

## ANNEX

Identi- fication number of the additive	Name of the additive	Composition, chemical formula, description, analytical method	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	End of period of authorisa- tion
					mg/kg of complete feed with 12 % moisture content			
Category: nutritional additives. Functional group: amino acids, their salts and analogues								
3c353	L-histidine	<b>Additive composition</b> L-histidine ≥ 90 % (on a dry matter basis) and a maximum content of 100 ppm histamine Solid form  <b>Characterisation of the active substance</b> L-histidine produced with <i>Corynebacterium glutamicum</i> KCCM 80389 IUPAC name: (2 <i>S</i> )-2-amino-3-(1 <i>H</i> -imidazol-5-yl) propanoic acid Chemical formula: C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub> CAS number: 71-00-1  <b>Analytical method<sup>1</sup></b> For the determination of histidine in the feed additive: – Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS or IEC-VIS/FLD) or – High performance liquid chromatography coupled with	All animal species	-	-	-	<ol style="list-style-type: none"><li>1. In the directions for use of the additive and premixtures, the storage conditions, the stability to heat treatment and in water for drinking shall be indicated.</li><li>2. The additive may be used via water for drinking.</li><li>3. Feed business operators shall ensure that L-histidine is rumen protected, when fed to ruminants.</li><li>4. The moisture content shall be indicated on the label of the additive.</li><li>5. The label of the additive and premixtures shall indicate the following: ‘The supplementation with L-histidine, in particular via water for drinking, shall take into account all essential and conditionally essential amino acids in order to avoid imbalances.’</li></ol>	[10 years from the date of entry into force of this Regulation. To be completed by the OP]

<sup>1</sup> Details of the analytical methods are available at the following address of the Reference Laboratory: [https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports\\_en](https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en).

		<p>spectrophotometric detection (HPLC-UV)</p> <p>For the determination of histidine in premixtures and compound feed: – Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS) – Commission Regulation (EC) No 152/2009</p> <p>For the determination of histidine in water for drinking: – Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS)</p> <p>For the determination of histamine in the feed additive: – High performance liquid chromatography coupled with spectrophotometric detection (HPLC-UV)</p>					<p>6. For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address potential risks resulting from their use. Where those risks cannot be eliminated by such procedures and measures, the additive and premixtures shall be used with personal breathing protective equipment.</p>	
3c354	L-histidine monohydrochloride monohydrate	<p><b>Additive composition</b></p> <p>L-histidine monohydrochloride monohydrate ≥ 98 % with a minimum content of 72% histidine (on a dry matter basis) and a maximum content of 100 ppm histamine</p> <p>Solid form</p> <p><b>Characterisation of the active substance</b></p> <p>L-histidine monohydrochloride monohydrate produced with <i>Corynebacterium glutamicum</i> KCCM 80389</p> <p>IUPAC name: (2S)-2-amino-3-(1H-imidazol-5-yl) propanoic acid hydrate hydrochloride</p> <p>Chemical formula: C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>·HCl·H<sub>2</sub>O</p>	All animal species	-	-	-	<ol style="list-style-type: none"> <li>1. In the directions for use of the additive and premixtures, the storage conditions, the stability to heat treatment and in water for drinking shall be indicated.</li> <li>2. The additive may be used via water for drinking.</li> <li>3. Feed business operators shall ensure that L-histidine monohydrochloride monohydrate is rumen protected, when fed to ruminants.</li> <li>4. The moisture content shall be indicated on the label of the additive.</li> <li>5. The label of the additive and</li> </ol>	<i>[10 years from the date of entry into force of this Regulation. To be completed by the OP]</i>

		<p>CAS number: 5934-29-2</p> <p><b>Analytical method<sup>2</sup></b></p> <p>For the determination of histidine in the feed additive:</p> <ul style="list-style-type: none"> <li>– Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS or IEC-VIS/FLD) or</li> <li>– High performance liquid chromatography coupled with spectrophotometric detection (HPLC-UV)</li> </ul> <p>For the determination of histidine in premixtures and compound feed:</p> <ul style="list-style-type: none"> <li>– Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS) – Commission Regulation (EC) No 152/2009</li> </ul> <p>For the determination of histidine in water for drinking:</p> <ul style="list-style-type: none"> <li>– Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS)</li> </ul> <p>For the determination of histamine in the feed additive:</p> <ul style="list-style-type: none"> <li>– High performance liquid chromatography coupled with spectrophotometric detection (HPLC-UV)</li> </ul>					<p>premixtures shall indicate the following: ‘The supplementation with L-histidine monohydrochloride monohydrate, in particular via water for drinking, shall take into account all essential and conditionally essential amino acids in order to avoid imbalances.’</p> <p>6. For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address potential risks resulting from their use. Where those risks cannot be eliminated by such procedures and measures, the additive and premixtures shall be used with personal breathing protective equipment.</p>	
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<sup>2</sup> Details of the analytical methods are available at the following address of the Reference Laboratory: [https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports\\_en](https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en).

Identi- fication number of the additive	Additive	Composition, chemical formula, description, analytical method	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	End of period of authorisa- tion
					mg/kg of complete feed with 12 % moisture content			
Category: Sensory additives. Functional group: Flavouring compounds								
3c353	L-histidine	<b>Additive composition</b> L-histidine ≥ 90 % (on a dry matter basis) and a maximum content of 100 ppm histamine Solid form  <b>Characterisation of the active substance</b> L-histidine produced with <i>Corynebacterium glutamicum</i> KCCM 80389 IUPAC name: (2S)-2-amino-3-(1H- imidazol-5-yl) propanoic acid Chemical formula: C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub> CAS number: 71-00-1  <b>Analytical method<sup>3</sup></b> For the determination of histidine in the feed additive: – Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS or IEC- VIS/FLD) or – High performance liquid chromatography coupled with spectrophotometric detection (HPLC-UV)	All animal species	-	-	-	<ol style="list-style-type: none"><li>1. The additive shall be incorporated into the feed in the form of a premixture.</li><li>2. In the directions for use of the additive and premixtures, the storage conditions and the stability to heat treatment shall be indicated.</li><li>3. 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.’</li><li>4. The functional group, the identification number, the name and the added amount of the active substance shall be indicated on the label of the premixture where the use level on the label of the premixture would result in exceeding the content referred to in point 3.</li><li>5. The moisture content shall be indicated on the label of the</li></ol>	<i>[10 years from the date of entry into force of this Regulation. To be completed by the OP]</i>

<sup>3</sup> Details of the analytical methods are available at the following address of the Reference Laboratory: [https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports\\_en](https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en).

		<p>For the determination of histidine in premixtures:</p> <ul style="list-style-type: none"> <li>– Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS) – Commission Regulation (EC) No 152/2009</li> </ul> <p>For the determination of histamine in the feed additive:</p> <ul style="list-style-type: none"> <li>– High performance liquid chromatography coupled with spectrophotometric detection (HPLC-UV)</li> </ul>					<p>additive.</p> <p>6. For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address potential risks resulting from their use. Where those risks cannot be eliminated by such procedures and measures, the additive and premixtures shall be used with personal breathing protective equipment.</p>	
3c354	L-histidine monohydrochloride monohydrate	<p><b>Additive composition</b></p> <p>L-histidine monohydrochloride monohydrate <math>\geq 98</math> % with a minimum content of 72% histidine (on a dry matter basis) and a maximum content of 100 ppm histamine</p> <p>Solid form</p> <p><b>Characterisation of the active substance</b></p> <p>L-histidine monohydrochloride monohydrate produced with <i>Corynebacterium glutamicum</i> KCCM 80389</p> <p>IUPAC name: (2S)-2-amino-3-(1H-imidazol-5-yl) propanoic acid hydrate hydrochloride</p> <p>Chemical formula: <math>C_6H_9N_3O_2 \cdot HCl \cdot H_2O</math></p> <p>CAS number: 5934-29-2</p>	All animal species	-	-	-	<ol style="list-style-type: none"> <li>1. The additive shall be incorporated into the feed in the form of a premixture.</li> <li>2. In the directions for use of the additive and premixtures, the storage conditions and the stability to heat treatment shall be indicated.</li> <li>3. 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.’</li> <li>4. The functional group, the identification number, the name and the added amount of the active substance shall be</li> </ol>	<i>[10 years from the date of entry into force of this Regulation. To be completed by the OP]</i>

		<p><b>Analytical method<sup>4</sup></b></p> <p>For the determination of histidine in the feed additive:</p> <ul style="list-style-type: none"> <li>– Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS or IEC-VIS/FLD) or</li> <li>– High performance liquid chromatography coupled with spectrophotometric detection (HPLC-UV)</li> </ul> <p>For the determination of histidine in premixtures:</p> <ul style="list-style-type: none"> <li>– Ion-exchange chromatography coupled with post-column derivatisation and optical detection (IEC-VIS) – Commission Regulation (EC) No 152/2009</li> </ul> <p>For the determination of histamine in the feed additive:</p> <ul style="list-style-type: none"> <li>– High performance liquid chromatography coupled with spectrophotometric detection (HPLC-UV)</li> </ul>					<p>indicated on the label of the premixture where the use level on the label of the premixture would result in exceeding the content referred to in point 3.</p> <p>5. The moisture content shall be indicated on the label of the additive.</p> <p>6. For users of the additive and premixtures, feed business operators shall establish operational procedures and organisational measures to address potential risks resulting from their use. Where those risks cannot be eliminated by such procedures and measures, the additive and premixtures shall be used with personal breathing protective equipment.</p>	
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<sup>4</sup> Details of the analytical methods are available at the following address of the Reference Laboratory: [https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports\\_en](https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en).