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JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
**European Union Reference Laboratory for Feed Additives**

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# EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

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| Dossier related to: | FAD-2010-0260<br>CRL/ 100259 |
| Name of Feed Additive: | L-Tyrosine |
| Active Agent (s): | L-Tyrosine |
| Rapporteur Laboratory: | European Union Reference Laboratory for Feed Additives (EURL-FA)<br>Geel, Belgium |
| Report prepared by: | Stefano Bellorini (EURL-FA) |
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| Report approved by:<br>Date: | Christoph von Holst<br>06/05/2011 |

## EXECUTIVE SUMMARY

In the current application authorisation is sought for *L-Tyrosine* under Articles 4(1), category 'nutritional additives' and functional group 3(c) 'amino acids, their salts and analogues' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *L-Tyrosine* for all animal species and categories. The *feed additive* is intended to be mixed either in *premixtures* or added directly to complete *feedingstuffs*. The Applicant suggested no minimum or maximum *L-Tyrosine* concentrations in *premixtures* and *feedingstuffs*.

For the determination of the *active substance* in the *feed additive* the Applicant submitted a single laboratory validated and further verified method based on UV-visible spectrophotometry. Nevertheless, the EURL suggests the internationally recognised European Pharmacopoeia titrimetric method for which no performance characteristics are provided. However, the EURL considers this method suitable to determine *L-Tyrosine* in the *feed additive* within the frame of official control.

For the determination of *Tyrosine* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009). The method applies for the determination of *free* (synthetic and natural) and *total* (peptide-bound and free) amino acids, using an amino acid analyzer or High Performance Liquid Chromatography (HPLC) equipment. The performance characteristics reported for a variety of amino acids are:

- a relative standard deviation for *repeatability* ($RSD_r$) ranging from 0.9 to 7.0 %;
- a relative standard deviation for *reproducibility* ($RSD_R$) ranging from 4.1 to 23.3 %.

Based on the performance characteristics presented, the EURL recommends for official control, the ring-trial validated Community method based on ion exchange chromatography coupled with post-column derivatisation and photometric detection to determine *Tyrosine* in *premixtures* and *feedingstuffs*.

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

## KEYWORDS

*L-Tyrosine*, nutritional additives, amino acids, all animal species and categories.

## 1. BACKGROUND

*L-Tyrosine* is already authorised as *feed additive* as "sensory additive", functional group "flavouring compounds", under the category "natural or corresponding synthetic chemically defined flavouring" [1]. In the current application authorisation is sought for *L-Tyrosine* under Articles 4(1), category of 'nutritional additives' functional group 3(c) 'amino acids, their salts and analogues' according to Annex I of Regulation (EC) No 1831/2003 [2]. According to the Applicant, *L-Tyrosine* is produced by extraction from a natural source (i.e. keratin present in poultry feathers) [3]. The *feed additive* is a beige fine crystalline powder with a minimum purity of 95.0 % of the active substance [4, 5].

Specifically, authorisation is sought for the use of *L-Tyrosine* for all animal species and categories. The *feed additive* is intended to be mixed either in *premixtures* or added directly to complete *feedingstuffs* [6]. The Applicant suggested no minimum or maximum *L-Tyrosine* concentrations in *premixtures* and *feedingstuffs* [7].

## 2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application or group of applications. For this dossier, the methods of analysis submitted in connection with *L-Tyrosine*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

## 3. EVALUATION

### *Identification /Characterisation of the feed additive*

*Qualitative and quantitative composition of impurities in the additive*

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [8].

***Description of the analytical methods for the determination of the active substances in feed additive, premixtures and feedingstuffs.***

For the determination of *L-Tyrosine* in the *feed additive*, the Applicant proposed to apply a single laboratory validated and further verified method based on UV-visible spectrophotometry. The absorbance of an UV spectrum of a diluted HCl solution containing the *feed additive* is compared with the absorbance of a standard solution of *L-Tyrosine*. However, the Applicant mentioned that amino acid impurities present in the L-Tyrosine product may interfere with the observed spectrum [9]. Therefore, the EURL suggests instead the internationally recognised European Pharmacopoeia methods [10] prescribing:

- optical rotation and infrared absorption spectrophotometry methods (test A & B respectively) for the identification of *L-Tyrosine* in the *feed additive*, and

- titrimetry for the determination of *L-Tyrosine* in the *feed additive*.

No performance characteristics of these methods are provided. However, the EURL considers these methods suitable to determine *L-Tyrosine* in the *feed additive* in the frame of official control.

For the determination of *Tyrosine* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated Community method [11]. This method applies for the determination of *free* (synthetic and natural) and of *total* (peptide-bound and free) amino acids including *L-Tyrosine*, using an amino acid analyzer or High Performance Liquid Chromatography (HPLC) equipment. The method does not distinguish between the salts and the amino acid enantiomers.

The *free* amino acids are extracted with diluted hydrochloric acid. Co-extracted nitrogenous macromolecules are precipitated with sulfosalicylic acid and removed by filtration. The solution is filtered and adjusted to pH 2.2. The amino acids are separated by ion exchange chromatography and determined after post column derivatisation with ninhydrin by photometric detection at 570 nm.

The procedure chosen for the determination of the *total* amino acids depends on the amino acids under investigation. *Tyrosine* must be determined in hydrolysates of unoxidised samples. The sample is hydrolysed with hydrochloric acid (6 mol/l) for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by ion exchange chromatography and determined by post column derivatisation with ninhydrin and photometric detection at 570 nm.

The Community method [1] was ring trial validated for the determination of a variety of *amino acids* [2] (excluding *Tyrosine*) in *premixture*s and *feedingstuffs*. This method was further ring-trial validated by twenty-three laboratories, resulting in the CEN – EN ISO 13903:2005 method [12]. The following performance characteristics concerning the determination of total *amino acids* content were reported:

- a relative standard deviation for *repeatability* ($RSD_r$) ranging from 0.9 to 7.0 %;

- a relative standard deviation for *reproducibility* ($RSD_R$) ranging from 4.1 to 23.3 %.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated Community method, based on ion exchange chromatography coupled with post-column derivatisation and photometric detection to determine *Tyrosine* in *premixtures* and *feedingstuffs*.

Furthermore, the EURL would like to underline that an additional interlaboratory comparison of the above-mentioned ISO-CEN method is scheduled for 2011 to prove its applicability to high content products (e.g. *feed additive*). When the corresponding validation results will be published, the EURL will consider this method suitable for the quantification of *Tyrosine* in the *feed additive* in the frame of official control.

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) is not considered necessary.

## 4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

- the European Pharmacopoeia 6.0, method 01/2008-1161, using titration to determine *L-Tyrosine* in *feed additive*;
- the ring trial validated Community method, using ion exchange chromatography coupled to post column derivatisation and photometric detection, to determine *Tyrosine* in *premixtures* and *feedingstuffs*.

[1] The **scope** of the method includes the following amino acids: cyst(e)ine, methionine, lysine, threonine, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, phenylalanine, proline, serine, *tyrosine* and valine.

[2] **Validation data** of the method are available for the following amino acids: threonine, cyst(e)ine, methionine, lysine, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, phenylalanine, proline, serine and valine.

***Recommended text for the register entry (analytical method)***

For the determination of *L-Tyrosine* in *feed additive*:

- Titrimetry, European Pharmacopoeia (Ph. Eur. 6.0, method 01/2008-1161)

For the determination of *Tyrosine* in *premixtures* and *feedingstuffs*:

- ion exchange chromatography method with post-column derivatisation and photometric detection: Commission Regulation (EC) No 152/2009 (Annex III, F)

## 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *L-Tyrosine* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

## 6. REFERENCES

[1] Council Directive 70/524/EEC concerning additives in feedingstuffs – List of authorised additives in feedingstuffs (2004/C50/01)
[2] *Application/Ref:SANCO/D/2:Forw.Appl.1831/0154-2010
[3] *Technical dossier, Section II: 2.3.1 Active substance(s)/agent(s)
[4] *Technical dossier, Section II: 2.1.5 Physical state of each form of the product
[5] *Technical dossier, Section II: 2.2.1. Description
[6] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
[7] *Application, (Annex A), FAD-2010-0261_Conditions of use_ *L-Tyrosine*
[8] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories
[9] *Technical dossier, Section II: 2.6.1 Method of analysis for the active substances
[10] European Pharmacopoeia 6.0, method 01/2008-1161
[11] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, O.J. L 54, 26.02.2009
[12] Animal feeding stuffs – Determination of amino acids content (CEN - EN ISO 13903:2005)
*Refers to Dossier no: FAD-2010-0261

## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

## 8. ACKNOWLEDGEMENTS

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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Speyer (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
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