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Safety and efficacy of Natugrain® TS (endo-1,4-β-xylanase and endo-1,4-β-glucanase) for chickens for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

The additive is a preparation of endo-1,4-β-xylanase and endo-1,4-β-glucanase produced by two genetically modified strains of *Aspergillus niger*. It is authorised for use in piglets (weaned) and pigs for fattening, poultry species and ornamental birds as a zootechnical additive, functional group of digestibility enhancers. The applicant is now seeking for the authorisation of the product as a feed additive for chickens for fattening under the functional group of other zootechnical additives in order to positively affect the welfare of the animals. Aspects other than the efficacy of the product under the new functional group have been addressed in previous opinions and the new proposed use would not change the previous conclusions. The dossier submitted also presented new data regarding the safety for the user. Therefore, the Panel assessed the data on the safety for the user and the data supporting the efficacy of the additive under the new functional group. The Panel concluded that the additive is a dermal sensitiser. Two efficacy trials were considered in the assessment; the addition of Natugrain® TS at the recommended dose in the diets reduced the lesions on the foot-pad and on the hock of chickens for fattening on day 42 of life in the two trials. However, considering the limitations of the studies regarding the type of diets used and the fact that three studies are required to demonstrate the efficacy of the additive, the Panel could not conclude on the efficacy of Natugrain® TS in chickens for fattening as a zootechnical additive, functional group of other zootechnical additives.

Keywords: zootechnical additive, other zootechnical additives, positively affects welfare, xylanase, glucanase, chickens

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Summary

Following a request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Natugrain® TS (endo-1,4-β-xylanase and endo-1,4-β-glucanase) for chickens for fattening as a zootechnical additive, functional group of other zootechnical additives.

The additive contains endo-1,4-β-xylanase and endo-1,4-β-glucanase, which are produced by two genetically modified strains of *Aspergillus niger*. The product is available in solid (Natugrain® TS) and liquid (Natugrain® TS L) forms and is authorised for use in piglets (weaned) and pigs for fattening, poultry species and ornamental birds as a zootechnical additive, functional group of digestibility enhancers. The European Food Safety Authority (EFSA) issued an opinion on the safety and efficacy of this enzyme preparation when used in piglets (weaned), chickens for fattening, laying hens, turkeys for fattening and ducks for fattening, including the assessment of the safety for the consumer, the user and the environment, as well as the safety aspects of the genetic modification. Three further opinions on the use of this product were issued by EFSA: one on the use in other avian species, one regarding its use in pigs for fattening and another one on the efficacy in laying hens. All these opinions dealt with the use of this additive as a zootechnical additive, functional group of digestibility enhancers.

The applicant is now seeking for the authorisation of the product as a feed additive for chickens for fattening as a zootechnical additive under the functional group of other zootechnical additives (favourably affects animal welfare). The additive is to be used at the dose range of 560–840 TXU and 250–375 TGU per kg feed in order to reduce foot-pad lesions in chickens for fattening. Aspects other than the efficacy under the new functional group have been addressed in the previous opinions and the Panel considered that the new proposed use would not change the previous conclusions. However, in the dossier submitted, there were new data on the safety for the user. Therefore, the Panel will assess the new data on the safety for the user and the efficacy of the product in chickens for fattening as a feed additive that favourably affects animal welfare.

Based on the results of two skin sensitisation studies, the Panel concludes that the additive is a dermal sensititser.

The applicant submitted a total of four long-term trials. In two of these trials, high mortalities/culling were registered and animals were medicated with antibiotics; due to this fact, the FEEDAP Panel did not consider these two studies in the assessment. In the other two trials submitted, the animals were under study for 42 days and the measurements performed included performance parameters of the birds, digesta viscosity, litter quality and moisture content and evaluation of the foot-pad and hock lesions. Results in the two trials showed that the additive permits to reduce the lesions on the foot-pad and on the hock of the birds when used in diets with a high content of wheat or wheat plus barley. The studies were carried out with diets containing high amounts of wheat or wheat plus barley in accordance with the conditions of use established by the applicant. These types of diets are known to have detrimental effects on the growth/general health of the birds and in commercial conditions such diets would only be offered to the birds if supplemented with non-starch polysaccharides (NSP) degrading enzymes such as the ones present in the additive. Consequently, the experimental design lacked of control diet that could be offered to the birds without the need of enzymes. The lack of this control did not permit the Panel to conclude on the effect of the enzyme in diets containing lower amounts of NSP.

Considering the limitations of the studies provided and the fact that three efficacy trials showing significant and positive effects on relevant parameters are needed in order to support the efficacy of a zootechnical additive, the Panel could not conclude on the efficacy of Natugrain® TS in chickens for fattening as a zootechnical additive, functional group of other zootechnical additives.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from BASF SE[2] for authorisation of the product Natugrain® TS, endo-1,4-β-xylanase and endo-1,4-β-glucanase, when used as a feed additive for chickens for fattening (category: zootechnical additive; functional group: other zootechnical additives).

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 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 5 June 2014.

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 safety for the target animals, consumer, user and the environment and on the efficacy of the product Natugrain® TS (endo-1,4-β-xylanase and endo-1,4-β-glucanase), when used under the proposed conditions of use (see Section 3).

1.2. Additional information

Natugrain® TS is a feed additive that contains endo-1,4-β-xylanase (EC 3.2.1.8; xylanase) and endo-1,4-β-glucanase (EC 3.2.1.4; glucanase), produced by two genetically modified strains of *Aspergillus niger* (CBS 109.713 and DSM 18404, respectively). The product is available in solid (Natugrain® TS) and liquid (Natugrain® TS L) forms. This additive is authorised for use in piglets (weaned) and pigs for fattening, chickens and turkeys for fattening, laying hens, ducks for fattening, chickens reared for laying, turkeys for breeding purposes, turkeys reared for breeding, other minor avian species (other than ducks for fattening) and ornamental birds as a zootechnical additive, functional group of digestibility enhancers.

The European Food Safety Authority (EFSA) issued an opinion on the safety and efficacy of this enzyme preparation when used in piglets (weaned), chickens for fattening, laying hens, turkeys for fattening and ducks for fattening, including the assessment of the safety for the consumer, the user and the environment, as well as the safety aspects of the genetic modification (EFSA, 2008). Three further opinions on the use of this product were issued by EFSA: one on the use in other avian species, one regarding its use in pigs for fattening and another one on the efficacy in laying hens (EFSA FEEDAP Panel, 2011a, 2013, 2014, respectively). All these opinions dealt with the use of this additive as a zootechnical additive, functional group of digestibility enhancers.

The applicant is now seeking the authorisation of the product as a feed additive for chickens for fattening as a zootechnical additive under the functional group of other zootechnical additives (favourably affects animal welfare). The additive is intended to be included in cereal-based diets in order to reduce foot-pad lesions. In chickens for fattening, contact dermatitis is commonly found on the breasts (breast blisters), hocks (hock burns) and feet (foot-pad dermatitis). In the initial phases of the dermatitis, discolouration of the skin can be seen; afterwards, dermal erosions may develop into ulcerations with inflammatory reactions of the subcutaneous tissue (Greene et al., 1985). The lesions may also become infected and permit the entrance of microorganisms that can spread elsewhere (Jensen et al., 1970) and cause, for instance, joint inflammations (Schulze Kersting, 1996). The lesions on the breast and hocks usually develop more slowly and are less frequent than foot-pad lesions (Stephenson et al., 1960), but show positive correlations (e.g. hock burns to foot-pad dermatitis, r = 0.76; Meluzzi et al., 2008). The presence of contact dermatitis and derived lesions is

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] BASF SE, G-ENL/R – F31, 68623 Lampertheim, Germany.

painful to the animals and may constitute a welfare issue. Reduction on the incidence/severity of such conditions may therefore determine an improvement on the welfare of the birds.

Several factors influence the presence of contact dermatitis. Factors linked to the animal (sex, body weight and strain), environmental and management factors and also dietary factors have been reported (reviewed by Sheperd and Fairchild, 2010). Among the environmental/management factors, an important one is the litter's moisture content which is influenced by several factors (type of material, ventilation, stock density, drinkers design and maintenance, diet, etc.). Increased moisture content may determine the development of foot-pad lesions especially when litter contains high moisture with sticky/viscous excreta. High levels of non-starch polysaccharides (NSP) in the diets, viscous substances, may increase the adherence of the bedding to the foot, therefore, the hydrolisis of such polysaccharides (e.g. by using enzymes) may permit to reduce the viscosity of the excreta and its adherence to foot.

2. Data and Methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of Natugrain® TS as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003 and the applicable EFSA guidance documents.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or peer-reviewed scientific papers to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of product/active substance is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b).

3. Assessment

The additive is to be used in feed for chickens for fattening at the minimum dose of 560 TXU and 250 TGU per kg feed in order to reduce foot-pad lesions in chickens for fattening. The maximum dose is 840 TXU and 375 TGU/kg feed (maximum recommended dose authorised). The additive is to be used in feed rich in NSP (mainly β-glucans and arabinoxylans) (e.g. containing more than 30% wheat, barley, rye and/or triticale). Aspects other than the efficacy under the new functional group have been addressed in the previous opinions (see Introduction) and the Panel considers that the new proposed use would not change the previous conclusions. However, in the dossier submitted, there were also new data on the safety for the user. Therefore, the Panel will assess the new data on the safety for the user and the efficacy of the product in chickens for fattening.

[3] FEED dossier reference: FAD-2014-0007.
[4] The full report is available on the EURL wesbite: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0034.pdf
[5] 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.

3.1. Safety for the user

In its previous opinion, based on acute dermal and eye irritation studies, the Panel concluded that the additive is not irritant to the eyes and skin. In the absence of specific studies, the Panel also concluded that 'the preparation must be considered as potential skin sensitiser'.

The applicant has now tested the skin sensitisation potential of each of the enzyme components in individual tests following OECD Guideline 406.[6] The test materials were considered to be sensitisers under the test conditions. Therefore, the FEEDAP Panel confirms its previous conclusion that the additive is not irritant to skin and eyes but must be considered a skin sensitiser.

3.2. Efficacy[7]

The applicant submitted four long-term trials. In two[8,9] of the trials, high mortality/culling were registered (12.1–13.5%) and the birds were treated with antibiotic during the study (3 or 4 days, respectively). Due to this fact, the FEEDAP Panel did not consider these two studies in the assessment.

The other two trials were performed in the same trial site, on the same dates and shared a common design.[10]

In each trial, a total of 360 one-day-old Ross 308 male chicks were penned in groups of 15 birds and allocated to three experimental treatments (representing eight replicates per treatment). Starter and grower diets were supplemented with Natugrain® TS to provide (xylanase/glucanase) 0/0, 280/125 or 560/250 TXU/TGU per kg feed. The enzyme activities were confirmed by analyses.[11] In trial 1, the diets were based on wheat and in trial 2, the diets were based on wheat and barley. Diets were provided *ad libitum* in pelleted form for 42 days. Health status was assessed daily, mortality and culls (including their weight) were recorded daily. Dead animals were subject to necropsy. Body weight of the birds (per pen) and feed intake were measured weekly and feed to gain ratio was calculated. On days 21 and 42, the quality of the litter was assessed visually by the same person and samples of litter material were collected from each pen in order to determine its dry matter content. On day 21, ileal digesta samples were collected and the viscosity of the supernatants was determined. On day 42, the foot-pad lesions and the hock burns were evaluated in six birds per pen. The foot-pad lesions and the hock burns were scored by persons on blind basis. An ANOVA was performed with the data obtained considering the pen as the experimental unit. Mean values were compared to the control diet using the Dunnett's t-test. Differences were considered significant at a level of at least $P < 0.05$.

Mortality and culling was low and not treatment related. In both studies, the supplementation with the additive at the recommended dose resulted in a significantly higher weight gain and in a better feed to gain ratio.

In trial one, the supplementation of Natugrain® TS at 560/250 TXU/TGU per kg feed resulted in a higher dry matter content of the litter on days 21 and 42. This effect was seen in trial 2 only on day 42 at the dose of 280/125 TXU/TGU per kg feed.

In trial 2, the additive reduced significantly the viscosity of the ileal contents on day 21 from the supplementation at 280/125 TXU/TGU per kg feed. No differences were found in trial one.

Animals receiving the additive had lower scores in the evaluation of foot-pad lesions and hock burns, at the dose of 560/250 TXU/TGU per kg feed in trial 1 and at the two dosages in trial 2.

Results in the two trials considered showed that the additive reduced the lesions on the foot-pad and on the hock of the birds when used in diets with a high content of wheat or wheat plus barley. The applicant established that the additive is to be used in feed rich in NSP (mainly β-glucans and arabinoxylans), e.g. containing more than 30% wheat, barley, rye and/or triticale. In the two trials, the basal diets used contained high amounts of wheat or wheat plus barley (reaching 60%), common

[6] Technical dossier/Section III/Annex III.13 and 14.
[7] This section has been amended following the confidentiality claims made by the applicant.
[8] Technical dossier/Section IV/Annex IV.11.
[9] Technical dossier/Supplementary information June 2015/Annex 6.
[10] Technical dossier/Section IV/Annex IV.9 and Annex IV.10 and Supplementary information June 2015.
[11] Technical dossier/Supplementary information June 2015/Annexes 1, 2, 4 and 5.

in European farming practices in contrast to other practices (e.g. USA) and therefore containing high amounts of (soluble/viscous) NSP. The studies were carried out in fact in accordance with the conditions of use established by the applicant. However, the Panel notes that in commercial conditions such diets would only be offered to the birds if supplemented with NSP degrading enzymes as the ones present in the additive. Indeed, use of such diets without NSP-ases would have detrimental effects on the growth/general health of the birds and the presence of viscous NSP would also increase the adherence of the bedding to the foot, favouring the onset of the lesions in the feet.

The Panel considers that the non-supplemented diet used in the two trials should be regarded as a negative control. The experimental design lacks, at least, a positive control diet that could be offered to the birds without the need of enzymes (i.e. with low levels of NSP). The lack of data on this type of diets do not permit the Panel to conclude on the effect of the enzyme in diets containing lower amounts of NSP (e.g. in diets containing mainly maize).

3.3. Conclusions on the efficacy

The addition of Natugrain® TS at the recommended dose in the diets reduced the lesions on the foot-pad and on the hock of chickens for fattening on day 42 of life in two trials. Considering the limitations of the studies provided and the fact that three efficacy trials showing significant and positive effects on relevant parameters are needed in order to support the efficacy of a zootechnical additive, the Panel cannot conclude on the efficacy of Natugrain® TS in chickens for fattening as a zootechnical additive, functional group of other zootechnical additives (favourably affects animal welfare).

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[12] and Good Manufacturing Practice.

4. Conclusions

Natugrain® TS is not an eye and dermal irritant but is a dermal sensitiser.

The efficacy of Natugrain® TS in chickens for fattening as an additive that favourably affects animal welfare cannot be established.

Documentation provided to EFSA

1. Natugrain® TS for chickens for fattening. April 2014. Submitted by BASF SE.
2. Natugrain® TS for chickens for fattening. Supplementary information. June 2015. Submitted by BASF SE.
3. Natugrain® TS for chickens for fattening. Supplementary information. November 2015. Submitted by BASF SE.
4. Comments from Member States received through the ScienceNet.

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[12] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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