procedure.

Notwithstanding the above-indicated uncertainties in the analytical dataset, the FEEDAP Panel has considered the residues in muscle and skin + fat to assess the chronic consumer exposure for menthol and carvone for which an acceptable daily intake (ADI) is established: 4 mg menthol/kg body weight (bw) for menthol (WHO, 1999) and 60 mg carvone/kg bw (EFSA Scientific Committee, 2014). For this assessment, the European food consumption data of different age classes from EFSA's Comprehensive European Food Consumption Database as detailed in the Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017); the Panel used the highest analysed data for menthol and carvacrol in muscle and skin + fat.[36] In all population groups, the chronic exposure to menthol and carvone was 4–5 orders of magnitude lower than the corresponding ADI.

For the rest of the components of the additive an ADI is not available and, thus, the procedure used for menthol and carvacrol as described above, cannot be applied. However, the FEEDAP Panel notes that those components (carvacrol, thymol and methyl salicylate) – and also the rest of the components of the additive: carvone and menthol – have been assessed and considered safe for use as flavourings, and they are currently authorised for food[33] and feed[34] uses.

3.2.2.3. Conclusions on safety for the consumer

The FEEDAP Panel concludes that the use of Biomin® DC-P in animal nutrition is considered safe for consumers of animal products under the proposed conditions of use.

[35] Technical Dossier/Supplementary Information/Annex (ii_01).
[36] Input data were: for menthol 49.77 μg/kg skin + fat and 27.45 μg/kg muscle, and for carvone 314.59 μg/kg skin + fat and 106.01 μg/kg muscle.

3.2.3. Safety for the user

The virtual absence of respirable particles (0.11 % of particles with diameters < 100 μm) and the very low dusting potential (0.03 g/m^3) would suggest that respiratory exposure of users is unlikely.

No specific studies on skin and eye irritation or skin sensitisation were provided. The applicant provided the safety datasheets of the additive's individual compounds where hazards for users are identified.[37]

The FEEDAP Panel considered that exposure to users by inhalation is unlikely. In the absence of data, the Panel cannot conclude on the effects of Biomin® DC-P on skin and eyes.

3.2.4. Safety for the environment

Biomin® DC-P is a feed additive composed of a mixture of individual chemical compounds. A literature search aiming to examine the natural occurrence of the single components of Biomin® DC-P was performed by using several databases (e.g. TNO VCF, Dukes Phytochemical and Ethnobotanical Databases, HSDB). The 'CAS numbers', 'chemical names', 'plant species' or 'synonyms' of the active substances of the additive were used as keywords.[38]

The literature search revealed that all the additive's components occur in various plants, which are native to the countries of the European Union. A number of these plants are important crops or occur at various sites, such as meadows or alpine regions. The concentrations of the chemical components naturally occurring in these plants are much higher than the corresponding levels intended to be used in feed.

The use of Biomin® DC-P in animal production is not expected to pose a risk for the environment.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

The applicant proposes that the additive improves performance of chickens for fattening, chickens reared for laying, minor avian species to the point of lay. To support the efficacy of the additive the applicant submitted four studies made with chickens for fattening performed in three Member States and at three different locations. All four involved a comparison between a control group given a basal diet and a group in which the basal diet was supplemented with the additive at the minimum recommended dose of 65 mg additive/kg complete feed. Details on the study designs are given in Table 4.

Table 4: Details of the designs of the three studies conducted with Biomin® DC-P with chickens for fattening

Study	Biomin® DC-P (mg/kg complete feed)		Total number of animals Replicates per treatment / Birds per replicate	Genotype Sex	Diet composition (form)	Duration of the study (days)
	Intended	Analysed(1)				
1(5)	0		760 19/20	Ross 308 ♂	Wheat–maize–soybean (mash)	35
	65	38-37(2)				
2(6)	0		544 16/17	Ross 308 ♂♀	Maize–soybean (mash)	42
	65	39-48				
3(7)	0		640 16/20	Ross 308 ♂♀	Maize–soybean (mash)(3)	42
	65	44-27				
4(8)	0		880 20/22	Ross 308 ♂	Maize–soybean (mash)(4)	42
	65	34-32-35				

(1): Diets were analysed for the active compounds shown in Table 2 and from this an estimate of additive addition calculated.
(2): Figure before the hyphen for the starter diet, and that after the hyphen for the grower/finisher diet. For Study 4 the three figures correspond to starter, grower and finisher diets, respectively.
(3): Diets include an enzyme and a coccidiostat.
(4): Diets included an enzyme.

[37] Technical Dossier/Section II/Annex_67.
[38] Technical Dossier/Section III/Annex_15.

(5): Technical Dossier/Section IV/ Annex IV_03 and Technical Dossier/Supplementary Information.
(6): Technical Dossier/Section IV/ Annex IV_07.
(7): Technical Dossier/Section IV/ Annex IV_22. Technical Dossier/Supplementary information/Annexes iv_01 and iv_02.
(8): Technical Dossier/Section IV/ Annex IV_15, Annex IV_17.

Analysis of feed for the five active compounds suggested that recoveries were lower than the intended values.

In Study 1, birds were monitored throughout the study and were weighed by pen at the start and on days 21 and 35. Feed intake per pen was measured for the periods 1–21 days and 21–35 days. From these data, average daily gain and feed to gain ratio were calculated. Data were analysed with a two-way ANOVA with the pen as the experimental unit. Treatment means were compared with Fisher's least significant differences after significant effects were confirmed by ANOVA.

In studies 2 and 3, birds were monitored throughout the study and were weighed by pen at the start and on days 14 and 28 and individually on days 35 and 42. Feed intake per pen was measured for the weighing periods. From these data average daily gain and feed to gain ratio were calculated. Data were checked for normal distribution by Shapiro–Wilk test. A non-parametric Mann–Whitney U-test was used for further testing of data that were not normally distributed, while parametric tests were used for normally distributed data. For parametric analysis, data were checked using Levene's test of homogeneity of variances, followed by Student's t-test taking into account the result of Levene's test. The pen was the experimental unit and two-sided tests were applied.

In study 4, weight gain, feed intake and feed to gain for each pen were recorded/calculated on days 1, 14, 35 and 42 for each feeding period and for the whole study. In addition, birds were individually weighed on day 42 to estimate the homogeneity of the population groups. Data were analysed by ANOVA.

The results of the four individual studies are shown in Table 5.

Table 5: Summary of the results of the four individual efficacy studies performed with the additive Biomin® DC-P at the minimum recommended dose (65 mg/kg feed) in diets of chickens for fattening

Study No	Duration	Treatment	Final body weight (kg)	Average daily gain (g)	Average daily feed intake (g)	Feed to Gain	Mortality (%)
1	35 days	0	2.00		2.99(1)	1.53	2.1
		Biomin®DC-P	2.04		3.01	1.51	3.2
2	35 days	0	1.75	48.7	98.6	2.03	2.9
		Biomin®DC-P	1.81	50.5	92.2	1.83*	5.5
	42 days	0	2.32	54.2	111	2.06	2.9
		Biomin®DC-P	2.41*	56.5*	108	1.92	5.5
3	35 days	0	2.03	56.9	96.5	1.70	4.2
		Biomin®DC-P	2.09	58.5	97.9	1.67	1.9
	42 days	0	2.72	63.8	114	1.78	4.2
		Biomin®DC-P	2.77	65.1	115	1.76	1.9
4	35 days	0	2.19	61.3	91.5	1.49	3.2
		Biomin®DC-P	2.21	61.9	91.6	1.48	4.3
	42 days	0	2.93	68.8	112	1.62	3.2
		Biomin®DC-P	2.97	69.6	111	1.60*	4.3

(1): For Study 1 this figure is total feed intake (kg).
*: Within a trial and a given time the control and treated groups are significantly different ($p < 0.05$).

Although in all four studies final body weight was numerically improved by the addition of the additive, this reached significance in only one study (Study 2). Similarly, there was a numerical improvement in feed to gain in all studies, but this effect again reached significance at the end of the study in only one (Study 4). The applicant undertook a meta-analysis of the data from all four studies (Section 3.3.2).

3.3.2. Meta-analysis of data

While the full duration of the four studies varied between 35 and 42 days, all studies contained a set of observations made on day 35.[39] These data sets were submitted to a meta-analysis. Following this analysis, Biomin® DC-P showed significant effects with respect to feed to gain ratio ($p = 0.003$ CI 95% −0.87 to −0.19) and body weight gain ($p = 0.004$ CI 95% 0.17 to 0.85).[40] Feed intake was not affected by treatment ($p > 0.05$).

3.3.3. Conclusions on efficacy for chickens for fattening

Based on a meta-analysis of four efficacy studies, Biomin® DC-P has a potential to increase the growth performance of chickens for fattening when incorporated into feed at a minimum concentration of 65 mg/kg complete feed.

3.3.4. Conclusions on efficacy for target species

Biomin®DC-P has a potential to increase the growth performance of chickens for fattening when incorporated into feed at a minimum concentration of 65 mg/kg complete feed. The conclusion can be extended to chickens reared for laying and extrapolated to minor poultry species reared up to the point of lay at the same minimum dose.

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

The results of the tolerance study show that Biomin® DC-P is safe for chickens for fattening at the maximum recommended application rate of 105 mg/kg complete feed; this conclusion is extended to include chickens reared for laying and extrapolated to minor poultry species.

The FEEDAP Panel concludes that the use of Biomin® DC-P in animal nutrition is considered safe for consumers of animal products under the proposed conditions of use.

The FEEDAP Panel considered that exposure of users by inhalation is unlikely. In the absence of data, the Panel cannot conclude on the effects of Biomin® DC-P on skin and eyes.

The use of Biomin® DC-P in animal production is not expected to pose a risk for the environment.

Biomin®DC-P has a potential to increase the growth performance of chickens for fattening when incorporated into feed at a minimum concentration of 65 mg/kg complete feed. The conclusion can be extended to chickens reared for laying and extrapolated to minor poultry species reared up to the point of lay at the same minimum dose.

5. Remark

The FEEDAP Panel notes that the EURL indicated in its report that 'Since the accurate determination of the Biomin® DC-P content added to premixtures and feedingstuffs is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to determine Biomin® DC-P in feedingstuffs'.

Documentation provided to EFSA/Chronology

Date	Event
27/04/2018	Dossier received by EFSA. Biomin® DC-P for chickens for fattening, chickens reared for laying, minor avian species to the point of lay. April 2018. Submitted by Biomin GmbH.
18/05/2018	Reception mandate from the European Commission

[39] Technical Dossier/Section IV/ Annex IV_02, Annex IV_01.
[40] Confidence intervals of the standardised mean differences between the two groups.
[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.

Date	Event
02/07/2018	Application validated by EFSA – Start of the scientific assessment
01/10/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for the consumer and efficacy*
02/10/2018	Comments received from Member States
02/10/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
29/01/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
15/05/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2008. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008,6(10):842, 28 pp. https://doi.org/10.2903/j.efsa.2008.842

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: Tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. https://doi.org/10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. https://doi.org/10.2903/j.efsa.2012.2536

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. https://doi.org/10.2903/j.efsa.2012.2537

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente M, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Chesson A, Gropp J, Martelli G, Renshaw D, López-Gálvez G and Mantovani A, 2019. Scientific Opinion on the safety and efficacy of Biomin®DC-C as a zootechnical feed additive for weaned piglets. EFSA Journal 2019;17(4):5688, 15 pp. https://doi.org/10.2903/j.efsa.2019.5688

EFSA Scientific Committee, 2014. Scientific Opinion on the safety assessment of carvone, considering all sources of exposure.. EFSA Journal 2014;12(7):3806, 74 pp. https://doi.org/10.2903/j.efsa.2014.3806

VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products), 2011. Impurities: Residual Solvents in new veterinary medicinal products, active substances and excipients (Revision). Available online: http://www.vichsec.org/component/attachments/attachments/154.html?task=download

WHO (World Health Organization), 1999. Safety evaluation of certain food additives. Prepared by the Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, WHO Food Additives Series, FAS 42.

Abbreviations

ADI	acceptable daily intake
ANOVA	analysis of variance
Bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CV	coefficients of variation
DGGE	denaturing gradient gel electrophoresis
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed

GC-FID	gas chromatography coupled to flame ionisation detection
LOD	limit of detection
LOQ	limit of quantification
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo(p)dioxins
PCDF	polychlorinated dibenzofurans
PCR	polymerase chain reaction
Rrec	*recovery* rate
RSDip	relative standard deviation for *intermediate precision*
RSDr	relative standard deviation for *repeatability*
TEQ	toxic equivalent
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Biomin® DC-P

In the current application authorisation is sought under article 4(1) for the *preparation of carvacrol, thymol, D-carvone, methyl salicylate and L-menthol (Biomin® DC-P)* under the category/ functional group (4 d) "zootechnical additives"/"other zootechnical additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for chickens for fattening and reared for laying and minor avian species other than laying species.

The *feed additive* is an off-white powder consisting of the following active substances (expressed as percentage mass fractions related to the *feed additive):* 10 to 16 % of *carvacrol,* 0.1 to 0.3 % *thymol,* 0.3 to 0.6 % *D-carvone,* 1 to 4.5 % *methyl salicylate* and 3 to 5.5 % *L-menthol.* In addition, it contains hydrogenated vegetable oil and silica as carriers.

The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* with a proposed *Biomin® DC-P* content ranging from 65 to 105 mg /kg *feedingstuffs.*

For the quantification of *carvacrol, thymol, D-carvone, methyl salicylate* and *L-menthol* in the *feed additive* the Applicant submitted a single-laboratory validated and further verified multianalyte method based on gas chromatography coupled to flame ionisation detection (GC-FID). The following performance characteristics were reported for the five analytes mentioned above: a relative standard deviation for *repeatability* (RSDr) ranging from 0.7 to 1.6 %; a relative standard deviation for *intermediate precision* (RSDip) ranging from 0.9 to 6.5 %; and a *recovery* rate (Rrec) ranging from 82 to 92 %.

Based on the experimental evidence available the EURL recommends for the official control the single-laboratory validated and further verified multi-analyte GC-FID method for the quantification of *carvacrol, thymol, D-carvone, methyl salicylate* and *L-menthol* in the *feed additive.*

Since the accurate determination of the *Biomin® DC-P* content added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to determine *Biomin® DC-P* in *feedingstuffs.*

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.