Safety and efficacy of the feed additive consisting of 6-phytase (produced by *Komagataella phaffii* CGMCC 7.19) (Nutrase P) for chickens for fattening, other poultry for fattening or reared for laying and ornamental birds (Nutrex N.V.)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fašmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Noël Dierick, Montserrat Anguita, Jaume Galobart, Jordi Ortuno and Elisa Pettenati

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of 6-phytase produced by a genetically modified strain of *Komagataella phaffii* (CGMCC 7.19) (Nutrase P) for chickens for fattening, other poultry for fattening or reared for laying and ornamental birds. The additive is available in solid (Nutrase PD 100,000, Nutrase PG 10,000 and Nutrase PTS 10,000) and liquid (Nutrase PL 10,000) forms. In 2020, the FEEDAP Panel issued an opinion on the safety and efficacy of the product and concluded that uncertainties remained on the presence of viable cells and recombinant DNA of the production strain in two of the formulations (Nutrase PL 10,000 and Nutrase PD 100,000). Moreover, the Panel could not conclude on the safety and the efficacy of the additive for the target species at the recommended level of 250 FTU/kg complete feed. The applicant provided supplementary information to address the limitations identified in that assessment and requested to increase the minimum recommended use level to 500 FTU/kg complete feed. No viable cells and recombinant DNA of the production strain were detected in Nutrase PL 10,000 and Nutrase PD 100,000. Therefore, the Panel concluded that the additive, in any formulations, does not pose any safety concern with regard to the production strain. Based on the results of a new tolerance trial, the Panel concluded that Nutrase P is safe for the target species. The additive in any formulation is safe for the consumers and the environment, but should be considered a respiratory sensitiser. Considering the two long-term trials in chickens for fattening previously evaluated and the new trial submitted, the Panel concluded that the additive is efficacious in chickens for fattening, other poultry species for fattening or reared for laying and ornamental birds at 1,500 FTU/kg complete feed.

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1. **Introduction**

1.1. **Background and Terms of Reference as provided by the requestor**

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 thereof defines the terms of the authorisation by the Commission.

The applicant Nutrex N.V. is seeking a Community authorisation of 6-phytase as a feed additive to be used as a zootechnical additive for chickens for fattening, ornamental birds and other poultry species for fattening and reared for laying (Table 1).

**Table 1:** Description of the additive

| Category of additive | Zootechnical additive |
|----------------------|-----------------------|
| Functional group of additive | Digestibility enhancers |
| Description | 6-phytase |
| Target animals | Chickens for fattening, ornamental birds and other poultry species for fattening and reared for laying |
| Applicant | Nutrex N.V. |
| Type of request | New opinion |

On 30 September 2020, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA), in its opinion on the safety and efficacy of the product, could not conclude on the safety and efficacy of 6-phytase as a feed additive for chickens for fattening, ornamental birds and other poultry species for fattening and reared for laying due to efficacy and safety of the additive.

The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. The new data have been received on 21 June 2021.

In view of the above, the Commission asks EFSA to deliver a new opinion on 6-phytase as a feed additive for chickens for fattening, ornamental birds and other poultry species for fattening and reared for laying, based on the supplementary data submitted by the applicant, in accordance with Article 29 (1)(a) of Regulation (EC) No 178/2002.

1.2. **Additional information**

The additive, with the trade name Nutrase P, is a preparation containing 6-phytase produced by a genetically modified strain of *Komagataella phaffii* (CGMCC 7.19).

The FEEDAP Panel adopted an opinion on this product in 2020 (EFSA FEEDAP Panel, 2020).

2. **Data and methodologies**

2.1. **Data**

The present assessment is based on data submitted by the applicant in the form of supplementary information to a previous application on the same product.¹

2.2. **Methodologies**

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of 6-phytase produced by *K. phaffii* CGMCC 7.19 (Nutrase P) is in line with the principles laid down in Regulation (EC) No 429/2008² and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a) and Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b).

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¹ FEED dossier reference: FAD-2019-0005.
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 applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
3. Assessment

The additive Nutrase P is an enzyme preparation with 6-phytase (IUBMB EC 3.1.3.26; phytase) as the active substance. The additive is available in solid (granulated and powder) and liquid formulations. The powder formulation (Nutrase PD) ensures a minimum phytase activity of 100,000 FTU/g of product, and the granulate (Nutrase PG), thermostable granulate (Nutrase PTS) and liquid (Nutrase PL) formulations ensure a minimum phytase activity of 10,000 FTU/g or mL of product. The additive is intended to be used as a zootechnical additive (functional group: digestibility enhancers) in feed for chickens for fattening, other poultry for fattening or reared for laying and ornamental birds.

The formulations of the additive and the production strain were characterised in a previous assessment (EFSA FEEDAP Panel, 2020). The 6-phytase is produced by a genetically modified strain of K. phaffii (CGMCC 7.19) four copies of an ampicillin resistance gene and one copy of a bleomycin resistance gene. The data previously submitted did not allow the Panel to exclude the presence of viable cells and DNA of the production strain in Nutrase PL 10,000 and Nutrase PD 100,000. Owing to this lack of information and considering that the production strain harbours antimicrobial resistance (AMR) genes, the Panel could not conclude on the safety aspects regarding the use of those two formulations of the additive for target species, consumers, users and the environment. In addition, the Panel could not conclude on the safety for the target species as the tolerance study in chickens for fattening submitted showed major limitations, nor on the efficacy of the additive at the minimum proposed use level of 250 FTU/kg.

The applicant has provided supplementary information to address the limitations regarding the potential presence of viable cells and recombinant DNA of the production strain in Nutrase PL 10,000 and Nutrase PD 100,000. Moreover, the applicant has requested for an increase of the minimum recommended use level in chickens for fattening, other poultry for fattening or reared for laying and ornamental birds from 250 to 500 FTU/kg feed and provided a new trial in chickens for fattening to support the safety and efficacy of the additive when added at 500 FTU/kg feed.

3.1. Presence of viable cells and DNA of the production strain in Nutrase PL 10,000 and Nutrase PD 100,000

The presence of viable cells of the production strain K. phaffii CGMCC 7.19 in Nutrase PL 10,000 and Nutrase PD 100,000 was investigated in three batches of the final formulations, each tested in triplicate. Positive and negative controls were included. No colonies were found in any of the samples tested. Therefore, it is concluded that no viable cells of the production strain were detected in Nutrase PL 10,000 or Nutrase PD 100,000.

The presence of recombinant DNA from the production strain K. phaffii CGMCC 7.19 was tested in three batches of Nutrase PL 10,000 and Nutrase PD 100,000, each tested in triplicate. The limit of detection in Nutrase PL 10,000 and Nutrase PD 100,000 samples spiked with genomic DNA of the production strain was 10 ng/mL and 1 ng/g, respectively. No recombinant DNA of the production strain was detected in either Nutrase PL 10,000 or Nutrase PD 100,000.

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3 Technical dossier FAD-2019-0005/Section II. Unit, one unit is defined as the amount of enzyme that releases 1 μmol of inorganic phosphate from phytate per minute at pH 5.5 and 37°C.
4 Technical dossier EFSA-Q-2021-00538/SIn_Annex 1.1 and SIn_Annex 1.2.
5 Technical dossier EFSA-Q-2021-00538/SIn_Annex 1.3 and SIn_Annex 1.4.
3.2. Safety

3.2.1. Safety of the production organism

The safety aspects of the genetic modification of the production strain have been previously assessed (EFSA FEEDAP Panel, 2020). In that opinion, considering that the production strain harboured AMR genes and uncertainties remained on the presence of viable cells and recombinant DNA of the production strain in Nutrase PL 10,000 (liquid) and Nutrase PD 100,000 (powder), the FEEDAP Panel could not conclude on the safety of those two formulations of the additive for the target species, consumer, user and environment.

The applicant has provided new data which demonstrated that viable cells and recombinant DNA of the production strain were not detected in Nutrase PL 10,000 and Nutrase PD 100,000. Therefore, the FEEDAP Panel concludes that the additive, in all its formulations, does not pose any safety concern with regard to the production strain.

3.2.2. Safety for the target species

The applicant provided a new tolerance study in chickens for fattening to support the safety of the additive for the target animals.

A total of 792 1-day-old female chickens for fattening (Ross 308) were distributed in 36 pens and allocated to four dietary treatments (nine replicates per treatment). Two basal diets (starter and grower/finisher) based on maize and soybean meal, with a total phosphorus/calcium (P/Ca) content of 0.57/0.78% and 0.44/0.56%, respectively, were either not supplemented (control) or supplemented with Nutrase PTS 10,000 to provide 250 FTU/kg (0.5× minimum recommended dose) or 100,000 FTU/kg (200×) or with Nutrase PD 100,000 to provide 500 FTU/kg (1×) (confirmed by analysis). The experimental diets included an external marker and were offered on ad libitum basis as a crumble (starter) or pellets (grower/finisher feed). The birds were under study until day 42 of life. Animal health was checked daily, mortality and the most probable cause of death recorded, and the dead animals weighed. Feed intake and body weight were measured at days 1, 14 and 41, and average daily feed intake, average daily gain and feed to gain ratio were calculated and corrected for mortality.

Data were analysed by one-way analysis of variance (ANOVA) considering the treatment as the effect and the pen as the experimental unit. Group means were compared with Tukey HSD test. Significance level was set at \( p \leq 0.05 \).

Mortality including culling was \( \leq 1\% \) in all treatments. The results are presented in Table 1 (see Section 3.3.1). The results showed no adverse effects on the performance of chickens for fattening from feeding the additive up to 200-fold the minimum recommended level.

3.2.3. Conclusions on safety for the target species

The results of the tolerance trial showed no adverse effects on the birds up to 200-fold the recommended dose.

Therefore, the FEEDAP Panel concludes that Nutrase P, in all its formulations, is safe for chickens for fattening at the recommended level of 500 FTU/kg feed with a margin of safety of 200. Based on this wide margin of safety, the conclusion reached in chickens for fattening is extrapolated to other poultry species for fattening or reared for laying and ornamental birds at the corresponding growing phases.

3.2.4. Safety for the consumers, users and the environment

In the previous opinion, no concerns were identified for consumers, users (other than respiratory sensitisation) and the environment linked to the product itself. However, due to the uncertainties regarding the presence of viable cells and recombinant DNA of the production strain in Nutrase PL 10,000 and Nutrase PD 100,000 formulations and the fact that the genetic modification introduced AMR genes in the production strain, the Panel could not conclude on the safety for the consumer, user and environment for these two formulations.

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6 Technical dossier EFSA-Q-2021-00538/SIn_Annex 2.1 and SIn_Annex 2.2.
7 Starter from day 1 to 14 and grower/finisher from day 15 to 42.
8 Technical dossier EFSA-Q-2021-00538/SIn_Annex 2.3.
The newly submitted data on the presence of viable cells and DNA in Nutrase PL 10,000 and Nutrase PD 100,000 formulations allow to conclude that there are no safety concerns as regard to the production strain for these formulations. Consequently, the Panel concludes that the additive, in any formulation, is safe for the consumers and the environment. The additive in any form is a respiratory sensitiser.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

In the previous opinion, the Panel evaluated two long-term trials in chickens for fattening which included measurements on the performance and on the phosphorus utilisation (EFSA FEEDAP Panel, 2020). In one of the trials (trial 1 in the previous opinion), the animals fed Nutrase P at 1,500 FTU/kg feed showed significantly higher phosphorus retention compared to those receiving the control diet. In the other trial (trial 2), a higher final body weight and body weight gain were found at a supplementation level of 250 FTU/kg feed, and a higher P retention at 250 FTU/kg feed and above.

The new tolerance/efficacy study in chickens for fattening described above (Section 3.2.2) evaluated the effect of the additive on the performance of the animals and included the study of the phosphorus retention and bone mineralisation in grower/finisher phase. From day 20 of life, samples of excreta were collected for 5 days and pooled per pen. Feed and excreta samples were analysed for P and Ca, and an external marker was used to calculate P and Ca retention. At day 42, two birds per pen were killed, the left tibia collected and analysed for dry matter, ash, P and Ca content. The results on the performance, P retention and bone mineralisation are shown in Table 2.

Table 2: Effects of Nutrase P on the performance, phosphorus retention and bone mineralisation in chickens for fattening

| Groups (FTU/kg feed) | Daily feed intake (g) | Final body weight (g) | Feed to gain ratio | P retention (%) | Bone mineralisation |
|----------------------|-----------------------|-----------------------|--------------------|-----------------|------------------|
| Intended Analysed (Grower/Finisher phase) | Daily feed intake (g) | Final body weight (g) | Feed to gain ratio | P retention (%) | Bone mineralisation |
| 0 | 16/218 | 88.6b | 2,353b | 1.57 | 48.3c | 49.3b | 17.7b |
| 250 | 286/254 | 92.8a | 2,419ab | 1.61 | 54.0b | 52.4a | 18.3a |
| 500 | 412/451 | 93.7a | 2,472ab | 1.58 | 57.6b | 53.7a | 18.4a |
| 2100,000 | 93,900/89,800 | 94.5a | 2,509a | 1.57 | 64.5a | 53.0a | 18.4a |

a,b: Mean values within a column with a different superscript are significantly different \( p < 0.05 \).

Significant and relevant effects on the performance of the birds were seen only in the final body weight at the level of 100,000 FTU/kg feed. The birds receiving the phytase under assessment at the minimum recommended level (500 FTU/kg) showed higher P retention, higher tibia ash and P content compared to the control.

3.3.2. Conclusions on efficacy

The results from two trials evaluated in the previous opinion showed positive effects in P retention at 250 or 1,500 FTU/kg feed. The results in the newly submitted study showed higher phosphorus retention and bone mineralisation at 500 FTU/kg feed and above. Therefore, the FEEDAP concludes that Nutrase P is efficacious in chickens for fattening at 1,500 FTU/kg complete feed.

The mode of action of the phytase is well known and can be considered to be similar in all poultry/avian species. Therefore, the conclusions drawn in chickens for fattening can be extrapolated to other poultry species for fattening or reared for laying and ornamental birds at the corresponding growing phases.

4. Conclusions

No viable cells and recombinant DNA of the production strain were detected in Nutrase PL 10,000 and Nutrase PD 100,000. The additive, in all its formulations, does not pose any safety concern with regard to the production strain.
Nutrase P is considered safe for chickens for fattening, other poultry species for fattening, reared for laying and ornamental birds. The Panel concludes that the additive, in any formulation, is safe for the consumers and the environment. The additive in any form is a respiratory sensitisier.

The additive is efficacious as a zootechnical additive in chickens for fattening, other poultry species for fattening or reared for laying and ornamental birds at 1,500 FTU/kg complete feed.

5. **Documentation provided to EFSA/Chronology**

| Date       | Event                                                                 |
|------------|----------------------------------------------------------------------|
| 23/06/2021 | Dossier received by EFSA. 6-phytase (Nutrase P) for chickens for fattening, other poultry for fattening, reared for laying and ornamental birds. Submitted by Nutrex N.V. |
| 16/08/2021 | Reception mandate from the European Commission                           |
| 01/10/2021 | Application validated by EFSA – Start of the scientific assessment      |
| 17/12/2021 | Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. Issues: characterisation, target species safety, efficacy |
| 17/01/2022 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 16/02/2022 | Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. Issues: characterisation |
| 28/02/2022 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 15/03/2022 | Request of supplementary information to the applicant in line with Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. Issues: target species safety, efficacy |
| 01/04/2022 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 04/05/2022 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment |

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**Abbreviations**

- ADFI average daily feed intake
- ADG average daily gain
AMR  antimicrobial resistance
ANOVA  analysis of variance
BW  body weight
CGMCC  China General microbiological Culture Collection Centre
DM  dry matter
FEEDAP  EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD  limit of detection
IUBMB  International Union of Biochemistry and Molecular Biology