CRITERIA FOR THE QUANTIFICATION OF THE ACTIVE AGENT(S) COMPOSING A FEED ADDITIVE

Endorsed by the FEEDAP Panel during the 170th Plenary meeting of 14-16 November 2023[1]

According to Commission Regulation (EC) No 429/2008,[2] a feed additive must be fully identified and characterised, and "*the qualitative and quantitative batch to batch variation of the active substance(s)/agent(s) shall be determined*". Specifications of the additive in terms of concentration of the active substance(s)/agent(s)[3] should be set by the applicant and compliance with such specifications confirmed, following the requirements of Commission Regulation (EC) No 429/2008 and the Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017).

Evidence should be provided by the analysis of at least five independent production batches that those specifications are satisfied in practice (EFSA FEEDAP Panel, 2017). The number of viable cells or spores of the active agent expressed as Colony Forming Unit (CFU) per gram should be determined. This should be done using laboratory-based studies by means of appropriate criteria as reflected in recognised acceptable methods. These methods should ensure the specificity against contaminating microbiota possibly occurring in the sample.

In general, counts of the active agent should be established based on a cultivation method coupled with identification of isolates with molecular methods, which represent the most accurate source of information for the unambiguous identification of a strain. The following criteria should be followed when designing the method:

- The culture-based method used to grow the active agent should be the optimal one to detect the microorganism under assessment.
- For each batch, the enumeration of the microorganism should be performed in triplicate. The dilution resulting in 30 to 300 colonies on the plate should be considered and at least five colonies should be randomly selected[4] and subject to molecular identification at strain level.
- The active agent under assessment should be included as a positive control.

In case of additives containing more than one active agent, the following is required: "*If the additive is a mixture of active substances or agents, each of which is clearly definable (qualitatively and quantitatively), the active substances/agents must be described and the proportions in the mixture given*" (EFSA FEEDAP Panel, 2017). Therefore, each active agent composing the additive should be unambiguously quantified to i) reach a full characterisation of the product in terms of ratio among the active agents and ii) confirm compliance with the specifications set for the individual counts. In order to choose the proper methodology, the applicant should refer to the requirements described above and provide evidence that the method is capable to discriminate between the active agents present in the additive.

[1] Available at: https://www.efsa.europa.eu/sites/default/files/2023-12/feedap_231114-16_m.pdf
[2] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of application and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1–65.
[3] Any viable microorganism intended to be used as/in a feed additive that provides the intended effect
[4] In case of additives containing more active agents, at least five colonies should be randomly selected for each active agent.

References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J and Innocenti ML, 2017. Guidance on the identity, characterisation and conditions of use of feed additives. EFSA Journal 2017;15(10):5023, 12 pp. https://doi.org/10.2903/j.efsa.2017.5023