European Food Safety Authority

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SCIENTIFIC OPINION

Modification of terms of authorisation of Natuphos® (3-phytase) as feed additive for pigs for fattening[1]

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2008-692)

Adopted on 7 July 2009

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the modification of terms of authorisation of Natuphos® for pigs for fattening. The additive Natuphos® is a preparation of 3-phytase produced by a genetically modified strain of *Aspergillus niger*.

The existing authorisation establishes a minimum recommended dose for pigs for fattening of 280 FTU kg^{-1} feed. With the present application the applicant requests to lower the minimum recommended dose to 100 FTU kg^{-1}.

In three digestibility studies made with animals of body weights of 40, 70 and 100 kg, significant improvements of faecal P digestibility were observed with the supplementation of Natuphos® at 100 FTU kg^{-1}.

The FEEDAP Panel concludes that there is sufficient evidence to support the efficacy of Natuphos® in pigs for fattening at the lower minimum recommended dose of 100 FTU kg^{-1}.

Key words: zootechnical additive, digestibility enhancer, enzyme, 3-phytase, efficacy, pigs for fattening

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BACKGROUND

Regulation (EC) No 1831/2003[2] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States.

The European Commission received a proposal from the company BASF SE[3] for a modification of the existing authorisation on Natuphos when used as feed additive in pigs for fattening.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 13(3) (modification of the authorisation of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[4] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 25 February 2009.

The additive Natuphos is a preparation of 3-phytase (EC 3.1.3.8) produced by *Aspergillus niger* (CBS 101.672) that it is presented in three forms. This product is authorised (4a 1600) for use in piglets, pigs for fattening and chickens for fattening (until 2 april 2017),[5] laying hens and turkeys for fattening (until 22 October 2017),[6] ducks (until 14 March 2018)[7] and sows (until 27 June 2018).[8]

The Scientific Committee on Animal Nutrition (SCAN) issued two opinions on the efficacy and safety of this enzyme preparation: one for piglets, pigs for fattening, sows, chickens for fattening and laying hens (SCAN 2000), and the other for the same target species and the user, consumer and environment (SCAN 2002). EFSA issued an opinion on the safety and efficacy of the enzyme preparation when the production strain was modified and for the use of new liquid and solid formulations with a double concentration compared to that of the preparation previously authorised (EFSA 2006); this opinion included the assessment of the safety for the target species, for the consumer, the user and the environment, as well as the safety aspects of the genetic modification. EFSA also issued opinions on the safety of the enzyme preparation for laying hens and turkeys for fattening (EFSA, 2007a) and for sows (EFSA, 2007c), and on the efficacy and safety of the product as a feed additive for ducks (EFSA, 2007b).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the efficacy and the safety for the target animals, user and consumer and the

[2] OJ L 268, 18.10.2003, p.29
[3] BASF SE, 67056 Ludwigshafen/Rhein, Germany
[4] Dossier reference: FAD-2008-0043
[5] OJ L 73, 13.3.2007, p.4
[6] OJ L 256, 2.10.2007, p.20
[7] OJ L 50, 23.2.2008, p.8
[8] OJ L 149, 7.6.2008, p.33

environment of the product Natuphos, 3-phytase (EC 3.1.3.8), when used under the conditions described in Table 1. Taking into consideration that this product has been assessed recently by EFSA (efficacy and safety for the target species, safety for the consumer, the user and the environment with a favourable outcome), the present assessment shall only focus on the proposed modification of the authorisation, i.e., the change in the lowest recommended dose for pigs for fattening from 280 to 100 FTU kg^{-1} complete feed.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Enzymes, Friedrich Schöne and Pieter Wester for the preparation of this opinion.

Table 1. **Description and conditions of use of the additive as proposed by the applicant**

Additive	3-phytase
Registration number/EC No/No (if appropriate)	4a 1600
Category of additive	Zootechnical
Functional group of additive	Digestibility enhancer

Description			
Composition, description	**Chemical formula**	**Purity criteria (if appropriate)**	**Method of analysis (if appropriate)**
3-phytase EC 3.1.3.8	Additive composition: Preparation 3-phytase produced by Aspergillus niger (CBS 101.672) having a minimum activity of: Solid form: 5000-10000 FTU[9] g^{-1} Liquid form: 5000-10000 FTU ml^{-1}		Analytical method[(2)]: Colorimetric method measuring inorganic phosphate released by the enzyme from phytate substrate [(2)] Details of the analytical method are available at the following addresss of the Community Reference Laboratory: www.irmm.jrc.be/html/crlfaa/

Trade name (if appropriate)	Natuphos®
Name of the holder of authorisation (if appropriate)	BASF SE, 67056 Ludwigshafen/Germany

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period (if appropriate)**
		Units kg^{-1} of complete feedingstuffs		
Pigs for fattening		100 FTU	-	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	1. In the directions of use of the additive and premixture, indicate the storage temperature, storage life and stability to pelleting. 2. Recommended dose per kilogram of complete feedingstuff: Pigs for fattening: 100 FTU 3. For use in compound feed containing more than 0.23% phytin bound phosphorus
Specific conditions or restrictions for handling (if appropriate)	Not appropriate

[9] 1 FTU is the amount of enzyme which liberates 1 micromole of inorganic phosphate per minute from sodium phytate at ph 5.5 and 37 °C

Post market monitoring (if appropriate)	BASF has a general traceability system and a complaint procedure in place. An emergency telephone number is printed on each label
Specific conditions for use in complementary feedingstuffs (if appropriate)	Not appropriate

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**
-	-	-	-

ASSESSMENT

1. Introduction

The additive Natuphos® is a preparation of 3-phytase (EC 3.1.3.8) produced by a genetically modified strain of *Aspergillus niger* (CBS 101.672, NPH54), presented in solid (granulated, G and powder) and liquid (L) forms. The three forms are presented with a concentration of 5000 FTU g^{-1} or mL^{-1} and the granulated and the liquid forms are also presented with a concentration of 10000 FTU g^{-1} or mL^{-1}, respectively. This product is already authorised for weaned piglets, pigs for fattening, sows, chickens and turkeys for fattening, laying hens and ducks. The safety of this enzyme preparation for the target species, the consumer, the user and the environment, including the safety assessment of the genetic modification, as well as its efficacy, have been the subject of several opinions from EFSA (see background). The current authorisation for pigs for fattening establishes a minimum recommended dose of 280 FTU kg^{-1}. The applicant is now asking to lower the minimum recommended dose to 100 FTU kg^{-1}. Therefore, the present opinion will deal only with efficacy aspects for pigs for fattening.

2. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the CRL report can be found in the Appendix.

3. Efficacy for pigs for fattening

In order to support the efficacy of Natuphos® in pigs for fattening, the applicant has studied the digestibility of P in three different stages of the growing-finishing period (40, 70 and 100 kg) in two different trials. All trials were carried out in the same place.

In trial 1,[10] 48 female crossbred pigs with an initial body weight of 30 kg were used for digestibility measurements when they reached 40 and 70 kg (two digestibility studies). In trial 2,[11] 40 crossbred pigs (24 male/16 female) with an initial body weight of 85 kg were used for digestibility measurements when they reached 100 kg. In both trials, the pigs were individually penned. The pigs received a basal diet based on maize, maize starch and soybean meal with some differences in each of the periods. The basal diets were supplemented with Natuphos® (5000 G) at 0, 100, 250, 500 FTU kg^{-1} (confirmed by analysis) and contained titanium dioxide as inert marker. Feeding was *ad libitum*. Levels of total and phytate P were 2.90/1.80, 3.28/1.97 and 2.81/1.40 g kg^{-1} for the periods ending at the weights of 40, 70 and 100 kg, respectively. For each period, the animals were fed the diets for ten days of adaptation and faecal samples were obtained twice per day for seven consecutive days. In trial 1, the animals were returned to normal diets containing adequate P levels for the period between the two experimental periods (20 days).

The results on organic matter, P and Ca digestibility for the three periods are shown in Table 2.

[10] Technical dossier/Section IV/Register 13
[11] Technical dossier/Section IV/Register 14

Table 2. **Effect of Natuphos® (5000 G) on the apparent faecal digestibility (%) of organic matter, P and Ca in growing and finishing pigs**

Natuphos® (FTU kg^{-1})	Organic Matter			P			Ca		
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
0	89^{b}	88	86	28^{d}	39^{d}	25^{b}	62^{b}	50^{b}	48
100	90^{a}	89	87	44^{c}	47^{c}	35^{a}	70^{a}	58^{a}	51
250	90^{a}	88	86	52^{b}	53^{b}	39^{a}	72^{a}	58^{a}	51
500	90^{a}	88	86	58^{a}	57^{a}	41^{a}	70^{a}	56^{ab}	48

$^{a, b, c, d}$: Means within the same column with a different superscript are different at least at $P < 0.01$

Faecal digestibility of P was improved in the three periods studied and Ca was improved in the first two (40 and 70 kg) when pigs received diets supplemented with Natuphos® at 100 FTU kg^{-1}.

CONCLUSIONS

Faecal digestibility of P was improved in three different stages of the growing-finishing period (at weights of 40, 70 and 100 kg) with the supplementation of Natuphos® at 100 FTU kg^{-1}. Therefore, the FEEDAP Panel concludes that there is sufficient evidence to support the efficacy of Natuphos®, in all formulations, in pigs for fattening at the lower minimum recommended dose of 100 FTU kg^{-1}.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Natuphos® for pigs for fattening. June 2008. Submitted by BASF SE.
2. Evaluation report of the Community Reference Laboratory for Feed Additives on the method of analysis for Natuphos® for pigs for fattening.

REFERENCES

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on Additives and Products or Substance used in Animal Feed and of the Scientific Panel on Genetically Modified Organism on the safety and efficacy of the enzyme preparation Natuphos® (3-phytase) produced by *Aspergillus niger*. *The EFSA Journal* (2006) 369, 1–19.
< http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620768985.htm >

EFSA (European Food Safety Authority), 2007a. Opinion of the Scientific Panel on Additives and Products or Substance used in Animal Feed on the safety of the enzyme preparation Natuphos® (3-phytase) produced by *Aspergillus niger* for laying hens and turkeys for fattening. *The EFSA Journal* (2007) 472, 1–4.
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EFSA (European Food Safety Authority), 2007b. Opinion of the Scientific Panel on Additives and Products or Substance used in Animal Feed on the safety and efficacy of the enzyme preparation Natuphos® (3-phytase) as feed additive for ducks. *The EFSA Journal* (2007) 544, 1–10.
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EFSA (European Food Safety Authority), 2007c. Opinion of the Scientific Panel on Additives and Products or Substance used in Animal Feed on the safety of the enzymatic preparation Natuphos® (3-phytase) for sows. *The EFSA Journal* (2007) 614, 1–5. < http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178675904916.htm >

SCAN (Scientific Committee on Animal Nutrition) 2000. Opinion of the Scientific Committee on Animal Nutrition on the use of enzymatic product Natuphos® 5000 (3-phytase; EC 3.1.3.8) as feed additive. < http://ec.europa.eu/food/fs/sc/scan/out44_en.pdf >

SCAN (Scientific Committee on Animal Nutrition) 2002. Opinion of the Scientific Committee on Animal Nutrition on the use of certain enzymes in animal feedingstuffs. < http://ec.europa.eu/food/fs/sc/scan/out96_en.pdf >

APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for Natuphos® for pigs for fattening

The current application authorisation is sought for 3-phytase (Natuphos) under the category 'zootechnical additives', group 4(a), digestibility enhancer for pigs for fattening according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for 3-phytase according to Article 13(3) of Regulation (EC) No 1831/2003.

The active agent of Natuphos is 3-phytase produced by submerged fermentation of Aspergillus niger (CBS 101.672). One FTU-unit is the amount of 3-phytase which liberates one µmol of inorganic phosphate per minute from sodium phytate at pH = 5.5 and 37 °C. The product is intended to be marketed in three forms, as powder (Natuphos 5000), as granulate (Natuphos 5000 G and 10000 G) and as liquid formulation (Natuphos 5000 L and 10000 L), containing, either 5000 or 10000 FTU/g or ml of product. These products are intended to be incorporated into premixtures and/or complete feedingstuffs to obtain an enzyme activity level of minimum 100 FTU/kg in complete feedingstuffs for pigs for fattening.

For the determination of 3-phytase activity in feed additives, premixtures and feedingstuffs the applicant proposes a colorimetric method measuring inorganic phosphate released by 3-phytase from phytate substrate at pH = 5.5 and 37 °C. The released inorganic phosphate forms a yellow complex with an acidic molybdate/vanadate reagent, which is measured at 415 nm and quantified against a standard curve of phosphate for feed additives; and is based on a reference enzyme Natuphos, available from the applicant, for premixtures and feedingstuffs.

The applicant submitted the validation data regarding the analytical method determining 3-phytase activity in feed additives, premixtures and feedingstuffs, which were obtained from collaborative studies organised by the Association of German Agricultural Analytical and Research Institutes (VDLUFA).

For the determination of 3-phytase activity in feed additives the following performance characteristics were reported: - a relative standard deviation for repeatability (RSDr) of 2.5 %, - a relative standard deviation for reproducibility (RSDR) of 4.9 % and - recovery rate (RR) ranging from 98 to 102 %. Based on these acceptable performance characteristics the proposed method is considered suitable for determination of 3-phytase activity in feed additives for pigs for fattening for official control purposes in the frame of authorisation.

For the determination of 3-phytase activity in premixtures the method performance characteristics obtained from feedingstuffs are applicable to the premixture samples which are diluted with ground corn meal and therefore behave as a matrix of feedingstuffs.

For the determination of 3-phytase activity in feedingstuffs the performance characteristics of the VDLUFA method, obtained at 600 U/kg enzymatic activity, were: - a limit of detection (LOD) of 45 FTU/kg of feedingstuffs, - a limit of quantification (LOQ) of 90 FTU/kg of feedingstuffs, - a RSDr ranging from 6.4 to 7.0 %, - a RSDR ranging from 11.1 to 12.3 % and RR ranging from 98 to 103 %. Upon request from the CRL the applicant provided additional precision data obtained at lower enzyme activity levels close to the minimum level in feedingstuffs (100 FTU/kg). For enzyme activity levels between 100 and 500 FTU/kg of feedingstuffs the reported relative standard deviation for intermediate precision (RSD) ranges from 11.8 to 15.2 %.

Several other ring trial validated methods for the determination of phytase activity in feedingstuffs exist. These include a colorimetric method which is developed by FEFANA (European Association of Feed Additive Manufacturers) and validated on various phytase products (including Natuphos products). The method is currently under evaluation to become a standard of the European Committee for Standardisation (CEN) and is very similar to the above mentioned VDLUFA method, but the quantification is based on the use of phosphate standard curve. The validation of draft CEN method included a phytase activity level ranging from 700 to 1500 U/kg of feedingstuffs, whereas the validation range of VDLUFA method was extended to cover the low phytase activities ranging from 100 to 500 FTU/kg of feedingstuffs. Therefore, the proposed VDLUFA method is found suitable for official controls for the determination of phytase activity at minimum proposed level (100 FTU/kg of feedingstuffs) in the frame of present authorisation.

Based on acceptable performance characteristics the applicant proposed VDLUFA method is recommended for official control purpose for the determination of 3-phytase activity in premixtures and feedingstuffs.

Further testing or validation is not considered necessary.