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CRL Evaluation Report on the Analytical Methods submitted in
connection with the Application for Authorised as Feed Additive
according to Regulation (EC) No 1831/2003

Dossier related to: FAD-2009-0036
CRL/090024

Name of Additive: Taminizer D

Active Substance(s): Dimethylglycine sodium salt

Rapporteur Laboratory: Community Reference Laboratory for
Feed Additives (CRL-FA)
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EXECUTIVE SUMMARY

Taminizer D is a product for which authorisation is sought as *feed additive* for chickens for fattening under the category 'zootechnical additives', functional group 'other zootechnical additives: performance enhancer'¹.

Taminizer D contains *dimethylglycine sodium salt* (DMG-Na) as active substance, at a minimum concentration of 97 % and the proposed recommended inclusion level of this product in complete feedingstuffs is 1 g/kg.

For the determination of DMG-Na in *feed additive* (purity) the applicant submitted an in-house validated method based on liquid chromatography with diode array detection.

For the determination of DMG-Na in the *premixtures*, the applicant proposed the same in-house validated method as for the *feed additive* with some slight modifications.

The methods were also verified by a second laboratory according to the CRL technical guide

The performance characteristics can be summarised as follows:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.45 to 0.53 % for the *feed additive* and ranging from 1.75 to 2.40 % for *premixtures*;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.45 to 0.57 % for the *feed additive* and ranging from 2.37 to 2.42 % for *premixtures*;
- a *recovery* rate ranging from 98.9 % to 100.8 % .

According to the applicant the method is applicable for the determination of the active substance in the range of 100 to 200 g/kg in *premixtures*.

For the quantitative determination of *DMG-Na* in *feedingstuffs* (broiler mash feed), the applicant submitted an in-house validated method based on capillary gas chromatography with pre-column derivatisation and flame ionisation detection. The method was verified by a second laboratory according to the CRL technical guide.

The reported performance characteristics were:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 1.26 to 1.76 %;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.88 to 3.95 %;
- a *recovery* rate ranging from 93 to 101 %;
- a *limit of quantification* (LOQ) of 0.103 g/kg;

¹ according to the classification system of Annex I of Regulation (EC) No 1831/2003.

- a *limit of detection* (LOD) of 0.006 g/kg.

According to the applicant the method is applicable for the determination of the active substance in the range of 0.10 to 1.50g/kg in feedingstuffs.

Based on the above mentioned performance characteristics, the CRL recommends for official control, the two in-house methods submitted by the applicant, DMG-Na in *feed additive*, *premixtures* and *feedingstuffs* within the concentration range indicated by the applicant.

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

Dimethylglycine, zootechnical additives, chickens for fattening

1. BACKGROUND

Taminizer D is product for which an authorisation as *feed additive* under the category 'zootechnical additives', functional group 'other zootechnical additives: performance enhancer' [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003 is sought.

The white micro-granules of *Taminizer D* contain minimum 97 % of dimethylglycine sodium salt (DMG-Na) as active agent, and maximum 3 % crystal bound water [2, 3].

In the current application submitted according to Article 4 (1) of Regulation (EC) No 1831/2003, the authorisation to be used for chickens for fattening at the recommended dose of 1 g/kg complete feed [1, 2] is sought.

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 Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Taminizer D* (EFSA-Q-2009-00881), 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, mercury and lead) are available at the respective Community Reference Laboratories (Commission Regulation (EC) No 776/2006).

Description of the analytical methods for the determination of the active substance in feed additive, premixtures and feedingstuffs

For the quantitative determination of DMG-Na in the *feed additive* (purity), the applicant submitted an in-house validated method based on liquid chromatography with diode array detection at 193 nm (with specific reference at 360 nm and bandwidth of 100 nm) [4, 5]. The feed additive sample is diluted in water and 1 g/kg betaine is added as internal standard. A calibration standard is prepared at the same time with a concentration of 1 g/kg dimethylglycine sodium salt and 1 g/kg internal standard. The calibration is based on bracket calibration, where a series of four injections is performed: after injection of the calibration standard, the sample solution is injected twice, followed by a second injection of the calibration standard. A mixture of acetonitrile and water 70/30 (% v/v) is used for the isocratic elution. The relative response factor for dimethylglycine sodium salt, which is the ratio of the response factor of the active substance to the response factor of the internal standard, is calculated as the mean relative response factor from the two injections of the calibration standard. The concentration of dimethylglycine sodium salt in the sample is derived from the peak areas of dimethylglycine sodium salt and of the internal standard in the chromatogram of the sample, the relative response factor calculated from the calibration standard and the dilution factor.

The method was in-house validated [6] and furthermore, a verification study by a second laboratory was performed [7], according to the CRL technical guide [8]. The performance characteristics are presented in Table 1. The method is applicable for the determination of total DMG in feed additives containing DMG-Na in a concentration range of 500 to 1000 g DMG-Na/Kg.

For the quantitative determination of DMG-Na in the *premixtures*, the applicant proposed the same method as for the *feed additive* with some slight modifications [4, 9]. DMG-Na is extracted in water from a 0.5 g sample of premixture and 1 g/kg betaine is added as internal standard. A calibration standard is prepared at the same time with a concentration of 0.7 g/kg dimethylglycine sodium salt and 1 g/kg internal standard. The target analyte is quantified applying the same principle as described for the feed additive method.

The method was in-house validated [10] and furthermore, a verification study by a second laboratory was performed [11], according to the CRL technical guide [8]. The performance characteristics are presented in Table 1. The method is applicable for the determination of total soluble DMG in premixtures with a concentration range 100 to 200 g DMG-Na salt /kg premixture.

Table 1 Performance characteristics of the analytical method for the determination of the *DMG-Na* in the *feed additive* and *premixtures*.

	RSD _r , % [9]		RSD _{ip} , % [9]		LOD g/kg [6]	LOQ g/kg [6]	Recovery rate % [6,7]
	Validation	Verification	Validation	Verification			
<i>Feed additive</i>	0.45	0.53	0.45	0.57	N/R	N/R	99.6 - 100
<i>Premixture</i>	2.40	1.75	2.42	2.37	N/R	N/R	98.9 – 100.8

RSD_r: relative standard deviation for *repeatability*; RSD_{ip}: relative standard deviation for *intermediate precision*
 LOD and LOQ: limit of detection and quantification; Target values of *DMG-Na* in the *feed additive*: 1000 g/kg, in *premixtures*: 150g/kg.

For the quantitative determination of *DMG-Na* in *feedingstuffs* (broiler mash feed), the applicant submitted an in-house validated method based on capillary gas chromatography with pre-column derivatisation and flame ionisation detection [4, 12]. *DMG-Na* is extracted in water. The supernatant is further extracted with hexane to remove residual fats and other interfering substances, whereas *DMG-Na* remains in the aqueous layer. Only a part of the aqueous phase is further treated. Diethylglycine sodium is added as internal standard. Subsequently an aliquote is taken from the aqueous phase, which is then completely evaporated by means of a nitrogen stream. After drying, N,O-bis(trimethylsilyl)trifluoroacetamide and dimethylformamide are added to produce a derivative of the target compound and the internal standard. Finally, the sample is injected in a gas chromatograph equipped with a split injector and flame ionisation detector. Calibration standards are prepared in a matrix, namely in extract of blank broiler mash feed. The results are calculated based on the internal standard calculation mode.

The method was in-house validated [13] and furthermore, a verification study by a second laboratory was performed [14], according to the CRL technical guide [8]. The performance characteristics are presented in Table 2. The method is applicable for the determination of total *DMG* in chicken feed with a concentration range 0.1 to 1.5 g *DMG-Na* salt /kg feed.

Table 2 Performance characteristics of the analytical method for the determination of the *DMG-Na* in *feedingstuffs*.

	RSD _r , % [9]		RSD _{ip} , % [9]		LOD g/kg [6]	LOQ g/kg [6]	Recovery rate % [6,7]
	Validation	Verification	Validation	Verification			
<i>Mash feed</i>	1.76	1.26	3.95	1.88	0.006	0.103	93 - 101

RSD_r: relative standard deviation for *repeatability*; RSD_{ip}: relative standard deviation for *intermediate precision*
 LOD and LOQ: limit of detection and quantification; Target values of *DMG-Na* in *chicken mash feed*: 1 g/kg.

4. CONCLUSIONS AND RECOMMENDATIONS

The CRL recommends for official control the two in-house validated methods submitted by the applicant, for the determination of *DMG-Na* in *feed additive*, *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.

Recommendations

HPLC-DAD method: Since the wavelength of the DAD detector is set at 193 nm, near the cut-off of acetonitrile, the CRL recommends the use UV grade quality solvents.

GC-FID method: When blank broiler mash feed is not available for the preparation of the calibration standards, the Multi Level Standard Addition technique should be used.

Recommended text for the register entry (analytical method)

For the determination of *DMG-Na* in *feed additive* and *premixtures*:

- liquid chromatography (HPLC) with diode array detection (DAD) detection at 193 nm

For the determination of *DMG-Na* in *feedingstuffs*:

- gas chromatography (GC) using pre-column derivatisation and flame ionisation detection (FID).

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Taminizer D* have been sent to the Community Reference Laboratory for Feed Additives.

The dossier has been made available to the CRL by EFSA.

6. REFERENCES

- [1] * Application, Reference SANCO/D/2 Forw. Appl. 1831/041-2009 and Annex I
 - [2] * Application, Proposal for Register Entry – Annex A
 - [3] * Technical dossier, Section II – 2.1.3 Qualitative and quantitative composition
 - [4] * Technical dossier, Section II – 2.6.1 Methods of analysis for the active substance
 - [5] * Technical dossier, Section II – Annex II 6a
 - [6] * Technical dossier, Section II – Annex II 6b
 - [7] * Technical dossier, Section II – Annex II 6c
 - [8] CRL-FA Technical Guide: Protocol for verification studies of single-laboratory/in-house validated methods
 - [9] * Technical dossier, Section II – Annex II 7a
 - [10] * Technical dossier, Section II – Annex II 7b
 - [11] * Technical dossier, Section II – Annex II 7c
 - [12] * Technical dossier, Section II – Annex II 8a
 - [13] * Technical dossier, Section II – Annex II 8b
 - [14] * Technical dossier, Section II – Annex II 8c
- * Refers to Dossier No. FAD-2009-0036

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Community 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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