

Commission Implementing Regulation (EU) 2020/1397 of 5 October 2020 concerning the renewal of the authorisation of L-isoleucine produced by *Escherichia coli* FERM ABP-10641 as a nutritional additive, its extension of use and the authorisation of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80189 as a feed additive for all animal species, and repealing Regulation (EU) No 348/2010 (Text with EEA relevance)

COMMISSION IMPLEMENTING REGULATION (EU) 2020/1397
of 5 October 2020

concerning the renewal of the authorisation of L-isoleucine produced by *Escherichia coli* FERM ABP-10641 as a nutritional additive, its extension of use and the authorisation of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80189 as a feed additive for all animal species, and repealing Regulation (EU) No 348/2010

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition⁽¹⁾, and in particular Article 9(2) thereof,

Whereas:

- (1) Regulation (EC) No 1831/2003 provides for the authorisation of additives for use in animal nutrition and for the grounds and procedures for granting and renewing such authorisation.
- (2) L-isoleucine produced by *Escherichia coli* FERM ABP-10641 was authorised for 10 years as a feed additive for all animal species by Commission Regulation (EU) No 348/2010⁽²⁾.
- (3) In accordance with Article 14 of Regulation (EC) No 1831/2003, an application was submitted for the renewal of the authorisation of L-isoleucine produced by *Escherichia coli* FERM ABP-10641 as a feed additive for all animal species, requesting this additive to be classified in the additive category ‘nutritional additives’, functional group ‘amino acids, their salts and analogues’. This application was accompanied by the particulars and documents required under Article 14(2) of Regulation (EC) No 1831/2003. Moreover, the application requested, in accordance with Article 7 of Regulation (EC) No 1831/2003, L-isoleucine produced by *Escherichia coli* FERM ABP-10641 to be authorised for use in water for drinking in the additive category ‘nutritional additives’, functional group ‘amino acids, their salts and analogues’ for all animal species, and for use in feed to be classified in the additive category ‘sensory additives’, functional group ‘flavouring compounds’. The application was accompanied

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by the particulars and documents required under Article 7(3) of Regulation (EC) No 1831/2003.

- (4) In accordance with Article 7 of Regulation (EC) No 1831/2003, an application was submitted for the authorisation of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80 189 as a feed additive for use in feed and in water for drinking for all animal species. The application concerns the authorisation of L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80 189 as a feed additive for all animal species to be classified in the additive category 'nutritional additives', functional group 'amino acids, their salts and analogues'. That application was accompanied by the particulars and documents required under Article 7(3) of Regulation (EC) No 1831/2003.
- (5) The European Food Safety Authority ('the Authority') concluded in its opinions of 28 January 2020⁽³⁾⁽⁴⁾ that, under the proposed conditions of use, L-isoleucine produced by *Escherichia coli* FERM ABP-10641 or *Corynebacterium glutamicum* KCCM 80 189 does not have an adverse effect on animal health, consumer health or the environment. It also stated that L-isoleucine produced by *Escherichia coli* FERM ABP-10641 was hazardous by inhalation for persons handling the additive. Therefore, the Commission considers that appropriate protective measures should be taken to prevent adverse effects on human health, in particular as regards the users of this additive. No adverse effects on user safety were stated for L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80 189. The Authority also concluded that the additive is an efficacious source of the essential amino acid isoleucine for non-ruminant animals and that for the supplemental L-isoleucine to be fully efficacious in ruminants, it should be protected against degradation in the rumen. The Authority expressed concerns on the simultaneous oral administration of the amino acid via water for drinking and feed. However, the Authority did not propose a maximum content for L-isoleucine. Thus, in the case of supplementation with L-isoleucine via water for drinking, it is appropriate to alert the user to take into account the dietary supply with all the essential and conditionally essential amino acids.
- (6) Restrictions and conditions should be provided for to allow for a better control for L-isoleucine when used as flavouring. For L-isoleucine used as flavouring, the recommended content should be indicated on the label. Where such content is exceeded, certain information should be indicated on the label of the additive and premixtures. As regards the use of L-isoleucine as a flavouring, the Authority states that no further demonstration of efficacy is necessary when the substance is used at the recommended dose level. The use of L-isoleucine as a flavouring compound is not authorised in water for drinking. The fact that the use of the L-isoleucine is not authorised for use as a flavouring in water for drinking, does not preclude its use in compound feed, which is administered via water.
- (7) The Authority does not consider that there is a need for specific requirements of post-market monitoring. It also verified the reports on the method of analysis of the feed additive in feed submitted by the Reference Laboratory set up by Regulation (EC) No 1831/2003.

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- (8) The assessment of L-isoleucine produced by *Escherichia coli* FERM ABP-10641 and by *Corynebacterium glutamicum* KCCM 80 189 shows that the conditions for authorisation, as provided for in Article 5 of Regulation (EC) No 1831/2003, are satisfied. Accordingly, the use of this additive should be authorised as specified in the Annex to this Regulation.
- (9) As a consequence of the renewal of the authorisation of L-isoleucine produced by *Escherichia coli* FERM ABP-10641 as a feed additive under the conditions laid down in the Annex to this Regulation, Regulation (EU) No 348/2010 should be repealed.
- (10) Since safety reasons do not require the immediate application of the modifications to the conditions of authorisation for L-isoleucine produced by *Escherichia coli* FERM ABP-10641, it is appropriate to provide a transitional period for interested parties to prepare themselves to meet the new requirements resulting from the renewal of the authorisation.
- (11) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on Plants, Animals, Food and Feed,

HAS ADOPTED THIS REGULATION:

Article 1

1 The authorisation of L-isoleucine produced by *Escherichia coli* FERM ABP-10641, belonging to the additive category ‘nutritional additives’ and to the functional group ‘amino acids, their salts and analogues’, is renewed, extended to its use via water for drinking, subject to the conditions laid down in the Annex.

L-isoleucine produced by *Escherichia coli* FERM ABP-10641 belonging to the additive category ‘sensory additives’ and to the functional group ‘flavouring compounds’, is authorised as an additive in animal nutrition, subject to the conditions laid down in the Annex.

2 L-isoleucine produced by *Corynebacterium glutamicum* KCCM 80 189, belonging to the additive category ‘nutritional additives’ and to the functional group ‘amino acids, their salts and analogues’, is authorised as an additive in animal nutrition, subject to the conditions laid down in the Annex.

Article 2

1 L-isoleucine produced by *Escherichia coli* FERM ABP-10641 and premixtures containing it, which are produced and labelled before 26 April 2021 in accordance with the rules applicable before 26 October 2020 may continue to be placed on the market and used until the existing stocks are exhausted.

2 Feed materials and compound feed containing L-isoleucine produced by *Escherichia coli* FERM ABP-10641 and premixtures containing it, which are produced and labelled before 26 October 2021 in accordance with the rules applicable before 26 October 2020 may continue to be placed on the market and used until the existing stocks are exhausted if they are intended for food-producing animals.

3 Feed materials and compound feed containing L-isoleucine produced by *Escherichia coli* FERM ABP-10641 and premixtures containing it, which are produced and labelled before 26 October 2022 in accordance with the rules applicable before 26 October 2020 may continue

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to be placed on the market and used until the existing stocks are exhausted if they are intended for non-food-producing animals.

Article 3

Regulation (EU) No 348/2010 is repealed.

Article 4

This Regulation shall enter into force on the twentieth day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 5 October 2020.

For the Commission

The President

Ursula VON DER LEYEN

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ANNEX

Identification number of the additive	Name of the holder of authorisation	Additive	Chemical composition, formula, analytical method.	Species, category, animal method.	Maximum age	Minimum content	Maximum content	Other provisions	End of period of authorisation

Category: nutritional additives. Functional group: amino acids, their salts and analogues.

3c381		L- isoleucine	<i>Additive composition</i> Powder with a minimum content of L-isoleucine of 93,4 % (on a dry matter basis)	All animal species				1.	26 October 2020
			<i>Characterisation of the active substance</i> L-isoleucine produced by fermentation with <i>Escherichia coli</i> FERM ABP-10641 IUPAC name: (2S,3S)-2-amino-3-methylpentanoic acid					2.	be placed on the market and used as an additive consisting of a preparation. In the directions for use of the additive and premixture, the storage conditions, the stability to heat

a Details of the analytical methods are available at the following address of the Reference Laboratory: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>

b Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).

c Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).

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		Chemical formula: C ₆ H ₁₃ NO ₂ CAS number: 73-32-5					treatment and in water shall be indicated.
		<i>Analytical method^a</i> For the identification of L-isoleucine in the feed additive: —	Food Chemical Codex 'L-isoleucine monograph'			3.	The additive may be used via water for drinking.
		For the quantification of isoleucine in the feed additive: —	ion exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS/FLD) or ion exchange chromatography			4.	Declaration to be made on the label of the additive and premixture: — 'The supplementation with L-isoleucine, in particular via water for drinking, shall take into account all essential and

a Details of the analytical methods are available at the following address of the Reference Laboratory: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>

b Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).

c Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).

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			coupled with post-column derivatisation and photometric detection (IEC-VIS)				conditionally essential amino acids in order to avoid imbalances.’ L-isoleucine content.
		For the quantification of isoleucine in premixtures:	— ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS/FLD)		5.	The endotoxin content of the additive and its dusting potential shall ensure a maximal endotoxin exposure of 1 600 IU endotoxins/m ³ air.	
			— ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS)		6.	For users of the additive and premixture, feed business operators shall	
			— Regulation (EC)				

a Details of the analytical methods are available at the following address of the Reference Laboratory: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>

b Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).

c Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).

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			<p>No 152/2009^b (Annex III, F)</p> <p>For the quantification of isoleucine in compound feed and feed materials: —</p> <p>ion exchange chromatography coupled with post- column derivatisation and photometric detection (IEC- VIS)</p> <p>— Regulation (EC) No 152/2009 (Annex III, F)</p> <p>For the quantification of isoleucine in water: —</p> <p>ion exchange chromatography coupled with</p>			<p>establish operational procedures and organisational measures to address potential risks by inhalation. Where those risks cannot be eliminated or reduced to a minimum by such procedures and measures, the additive and premixure shall be used with personal protective equipment.</p>
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a Details of the analytical methods are available at the following address of the Reference Laboratory: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>

b Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).

c Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).

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				post-column derivatisation and photometric detection (IEC-VIS)				
3c383		L- isoleucine	<p><i>Additive composition</i> Powder with a minimum content of L- isoleucine of 90 %</p> <p><i>Characterisation of the active substance</i> L- isoleucine produced by fermentation with <i>Corynebacterium glutamicum</i> KCCM 80189 IUPAC name: (2S,3S)-2- amino-3- methylpentanoic acid Chemical formula: C₆H₁₃NO₂ CAS number: 73-32-5</p> <p><i>Analytical method^a</i></p>	All animal species			<p>1.</p> <p>2.</p>	<p>26 0000 be placed on the market and used as an additive consisting of a preparation. In the directions for use of the additive and premixture, the storage conditions, the stability to heat treatment and in water</p>

a Details of the analytical methods are available at the following address of the Reference Laboratory: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>

b Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).

c Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).

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			For the identification of L- isoleucine in the feed additive: —	Food Chemical Codex 'L- isoleucine monograph'			3.	shall be indicated. The additive may be used via water for drinking.
			For the quantification of isoleucine in the feed additive: —	ion exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS/FLD)			4.	Declaration to be made on the label of the additive and premixture: — 'The supplementation with L- isoleucine, in particular via water for drinking, shall take into account all essential and conditionally essential amino acids in
			—	ion exchange chromatography coupled with post-column derivatisation and photometric detection				
a	Details of the analytical methods are available at the following address of the Reference Laboratory: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports							
b	Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).							
c	Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).							

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			<p>(IEC-VIS)</p> <p>For the quantification of isoleucine in premixtures:</p> <p>— ion-exchange chromatography coupled with post-column derivatisation and optical detection</p> <p>(IEC-VIS/FLD)</p> <p>or</p> <p>— ion exchange chromatography coupled with post-column derivatisation and photometric detection</p> <p>(IEC-VIS)</p> <p>—</p> <p>Regulation (EC) No 152/2009 (Annex III, F)</p> <p>For the quantification of</p>					<p>order to avoid imbalances.’</p> <p>—</p> <p>L-isoleucine content.</p>
a	Details of the analytical methods are available at the following address of the Reference Laboratory: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports							
b	Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).							
c	Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).							

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		<p>isoleucine in compound feed and feed materials: —</p> <p>ion exchange chromatography coupled with post- column derivatisation and photometric detection (IEC- VIS)</p> <p>— Regulation (EC) No 152/2009 (Annex III, F)</p> <p>For the quantification of isoleucine in water: —</p> <p>ion exchange chromatography coupled with post- column derivatisation and photometric detection</p>				
a	Details of the analytical methods are available at the following address of the Reference Laboratory: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports					
b	Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).					
c	Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).					

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			(IEC-VIS)					
Category: Sensory additives. Functional group: Flavouring compounds								
3c381		L- isoleucine	<i>Additive composition</i> Powder with a minimum content of L-isoleucine of 93,4 % (on a dry matter basis)	All animal species				1. 26 0000000000 be placed on the market and used as an additive consisting of a preparation. The additive shall be incorporated into the feed in the form of a premixture. In the directions for use of the additive and the storage
			<i>Characterisation of the active substance</i> L-isoleucine) produced by fermentation with <i>Escherichia coli</i> FERM ABP-10641 IUPAC name: (2S,3S)-2-amino-3-methylpentanoic acid Chemical formula: C ₆ H ₁₃ NO ₂ CAS number: 73-32-5					2.
			<i>Analytical method^a</i>					3.
a	Details of the analytical methods are available at the following address of the Reference Laboratory: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports							
b	Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).							
c	Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).							

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			For the identification of L- isoleucine in the feed additive: —	Food Chemical Codex 'L- isoleucine monograph'		4.	conditions and the stability to heat treatment shall be indicated. On the label of the additive the following shall be indicated: 'Recommended maximum content of the active substance of complete feedingstuff with a moisture content of 12 %: 25 mg/kg.'
			For the quantification of isoleucine in the feed additive: —	ion exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS/FLD)			
			—	ion exchange chromatography coupled with post-column derivatisation and photometric detection		5.	The functional group, the identification number,
a	Details of the analytical methods are available at the following address of the Reference Laboratory: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports						
b	Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).						
c	Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).						

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			For the quantification of isoleucine in premixtures: — ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS/FLD) or — ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS) — Regulation (EC) No 152/2009 (Annex III, F))			the name and the added amount of the active substance shall be indicated on the label of the premixtures, if the following content of the active substance in complete feedingstuff with a moisture content of 12 % is exceeded: 25 mg/kg. The endotoxin content of the
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a Details of the analytical methods are available at the following address of the Reference Laboratory: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>

b Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).

c Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).

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								or reduced to a minimum by such procedures and measures, the additive and premixture shall be used with personal protective equipment.
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- a** Details of the analytical methods are available at the following address of the Reference Laboratory: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>
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- b** Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).
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- c** Exposure calculated based on the endotoxin level and the dusting potential of the additive according to the method used by EFSA (EFSA Journal 2020;18(2):6022); analytical method: European Pharmacopoeia 2.6.14. (bacterial endotoxins).
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- (1) [OJ L 268, 18.10.2003, p. 29.](#)
- (2) Commission Regulation (EU) No 348/2010 of 23 April 2010 concerning the authorisation of L-
isoleucine as a feed additive for all animal species ([OJ L 104, 24.4.2010, p. 29.](#)).
- (3) EFSA Journal 2020;18(2):6022.
- (4) EFSA Journal 2020;18(2):6021.

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