Safety and efficacy of RONOZYME® WX (endo-1,4-β-xylanase) as a feed additive for laying hens

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Abstract

RONOZYME® WX is an additive that contains endo-1,4-β-xylanase which is authorised for use as a feed additive in poultry for fattening, weaned piglets and pigs for fattening. The applicant has requested for an extension of the use to laying hens. The Panel on additives and Products or Substances used in Animal Feed (FEEDAP) delivered in 2012 an opinion on the safety and efficacy of RONOZYME® WX when used as a feed additive for poultry, piglets (weaned) and pigs for fattening and another one in 2016 on the change of the production strain proposed by the applicant. In those opinions, the FEEDAP Panel concluded that the use of the product as a feed additive raises no concerns for consumer safety or for the environment. Considering the safety for the user, the Panel concluded that the additive is not a skin or eye irritant but could not conclude on its skin sensitisation potential; however, the additive was considered a potential respiratory sensitisier. The Panel considered that the new use of RONOZYME® WX would not modify those conclusions. The applicant submitted a tolerance study in laying hens the results of which permitted the Panel to conclude that the additive is safe for laying hens at the recommended dose of 100 FXU/kg feed. In order to support the efficacy of the additive in laying hens, the applicant submitted three long-term trials. In all three trials, the groups receiving the xylanase at the recommended dose showed a lower feed intake and a better feed to egg mass ratio. However, in one of the three trials, these reductions were seen concurrently with a decrease in the laying rate. This result casts doubts on the efficacy of the additive, and therefore, the Panel considered that there was not sufficient evidence to conclude on the efficacy of the product.

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Keywords: safety, efficacy, zootechnical additives, endo-1, 4-β-xylanase, laying hens

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Table of contents

Abstract...................................................................................................................................................... 1
1. Introduction.................................................................................................................................... 4
   1.1. Background and Terms of Reference................................................................................................. 4
   1.2. Additional information...................................................................................................................... 4
2. Data and methodologies.................................................................................................................. 4
   2.1. Data............................................................................................................................................... 4
   2.2. Methodologies................................................................................................................................. 4
3. Assessment..................................................................................................................................... 5
   3.1. Characterisation .............................................................................................................................. 5
   3.2. Safety ............................................................................................................................................ 5
   3.2.1. Safety for laying hens...................................................................................................................... 5
   3.2.1.1. Conclusions on the safety for laying hens.......................................................................................... 6
   3.3. Efficacy for laying hens.................................................................................................................... 6
   3.4. Post-market monitoring.................................................................................................................... 7
4. Conclusions..................................................................................................................................... 7
   Documentation provided to EFSA ................................................................................................................. 7
   References.................................................................................................................................................. 7
   Abbreviations .............................................................................................................................................. 8
1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003 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 DSM Nutritional Products Ltd., Switzerland, for authorisation of the product RONOZYME® WX (endo-1,4-β-xylanase), when used as a feed additive for laying hens (category: zootechnical additives; functional group: digestibility enhancers).

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 31 January 2017.

According to Article 8 of Regulation (EC) No 1831/2003, 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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product RONOZYME® WX (endo-1,4-β-xylanase), when used under the proposed conditions of use (see Section 3).

1.2. Additional information

The additive RONOZYME® WX (also known as BioFeed® Wheat) is a preparation of endo-1,4-β-xylanase which is produced by a genetically modified strain of Aspergillus oryzae (DSM 10287) and is authorised in the European Union (EU) as a feed additive for poultry for fattening, weaned piglets and pigs for fattening.

EFSA delivered an opinion on the safety and efficacy of RONOZYME® WX when used as a feed additive for poultry, piglets (weaned) and pigs for fattening (EFSA FEEDAP Panel, 2012a). The authorisation of this additive is currently linked to the production strain Aspergillus oryzae DSM 10287. In 2016, the Panel on additives and Products or Substances used in Animal Feed (FEEDAP) assessed the proposal made by the applicant in order to change the production strain to Aspergillus oryzae DSM 26372 (EFSA FEEDAP Panel, 2016). The applicant has requested for an extension of use of the additive to laying hens.

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 in support of the authorisation request for the use of active substance as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008 and the applicable EFSA guidance documents.

The European Union Reference Laboratory considered that the conclusions and recommendations reached in a previous assessment are valid and applicable for the current application.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of RONOZYME® WX is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012b) and Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011).

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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 Represented in EU by Novozymes A/S, Krogshejlev 36, 2880 Bagsvaerd, Denmark.
3 To put the relevant Regulation, the current one is to be updated after our opinion from 2016, check before adoption.
4 FEED dossier reference: FAD-2016-0073.
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.
6 The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/fnrep-fad-2013-0047-ronozymewx.pdf
3. **Assessment**

This assessment deals with a request from the applicant to extend the use of RONOZYMÉ® WX in feed for laying hens at a dose of 100 FXU/kg feed.

3.1. **Characterisation**

The additive RONOZYMÉ® WX is a preparation of endo-1,4-β-xylanase produced by a genetically modified strain of *A. oryzae*. The current authorisation of this additive is linked to the production strain *Aspergillus oryzae* DSM 10287. In 2016, the FEEDAP Panel assessed the proposal made by the applicant in order to change the production strain to *Aspergillus oryzae* DSM 26372 (EFSA FEEDAP Panel, 2016). In that assessment, the strain *Aspergillus oryzae* DSM 26372 was characterised in full.

The additive is available in two forms, RONOZYMÉ® WX (CT) and RONOZYMÉ® WX (L); these two formulations were fully characterised in previous opinions (EFSA FEEDAP Panel 2012a, 2016). RONOZYMÉ® WX (CT) is a coated thermotolerant formulation which ensures a minimum activity of xylanase of 1,000 FXU/g and RONOZYMÉ® WX (L) is a liquid formulation which ensures a minimum activity of xylanase of 650 FXU/mL.

3.2. **Safety**

Safety aspects regarding the use of this additive in feed including the safety of the genetic modification of the production strains, the safety for the consumers, for the users and for the environment have been previously evaluated (EFSA FEEDAP Panel, 2012a, 2016). The FEEDAP Panel concluded that there are no concerns for consumer safety and no risks for the environment are expected from the use of the additive in animal nutrition. Considering the safety for the user, the Panel concluded that the additive is not a skin or eye irritant but could not conclude on the skin sensitisation potential of the additive. Because of the proteinaceous nature of the active substance, the additive was considered a potential respiratory sensitiser. The FEEDAP Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously regarding the safety of the additive for the consumer, environment and user. Moreover, the FEEDAP Panel considers that the new use in laying hens requested by the applicant would not modify the above conclusions.

In order to support the safety of the additive for the new target species/category, the applicant provided a tolerance trial in laying hens.

3.2.1. **Safety for laying hens**

A total of 2,304 hens (Lohmann Brown, 27-week-old) were allocated to a total of four groups with nine replicates each.7 The hens were caged, four hens per cage and each replicate was composed of 16 cages. A basal diet based on wheat, rye and soybean meal was either not supplemented (control) or supplemented with xylanase from RONOZYMÉ® WX (production strain *Aspergillus oryzae* DSM 26372, liquid concentrated form) to provide 100 (1×) or 4,000 (40×) FXU/kg feed. Another group containing 100 FXU/kg feed was also considered, the xylanase in this group was the one obtained with the production strain DSM 10287. The enzyme activities were confirmed by analysis. The control feed was offered as crumbs and *ad libitum* to all hens from week 27 to week 29, then the hens received the different diets for 26 weeks. Mortality and general health were monitored throughout the study. Body weight was recorded for eight cages per replicate on week 27 of life and at the end of the trial. Feed and water consumption were recorded throughout the study period. Eggs were collected and weighed daily; egg production and feed to egg mass ratio were calculated. At the end of the experiment, nine hens per treatment8 were necropsied and liver, spleen, heart, lungs and kidney were weighed. Blood samples were collected from the same hens and analysed for haematological9 and biochemical10 parameters. An analysis of variance (ANOVA) was done with the data obtained, replicate

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7 Technical dossier/Section III/Annex III.3.2.
8 Except for the group receiving 100 FXU/kg feed from *Aspergillus oryzae* DSM 10287.
9 Including: erythrocytes, leucocytes (and formula), haemolysis index, haemoglobin, haematocrit, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume.
10 Including: calcium, chloride, phosphorus, potassium, magnesium, sodium, bilirubin, bile salts, total protein, albumin, creatinine, urea, cholesterol, glucose, and triglycerides, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transpeptidase, glutamate dehydrogenase, creatinine phosphokinase, lactate dehydrogenase.
basis, and the differences between treatment groups were analysed using the Tukey’s test. Differences were considered significant at $p < 0.05$.

Mortality was below 5% and no differences were found between treatments. The mean daily feed intake of the hens was 126 g, laying rate was 92.3%, daily egg mass production was 59.0 g and the feed to egg mass ratio was 2.13 (for details, see Table 1 in the efficacy section). No differences between the groups were identified on the body weight, laying rate and egg weight. A lower feed intake and a significantly better feed to egg mass ratio were found in the groups receiving the xylanase. The weight of the organs was not different between the treatments and no differences were found in the blood parameters investigated with the exception of potassium, which was increased in the 40-fold diet as compared to control (23 vs 19 mmol/L). The modification in the potassium raises no concerns.

Feeding the hens up to 40-fold, the recommended dose did not have adverse effects on the feed intake, laying performance and the blood parameters investigated in hens. The study submitted investigated the tolerance of laying hens to the xylanase produced by the strain \textit{A. oryzae} DSM 26372. However, taking into account the manufacturing process, the characteristics of the production strain, the xylanase and the resulting additive the Panel consider that the conclusions would also apply to the production strain \textit{A. oryzae} DSM 10287.

### 3.2.1.1. Conclusions on the safety for laying hens

The FEEDAP Panel considers that the additive is safe for laying hens at the recommended dose of 100 FXU/kg feed.

### 3.3. Efficacy for laying hens

Three trials were submitted by the applicant. In the studies, the xylanase used was obtained either from \textit{Aspergillus oryzae} DSM 10287 or from \textit{Aspergillus oryzae} DSM 26372. Considering that both strains produce the same xylanase and that the manufacturing process and composition of the additive are the same, the Panel considers that studies done with the xylanase produced by either strain can be used to support the efficacy of the additive.

The first trial is the tolerance trial and the description has been presented above.

The second and third trials had a similar design; in the second trial, a total of 1,152 hens (Lohmann Brown, 28-week-old) were caged, four hens per cage, and each experimental unit considered 16 cages, representing nine replicates per group. In the third trial, a total of 384 hens (Hy-Line Brown, 26-week-old) were caged, two hens per cage and each experimental unit considered 4 cages, representing 24 replicates per group. In the two trials, a basal diet based on wheat, rye and soybean meal was either not supplemented (control) or supplemented with the xylanase present in the additive to provide 100 FXU/kg feed. The enzyme activities were confirmed by analysis. The feed was offered as crumbs and \textit{ad libitum} for 26 weeks. Mortality and general health were monitored throughout the study period. Individual body weight was recorded at the beginning and at the end of the trial. Feed consumption (and water in trial 3) was recorded throughout the study period. Eggs were collected daily and egg production was calculated. In trial 3, all eggs were weighed daily to monitor egg mass production. In trial 2, weekly egg mass was estimated by weighing all eggs for two consecutive days at the end of each week and considering the weekly egg production. Feed to egg mass ratio was calculated in the two studies. An ANOVA was done with the data obtained, replicate basis. Differences were considered significant at a level of at least $p < 0.05$.

Mortality was low and not affected by the treatments. In all three trials, hens fed RONOZYMEXWX had a significantly lower feed intake and feed to egg mass ratio. In trials 1 and 3, the laying rate of the hens was not different between groups; however, in trial 2, the hens fed the xylanase showed a significantly lower laying rate compared to the control. The Panel considers that the reduction in the laying rate in trial 2 casts doubts on the overall efficacy of the additive.

Therefore, the Panel concludes that there is not sufficient evidence to conclude on the efficacy of RONOZYMEXWX in laying hens.

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11 Technical dossier/Supplementary information July 2017.
12 Technical dossier/Section IV/Annex 4.1 and supplementary information July 2017 Annex 1.
13 Technical dossier/Section IV/Annex 4.3.
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\(^\text{14}\) and Good Manufacturing Practice.

4. Conclusions

There are no concerns for consumer safety and no risks for the environment are expected from the use of the product as a feed additive. The additive is not a skin or eye irritant, no conclusions can be drawn regarding the dermal sensitisation potential of the additive, but the additive should be considered a potential respiratory sensitisier.

The additive is safe for the laying hens at the recommended dose of 100 FXU/kg feed. However, there is not sufficient evidence to conclude on the efficacy of RONOZYME\(^\text{®}\) WX in laying hens.

### Documentation provided to EFSA

1) RONOZYME\(^\text{®}\) WX for laying hens. December 2016. Submitted by Novozymes A/S on behalf of DSM Nutritional Products Ltd.
2) RONOZYME\(^\text{®}\) WX for laying hens. Supplementary information. July 2017 Year. Novozymes A/S on behalf of DSM Nutritional Products Ltd.
3) Comments from Member States.

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Scientific Opinion on Ronozyme WX (endo-1,4-\(\beta\)-xylanase) as a feed additive for poultry, piglets (weaned and pigs for fattening. EFSA Journal 2012;10(7):2790, 21 pp. https://doi.org/10.2903/j.efsa.2012.2790

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 14 pp. https://doi.org/10.2903/j.efsa.2012.2536

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of RONOZYME\(^\text{®}\) WX (endo-1,4-\(\beta\)-xylanase) as a feed additive for chickens and turkeys for fattening, minor poultry species for fattening, weaned piglets and pigs for fattening. EFSA Journal 2016;14(9):4564, 2 pp. https://doi.org/10.2903/j.efsa.2016.4564

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\(^{14}\) 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.
Abbreviations

ANOVA analysis of variance
FEEDAP Panel on Additives and Products or Substances used in Animal Feed