REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment on the Safety and Efficacy of a Feed Additive Consisting of L-isoleucine Produced by *Corynebacterium Glutamicum* KCCM 80185 for All Animal Species (CJ Europe GmbH) (RP2212)

Food Standards Agency[1], Food Standards Scotland[2]

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FSA Research and Evidence

An application was submitted to the Food Standards Agency and Food Standards Scotland in April 2024 from CJ Europe GmbH ("the applicant") for the new authorisation of an additive consisting of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185, under the category 'nutritional additives', functional group 'amino acids, their salts and analogues', for all animal species.

In 2021, the European Food Safety Authority (EFSA) published a scientific opinion on the safety and efficacy of a feed additive consisting of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185. The FEEDAP Panel concluded that the genetically modified production strain does not carry acquired antimicrobial resistance genes and no viable cells of the production strain were detected in the final additive. The presence of recombinant DNA from the production strain in the final additive could not be excluded. However, safety concerns regarding the potential presence of recombinant DNA from the production strain in the additive were not raised as no sequences of concern remain in the final production strain. Further, the FEEDAP Panel concluded that the additive is safe for the target species, the consumer and the environment under the proposed conditions of use. However, concern was raised regarding the simultaneous use of L-isoleucine in drinking water and feed due to the risk of nutritional imbalances, and hygienic reasons. Furthermore, the FEEDAP Panel concluded that L-isoleucine produced by *C. glutamicum* KCCM 80185 is considered non-toxic by inhalation, is not an irritant to the eyes or skin and is not a skin sensitiser. However, exposure to dust may be a risk for the user due to the high dusting potential of the additive. The additive is considered an efficacious source of the essential amino acid L-isoleucine for non-ruminant animal species. Protection against degradation in the rumen is required for the additive to be as efficacious in ruminants as in non-ruminant species.

Following the 2021 scientific opinion, EFSA published a scientific opinion on the presence of DNA in the feed additive consisting of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185 in 2023. The FEEDAP Panel assessed supplementary data to exclude the presence of recombinant DNA from the production organism in the additive and concluded that DNA of the production strain *C. glutamicum* KCCM 80185 was not detected in the additive.

FSA/FSS has reviewed the authorisation application, supporting documentation, and other regulators' risk assessments, most notably the EFSA risk assessment opinion, and considers that sufficient evidence has been demonstrated to conclude without the need for further questions or risk assessment.

This is a joint FSA and FSS publication.

1. Introduction

The FSA and FSS have undertaken an assessment of a feed additive submitted by CJ Europe GmbH (Unterschweinstiege 2-14, Frankfurt am Main, 60549, Germany) of L-isoleucine produced by the genetically modified strain *Corynebacterium glutamicum* KCCM 80185, under Assimilated Regulation (EC) No 1831/2003 (EC, 2003) in each nation of Great Britain (GB). The applicant seeks new authorisation under the category 'nutritional additive', functional group 'amino acids, their salts and analogues', for its use in feed and drinking water for all animal species.

In line with Article 8 of Assimilated Regulation No 1831/2003 (EC, 2003), the assessment has considered and concluded whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by the European Food Safety Authority (EFSA) for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

To ensure the regulatory systems of FSA/FSS are risk proportionate and resources are used effectively, the FSA and FSS have used the evidence submitted by the applicant and other information in the public domain, including the EFSA risk assessment opinion, to provide a summary assessment of the evidence of safety presented in this report.

In 2021, EFSA published a risk assessment opinion on the safety and efficacy of a feed additive consisting of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185 for all animal species (EFSA FEEDAP Panel, 2021). Following the 2021 opinion, EFSA published in 2023 a scientific opinion on the presence of DNA in the feed additive consisting of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185 for all animal species (EFSA FEEDAP Panel, 2023). These opinions have been reviewed by FSA/FSS risk assessors. It has been verified that the standard approach, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion.

The result of the assessment is that there is sufficient evidence of safety and efficacy for the UK to conclude this assessment at this time. This assessment represents the opinion of the FSA and FSS.

Table 1. Table showing products included in this assessment.

Title	Product type	Intended use/s	Intended dose/intake
L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185	Feed additive	Nutritional additive / amino acids, their salts and analogues	Direct use in feedingstuffs, complementary feedingstuffs or via premixture or drinking water in all animal species.

2. Assessment

2.1. Details of other regulators' opinions

2.1.1. Current authorisation

Under Commission Implementing Regulation (EU) No 2023/2583 (EC, 2023), following the EFSA 2023 opinion, the additive is authorised in the European Union (EU) for use in all species. The additive produced by *Corynebacterium glutamicum* KCCM 80185 has not been authorised as feed additive in Great Britain (GB).

2.1.2. Other regulators' opinions

In 2020, the EFSA published a scientific opinion on the safety and efficacy of L-isoleucine produced by fermentation using non-genetically modified *Corynebacterium glutamicum* KCCM 80189 when used as a nutritional additive in feed and drinking water for all animal species (EFSA FEEDAP Panel, 2020). No viable cells of *C. glutamicum* KCCM 80189 were detected in the final additive, and the additive did not raise any safety concern regarding the production strain. The FEEDAP Panel concluded that L-isoleucine produced by *C. glutamicum* KCCM 80189 is considered safe

for the target species, the consumer and the environment. The active substance of the additive is not considered a skin sensitiser or an irritant to the eyes and skin and is not toxic by inhalation. The concern was reiterated regarding the simultaneous use of L-isoleucine in drinking water and feed due to the risk of nutritional imbalances and hygienic reasons. The additive is considered an efficacious source of L-isoleucine for non-ruminant animal species.

The FEEDAP Panel concluded that the results of the studies regarding safety of the user performed with L-isoleucine produced by *C. glutamicum* KCCM 80189 in the 2020 EFSA opinion can be used to support the safety for the user in the assessment of L-isoleucine produced by *C. glutamicum* KCCM 80185 (current assessment) as the composition, purity and physicochemical characteristics of the active substance are the same in both assessments and the production process is very similar. In addition, the FEEDAP Panel concluded that the results of the shelf life and stability studies with an L-isoleucine produced by *C. glutamicum* KCCM 80189 (2020 EFSA assessment) can be applicable to the additive produced by *C. glutamicum* KCCM 80185 (current assessment).

In 2021, EFSA published a risk assessment opinion on the safety and efficacy of a feed additive consisting of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185 for all animal species. In 2023, EFSA published a follow-up scientific opinion on the presence of DNA in the feed additive consisting of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80185 for all animal species.

These opinions have been reviewed by FSA/FSS risk assessors. The 2021 (EFSA FEEDAP Panel, 2021) and 2023 (EFSA FEEDAP Panel, 2023) EFSA opinions assess the same product that is under current assessment by the FSA and FSS. It has been verified that the standard approach, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion.

2.1.3. Methodology applied in the EFSA opinion

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed the safety and the efficacy of the additive, in accordance with guidance documents:

- Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017c);
- Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a);

- Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b);
- Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a);
- Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b);
- Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012);
- Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019);

and principles in Assimilated Regulation (EC) No 429/2008.

These guidance documents were developed and implemented prior to the UK's exit from the EU and were also adopted by the FSA and FSS on exit and remain relevant for this assessment.

2.2. Section II: Identity, characterisation and conditions of use

2.2.1. Characterisation of the production microorganism

A genetically modified strain of *C. glutamicum* is deposited at the Korean Culture Centre of Microorganisms (KCCM). The taxonomic identity of the production strain was confirmed.

The production strain was tested for its susceptibility to the relevant antibiotics recommended in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) for *Corynebacterium* and other Gram-positive bacteria. The FEEDAP Panel concluded that the production strain is considered susceptible to the relevant antibiotics as all the minimum inhibitory concentration (MIC) values were below or equal to the corresponding cut-off values (EFSA FEEDAP Panel, 2021).

The whole genome sequence (WGS) of the production strain was interrogated for the presence of antimicrobial resistance genes (AMR) with no genes of concern identified.

The WGS of the *C. glutamicum* KCCM 80185 was queried for the presence of genes coding for toxins and virulence, with no concerns identified.

2.2.1.1 Information related to the genetic modification

The applicant provided characterisation of the recipient or parental microorganism, characterisation of the donor organism and description of the genetic modification, which were assessed by the FEEDAP Panel. The submitted information was considered sufficient by the FSA and FSS.

2.2.2. Manufacturing process

The applicant stated that the L-isoleucine is produced by fermentation with *C. glutamicum* KCCM 80185. The applicant provided a description of the manufacturing process, which was assessed by the FEEDAP Panel in 2021. The submitted information was considered sufficient by the FSA and FSS.

2.2.3. Characterisation of the additive

The active substance of the additive, L-isoleucine (International Union of Pure and Applied Chemistry (IUPAC) name: (2S,3S)-2-amino-3-methylpentanoic acid; the Chemical Abstracts Service (CAS) No 73-32-5; the European Inventory of Existing Commercial Chemical Substances (EINECS) No 200-798-2), has a molecular mass of 131.2 g/mol.

Analysis of 5 batches of the additive showed compliance with the predefined specifications with an average L-isoleucine content of 91.9% (range 91.8 – 91.9%) on a dry matter (DM) basis, moisture content of 0.21 – 0.27% and average ash content of 0.08% (range 0.07 – 0.09%) (EFSA FEEDAP Panel, 2021). Other components included in the analysis were free amino acids other than isoleucine (most of them were not detected): 5.84% of α-amino-butyric acid (range 5.81-5.86%), 0.13% of alanine (range 0.12-0.13%), 0.44% of valine (range 0.43-0.46%) and 1.02% of phenylalanine (range 1.02-1.03%); nitrogen containing components: 0.21% of ammonium (range 0.19-0.22%), nitrates, nitrites and betaine not detected; organic acids: formic, acetic, citric, malic, succinic, lactic, not detected; inorganic components: 0.03% of sodium (range 0.03-0.04%), 0.13% of sulfate (range 0.12-0.14%), potassium, magnesium, calcium, fluoride, bromide, chloride, phosphate not detected. On DM basis, the sum of identified compounds was above 99%.

The average specific optical rotation measured in five batches was +39.2° (range +39.0° to +39.4°) which complies with specifications set by the applicant (+38.5° to +41.5°). With respect to the European Pharmacopoeia (PhEur, 2019), a deviation is noted as a range of the specific optical rotation for this substance is +40° to +43°.

Analysis of three batches of the additive showed concentrations of arsenic, cadmium, lead, mercury and nickel as well as aflatoxins (B1, B2, G1 and G2), ochratoxin A, zearalenone and deoxynivalenol all below their corresponding limit of quantification (LOQ). The level of zinc was below its

LOQ with the exception of one batch (0.65 mg/kg) and levels of chromium ranged from 0.21 to 0.39 mg/kg. Further, analysis of three batches of the additive showed polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar dioxin-like polychlorinated biphenyls (co-planar PCBs) below the corresponding LOQ. In all three batches, the calculated upper bound levels of dioxins and the sum of dioxins and dioxin-like-PCBs were 0.07 ng WHO-PCDD/F-TEQ/kg DM and 0.14 ng WHO-PCDD/F-PCB-TEQ/kg DM, respectively and the calculated upper bound levels of non-dioxin-like PCBs (ICES-6) were 0.6 µg/kg. Analysis of three batches of the additive showed levels of Enterobacteriaceae and *Escherichia coli* ˂ 10 colony forming units (CFU)/g, yeast and moulds < 100 CFU/g and *Salmonella* spp. not detected in 25 g. The FEEDAP Panel concluded that the detected amounts of the impurities do not raise safety concerns.

Three batches of the final product (each batch tested in triplicate) were investigated for the presence of viable cells of the production strain with no cells of *C. glutamicum* detected.

Three batches of L-isoleucine (tested in triplicate) were analysed for the presence of recombinant DNA of the production strain in the final product. No DNA from the production strain was detected in the samples. The FEEDAP Panel could not exclude the presence of recombinant DNA of the production strain in the product as the lysis buffers used to extract the DNA may not ensure the recovery of the DNA from non-viable cells of the production strain, and they could potentially remain in the product.

Supplementary information was provided by the applicant in 2023 to exclude the potential presence of recombinant DNA of the production strain in the final additive (EFSA FEEDAP Panel, 2023). The polymerase chain reaction (PCR) method in three independent batches of L-isoleucine was tested in triplicate, to analyse the presence of recombinant DNA of the production strain. The FEEDAP Panel concluded that no DNA of the production strain was detected in the final product based on the results of the PCR analysis.

The additive is a white to yellowish crystalline powder with melting point of 270-290°C, solubility in water is 3.0-3.5 g/100 g water and bulk density of 0.6 -0.85 g/mL. Particle size distribution was determined in three batches by the sieving method showing 63-70% particles with diameter < 105 µm and 10-17% of particles < 62 µm. The dusting potential determined in three batches by the Stauber Heubach method ranged from 5.7 – 6.2 g/m^3.

The applicant did not provide information on the shelf-life, stability (in premixtures, feedingstuffs and water for drinking) and capacity of the additive to distribute homogeneously in feed under assessment. The applicant provided information on the shelf life and stability of the additive in a vitamin and mineral premixture, in a mash and pelleted forms of

compound feed for chickens and in drinking water with an L-isoleucine produced by a different strain *C. glutamicum* KCCM 80189 of the same producer. The studies were previously assessed in a 2020 EFSA opinion (EFSA FEEDAP Panel, 2020). The FEEDAP Panel concluded that the results of those studies are applicable to the current assessment as the production process is the same and the characteristics of the additive are very similar.

The FSA and FSS agree with the conclusions reached for the characterisation of the additive and production strain. The amounts of the detected impurities as well as microbial contamination, do not raise safety concerns. The certificates of analysis were reviewed by the FSA and FSS and confirmed compliance with the specifications. The FSA and FSS agrees with the conclusions of the 2023 assessment that DNA of the production strain was not detected in the final product based on the results of the PCR analysis, and that this is sufficient evidence to demonstrate the absence of the production strain in the final product.

2.2.4. Conditions of use

The additive is intended to be used directly in feedingstuffs, complementary feedingstuffs, via premixture or via drinking water in all animal species. As the optimal daily allowance in quantitative terms depends on the species, the physiological state of the animal, the performance level and the environmental conditions, in particular on the amino acid composition of the unsupplemented diet, no proposed inclusion levels are provided.

The applicant requested the same conditions of use for GB as EFSA evaluated in their 2021 opinion (EFSA, 2021). The FSA/FSS agrees with proposed conditions of use by the applicant.

2.2.5. Conclusion on Section II: Identity, characterisation and conditions of use

The additive was fully characterised, and the identity of the production strain confirmed. The applicant provided characterisation of the recipient or parental microorganism, characterisation of the donor organism and description of the genetic modification, which were assessed by the FEEDAP Panel in 2021.

The FEEDAP Panel concluded that the amounts of the detected impurities, as well as microbial contamination, do not raise safety concerns. The FEEDAP Panel concluded that the information on the shelf life and stability of the additive with L-isoleucine produced by *C. glutamicum* KCCM 80189 are applicable to the current assessment.

The FSA and FSS agree with the conclusions reached for the characterisation of the additive and active agent. The FSA/FSS agree with the conditions of use proposed by the applicant. The FSA and FSS agrees with the conclusions of the 2023 assessment that DNA of the production strain was not detected in the final product based on the results of the PCR analysis.

2.3. Section III: Safety

2.3.1. Safety of the production strain

The production strain *C. glutamicum* KCCM 80185, developed to increase production of L-isoleucine, belongs to a *C. glutamicum*. When used for production purposes (EFSA BIOHAZ Panel, 2020), the production strain qualifies for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007). The FEEDAP Panel concluded that the strain was unambiguously identified as *C. glutamicum,* the genetic modification does not raise safety concerns and no acquired antimicrobial resistance genes are carried in the strain. Further, *C. glutamicum* KCCM 80185 was not detected in the final additive. The FEEDAP Panel could not exclude the presence of recombinant DNA from the production strain in the product in their 2021 assessment. However, it was concluded that there are no safety concerns from the potential presence of recombinant DNA in the final product.

Supplementary information was provided by the applicant in 2023 to exclude the potential presence of recombinant DNA of the production strain in the final additive (EFSA FEEDAP Panel, 2023). The FEEDAP Panel concluded that no DNA of the production strain was detected in the final product based on the results of the PCR analysis.

The FSA and FSS agree with the conclusions reached for the safety of the production strains, including the use of the QPS approach and that clear strain identification has been provided. Therefore, the production strain does not raise any safety concerns.

2.3.2. Safety for the target species, consumer and the environment

The FEEDAP Panel concluded that the potential presence of recombinant DNA in the final additive would not pose a safety concern as the production strain qualifies for the QPS approach to safety assessment (EFSA, 2007). In addition, the L-isoleucine under assessment is highly purified (less than 1% unidentified material) and therefore no safety concerns for target animals, consumers or the environment would rise from the fermentation material

present in the final additive. FEEDAP Panel concluded that DNA of the production strain was not detected in the final product (EFSA FEEDAP Panel, 2023).

The interaction of branched chain amino acid (BCAA), leucine, L-isoleucine and valine, fed at excessive levels, has already been described in previous EFSA opinions (EFSA, 2008; EFSA FEEDAP Panel, 2013, 2020). Valine, isoleucine and leucine exert a strong antagonism on each other, which result in an alteration of the plasma and brain amino acid concentrations (Harper et al., 1984). This imbalance is responsible for a reduced feed intake with impaired feed efficiency and weight gain. When the concentrations of the other BCAA were slightly above the requirement levels, the addition of 10% of leucine, valine or isoleucine to semi-purified diets showed no adverse effects in kittens (Hargrove et al., 1988).

The FEEDAP Panel raised concerns regarding simultaneous use of isoleucine-containing additives via drinking water and complete diets with a well-balanced amino acid profile as it may present a risk for the target species due to nutritional imbalances, hygienic reasons and increased nitrogen excretion via urine (EFSA FEEDAP Panel, 2010). Therefore, the FEEDAP Panel has concerns on the safety of the simultaneous oral administration of isoleucine-containing additives via feed and water for drinking.

Supplemented to feed, L-isoleucine will be incorporated into proteins of tissues and products of animal origin, but any potential excess will be metabolised and excreted as urea/uric acid and as carbon dioxide. Therefore, the use of L-isoleucine in animal nutrition will not alter the composition of tissues and products of animal origin.

L-isoleucine, a natural component of the proteins of living organisms, will be absorbed or excreted as a part of the intestinal microbial mass after consumption. Any localised increase of its concentration in the environment is not expected from the use of the additive in animal nutrition. Viable cells of the C. *glutamicum* KCCM 80185 were not detected in the final product. The FEEDAP Panel concluded that a risk for the environment is not foreseen from the use of the additive in animal nutrition.

The FEEDAP Panel concluded that the L-isoleucine produced by *C. glutamicum* KCCM 80185 is safe for the target species, the consumer and the environment when used in animal nutrition to cover nutritional needs of animals (EFSA FEEDAP Panel, 2021).

The FSA and FSS agree with the conclusions reached on the safety for the target species, consumer and the environment. The FSA and FSS agrees that the production strain qualifies for the QPS approach to safety assessment, the strain was unambiguously identified as *C. glutamicum,*

the genetic modification does not raise safety concern, and no acquired antimicrobial resistance genes are carried in the strain. The FSA and FSS agrees with the conclusions of the 2023 assessment that DNA of the production strain was not detected in the final product based on the results of the PCR analysis.

2.3.2.1. Conclusion on safety for the target species, consumer and the environment

The FEEDAP Panel concluded that the L-isoleucine produced by *C. glutamicum* KCCM 80185 is safe for the target species, the consumer and the environment when used in animal nutrition. The FEEDAP Panel concluded that the production strain qualifies for the QPS approach to safety assessment (EFSA, 2007). In addition, the L-isoleucine under assessment is highly purified (less than 1% unidentified material) and therefore no safety concerns for target animals, consumers and the environment would rise from the fermentation material present in the final additive.

The FEEDAP Panel raised a concern regarding simultaneous use of isoleucine-containing additives via drinking water and complete diets with a well-balanced amino acid profile as it may present a risk for the target species due to nutritional imbalances, hygienic reasons and increased nitrogen excretion via urine (EFSA FEEDAP Panel, 2010).

The FSA and FSS agree with the conclusions reached on the safety for the target species, consumer and the environment. The FSA and FSS agrees that the production strain qualifies for the QPS approach to safety assessment, that the strain was unambiguously identified as *C. glutamicum,* the genetic modification does not raise safety concern and no acquired antimicrobial resistance genes are carried in the strain.

2.3.3. Safety for the user

No studies using the additive under assessment to assess the safety for the user were provided by the applicant. An acute inhalation test following OECD guideline 403, a skin sensitisation test following OECD guideline 429, an eye irritation test following OECD guideline 437 and a skin irritation test following OECD guideline 439 performed using L-isoleucine produced by a different strain *C. glutamicum* KCCM 80189 were provided by the applicant. The FEEDAP Panel concluded that the additive should be considered not a skin sensitiser, not an irritant to eyes and skin, as well as not toxic by inhalation. However, the exposure to dust might be a risk for the user due to a high dusting potential (up to 6.2 g/m^3). These tests have been assessed in a previous EFSA opinion in 2020 (EFSA FEEDAP Panel, 2020). The FEEDAP Panel considers that the results of the studies performed with L-isoleucine produced by *C. glutamicum* KCCM 80189 can be used to support the safety

for the user in the current assessment as the composition, purity and physicochemical characteristics of the active substance in the current and 2020 assessment are the same and the production process is very similar.

The FSA and FSS agree with the conclusions reached on the safety for the user. The studies were reviewed by EFSA in 2020, prior to the UK's exit from the EU; thus, this opinion is applicable to GB. The FSA and FSS agree that the results of the studies performed with L-isoleucine produced by *C. glutamicum* KCCM 80189 can be used to support the safety for the user in the current assessment as the data are owned by the same applicant and the two additives are sufficiently comparable to support this extrapolation.

2.3.4. Conclusion on Section III: Safety

The FEEDAP Panel concluded that L-isoleucine produced by *C. glutamicum* KCCM 80185 is safe for the target species, the consumer and the environment when used in animal nutrition. However, the FEEDAP Panel raised concerns regarding simultaneous use of isoleucine-containing additives via drinking water and complete diets with a well-balanced amino acid profile as it may present a risk for the target species due to nutritional imbalances, hygienic reasons and increased nitrogen excretion via urine.

In 2021, the FEEDAP Panel concluded that the potential presence of recombinant DNA in the final additive would not pose a safety concern as the production strain qualifies for the QPS approach to safety assessment (EFSA, 2007). In addition, the L-isoleucine under assessment is highly purified (less than 1% unidentified material) and therefore no safety concerns for target animals, consumers and the environment would rise from the fermentation material present in the final additive. After supplementary information was provided by the applicant in 2023, the FEEDAP Panel concluded that DNA of the production strain was not detected in the final product.

With respect to safety for the user, The FEEDAP Panel concluded that the additive should be considered not a skin sensitiser, not an irritant to eyes and skin as well as not toxic by inhalation. However, the exposure to dust might be a risk for the user due to a high dusting potential (up to 6.2 g/m^3).

The FSA and FSS agree with the conclusions reached on the safety of the additive for target species, the consumer, user and the environment.

2.4. Section IV: Efficacy as a nutritional additive

Use of amino acids naturally occurring in proteins of plants and animals in animal nutrition does not require efficacy studies. The role of the L-isoleucine in nutrition is well established in the scientific literature. L-isoleucine produced by *C. glutamicum* KCCM 80185 is considered an

efficacious source of isoleucine for non-ruminant animal species. Protection against degradation in the rumen is required, for the additive to be as efficacious in ruminants as in non-ruminant species.

The FSA and FSS agree with the conclusions reached on the efficacy, which is supported by the guidance that is also applicable in GB. This demonstrates that the additive satisfies the nutritional needs of animals.

3. Analytical method evaluation

The FSA/FSS evaluated the EURL analytical method evaluation, noting it was carried out in 2020, when the UK was still part of the EU and would have participated in their approval. No concerns are raised at this stage for the validity of the methods for UK/GB use, and therefore the FSA/FSS accept the EURL analytical method evaluation report (EURL, 2020). The FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

In 2021, the FEEDAP Panel concluded that L-isoleucine produced by *C. glutamicum* KCCM 80185 is considered to be safe for the target species, the consumer and the environment. The genetically modified strain of *C. glutamicum* KCCM 80185 does not carry acquired antimicrobial resistance genes. In addition, no viable cells of the production strain were detected in the final additive.

However, the FEEDAP Panel reiterated concerns for the target species regarding simultaneous use of L-isoleucine via drinking water and feed due to the risk of nutritional imbalances and hygienic reasons. Furthermore, the FEEDAP Panel concluded in 2021 that the additive should be considered not a skin sensitiser, not an irritant to eyes and skin as well as not toxic by inhalation. However, the exposure to dust might be a risk for the user due to a high dusting potential. The additive is considered an efficacious source of isoleucine for non-ruminant animal species. Protection against degradation in the rumen is required for the additive to be as efficacious in ruminants as in non-ruminant species.

In 2021, the FEEDAP Panel could not exclude the presence of recombinant DNA from the production strain in the final additive. However, this did not raise any safety concerns. Following the 2021 EFSA opinion, the applicant provided supplementary information to exclude the potential presence of recombinant DNA of the production strain in the final additive. The FEEDAP Panel concluded in 2023 that DNA of the production strain was not detected in the final product.

5. Caveats and uncertainties

Simultaneous use of isoleucine-containing additives via drinking water and complete diets with a well-balanced amino acid profile may present a risk for the target species due to nutritional imbalances, hygienic reasons and increased nitrogen excretion via urine.

As the optimal daily allowance in quantitative terms depends on the species, the physiological state of the animal, the performance level and the environmental conditions, in particular on the amino acid composition of the unsupplemented diet, no proposed inclusion levels are provided.

6. FSA/FSS conclusions for GB risk analysis

The application has been assessed in line with the applicable guidance and is partially based on considerations of detailed proprietary information available to the Panel, which were also submitted to the FSA and FSS. The EFSA opinion (EFSA FEEDAP Panel, 2020, 2021, 2023) identify and characterise the hazards present from the proposed use and conclude there is sufficient information to enable an assessment of exposure, which is also relevant to GB. The conclusions of the EFSA opinion have been reviewed in detail by the FSA and FSS, and are considered appropriate and consistent, including the caveats and uncertainties identified in the opinion which are applicable to GB. Sufficient evidence has been demonstrated to conclude without further questions or risk assessment.

Abbreviations

Abbreviation	Definition
AMR	Antimicrobial resistance
CAS	Chemical Abstracts Service
CFU	Colony forming units
CV	Coefficient of variation
IUEC	European Commission
EU	European Union
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
EINECS	European Inventory of Existing Commercial Chemical Substances
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland
GB	Great Britain
IUPAC	International Union of Pure and Applied Chemistry
KCCM	Korean Culture Centre of Microorganisms
LOD	Limit of detection
LOQ	Limit of quantification

Abbreviation	Definition
MIC	Minimum inhibitory concentration
RP	Regulated product
U	Units
UK	United Kingdom
WGS	Whole genome sequence

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References

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