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

JRC.D.5/FSQ/CvH/CMP/ag/ARES(2012)510214

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

Dossier related to:	FAD-2011-0036 CRL/110010
Name of Product:	Biostrong 510
Active Substance(s):	Thymol
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
Report prepared by:	Carlos Magno Pinto (EURL-FA)
Report revised by: Date:	Piotr Robouch and Dijana Mitic (EURL-FA) 24/04/2012
Report approved by: Date:	Christoph von Holst 25/04/2012

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *BIOSTRONG® 510* under the category/functional group 4(a)&(d) "zootechnical additives/digestibility enhancers & other zootechnical additives" according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for chickens and minor avian for fattening or rearing to point of lay. BIOSTRONG® *510* is a preparation of partially micro-encapsulated essential oils of thyme and star anise, with a guaranteed minimum content of the active substance (*Thymol*) of 2 g/kg in an excipient based on mixed dried herbs and spices, and other bulking and anti-caking agents. The *feed additive* is intended to be incorporated in complete or complementary *feedingstuffs* through *premixtures*. The Applicant proposed a dosage of 150 mg BIOSTRONG® *510* per kilogram *feedingstuffs*, which corresponds to 0.3 mg/kg of *Thymol* in *feedingstuffs*.

For the determination of *Thymol* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed a single-laboratory validated and further verified method based on gas chromatography-mass spectrometry (GC-MS). The following performance characteristics were reported:

- a standard deviation for *repeatability* (RSD_r) and for *intermediate precision* (RSD_{ip}) ranging from 1.4 to 9.2 %;
- a *recovery rate* (R_{rec}) ranging from 98.3 to 119%; and
- a limit of *quantification* (LOQ) of 21 µg/kg.

Based on these performance characteristics, the EURL recommends for official control the single-laboratory validated and further verified method based on gas chromatography- mass spectrometry (GC-MS) for the determination of *Thymol* in the *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.

KEYWORDS

BIOSTRONG® *510, Thymol,* zootechnical additives, other zootechnical additives, digestibility enhancers, chicken and other avian for fattening and rearing.

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new use) for *BIOSTRONG® 510* under the category/functional group 4(a)&(d) "zootechnical additives/digestibility enhancers & other zootechnical additives" according to Annex I of Regulation (EC) No 1831/2003 [1]. Authorisation is sought for the use of the *feed additive* for chickens and minor avian for fattening or rearing to point of lay [2].

According to the Applicant, BIOSTRONG® *510* is a preparation of partially micro-encapsulated essential oils of thyme and star anise with a guaranteed minimum content of the active substance (*Thymol*) of 2 g/kg in an excipient based on mixed dried herbs and spices, and other bulking and anti-caking agents [3]. The *feed additive* is intended to be incorporated in complete or complementary *feedingstuffs* through *premixtures*. The Applicant proposed a dosage of 150 mg BIOSTRONG® *510* per kilogram of *feedingstuffs*, which corresponds to 0.3 mg/kg of *Thymol* in *feedingstuffs* [4,5].

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 particular dossier, the methods of analysis submitted in connection with *BIOSTRONG® 510*, 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 [6].

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

For the determination of *Thymol* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed a single-laboratory validated [7,10] and further verified [8] method based on gas chromatography-mass spectrometry (GC-MS). The samples (33 mg for *feed additive*, 0.2 g for *premixtures*, or 1 g for *feedingstuffs*) are weighed in a vial and ethanol with biphenyl (used as internal standard) is added. *Thymol* is extracted by tempered ultrasound for 60 minutes at 50 °C. The sample is then centrifuged and filtrated. The resulting clear solution is then transferred into a GC-vial. *Thymol* is separated from other volatile components by gas chromatography with a retention time of 20.76 min and detected with a mass spectrometer [9].

Upon request by the EURL, the Applicant provided the detailed validation data that was absent from the original dossier [10]. The EURL recalculated the corresponding performance characteristics obtained in the frame of the validation study [11]. The performance characteristics derived from the validation [11] and verification [8] studies are reported in Table 1. Furthermore the Applicant reported a limit of detection and quantification (LOD and LOQ) of 6 and 21 µg/kg in *feedingstuffs*, respectively [7].

Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified method based on gas chromatography-mass spectrometry (GC-MS) for the determination of *Thymol* in the *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.

Table 1: Performance characteristics for the determination of *Thymol* in the *feed additive* (FA), *premixtures* (PM) and *feedingstuffs* (FS)

	Conc. (mg/kg)	RSD_r (%)		RSD_{ip} (%)		R_{rec} (%)	
		Validation* [11]	Verification [8]	Validation* [11]	Verification [8]	Validation [7]	Verification [8]
FA	3195-3408	2.2	1.4	2.2	2.3	98.3	99.0
PM	81.3-142.6	4.0	4.6	4.0	4.4	101	98.3
FS	0.33-0.42	9.2	3.7-4.3	9.2	6.4-7.0	--	101-119

RSD_r= standard deviation for repeatability; RSD_{ip} = standard deviation for intermediate precision;

R_{rec} = recovery rate; (*) Recalculated by the EURL

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation, the EURL recommends for official control the single-laboratory validated and further verified method based on gas chromatography-mass spectrometry (GC-MS) for the determination of *Thymol* in the *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the quantification of *Thymol* in the *feed additive*, *premixtures* and *feedingstuffs:*

- Gas chromatography-mass spectrometry (GC-MS)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *BIOSTRONG® 510* 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] *Application, Reference SANCO/G1: Forw. Appl. 1831/0082-2011

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II,

[4] *Technical dossier, Section II, 2.5.1. Proposed mode of use in animal nutrition

[5] * Technical Dossier, Section II,Annex_II_4_1_2.pdf

[6] 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

[7] *Technical Dossier, Section II, Annex_II.6.2

[8] *Technical Dossier, Section I,Annex_II.6.3

[9] * Technical Dossier, Section II, Annex_II.6.1

[10] *Supplementary information, Info by appl 19_03_12

[11] *Supplementary information, Biostrong-EURL-calculation

* Refers to Dossier No. FAD-2011-0036

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

The following National Reference Laboratories contributed to this report:

- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)