Safety and efficacy of a feed additive consisting of a preparation of carvacrol, thymol, D-carvone, methyl salicylate and L-menthol (Biomin® DC-P) for all poultry species (Biomin GmbH)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Montserrat Anguita, Rosella Brozzi, Jaume Galobart, Elisa Petenatti, Fabiola Pizzo, Joana Revez, Jordi Tarrés-Call and Jordi Ortuño

Abstract
Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of a preparation of carvacrol, thymol, D-carvone, methyl salicylate and L-menthol (Biomin® DC-P) for all poultry species. The additive is authorised for use in feed for chickens for fattening, chickens reared for laying and minor poultry species reared to the point of lay. The safety and efficacy of the additive for those species have been previously evaluated by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel). The current application is for an extension of use of the additive for all poultry species under the same conditions of use (recommended level ranging between 65 and 105 mg/kg complete feed). The FEEDAP Panel concluded that the additive Biomin® DC-P is safe for poultry species for fattening or reared for laying, but in the absence of adequate data cannot conclude on the safety for laying/breeding birds. The use of Biomin® DC-P in feed for all poultry species under the proposed conditions of use was considered safe for consumers and the environment. The FEEDAP Panel considered that exposure of users by inhalation is unlikely. In the absence of data, the Panel could not conclude on the effects of Biomin® DC-P on skin and eyes. Biomin® DC-P has the potential to be efficacious in poultry species for fattening or reared for laying when incorporated into feed at a minimum concentration of 65 mg/kg complete feed. In the absence of sufficient data, the Panel could not conclude on the efficacy for laying hens or for other poultry species for laying/breeding.

Keywords: zootechnical additives, other zootechnical additives, Biomin® DC-P, carvacrol, safety, efficacy, all poultry species

Requestor: European Commission
Question number: EFSA-Q-2020-00835
Correspondence: feedap@efsa.europa.eu
Panel members: Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fasmon Durjava, Maryline Koubâ, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003 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 Biomin GmbH for the authorisation of the additive consisting of a preparation of carvacrol, thymol, D-carvone, methyl salicylate and L-menthol (Biomin® DC-P) when used as a feed additive for all poultry species (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). The particulars and documents in support of the application were considered valid by EFSA as of 8 March 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 preparation of carvacrol, thymol, D-carvone, methyl salicylate and L-menthol (Biomin® DC-P), when used under the proposed conditions of use (see Section 3.2.1).

1.2. Additional information

EFSA issued one opinion on the safety and efficacy of this product when used in feed for chickens for fattening, chickens reared for laying and minor poultry species reared to the point of lay (EFSA FEEDAP Panel, 2019a). The additive is currently authorised for use in those species and categories (4d20).3

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 in support of the authorisation request for the use of a preparation of carvacrol, thymol, D-carvone, methyl salicylate and L-menthol (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 active substances in animal feed. The Executive Summary of the EURL report can be found in Annex A.5

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of a preparation of carvacrol, thymol, D-carvone, methyl salicylate and L-menthol (Biomin® DC-P) is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012),

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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 Erber Campus 1, 3131, Getzersdorf, Austria.
3 COMMISSION IMPLEMENTING REGULATION (EU) 2020/996 of 9 July 2020 concerning the authorisation of the preparation of carvacrol, thymol, D-carvone, methyl salicylate and L-menthol as a feed additive for chickens for fattening, chickens reared for laying and minor poultry species reared to the point of lay (holder of authorisation Biomin GmbH) OJ L 221 10.07.2020 p. 87.
4 FEED dossier reference: FAD-2020-0070.
5 The full report is available on the EU science HUB webpage: https://joint-research-centre.ec.europa.eu/system/files/2021-07/finrep_fad-2020-0070-biomin-dc-p.pdf
6 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.
Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019b).

3. Assessment

The additive, hereinafter referred as Biomin® DC-P, is a preparation of carvacrol, thymol, $\alpha$-carvone, methyl salicylate and $\lambda$-menthol intended to be used as a zootechnical feed additive (functional group: other zootechnical additives) for all poultry species. The additive is currently authorised for use in feed for chickens for fattening, chickens reared for laying and minor poultry species reared to the point of lay.

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive is a blend of five individual compounds (carvacrol, thymol, $\alpha$-carvone, methyl salicylate and $\lambda$-menthol) encapsulated with a hydrogenated vegetable oil. Amorphous silica is added as a carrier. The typical additive composition as it is specified in the authorisation \(^{8}\) is shown in Table 1.

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

The information relating to the characterisation of the additive and its manufacturing process has been assessed by the FEEDAP Panel in a previous opinion (EFSA FEEDAP Panel, 2019a) and is considered to apply to the present assessment.

3.1.2. Conditions of use

The additive is intended to be used in feed for all poultry species at a minimum recommended level of 65 mg/kg complete feed and a maximum recommended level of 105 mg/kg complete feed. The additive is intended to be incorporated directly to feed or via premixture.

3.2. Safety

The safety of the product for the user and environment was evaluated in the context of the previous opinion (EFSA FEEDAP Panel, 2019a). The FEEDAP Panel concluded that the use of the additive under the conditions of use proposed would raise no concerns for the environment. Concerning the safety for the users, the FEEDAP Panel considered that exposure of Biomin® DC-P by inhalation is unlikely. In the absence of data, the Panel could not conclude on the effects on skin and eyes.

The applicant did not provide any new data in the current application and the FEEDAP Panel is not aware of new information that would lead to reconsider the previous conclusions on the safety for the

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\(^{7}\) COMMISSION IMPLEMENTING REGULATION (EU) 2020/996 of 9 July 2020 concerning the authorisation of the preparation of carvacrol, thymol, $\alpha$-carvone, methyl salicylate and $\lambda$-menthol as a feed additive for chickens for fattening, chickens reared for laying and minor poultry species reared for laying (holder of authorisation Biomin GmbH) OJ L 221 10.07.2020 p. 87.
environment and users. In addition, the proposed extension of use to the new species/categories would not introduce risks not already considered in the previous assessment. Therefore, the FEEDAP Panel considers that the conclusions of the previous opinion regarding the safety for the environment and the users still apply to the present assessment.

### 3.2.1. Safety for the target species

In a previous opinion, the Panel evaluated a tolerance study in chickens for fattening (EFSA FEEDAP Panel, 2019a). Based on that study, the Panel concluded that the additive is safe for chickens for fattening at the maximum recommended level of 105 mg/kg complete feed; this conclusion was extended to chickens reared for laying and extrapolated to minor poultry species to the point of lay. The Panel considers that the conclusions can also be extrapolated to other physiologically related poultry species, namely turkeys.

The applicant submitted a new tolerance study in laying hens, including data on the deposition of the active compounds in the eggs, to support the safety for the target species.

#### 3.2.1.1. Tolerance study in laying hens

A total of 600 20-week-old hens (ISA Brown) were distributed in 60 cages of 10 hens each and allocated to four dietary treatments (24 replicates (cages) for control diet and 12 replicates per treatment). A basal diet based on maize and soyabean meal was either not supplemented (control) or supplemented with Biomin® DC-P to provide 105 (1× maximum recommended level), 525 (5×) and 1,050 (10×) mg/kg feed. The 5× and 10× doses could not be confirmed analytically, reaching 68% and 50% of the intended additive concentration in the final feed, respectively. Water and feed (in mash form) were offered ad libitum for 56 days. Mortality and general health were monitored throughout the study. Individual body weight was recorded at the beginning and at the end of the trial. Feed intake, laying performance and egg quality parameters were recorded at days 28 and 56. At the end of the study, eggs were collected for the analysis of the active substances to evaluate possible residues (see Section 3.2.2.2) and blood samples from 12 hens per dietary treatment were taken and the animals killed. Haematological and biochemical parameters were analysed, and the weight of liver, kidneys, lungs and heart, as well as the length of jejunum and ileum were recorded.

The maximum intended overdose level applied in the study, which could not be demonstrated analytically, was of 10× the maximum recommended level. Therefore, the battery of observations included in the tolerance study provided showed limitations that prevent the Panel to perform a complete assessment of the safety of the additive for laying hens: several relevant blood parameters were not measured (prothrombin time, fibrinogen, magnesium, cholesterol, acute phase protein, amylase and creatine kinase) and a complete necropsy was not performed at the end of the study. Therefore, in the absence of an adequate tolerance study, the FEEDAP Panel cannot conclude on the safety of the additive for laying hens.

#### 3.2.1.2. Conclusions on the safety for the target species

The FEEDAP Panel concludes that the additive Biomin® DC-P is safe for poultry species for fattening or reared for laying/breeding. In the absence of adequate data, the FEEDAP Panel cannot conclude on the safety for laying/breeding birds.

### 3.2.2. Safety for the consumer

#### 3.2.2.1. Toxicological studies

The FEEDAP Panel assessed in its previous opinion on Biomin® DC-P genotoxicity studies (bacterial reverse mutation test and a mammalian chromosome aberration test) and a sub-chronic oral toxicity study (90-day toxicity study) performed with a concentrated preparation of the active compounds of Biomin® DC-C, which includes all the active compounds of Biomin® DC-P at a concentration ca. 3–4

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9 Technical dossier/Section III/Annex 01.
10 Technical dossier/Section III/Annex 03.
11 Technical dossier/Section III/Annex 01: Haematology parameters analysed were total erythrocyte count, total leucocyte count, haemoglobin concentration, haematocrit, mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count and differential leucocyte count. Blood chemistry consisted of analysis of concentrations of glucose, urea, total protein, albumin, albumin/globulin ratio, creatinine, total bilirubin, alkaline phosphate activity (ALP), alanine aminotransferase activity (ALT), lactate dehydrogenase activity (LDH), aspartate aminotransferase activity (AST), gamma glutamyl transferase (GGT), calcium, phosphorus, chloride, sodium and potassium.
times higher than in the additive plus linalool (EFSA FEEDAP Panel, 2019a,c). Based on the
genotoxicity studies and considering that all the identified components of the additive (α-carvone, carvacrol, thymol, methyl salicylate and l-menthol) were already assessed and authorised for use in food and feed as flavouring compounds, the Panel concluded that the additive under assessment was not genotoxic. The 90-day study was not further considered as it did not comply with the requirements of the relevant Organisation for Economic Co-operation and Development (OECD) Testing Guideline (TG).

In the current dossier, to support the lack of data for the assessment of the clastogenic and aneugenic potential of the additive, the applicant submitted a new in vivo mammalian micronucleus test in accordance with OECD TG 474 and following Good Laboratory Practices (GLP) performed in peripheral blood reticulocytes of NMRI mice. The Panel noted that the test item used was the same concentrated mixture of the active compounds from Biomin® DC-C used in the abovementioned toxicological tests. Based on the results of a dose-finding test showing mild toxicity signs at 2,000 mg/kg body weight (bw), animals were treated twice by oral gavage, 2 h apart, at 500, 1,000 and 2,000 mg/kg bw and sacrificed 44 and 68 h after last administration. Flow cytometric analysis showed a dose-related, statistically significant increase in the percentage of immature reticulocytes (also called polychromatic erythrocytes, PCE) induced by the mixture of active compounds of Biomin® DC-C, confirming the exposure of the target tissue. No increase in the frequency of micronuclei was observed in treated animals compared to the negative control group. Based on the results of this study, the Panel concluded that the test item did not induce structural and numerical chromosome aberrations in vivo when tested up to 2,000 mg/kg bw.

The FEEDAP Panel considered that the existing genotoxicity studies performed with a concentrated preparation of the active compounds of Biomin® DC-C are relevant for the assessment of the additive currently under assessment and, therefore, concluded that Biomin® DC-P is not genotoxic.

### 3.2.2.2. Residue study

In the previous opinion, the Panel concluded that the use of Biomin® DC-P in feed for chickens for fattening and reared for laying and minor avian species under the proposed conditions of use is safe for the consumers (EFSA FEEDAP Panel, 2019a). The applicant provided new data in laying hens based on the analyses of the active compounds’ residues (l-menthol, α-carvone, methyl salicylate, thymol and carvacrol) in egg samples taken from the tolerance study (see Section 3.2.1.1) performed with laying hens.12

Six eggs from each of the twelve replicates (cages) were collected in 3 consecutive days (2 eggs/cage/day from control, 105 and 525 mg/kg groups). Yolks and albumens were separated and samples coming from the same cage were pooled to form 12 independent yolk and albumen samples from each treatment. The marker residues (l-menthol, α-carvone, methyl salicylate, thymol and carvacrol) were analysed by gas chromatography-tandem mass spectrometry (GC-MS/MS) with a limit of quantification (LOQ) of 20 μg/kg for each substance. Data showed non-normal distribution (Shapiro-Wilk test). Therefore, statistical comparison between treatments were done by Wilcoxon Rank-Sum-Test. Significance level was set at 0.05.

Regardless of the use level tested, no residue above the LOQ (20 μg/kg) was found for any of the analytes in the yolk samples, and in the albumen for α-carvone and thymol. In albumen, l-menthol and carvacrol were only detected in 2 out of the 12 replicates in the group supplemented with the maximum recommended level of Biomin® DC-P (105 mg/kg complete feed), with a maximum content of 23.5 and 23.9 μg/kg, respectively. In contrast, methyl salicylate was detected in all samples of the 105 mg/kg group, with an average content of 30.0 (range from 23.6 to 43.9) μg/kg.

The FEEDAP Panel has considered the residues in eggs from the study above and of muscle and skin + fat from the previous opinion on the same additive (EFSA FEEDAP Panel, 2019a) to assess the chronic consumer exposure for l-menthol and α-carvone for which an acceptable daily intake (ADI) is established: 4 mg l-menthol/kg bw (WHO, 1999) and 60 mg α-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 was used as detailed in the Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a). Table 2 shows the input data used for the calculation by the Panel, based on the highest analysed value and/or the LOQ for l-menthol and α-carvone in eggs, muscle and skin + fat.

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12 Technical dossier/Section III/Annex 08.
The results of the chronic dietary exposure to menthol and carvone are summarized in Table 3, while the detailed results are given in Appendix A.

As it can be seen in Table 3, for all population groups, the chronic exposure to L-menthol and D-carvone was at least 4–5 orders of magnitude lower than the corresponding ADI. The Panel notes that no data on residues of L-menthol and D-carvone in liver were available. However, considering the low contribution to the ADI of the residues in other tissues and products, it is not expected that any potential residue from liver would significantly increase the consumer exposure. For the rest of the components of the additive, an ADI is not available and, thus, the procedure used for L-menthol and D-carvone cannot be applied. However, the FEEDAP Panel notes that those components (carvacrol, thymol and methyl salicylate) – and the rest of the components of the additive: D-carvone and L-menthol – have been assessed and considered safe for use as flavouring compounds, and they are currently authorised for food and feed uses.

**Table 2:** Input data on concentration (mg/kg) of L-menthol and D-carvone in eggs of hens, breast meat and skin+fat of chickens for fattening used for the consumer exposure assessment

| Compound  | Eggs(1) | Meat(2) | Skin + Fat |
|-----------|---------|---------|------------|
| L-Menthol | 0.022   | 0.030   | 0.050      |
| D-Carvone | 0.020   | 0.127   | 0.315      |

(1): Calculated by default as 66% albumen and 34% yolk (w/w).
(2): Calculated by default as 90% muscle and 10% skin + fat (w/w).

The results of the chronic dietary exposure to menthol and carvone are summarized in Table 3, while the detailed results are given in Appendix A.

**Table 3:** Chronic dietary exposure of consumers to L-menthol and D-carvone total residues based on residue data in chicken tissues and hen eggs - Summary statistics across European dietary surveys

| Active substance | Population class | Maximum HRP(1) (mg/kg bw per day) | % ADI |
|------------------|------------------|----------------------------------|-------|
| L-Menthol Infants | 0.00023          | 0.006               |       |
| Toddlers         | 0.00027          | 0.007               |       |
| Other children   | 0.00023          | 0.006               |       |
| Adolescents      | 0.00014          | 0.004               |       |
| Adults           | 0.00008          | 0.002               |       |
| Elderly          | 0.00008          | 0.002               |       |
| Very elderly     | 0.00007          | 0.002               |       |
| D-Carvone Infants | 0.00086          | 0.001               |       |
| Toddlers         | 0.00100          | 0.002               |       |
| Other children   | 0.00082          | 0.001               |       |
| Adolescents      | 0.00058          | <0.001              |       |
| Adults           | 0.00030          | <0.001              |       |
| Elderly          | 0.00027          | <0.001              |       |
| Very elderly     | 0.00026          | <0.001              |       |

(1): Highest Reliable Percentile = the highest percentile that is considered statistically robust for combinations of dietary survey, age class and possible raw primary commodity, considering that a minimum of 5, 12, 30 and 61 observations are respectively required to derive 50th, 75th, 90th and 95th percentile estimates.

As it can be seen in Table 3, for all population groups, the chronic exposure to L-menthol and D-carvone was at least 4–5 orders of magnitude lower than the corresponding ADI. The Panel notes that no data on residues of L-menthol and D-carvone in liver were available. However, considering the low contribution to the ADI of the residues in other tissues and products, it is not expected that any potential residue from liver would significantly increase the consumer exposure. For the rest of the components of the additive, an ADI is not available and, thus, the procedure used for L-menthol and D-carvone cannot be applied. However, the FEEDAP Panel notes that those components (carvacrol, thymol and methyl salicylate) – and the rest of the components of the additive: D-carvone and L-menthol – have been assessed and considered safe for use as flavouring compounds, and they are currently authorised for food and feed uses.

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13 Samples taken from the hens and chickens used in the tolerance studies from the current and previous Biomin DC-P opinion (EFSA FEEDAP Panel, 2019a).
14 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 of 03 L 267, 2.10.2012, p. 1.
15 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
3.2.2.3. Conclusions on safety for the consumer

The FEEDAP Panel concludes that the use of Biomin® DC-P in feed for all poultry species under the proposed conditions of use is considered safe for consumers.

3.3. Efficacy

The efficacy of Biomin® DC-P for chickens for fattening was evaluated by the Panel in a previous opinion (EFSA FEEDAP Panel, 2019a). The Panel concluded that Biomin® DC-P has 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 was extended to chickens reared for laying and extrapolated to minor poultry species reared up to the point of lay at the minimum concentration level. The Panel considers that the conclusions can also be extrapolated to other physiologically related poultry species, namely turkeys for fattening/reared for breeding.

3.3.1. Efficacy for laying hens

A total of 3 long-term efficacy trials with laying hens were submitted. However, one of the studies was not further considered as no information on the initial and final body weight of the hens was provided. The other two studies shared a common design (Table 4) and the main results are reported in Table 5.

Table 4: Trial design and use level of the efficacy trials performed in laying hens

| Trial | Total n° of animals (animals × replicate) Replicates × treatment | Breed start age (duration) | Composition feed (form) | Groups (mg/kg feed) |
|-------|-----------------------------------------------------------------|---------------------------|-------------------------|---------------------|
|       | Replicates                                                      |                           |                         | Intended | Analysed |
| 16    | 600 (10)                                                        | Tetra SL 22 weeks (84 days)| Maize, soyabean meal and wheat (mash) | 0 65  | – 52   |
| 17    | 600 (10)                                                        | Tetra SL 22 weeks (84 days)| Maize, soyabean meal and wheat (mash) | 0 65  | – 52   |

In both trials, 22-week-old laying hens were distributed in cages and allocated to two different treatments. The animals were fed either a non-supplemented diet (control) or a diet containing Biomin® DC-P at the minimum recommended use level of 65 mg/kg feed (52 mg/kg analytically confirmed for both studies) for 84 days. The health and mortality were monitored throughout the study and the initial and final body weight, feed intake and egg weight were recorded. Average daily feed intake, egg mass per hen and day and the feed to egg mass ratio were calculated. The data were analysed with t-test, using the cage as experimental unit. Significance level was set at 0.05.

Table 5: Effects of Biomin® DC-P on the performance of laying hens

| Trial | Groups (mg/kg feed) | Initial body weight (g) | Final body weight (g) | Average daily feed intake (g) | Laying rate (%) | Daily egg mass per hen (g/hen per day) | Egg weight (g) | Feed to egg mass ratio |
|-------|---------------------|-------------------------|-----------------------|-----------------------------|----------------|-----------------------------------------|---------------|------------------------|
| 1     | 0                   | 1,784                   | 2,038                 | 117a                        | 93.9b          | 55.2b                                   | 58.8b         | 2.12b                  |
|       | 65                  | 1,782                   | 2,037                 | 116b                        | 94.8a          | 55.8a                                   | 58.9a         | 2.08b                  |
| 2     | 0                   | 1,790                   | 2,040                 | 122a                        | 93.3b          | 54.8b                                   | 58.6b         | 2.23b                  |
|       | 65                  | 1,792                   | 2,027                 | 121b                        | 93.8b          | 55.2b                                   | 58.9a         | 2.18b                  |

a,b: Mean values within a trial and within a column with a different superscript are significantly different p < 0.05.

No hens died during the experiment. The inclusion of the minimum use level of Biomin® DC-P in both trials resulted in significantly higher laying rate and average egg mass, which, together with a reduction of the feed intake, was translated into an improved feed to egg mass ratio.

16 Technical dossier/Section IV/Annex 06.
17 Technical dossier/Section IV/Annex 09.
3.3.2. Conclusions on efficacy

Biomin® DC-P has the potential to be efficacious in poultry species for fattening or reared for laying/breeding when incorporated into feed at a minimum concentration of 65 mg/kg complete feed. Positive effects of the supplementation of the hen’s diets with Biomin® DC-P at the minimum recommended use level on the performance of the hens were observed in two studies. In the absence of a third positive study, the Panel cannot conclude on the efficacy for laying hens or for other poultry species for laying/breeding.

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 Regulation¹⁸ and Good Manufacturing Practice.

4. Conclusions

The FEEDAP Panel concludes that the additive Biomin® DC-P is safe for poultry species for fattening or reared for laying/breeding, but in the absence of adequate data, cannot conclude on the safety for laying/breeding birds.

The use of Biomin® DC-P in feed for all poultry species under the proposed conditions of use is considered safe for consumers of animal products and for the environment.

Biomin® DC-P has the potential to be efficacious in poultry species for fattening or reared for laying/breeding when incorporated into feed at a minimum concentration of 65 mg/kg complete feed. In the absence of sufficient data, the Panel cannot conclude on the efficacy for laying hens or for other poultry species for laying/breeding.

5. Documentation provided to EFSA/Chronology

| Date       | Event                                                                 |
|------------|------------------------------------------------------------------------|
| 30/09/2020 | Dossier received by EFSA. FAD-2020-0070 Biomin® DC-P for all poultry species. Submitted by Biomin GmbH. |
| 17/12/2020 | Reception mandate from the European Commission                          |
| 08/03/2021 | Application validated by EFSA – Start of the scientific assessment      |
| 01/06/2021 | 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 of the additive/safety for the target species/user safety/efficacy |
| 09/06/2021 | Comments received from Member States                                   |
| 14/06/2021 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives |
| 12/11/2021 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 18/01/2022 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: toxicological studies |
| 22/03/2022 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 29/06/2022 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment |

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Abbreviations

ADFI average daily feed intake  
ADI acceptable daily intake  
ALP alkaline phosphate activity  
ALT alanine aminotransferase activity  
AST aspartate aminotransferase activity  
BW body weight  
CAS Chemical Abstracts Service  
DM dry matter  
EC European Commission  
EURL European Union Reference Laboratory  
FEEDAP EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed  
GC-MS/MS gas chromatography-mass spectrometry/mass spectrometry  
GGT gamma glutamyl transferase  
GLP good laboratory practices
| Abbreviation | Description |
|--------------|-------------|
| HRP          | highest reliable percentile |
| LDH          | lactate dehydrogenase activity |
| LOQ          | limit of quantification |
| MCH          | mean corpuscular haemoglobin |
| MCHC         | mean corpuscular haemoglobin concentration |
| MCV          | mean corpuscular volume |
| OECD         | Organisation for Economic Co-operation and Development |
| PCE          | polychromatic erythrocytes |
| TG           | Test guidelines |
| WHO          | World Health Organization |
### Table A.1: Chronic dietary exposure per population class, country and survey (mg/kg bw per day) of consumers to l-menthol based on residue data

| Population class | Survey's country | Number of subjects | HRP value  | HRP description |
|------------------|------------------|--------------------|------------|-----------------|
| Infants          | Bulgaria         | 523                | 0.0020844786 | 95th            |
| Infants          | Germany          | 142                | 0.0003821946 | 95th            |
| Infants          | Denmark          | 799                | 0.0004675338 | 95th            |
| Infants          | Finland          | 427                | 0.0006973620 | 95th            |
| Infants          | Italy            | 9                  | 0.0000000000 | 50th            |
| Infants          | United Kingdom(1)| 1,251              | 0.0009328590 | 95th            |
| Toddlers         | Belgium          | 36                 | 0.0010382479 | 90th            |
| Toddlers         | Bulgaria         | 428                | 0.0024357064 | 95th            |
| Toddlers         | Germany          | 348                | 0.0006838511 | 95th            |
| Toddlers         | Denmark          | 917                | 0.0005350626 | 95th            |
| Toddlers         | Spain            | 17                 | 0.0011418147 | 75th            |
| Toddlers         | Finland          | 500                | 0.0011146343 | 95th            |
| Toddlers         | Italy            | 36                 | 0.0009356891 | 90th            |
| Toddlers         | United Kingdom   | 1,314              | 0.0010838867 | 95th            |
| Toddlers         | United Kingdom   | 185                | 0.0010899219 | 95th            |
| Other children   | Austria          | 128                | 0.0009212682 | 95th            |
| Other children   | Belgium          | 625                | 0.0011893324 | 95th            |
| Other children   | Bulgaria         | 433                | 0.0020160104 | 95th            |
| Other children   | Germany          | 293                | 0.0007059453 | 95th            |
| Other children   | Germany          | 835                | 0.0007247079 | 95th            |
| Other children   | Denmark          | 298                | 0.0005945892 | 95th            |
| Other children   | Spain            | 399                | 0.0013405985 | 95th            |
| Other children   | Spain            | 156                | 0.0019013278 | 95th            |
| Other children   | Finland          | 750                | 0.0009430660 | 95th            |
| Other children   | France           | 482                | 0.0008461731 | 95th            |
| Other children   | Greece           | 838                | 0.0009499360 | 95th            |
| Other children   | Italy            | 193                | 0.0010039080 | 95th            |
| Other children   | Latvia           | 187                | 0.0010692526 | 95th            |
| Other children   | Netherlands      | 957                | 0.0008259735 | 95th            |
| Other children   | Netherlands      | 447                | 0.0010531076 | 95th            |
| Other children   | Sweden           | 1,473              | 0.0007864807 | 95th            |
| Other children   | Czechia          | 389                | 0.0019119224 | 95th            |
| Other children   | United Kingdom   | 651                | 0.0009542122 | 95th            |
| Adolescents      | Austria          | 237                | 0.0006222662 | 95th            |
| Adolescents      | Belgium          | 576                | 0.0005656936 | 95th            |
| Adolescents      | Cyprus           | 303                | 0.0005994523 | 95th            |
| Adolescents      | Germany          | 393                | 0.0005472404 | 95th            |
| Adolescents      | Germany          | 1,011              | 0.0004424992 | 95th            |
| Adolescents      | Denmark          | 377                | 0.0004544047 | 95th            |
| Adolescents      | Spain            | 651                | 0.0007764022 | 95th            |
| Adolescents      | Spain            | 209                | 0.0010379790 | 95th            |
| Adolescents      | Spain            | 86                 | 0.0008458131 | 95th            |
| Adolescents      | Finland          | 306                | 0.0005733529 | 95th            |
| Adolescents      | France           | 973                | 0.0005170738 | 95th            |
| Population class | Survey’s country | Number of subjects | HRP value   | HRP description |
|------------------|------------------|--------------------|-------------|-----------------|
| Adolescents      | Italy            | 247                | 0.0004526719 | 95th            |
| Adolescents      | Latvia           | 453                | 0.0006617021 | 95th            |
| Adolescents      | Netherlands      | 1,142              | 0.0007808804 | 95th            |
| Adolescents      | Sweden           | 1,018              | 0.0005901800 | 95th            |
| Adolescents      | Czechia          | 298                | 0.0014051628 | 95th            |
| Adolescents      | United Kingdom   | 666                | 0.0007197633 | 95th            |
| Adults           | Austria          | 308                | 0.0007067125 | 95th            |
| Adults           | Belgium          | 1,292              | 0.0005383391 | 95th            |
| Adults           | Germany          | 10,419             | 0.0004346363 | 95th            |
| Adults           | Denmark          | 1,739              | 0.0003019610 | 95th            |
| Adults           | Spain            | 981                | 0.0007150283 | 95th            |
| Adults           | Spain            | 410                | 0.0007197629 | 95th            |
| Adults           | Finland          | 1,295              | 0.0005643747 | 95th            |
| Adults           | France           | 2,276              | 0.0004311251 | 95th            |
| Adults           | Hungary          | 1,074              | 0.0006386299 | 95th            |
| Adults           | Ireland          | 1,274              | 0.0007027243 | 95th            |
| Adults           | Italy            | 2,313              | 0.0003809462 | 95th            |
| Adults           | Latvia           | 1,271              | 0.0005633623 | 95th            |
| Adults           | Netherlands      | 2,055              | 0.0006400050 | 95th            |
| Adults           | Romania          | 1,254              | 0.0007232285 | 95th            |
| Adults           | Sweden           | 1,430              | 0.0006099221 | 95th            |
| Adults           | Czechia          | 1,666              | 0.0006881914 | 95th            |
| Adults           | United Kingdom   | 1,265              | 0.0005216291 | 95th            |
| Elderly          | Austria          | 67                 | 0.0005605616 | 95th            |
| Elderly          | Belgium          | 511                | 0.0004362619 | 95th            |
| Elderly          | Germany          | 2,006              | 0.000342854  | 95th            |
| Elderly          | Denmark          | 274                | 0.0002462935 | 95th            |
| Elderly          | Finland          | 413                | 0.000464047  | 95th            |
| Elderly          | France           | 264                | 0.0003588130 | 95th            |
| Elderly          | Hungary          | 206                | 0.0004832871 | 95th            |
| Elderly          | Ireland          | 149                | 0.0005730619 | 95th            |
| Elderly          | Italy            | 289                | 0.0003685168 | 95th            |
| Elderly          | Netherlands      | 173                | 0.0004911107 | 95th            |
| Elderly          | Netherlands      | 289                | 0.0004152578 | 95th            |
| Elderly          | Romania          | 83                 | 0.0006453365 | 95th            |
| Elderly          | Sweden           | 295                | 0.0005382478 | 95th            |
| Elderly          | United Kingdom   | 166                | 0.0004162738 | 95th            |
| Very elderly     | Austria          | 25                 | 0.0001508615 | 75th            |
| Very elderly     | Belgium          | 704                | 0.0005028246 | 95th            |
| Very elderly     | Germany          | 490                | 0.0003623556 | 95th            |
| Very elderly     | Denmark          | 12                 | 0.0001281694 | 75th            |
| Very elderly     | France           | 84                 | 0.0004071186 | 95th            |
| Very elderly     | Hungary          | 80                 | 0.0004687121 | 95th            |
| Very elderly     | Ireland          | 77                 | 0.0005805875 | 95th            |
| Very elderly     | Italy            | 228                | 0.0003751192 | 95th            |
| Very elderly     | Netherlands      | 450                | 0.0004121388 | 95th            |
| Very elderly     | Romania          | 45                 | 0.0006530012 | 90th            |
| Very elderly     | Sweden           | 72                 | 0.0004376318 | 95th            |
| Very elderly     | United Kingdom   | 139                | 0.0003284572 | 95th            |

(1): Dietary data from the UK were included in FACE when the UK was a member of the European Union.
Table A.2: Chronic dietary exposure per population class, country and survey (mg/kg bw per day) of consumers to d-carvone based on residue data

| Population class | Survey’s country | Number of subjects | HRP value  | HRP description |
|------------------|------------------|--------------------|------------|-----------------|
| Infants          | Bulgaria         | 523                | 0.0007253282 | 95th            |
| Infants          | Germany          | 142                | 0.0001307449 | 95th            |
| Infants          | Denmark          | 799                | 0.0001604675 | 95th            |
| Infants          | Finland          | 427                | 0.0002349959 | 95th            |
| Infants          | Italy            | 9                  | 0.0000000000 | 50th            |
| Infants          | United Kingdom(1)| 1,251              | 0.0003239569 | 95th            |
| Toddlers         | Belgium          | 36                 | 0.0003553390 | 90th            |
| Toddlers         | Bulgaria         | 428                | 0.0008399293 | 95th            |
| Toddlers         | Germany          | 348                | 0.0002493247 | 95th            |
| Toddlers         | Denmark          | 917                | 0.0001903070 | 95th            |
| Toddlers         | Spain            | 17                 | 0.0004606023 | 75th            |
| Toddlers         | Finland          | 500                | 0.0003837877 | 95th            |
| Toddlers         | Italy            | 36                 | 0.0003249307 | 90th            |
| Toddlers         | Netherlands      | 322                | 0.0003804529 | 95th            |
| Toddlers         | United Kingdom   | 1,314              | 0.0003762606 | 95th            |
| Toddlers         | United Kingdom   | 185                | 0.0003754682 | 95th            |
| Other children   | Austria          | 128                | 0.0003276236 | 95th            |
| Other children   | Belgium          | 625                | 0.0004028917 | 95th            |
| Other children   | Bulgaria         | 433                | 0.0006898541 | 95th            |
| Other children   | Germany          | 293                | 0.0002465699 | 95th            |
| Other children   | Germany          | 835                | 0.0002603152 | 95th            |
| Other children   | Denmark          | 298                | 0.0002110713 | 95th            |
| Other children   | Spain            | 399                | 0.0004601920 | 95th            |
| Other children   | Spain            | 156                | 0.0006699533 | 95th            |
| Other children   | Finland          | 750                | 0.0003274705 | 95th            |
| Other children   | France           | 482                | 0.0003033864 | 95th            |
| Other children   | Greece           | 838                | 0.000318328 | 95th            |
| Other children   | Italy            | 193                | 0.0003577633 | 95th            |
| Other children   | Latvia           | 187                | 0.0003632254 | 95th            |
| Other children   | Netherlands      | 957                | 0.0002942890 | 95th            |
| Other children   | Netherlands      | 447                | 0.0003550567 | 95th            |
| Other children   | Sweden           | 1,473              | 0.0002712973 | 95th            |
| Other children   | Czechia          | 389                | 0.0006511642 | 95th            |
| Other children   | United Kingdom   | 651                | 0.0003343055 | 95th            |
| Adolescents      | Austria          | 237                | 0.0002194632 | 95th            |
| Adolescents      | Belgium          | 576                | 0.0001972614 | 95th            |
| Adolescents      | Cyprus           | 303                | 0.0002041877 | 95th            |
| Adolescents      | Germany          | 393                | 0.0001981068 | 95th            |
| Adolescents      | Germany          | 1,011              | 0.0001540753 | 95th            |
| Adolescents      | Denmark          | 377                | 0.0001565004 | 95th            |
| Adolescents      | Spain            | 651                | 0.0002712899 | 95th            |
| Adolescents      | Spain            | 209                | 0.0003773982 | 95th            |
| Adolescents      | Spain            | 86                 | 0.0002927712 | 95th            |
| Adolescents      | Finland          | 306                | 0.0001969166 | 95th            |
| Adolescents      | France           | 973                | 0.0001898801 | 95th            |
| Adolescents      | Italy            | 247                | 0.0001593830 | 95th            |
| Adolescents      | Latvia           | 453                | 0.0002324103 | 95th            |
| Adolescents      | Netherlands      | 1,142              | 0.0002677546 | 95th            |
| Population class | Survey’s country | Number of subjects | HRP value   | HRP description |
|------------------|------------------|--------------------|-------------|-----------------|
| Adolescents      | Sweden           | 1,018              | 0.0002021040 | 95th            |
| Adolescents      | Czechia          | 298                | 0.0004859321 | 95th            |
| Adolescents      | United Kingdom   | 666                | 0.0002473109 | 95th            |
| Adults           | Austria          | 308                | 0.0002385432 | 95th            |
| Adults           | Belgium          | 1,292              | 0.0001843464 | 95th            |
| Adults           | Germany          | 10,419             | 0.0001506788 | 95th            |
| Adults           | Denmark          | 1,739              | 0.0001055213 | 95th            |
| Adults           | Spain            | 981                | 0.0002463967 | 95th            |
| Adults           | Spain            | 410                | 0.0002471020 | 95th            |
| Adults           | Finland          | 1,295              | 0.0001944203 | 95th            |
| Adults           | France           | 2,276              | 0.0001538804 | 95th            |
| Adults           | Hungary          | 1,074              | 0.0002228277 | 95th            |
| Adults           | Ireland          | 1,274              | 0.0002409213 | 95th            |
| Adults           | Italy            | 2,313              | 0.0001333074 | 95th            |
| Adults           | Latvia           | 1,271              | 0.0002025854 | 95th            |
| Adults           | Netherlands      | 2,055              | 0.0002194633 | 95th            |
| Adults           | Romania          | 1,254              | 0.0002526306 | 95th            |
| Adults           | Sweden           | 1,430              | 0.0002167512 | 95th            |
| Adults           | Czechia          | 1,666              | 0.0002341881 | 95th            |
| Adults           | United Kingdom   | 1,265              | 0.0001783861 | 95th            |
| Elderly          | Austria          | 67                 | 0.0001889250 | 95th            |
| Elderly          | Belgium          | 511                | 0.0001508964 | 95th            |
| Elderly          | Germany          | 2,006              | 0.0001173208 | 95th            |
| Elderly          | Denmark          | 274                | 0.0000866998 | 95th            |
| Elderly          | Finland          | 413                | 0.0001617826 | 95th            |
| Elderly          | France           | 264                | 0.0001300537 | 95th            |
| Elderly          | Hungary          | 206                | 0.0001904336 | 95th            |
| Elderly          | Ireland          | 149                | 0.0001961810 | 95th            |
| Elderly          | Italy            | 289                | 0.0001286382 | 95th            |
| Elderly          | Netherlands      | 173                | 0.0001676834 | 95th            |
| Elderly          | Nederland        | 289                | 0.0001412697 | 95th            |
| Elderly          | Romania          | 83                 | 0.0002295353 | 95th            |
| Elderly          | Sweden           | 295                | 0.0001824187 | 95th            |
| Elderly          | United Kingdom   | 166                | 0.0001432642 | 95th            |
| Very elderly     | Austria          | 25                 | 0.0000536653 | 75th            |
| Very elderly     | Belgium          | 704                | 0.0001762514 | 95th            |
| Very elderly     | Germany          | 490                | 0.0001251077 | 95th            |
| Very elderly     | Denmark          | 12                 | 0.0000478329 | 75th            |
| Very elderly     | France           | 84                 | 0.0001443068 | 95th            |
| Very elderly     | Hungary          | 80                 | 0.0001667012 | 95th            |
| Very elderly     | Ireland          | 77                 | 0.0001986284 | 95th            |
| Very elderly     | Italy            | 228                | 0.0001264070 | 95th            |
| Very elderly     | Netherlands      | 450                | 0.0001422361 | 95th            |
| Very elderly     | Romania          | 45                 | 0.0002200472 | 90th            |
| Very elderly     | Sweden           | 72                 | 0.0001549586 | 95th            |
| Very elderly     | United Kingdom   | 139                | 0.0001141547 | 95th            |

(1): Dietary data from the UK were included in FACE when the UK was a member of the European Union.
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for carvacrol, thymol, d-carvone, methyl salicylate and L-menthol

In the current application an authorisation is sought under Article 4(1) for a currently authorised preparation of carvacrol, thymol, d-carvone, methyl salicylate and L-menthol (Biomin® DC-P) under the category/functional group (4d) "zootechnical additives"/"other zootechnical additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the feed additive for all poultry species. The feed additive is an off-white powder composed of a mixture of the following active substances: 12–16% (w/w) of carvacrol, 0.1–0.3% (w/w) of thymol, 0.3–0.6% (w/w) of d-carvone, 1.0–3.5% (w/w) of methyl salicylate and 3.0–5.5% (w/w) of L-menthol. In addition, Biomin® DC-P contains hydrogenated vegetable oil and silica as carriers.

The feed additive is intended to be incorporated through premixtures or directly into feedingstuffs at a recommended 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).

Based on the experimental evidence available the EURL recommends for the official control the GC-FID method for the quantification of carvacrol, thymol, d-carvone, methyl salicylate and L-menthol in the feed additive.

Additionally, the Applicant submitted a single-laboratory validated and further verified multianalyte method based on gas chromatography coupled to mass spectrometry (GC-MS) for the quantification of carvacrol, thymol, d-carvone, methyl salicylate and L-menthol in premixtures and feedingstuffs. The EURL considers the GC-MS method proposed by the Applicant fit-for-purpose for the quantification of the total content of each active substances, namely carvacrol, thymol, d-carvone, methyl salicylate and L-menthol, in premixtures and feedingstuffs at the content levels investigated in the frame of the validation and verification studies.

However, as the accurate determination of the Biomin® DC-P content added to premixtures and feedingstuffs is not achievable experimentally, the EURL cannot evaluate or recommend any method for official control to determine Biomin® DC-P in premixtures and feedingstuffs.

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