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Safety and efficacy of Biomin® DC-C as a zootechnical feed additive for weaned piglets

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Abstract

The additive (trade name Biomin® DC-C) is a blend of essential oils from oregano (*Origanum vulgare* L.) and from caraway seed (*Carum carvi* L.) and three individual compounds (carvacrol, methyl salicylate and L-menthol) encapsulated with a hydrogenated vegetable oil. The additive is intended for use in feed for weaned piglets at a minimum concentration of 75 mg/kg complete feed and a recommended maximum level of 125 mg/kg complete feed. A tolerance test in which piglets were exposed to feed containing up to an intended 1,250 mg additive/kg complete feed showed that additive is safe for piglets at the maximum recommended level with at least a sixfold margin of safety. The active components of the additive are not genotoxic and from the available residue study, based on the detection of five marker compounds, no measurable exposure of the consumers is foreseen; consequently, the use of the additive is considered safe for consumers of animal products. 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-C on skin and eyes. The use of the additive in animal production is not expected to pose a risk for the terrestrial or aquatic environments. Based on the results of three efficacy studies in which positive benefits were seen, the additive has a potential to improve the growth performance of weaned piglets at a minimum application rate of 75 mg/kg complete feed.

Keywords: zootechnical additive, other zootechnical additives, Biomin® DC-C, origanum oil, caraway oil, carvacrol, weaned piglets

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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-C, when used as a feed additive for weaned piglets (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 18 July 2017.

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-C, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

Biomin® DC-C has not been previously assessed in the European Union (EU).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of Biomin® DC-C as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[4] and the applicable EFSA guidance documents.

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-C as a zootechnical feed additive for weaned piglets. 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 Biomin® DC-C is in line with the principles laid down in Regulation (EC) No 429/2008 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-2017-0026.
[4] 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.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2017-0026_biomin.pdf

3. Assessment

Biomin® DC-C is a feed additive composed of a mixture of botanical extracts and individual compounds intended to be used as a zootechnical feed additive for weaned piglets. It has not been previously assessed in the EU.

3.1. Characterisation

3.1.1. Characterisation of the additive

Biomin® DC-C is a blend of an essential oil from oregano (*Origanum vulgare* L.), an essential oil from caraway seed (*Carum carvi* L.) and three individual compounds (carvacrol, methyl salicylate and L-menthol). 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.[6]

Table 1: Typical composition of Biomin® DC-C

Ingredient (CAS number)	Content (mg/g additive)
Oregano oil (8007-11-2)	60–80
Caraway oil (8000-42-8)	5–10
Carvacrol (499-75-2)	60–80
Methyl salicylate (119-36-8)	10–40
L-Menthol (2216-51-8)	30–55
Amorphous silica (68611-44-9)	Max 100
Hydrogenated vegetable oil	Max 700

CAS: Chemical Abstracts Service.

The applicant is responsible for the manufacture of the additive itself (full details provided including evidence of compliance with ISO standard 9001:2015 and Hazard Analysis and Critical Control Points (HACCP)), but raw materials are sourced from third party suppliers. In each case, raw materials are purchased to a specification defined by the applicant as indicated below.

Oregano oil. [redacted].

Caraway oil. [redacted].

Carvacrol and *methyl salicylate* [redacted].

L-menthol [redacted]

Silica and *vegetable oil*: the sources and specifications of pure silica [redacted] and hydrogenated vegetable oil [redacted] are described in the technical dossier.

[6] Technical Dossier/Section II.

[redacted]

Data on the content of the active components were shown for five batches of the additive (Table 2). Since it is not possible to assay directly for the essential oils, linalool was taken as a marker for the oregano oil and D-carvone as a marker for the caraway oil. The content of carvacrol, the most prevalent component of oregano oil, was also monitored, but could not be used as a marker as additional free carvacrol is added during manufacture.[14,15,16,17,18]

Table 2: Marker compounds of Biomin® DC-C: variation in the content of five standardised batches and typical content

Marker compounds/ Active substances	Content (mg/g additive)		Typical content (mg/g additive)
	Mean	Range	
Linalool[(a)]	6.4	5.6–7.7	1.8–16
D-carvone[(b)]		3.8–4.2	2.5–6.5
Carvacrol[(c)]	127.7	119.5–131.4	95–140
Methyl salicylate	25.8	23.1–30.4	10–40
L-Menthol	47.4	44.8–48.2	30–55

(a): Linalool is a marker compound for oregano oil.
(b): D-carvone is a marker compound for caraway oil.
(c): Carvacrol content is the sum of carvacrol in oregano oil and pure carvacrol.

Analysis of three batches of the additive for heavy metals (lead (Pb), cadmium (Cd) and mercury (Hg)) and arsenic (As) was provided. Data showed that Cd,[19] Hg[20] and As[21] content was below the respective limits of quantification (LOQ), which were 0.5, 0.01 and 0.5 mg/kg, respectively. Lead was detected in all three batches at a maximum concentration of 2 mg/kg additive and can be regarded as of no concern.[22]

Since a significant part of the additive is formed from materials of plant origin, a further set of three batches of the product was analysed for the presence of mycotoxins,[23] pesticide residues[24] and dioxins and dioxin-like PCBs.[25,26,27] All of the mycotoxins tested (deoxynivalenol, zearalenone, aflatoxins (B1, B2, G1, G2), ochratoxin A, fumonisin (B1, B2), HT-2 toxin and T-2 toxin) were below their respective limits of detection (LOD). Similarly, no pesticide residues could be detected. Dioxins were detected in all three batches but at concentration far below those which would raise concerns (0.06–0.07 ng WHO-PCDD/F-TEQ/kg); in each case the sum of dioxins and dioxin-like PCBs was at maximum 0.12 ng WHO-PCDD/F-PCB-TEQ/kg additive.

Another set of three batches was analysed for residual amounts of methanol used as a solvent (from manufacture of the individual components).[28] Methanol was found at a mean concentration of 120 μg/g additive (range 81–198 μg/g additive), substantially below the maximum limit of 3,000 μg/g product set in the Guidance document of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH, 2011).

Limits are set for microbial contamination, which is specified not to exceed for total coliforms 30 colonies/g additive, and for yeasts and filamentous fungi 100 colonies/g additive. In addition, no

[14] Technical Dossier/Section II/Annex_02.
[15] Technical Dossier/Section II/Annex_03.
[16] Technical Dossier/Section II/Annex_04.
[17] Technical Dossier/Section II/Annex_05.
[18] Technical Dossier/Section II/Annex_06.
[19] Technical Dossier/Section II/Annex_08.
[20] Technical Dossier/Section II/Annex_09.
[21] Technical Dossier/Section II/Annex_07.
[22] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.
[23] The LODs were (in μg/kg): 20 for deoxynivalenol, 4 for zearalenone, 0.2 for aflatoxins (B1, B2, G1, G2) and ochratoxin A, 20 for fumonisin (B1 and B2) and 2 for HT-2 toxin and T-2 toxin.
[24] The battery of pesticides analysed included: aldrin, dieldrin, chlordane, DDT, endosulfan, endrin, heptachlor, hexachlorobenzene, hexachlorohexane, hexachlorohexane-α, hexachlorohexane-β and hexachlorohexane-γ.
[25] Technical Dossier/Section II/Annex_14.
[26] Technical Dossier/Section II/Annex_15.
[27] Technical Dossier/Section II/Annex_16.
[28] Technical Dossier/Section II/Annex_20.

Escherichia coli or *Salmonella* should be detected in 25 g additive. The analysis of three batches of the additive showed compliance with these specifications.[29]

Three batches of the additive were examined for particle size distribution by laser diffraction and for dusting potential using the Stauber–Heubach method. Only 0.04% of particles with diameters < 100 μm could be detected and none with diameters < 10 μm. The measurements of dusting potential gave a mean value of 0.03 g/m^3.[30]

3.1.2. Stability and homogeneity

In all of the following studies, the stability of the additive and its distribution and maintenance in feed were assessed by following the concentration of the five marker compounds shown in Table 2.[31]

3.1.2.1. Shelf-life

The shelf-life of the additive was assessed using three batches of the additive stored in sealed containers at either 22 ± 2°C or 37 ± 0.5°C for 12 months. Recoveries of the marker compounds were > 95% at the lower storage temperature and > 90% at the higher temperature.[32]

3.1.2.2. Stability in premixtures and feed

Biomin® DC-C (three batches) was incorporated into a commercial premixture containing minerals, vitamins and phytase and stored in sealed containers for 6 months at 22 ± 2°C. Recoveries of the marker compounds at the end of the experiment varied between 94.6% and 86.8% with methyl salicylate appearing the least stable.[33]

In a similar study, Biomin® DC-C (single batch) was incorporated into a mash feed for piglets based on wheat, barley and soybean at 75 mg/kg complete feed (the minimum recommended dose). The feed was stored in a sealed container at 22 ± 2°C for 5 months then analysed for the marker compounds. Recoveries varied between 96.9% to 87.0% of that present at the start of the study with methyl salicylate again appearing to be the least stable.

The additive was incorporated into a mash feed (wheat and soybean based) at the minimum recommended inclusion level and the feed then pelleted at temperatures of 80 and 90°C (conditioning time of 30 s, pelleting time 30 s); the feed were then stored at 21°C for 5 months. The concentration of the five marker compounds were measured in the mash feed prior to pelleting, immediately after pelleting and after the 5-month storage period.[34] Recoveries immediately after pelleting ranged between 65.7% and 89.6% at 80°C and 58.7% and 85.2% at 90°C, with linalool showing the lowest recovery. Subsequent storage of the pellets resulted in further losses of around 20%.

3.1.2.3. Homogeneity

One tonne of a mash feed for piglets was prepared incorporating Biomin® DC-C at the minimum recommended inclusion level of 75 mg/kg complete feed. The feed was distributed in 25 kg bags and samples taken from ten randomly selected bags. Samples were then analysed for the concentrations of the five marker compounds. The resulting coefficients of variation for the individual compounds were, in ascending order: 8% for each linalool and carvacrol, 9% for methyl salicylate, 10% L-menthol and 13% D-carvone.[35]

3.1.3. Conditions of use

Biomin® DC-C is intended for use as a zootechnical additive (functional group: other zootechnical additives) in feed for weaned piglets at a minimum concentration of 75 mg/kg complete feed and a maximum recommended level of 125 mg/kg complete feed. No withdrawal period is foreseen.

[29] Technical Dossier/Section II/Annex_11, 12 and 13.
[30] Technical Dossier/Section II/Annex_21.
[31] Technical Dossier/Section II/Annex_65.
[32] Technical Dossier/Section II/Annex_58.
[33] Technical Dossier/Section II/Annex_67.
[34] Technical Dossier/Section II/Annex_61.
[35] Technical Dossier/Section II/Annex_62.

3.2. Safety

3.2.1. Safety for piglets

3.2.1.1. Tolerance study

A tolerance study was made with 288 piglets (Tempo × Topigs 20) of approximately 26 days of age at the start and a mean weight of 7 kg.[36] The study followed a randomised complete block design with four treatments and 12 replicate pens/treatment. Each pen housed six pigs (three males and three females). Animals were fed a control diet or one of three experimental diets supplemented with 125 mg (×1 the maximum recommended use level), 625 mg (×5) or 1,250 mg (×10) Biomin® DC-C/kg complete feed for 42 days. The inclusion levels in feed were measured by the analysis of feed for the five marker compounds shown in Table 2 and from these data an estimate of additive inclusion made; the additive concentration in each of the prepared feeds was approximately 60% of the intended dose. The basal diet was supplied *ad libitum* in mash form and was based on wheat/barley/maize and soybean.

Animals were monitored daily for general health status. Piglets were individually weighed at the start of the trial and then at 14, 28 and 42 days under study. Feed intake was measured per pen in the period days 1–14, 14–28 and 28–42. Total body weight gain/pen and total feed intake/pen were used to calculate the feed to gain ratio for the corresponding periods including the overall. Faecal consistency was subjectively scored twice weekly on pen basis using a score of 1–10, where 1 represented liquid faeces and 10 hard dry faeces. On day 42, one pig with the average weight of the pen was selected from 6 of the 12 replicate pens and sampled for blood (haematology[37] and clinical chemistry analysis[38]) killed and necropsied; tissues and organs were sampled for residue analysis, and also digesta samples were collected. The animals selected for the necropsy were 50% female and 50% male. One animal with the average weight of the pen was also selected from the remaining six pens and a blood sample was taken. Samples of jejunum from piglets taken for gross pathology were sectioned and stained to various histomorphological parameters (villus height, crypt depth, goblet cell numbers/villus).[39]

Data were subjected to analysis of variance (ANOVA); treatment means were compared with least significant difference (LSD) after significant effects were confirmed by the Fisher's test. The pen was the experimental unit for all performance data except the growth of the individual pigs, blood haematology and clinical chemistry data.

No mortalities were recorded and animals on all treatments reached an average final body weight of approximately 23.5 kg (range 23.4–23.8 kg). No significant differences between treatments were found for average daily gain (0.41 kg), average daily feed intake (0.67 kg), feed to gain ratio (1.63) or for the mean faecal score (5.6–5.9). No significant differences were seen in the haematology results; the only difference observed in the clinical chemistry parameters was a significant reduction in alkaline phosphatase in the ×1 group compared to the control group, which was not treatment related. Gross pathology revealed no treatment related effects and no significant differences were seen in the histomorphological measurements of jejunal tissue.

3.2.1.2. Gut microbiota

The potential effect of Biomin® DC-C on the intestinal microbiota of digesta taken from the weaned piglets 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.[40] This provided a fingerprint of the dominant bacterial strains present. Computational analysis of DGGE patterns showed 29–41 prominent bands. The number of dominant bands (sequence 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.

[36] Technical Dossier/Section III/Annex_01.
[37] Haematocrit, leucocytes, erythrocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocytes, neutrophils, monocytes, eosinophils, basophils.
[38] Alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CPK), lactate dehydrogenase (LDH), albumin, urea, creatinine, bilirubin, phosphate triglycerides, Ca, Na, K, Cl.
[39] Technical Dossier/Section III/Annex_03.
[40] Technical Dossier/Section III/Annex_04.

3.2.1.3. Conclusions on the safety for target species

The results of the tolerance study show that the additive is safe for piglets at the maximum recommended application rate of 125 mg/g complete feed. Even allowing for the loss of volatile components from the additive after incorporation into feed, the margin of safety is at least six times the maximum application rate.

3.2.2. Safety for the consumer

3.2.2.1. Toxicological studies

3.2.2.1.1. Genotoxicity

A bacterial reverse mutation test (Ames test) was made following OECD 471 guideline and the principles of Good Laboratory Practices (GLP) using the *Salmonella* Typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 102.[41] The test item used was a preparation of the 'active' components of Biomin® DC-C (carvacrol, thymol, linalool, methyl-salicylate, carvone and menthol; in comparable concentrations to those of the additive) dissolved in dimethylsulfoxide (DMSO). The test item was introduced at concentrations between 1 and 1,000 μg/plate – after initial studies showed that higher concentrations fully inhibited bacterial growth. The study was made in duplicate in the presence and absence of S9 metabolic activation. The appropriate positive controls were included. No relevant increases in revertant colony numbers of any of the five tester strains were seen following treatment with the active components of Biomin® DC-C at any concentration tested in the presence or absence of metabolic activation. Consequently, the active components of Biomin® DC-C are considered to be non-mutagenic under the conditions of the assay.

An *in vitro* mammalian chromosome aberration test was made according to OECD guideline 473 and the principles of GLP in Chinese hamster V79 cells and using the same test item used in the test described above.[42] The metaphases were prepared 21 h after the start of treatment with the test item. The treatment interval was 4 h with and without metabolic activation (experiment 1) or 21 h without metabolic activation (experiment 2). The concentrations of the test item tested were 20, 50 and 75 μg/mL without metabolic activation in both experiment; 50, 75 and 100 μg/mL with activation in experiment 1. Evidence of precipitation of the test item and cytotoxicity was seen at concentrations > 100 μg/mL. One hundred and fifty metaphases per culture were scored for structural chromosomal aberrations. In the different experiments, some increase of structural chromosomal aberration was seen only at the highest concentration tested with concurrent evidence of cytotoxicity; therefore these findings were considered of no biological relevance. Consequently, it is concluded that the active compounds present in the additive can be considered non-clastogenic under the conditions of the test.

The FEEDAP Panel notes that for fully defined mixtures, the EFSA Scientific Committee recommends applying a component-based approach, i.e. assessing all components individually for their genotoxic potential (EFSA Scientific Committee, 2019). The Panel also notes 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 they are currently authorised for food[43] and feed[44] uses.

3.2.2.1.2. Repeated dose oral toxicity study

A subchronic oral toxicity study in Wistar rats was made following OECD 408 guidelines and the principles of GLP.[45] The test item used was a preparation of the 'active' components of Biomin® DC-C (carvacrol, thymol, linalool, methyl-salicylate, carvone and menthol; in comparable concentrations to those of the additive). Groups of 10 male and 10 female rats were given a basal diet supplemented with 20, 100 or 200 mg/kg body weight (bw) and day of the test item. A fourth group acted as the control group and was given the vehicle (olive oil) at the highest amount used. Rats were housed in

[41] Technical Dossier/Section III/Annex_III_08.
[42] Technical Dossier/Section III/Annex_III_09.
[43] 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.
[44] 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
[45] Technical Dossier/Section III/Annex_III_10; Technical Dossier/Supplementary Information/September 2017.

groups of five of the same sex per cage. Rats were monitored daily, and body weights and food and water intake recorded throughout the study. Blood was collected from all animals in the top-dose and control groups on day 90 for haematology[46] and clinical chemistry[47] examination. Animals from all groups were then killed and organ weights recorded; tissues from all animals were retained for histology, but only tissues from the control and top-dose groups (five male and five females per treatment) were examined.

The FEEDAP Panel notes that this study did not follow in full the OECD 408 guidelines, i.e. only the data and statistical comparison for the control and the high-dose groups were presented for haematology and biochemistry parameters. Therefore, the study is not further considered for the assessment.

3.2.2.2. Residue study

The applicant provided a residue study. Samples of muscle and skin plus fat were taken from each of the six animals from the control group and from the group given the maximum recommended level of the additive in the tolerance study (Section 3.2.2) were analysed for residues derived from the use of the additive.

The concentration in tissues of the marker compounds (carvacrol, methyl salicylate, D-carvone and L-menthol and thymol) was made after an enzymatic treatment to release parent compound from their glucuronidated or sulfated conjugates using a validated headspace-solid phase microextraction–gas chromatography–mass spectrometry (HS-SPME–GC–MS) method.[48]

Methyl salicylate, D-carvone and L-menthol were detected in all muscle samples at concentrations close to the LOD[49] with no differences evident between the background values seen in control samples and those from treated animals; carvacrol and thymol were below the LOD.[50] None of the marker compounds could be detected in the skin plus fat samples.

3.2.2.3. Conclusions on safety for the consumer

The active components of Biomin® DC-C were shown to be not genotoxic. From the available residue study no measurable exposure of the consumers is foreseen. Consequently, the use of the additive in animal nutrition is considered safe for consumers of animal products under the proposed conditions of use.

3.2.3. Safety for the user

The virtual absence of respirable particles (0.04% 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.[51]

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-C on skin and eyes.

3.2.4. Safety for the environment

Biomin® DC-C is a feed additive composed of a mixture of botanical extracts and individual compounds. A literature search aiming to examine the natural occurrence of the single components of Biomin® DC-C 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.[52]

The literature search revealed that all the additive's components occur in various plants, which are native to the countries of the EU. A number of these plants are important crops or occur at various

[46] Leucocyte count (WBC), erythrocyte count (RBC), haematocrit (HT), haemoglobin concentration (Hb) and platelet count (PL).
[47] Total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), creatinine (CREA), urea nitrogen, fasting blood glucose, total bilirubin (TBIL), Ca, P, Na, K, Cl.
[48] Technical Dossier/Section III/Annex_06.
[49] LOD for methyl salicylate: 1.15 μg/kg, for D-carvone: 0.53 μg/kg and for L-menthol: 0.98 μg/kg.
[50] LOD for carvacrol: 0.49 μg/kg and for thymol: 0.09 μg/kg.
[51] Oregano and caraway seed essential oils, carvacrol and L-menthol are irritant to skin and eyes.
[52] Technical Dossier/Section III/Annex_21.

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-C in animal production is not expected to pose a risk for the environment.

3.3. Efficacy for weaned piglets

The applicant proposes that the additive improves performance of weaned piglets.

To support efficacy of the additive, three efficacy studies with weaned piglets performed in two Member States were submitted. The design of the three studies was very similar, with a control group given the basal diet compared to a treated group given the basal diet supplemented with Biomin® DC-C at the minimum recommended level of 75 mg/kg complete feed. In all studies, the basal feed provided was based on mixed cereal/soybean in mash form. Details on the study designs are given in Table 3.

Table 3: Details of the designs of the three studies conducted with Biomin® DC-C with weaned piglets

Study	Substudy	Biomin® DC-C (mg/kg complete	Total number of animals Replicates per treatment/ piglets per replicate	Genotype Sex		Duration of the study (days)
		Intended	Analysed[a]			
1[e]	A	0		144 12/6	Ö-HYB-F1 ♂♀	42
		75	51–33[b]			
	B	0		192 12/10-6[c]	Ö-HYB-F1 ♂♀	42
		75	35–31			
2[f]		0		216 12-3/6-12[d]	Ö-HYB-F1 ♂♀	56
		75	60–56			
3[g]		0		192 16/6	DanAvl ♂♀	42
		75	64–44			

(a): Diets were analysed for the marker compounds shown in Table 2 and from this an estimate of additive addition calculated.
(b): Figure before the hyphen for the starter diet, and that after the hyphen for the grower diet.
(c): The substudy was conducted in two rooms, each with 6 pens/treatment, with 10 animals/pen in one house and 6 animals/pen in the other.
(d): The study was conducted in three runs. In both the first and the second run, 6 pens for control and 6 pens for the test group were used (summing up a total of 12 pens/treatment), each with 6 piglets/pen. In the third run, 3 pens for control and 3 pens for the test group were used, each with 12 piglets.
(e): Technical Dossier/Section IV/Annex_01 (Substudy A) and Technical Dossier/Section IV/Annex_09 (Substudy B). Technical Dossier/Supplementary Information/February 2019/Annex (i_ii).
(f): Technical Dossier/Supplementary Information/November 2018/Annex (iii_01).
(g): Technical Dossier/Section IV/Annex_17.

Piglets were weighed at the start of the experiments and on days 14, 28 and 42 for studies 1 and 3 and on days 15 and 56 for study 2. Feed consumption over the same periods was recorded. From these data average daily weight gain and feed to gain ratio was calculated.

In study 1, the data of both substudies were pooled and either ANOVA or non-parametric tests were used, depending on the statistical distribution of data for each variable. In study 2, the non-parametric Mann–Whitney test was applied to data not normally distributed, while normally distributed data were subjected to ANOVA. In study 3, data were subjected to ANOVA to test the significance of the differences between means. In all trials, the pen was the experimental unit and all tests were two-sided.

Mortality/culling was low in all studies (less than 6.5%, see Table 4) and there was no report of a need for veterinary intervention, other than exclusion of sick animals (included in the mortality/culling rate). No significant differences in mortality were identified between treatments. The performance results of the three studies are summarised in Table 4.

Table 4: Summary of the effects on performance of piglets given Biomin® DC-C at 75 mg/kg complete feed

Study	Biomin® DC-C (mg additive/kg complete feed)	Feed intake(b) (kg)	Body weight (kg)		Average daily weight gain (g)	Feed to Gain	Mortality/ Culling %
			Initial	Final			
1(a)	0	0.59	7.60	23.1	370	1.65	0.6
	75	0.61*	7.78	24.4*	397*	1.60	1.8
2	0	309	7.48	35.1	492	1.71	5.6
	75	353	7.51	36.1*	510*	1.65*	3.7
3	0	0.68	7.28	25.7	437	1.56	6.3
	75	0.70	7.27	27.0	463	1.50*	2.1

*: Within a trial, the control and treated groups are significantly different ($p < 0.05$).
(a): Includes pooled data of performance parameters from substudies A and B.
(b): For studies 1 and 3, average daily feed intake per piglet. For study 2, the total feed intake per pen is reported.

In all three studies, there was an increase in final body weight and average daily gain in the animals that received the additive compared with the control, but this reached significance only in studies 1 and 2. Feed intake was also higher in the animals supplemented Biomin® DC-C, but was only significant in study 1. Feed to gain ratio was numerically improved in the three studies, reaching significance in studies 2 and 3. Therefore, the additive showed positive effects on the performance of the piglets in three studies.

3.3.1. Conclusions on efficacy for piglets

Biomin® DC-C has a potential to improve the growth performance of weaned piglets at a minimum application rate of 75 mg/kg complete feed.

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[53] and Good Manufacturing Practice.

4. Conclusions

Biomin® DC-C is a mixture of naturally occurring compounds. The additive is safe for piglets at the maximum recommended application rate of 125 mg/g complete feed with a margin of safety of at least six times the maximum application rate.

The active components of the additive are not genotoxic. From the available residue study, no measurable exposure of the consumers is expected. Consequently, the use of the additive in animal nutrition is considered safe for consumers of animal products.

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

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

Biomin® DC-C has a potential to improve the performance of weaned piglets at a minimum application rate of 75 mg/kg complete feed.

5. Recommendations

The FEEDAP Panel recommends setting specification values for the marker compounds of the additive, which could be derived from those identified in analytical data.

[53] 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.

Documentation provided to EFSA/Chronology

Date	Event
04/05/2017	Dossier received by EFSA. Biomin® DC-C for weaned piglets. Submitted by Biomin GmbH
06/06/2017	Reception mandate from the European Commission
18/07/2017	Application validated by EFSA – Start of the scientific assessment
13/09/2017	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the consumer*
29/09/2017	Reception of supplementary information from the applicant - Scientific assessment re-started
19/10/2017	Comments received from Member States
18/11/2017	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
13/03/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: efficacy*
13/11/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
20/12/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: efficacy*
26/02/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
02/04/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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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

Abbreviations

ANOVA	analysis of variance
bw	body weight
CAS	Chemical Abstracts Service
DGGE	denaturing gradient gel electrophoresis
DMSO	dimethylsulfoxide
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GC-FID	gas chromatography coupled with flame ionisation detection
GLP	Good Laboratory Practices
HACCP	Hazard Analysis and Critical Control Points

HS-SPME–GC–MS	headspace-solid phase microextraction–gas chromatography–mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
LSD	least significant difference
OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyls
PCDD/F	polychlorinated dibenzo-*p*-dioxins and 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-C

In the current application authorisation is sought under article 4(1) for a *preparation of oregano oil, caraway oil, carvacrol, methyl salicylate and L-menthol (Biomin® DC-C)* 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 weaned piglets.

The *feed additive* is an off-white powder consisting of the following active substances (marker compounds) expressed as percentage mass fractions related to the feed additive: 9.5 to 14% of *carvacrol*; 3 to 5.5% of *L-menthol*; 1 to 4% of *methyl salicylate*; 6 to 8% of *oregano oil* (0.18 to 1.6% of *linalool*); and 0.5 to 1% of *caraway oil* (0.25 to 0.65% of *D-carvone*). 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-C* content ranging from 75 to 125 mg/kg feedingstuffs.

For the quantification of *carvacrol*, *L-menthol*, *methyl salicylate*, *linalool* and *D-carvone* in the *feed additive* the Applicant submitted a single-laboratory validated and further verified multi-analyte method based on gas chromatography coupled with 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 0.1 to 1.3%; a relative standard deviation for *intermediate precision* (RSDip) ranging from 0.5 to 4.1%; and a *recovery rate* (Rrec) ranging from 82 to 101%.

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*, *L-menthol*, *methyl salicylate*, *linalool* and *D-carvone* in the *feed additive*.

Since the accurate determination of the *Biomin® DC-C* 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-C* 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.