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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Danisco Xylanase 40000 G/L (endo-1,4,-beta-xylanase) for laying hens and poultry minor species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

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ABSTRACT

Danisco Xylanase 40000 G/L is a feed additive that contains endo-1,4-beta-xylanase produced by a genetically modified strain of *Trichoderma reesei*. The additive is intended for use in minor poultry species. The applicant is also seeking a modification of the current authorisation for laying hens (reduction of the minimum dose). The additive is intended for use in laying hens and minor poultry species at a dose range of 625–2 500 U/kg complete feedingstuffs. The efficacy and safety of this product for the target species, consumer, user and the environment, as well as the safety aspects of the genetic modification, were previously assessed by the EFSA. The solid and liquid forms of the product are considered to be equivalent in terms of efficacy and safety for the target animal. Based on the data from three tolerance studies with major poultry species, the FEEDAP Panel concludes that Danisco Xylanase is safe for all minor poultry species (fattening and laying) at the maximum recommended dose (2 500 U/kg complete feed). The FEEDAP Panel considers, based on the results of a meta-analysis of five trials, that Danisco Xylanase at the new proposed minimum dose of 625 U/kg complete feed has the potential to be efficacious in laying hens. Since the additive has shown to be effective at the minimum dose of 625 U/kg in the major poultry species, and considering that the mode of action of xylanases can be reasonably assumed to be the same in all poultry species, the conclusions on efficacy for major poultry species can be extrapolated to all minor poultry species (fattening and laying) at the dose of 625 U/kg complete feed.

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KEY WORDS

Zootechnical additive, digestibility enhancer, xylanase, laying hens, efficacy, minor poultry species, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2011-01171, adopted on 22 May 2012.

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on safety and efficacy of Danisco Xylanase 40000 G/L as a feed additive for minor poultry species. The applicant is also seeking a modification of the current authorisation for laying hens (reduction of the minimum dose). Danisco Xylanase 40000 G/L is a feed additive that contains endo-1,4-beta-xylanase produced by a genetically modified strain of *Trichoderma reesei*. The additive is intended for use in laying hens and minor poultry species at a dose range of 625–2 500 U/kg complete feedingstuffs.

The efficacy and safety of this product for the target species, consumer, user and the environment, as well as the safety aspects of the genetic modification, were previously assessed by EFSA. Therefore, the present opinion focuses on the safety and efficacy of this enzyme preparation for the new target species and the efficacy of the lower proposed dose for laying hens.

The solid and liquid forms of the product are considered to be equivalent in terms of efficacy and safety for the target animal.

Based on the data from three tolerance studies with major poultry species, the FEEDAP Panel concludes that Danisco Xylanase is safe for all minor poultry species (fattening and laying) at the maximum recommended dose (2 500 U/kg complete feed).

The FEEDAP Panel considers, based on the results of a meta-analysis of five trials, that Danisco Xylanase at the new proposed minimum dose of 625 U/kg complete feed has the potential to be efficacious in laying hens.

Since the additive has shown to be effective at the minimum dose of 625 U/kg in the major poultry species, and considering that the mode of action of xylanases can be reasonably assumed to be the same in all poultry species, the conclusions on efficacy for major poultry species can be extrapolated to all minor poultry species (fattening and laying) at the dose of 625 U/kg complete feed.

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BACKGROUND

Regulation (EC) No 1831/2003[4] 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. In addition, 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 request from the company Danisco (UK) Ltd [5] for authorisation of the product Danisco Xylanase 40000 G/L, endo-1,4,-beta-xylanase, when used as a feed additive for laying hens and poultry minor species (category: zootechnical; functional group: digestibility enhancer) under the conditions mentioned in Table 1.

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) and 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.[6] 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 10 January 2012.

The additive Danisco Xylanase 40000 G/L is a preparation of endo-1,4-beta xylanase produced by *Trichoderma reesei* (ATCC PTA 5588). This product (4a11) is authorised for use in chickens for fattening, laying hens, ducks and turkeys for fattening, weaned piglets and pigs for fattening.[7,8]

EFSA has issued three opinions on on the safety and efficacy of this additive, one for chickens for fattening, laying hens and ducks for fattening (EFSA, 2007), one for turkeys for fattening (EFSA, 2008) and one for piglets and pigs for fattening (EFSA, 2011).

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 safety for the target animals, consumer, user and the environment and the efficacy of the product Danisco Xylanase 40000 G/L (endo-1,4-beta xylanase), when used under the conditions described in Table 1.

[4] 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.
[5] Danisco (UK) Ltd (trading as Danisco Animal Nutrition), SN8 1XN Marlborough, Wiltshire (UK).
[6] EFSA Dossier reference: FAD-2011-0030.
[7] Commission regulation (EU) No 9/2010, OJ L3/10.
[8] Commission regulation (EU) No 528/2011, OJ L 143/10.

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

Additive	Endo-1,4-beta-xylanase			
Registration number/EC No/No (if appropriate)	4a11			
Category(ies) of additive	Zootechnical additive			
Functional group(s) of additive	Digestibility enhancers			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
Endo-1,4 beta-xylanase derived from *Trichoderma reesei* (ATCC PTA 5588)	N/A	a guaranteed minimum activity of 40000 U/g endo-1,4 beta-xylanase	One unit of xylanase activity will, under defined assay conditions, hydrolyse 0.5 µmol of reducing sugar (expressed as xylose equivalents) from a oat-spelt-xylan substrate at pH 5.3 and 50°C per minute.	
Trade name (if appropriate)	Danisco Xylanase 40000G/Danisco Xylanase 40000 L			
Name of the holder of authorisation (if appropriate)	**Danisco (UK) Ltd (trading as Danisco Animal Nutrition)**			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		Units of activity/kg of complete feedingstuffs		
Laying hens & all poultry minor species	N/A	625	2500	N/A
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use (if appropriate)	In the directions for use of the additive, indicate the storage temperature, storage life and stability to pelleting. For use in compound feed rich in starch and non-starch polysaccharides (mainly arabinoxylans and beta-glucans).			
Specific conditions or restrictions for handling (if appropriate)	DANISCO XYLANASE 40000 G Harmful: May cause sensitisation by inhalation. Do not breathe dust. Avoid contact with skin. Wear suitable protective clothing and gloves. DANISCO XYLANASE 40000 L Harmful: May cause sensitisation by inhalation. Do not breathe spray. Avoid contact with skin. Wear suitable protective clothing and gloves.			
Post-market monitoring (if appropriate)	All batches of Danisco Xylanase 40000 G and Danisco Xylanase 40000 L are shipped in closed containers and the label information includes the name and address of the producer, a batch number and a bar code. All customers are supplied with copies of the Material Safety Datasheet for the product that includes an emergency contact number. This enables any user to contact the company and provide batch specific information on the use of a product. In addition batch traceability and			

	complaints systems are in place so as to enable rapid investigation and resolution of any negative reports of usage or complaints. The traceability system ensures that any person to whom we have supplied the product can be rapidly identified
Specific conditions for use in complementary feedingstuffs (if appropriate)	N/A

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 Danisco Xylanase 40000 G/L is a preparation of endo-1,4-beta xylanase produced by a genetically modified strain of *Trichoderma reesei* (ATTC PTA 5588) and is presented in granular powder form (G) or in a liquid form (L). This product (4a11) is authorised for use in chickens for fattening, laying hens, ducks and turkeys for fattening, weaned piglets and pigs for fattening. The applicant is seeking a modification of the current authorisation for laying hens (reduction of the minimum dose) and an extension of the authorisation to all poultry minor species.

The efficacy and safety of this product for the target species, consumer, user and the environment, as well as the safety aspects of the genetic modification, were assessed by EFSA (EFSA, 2007, 2008, 2011). Therefore, the present opinion focuses on the safety and efficacy of this enzyme preparation for the new target species and the efficacy of the lower proposed dose for laying hens.

2. Characterisation

2.1. Conditions of use

The additive is intended to be used in diets for laying hens and minor poultry species at a minimum dose of 625 U/kg complete feed and at a maximum recommended dose of 2 500 U/kg complete feed.

2.2. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

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

3. Safety

3.1. Safety for the target species

The safety of Danisco Xylanase 40000 G/L for chickens for fattening, turkeys for fattening, laying hens and ducks for fattening at the maximum recommended dose (2 500 U/kg complete feed) has been established in the previous opinions (EFSA, 2007, 2008). The FEEDAP Panel considers that the conclusions reached for the major poultry species can be extrapolated to all minor poultry species.

4. Efficacy

4.1. Efficacy for laying hens

Five efficacy trials with laying hens have been carried out, four of them outside the European Union. The design of the different trials is summarised in Table 2.

All trials shared a similar design in which the laying hens (aged 18 weeks in trial 3, 26 weeks in trials 1, 4 and 5, and 28 weeks in trial 2) were randomly allocated to a control group or to a group treated with Danisco Xylanase 40000 G at either 625 or 2 500 U/kg complete feed (confirmed by analysis). Egg production, egg mass and feed to egg mass ratio were measured and calculated. Data were analysed using the generalised linear model (GLM) procedure with the pen as an experimental unit.

Table 2: Trial design and dosage confirmation for the efficacy trials

Trial	Breed Age at start Duration	Total no of animals Animals per replicate Replicates per treatment	Diet composition	Intended dose(U/kg)	Analysed dose(U/kg)
1[9]	ISA Brown 26 weeks 42 weeks	270 3 30	Wheat/triticale/ soybean meal	0 625 2500	243 739 2079
2[10]	Hy-Line Brown 28 weeks 42 weeks	624 16 13	Soft wheat/soybean meal	0 625 2500	36 672 2928
3[11]	Lohmann Brown 20 weeks 42 weeks	384 4 32	Soft wheat/rye/ soybean meal	0 625 2 500	0 647 2 689
4[12]	Hy-Line Brown 26 weeks 28 weeks	80 2 20	Hard wheat/rye/ soybean meal	0 625	123 720
5[13]	Hy-Line Brown 26 weeks 28 weeks	80 2 20	Soft wheat/rye/ soybean meal	0 625	168 817

Results of the five trials are summarised in Table 3. In all trials, the hens were in good health during the trial and the overall mortality was low. Supplementation with Danisco Xylanase at 625 U/kg significantly increased laying rate in one trial (trial 3), egg mass in two trials (3 and 4) and decreased feed to egg mass ratio in one trial (4).

Table 3: Effect of Danisco Xylanase 40000 on the performance of laying hens

Trial	Intended dose(U/kg)	Feed intake(g/hen/ day)[1]	Laying rate[1] (%)	Egg mass (g/hen/day)	Feed to egg mass ratio (g feed/g egg)[1]	Mortality/ culling(%)
1	0 625 2500	113 113 112	92.7^{b} 94.0^{ab} 94.8^{a}	59.4 60.7 60.8	1.88 1.86 1.84	1.1 1.1 0
2	0 625 2500	111 110 109	89.0 89.7 90,3	54.8 55.2 55.6	2.04^{a} 2.00^{a} 1.96^{b}	1.9 1.0 2.9
3	0 625 2500	127^{b} 127^{ab} 129^{a}	87.0^{c} 88.8^{b} 90.6^{a}	54.9^{c} 55.9^{b} 57.3^{a}	2.33^{b} 2.27^{b} 2.22^{a}	1.0[2]
4	0 625	110 110	91.1 92.9	57.3^{b} 59.1^{a}	1.94^{a} 1.87^{b}	1.9[2]
5	0 625	110 110	93.2 94.6	59.7 59.9	1.82 1.81	1.9[2]

[1] Calculated on a hen per day basis.
[2] Overall mortality/culling – treatment group not defined .
[a,b,c]: Different superscripts in the same column indicate a significant difference ($P < 0.05$).

[9] Technical dossier/Section IV/Annex IV/D6.
[10] Technical dossier/Section IV/Annex IV/D7.
[11] Technical dossier/Section IV/Annex IV/D8.
[12] Technical dossier/Section IV/Annex IV/D10.
[13] Technical dossier/Section IV/Annex IV/D11.

A meta-analysis of the five efficacy trials was also carried out. The suitability of the data for meta-analysis was tested by performing the normality test (goodness-of-fit test) and homogeneity by Bartlett's test. The results of the meta-analysis are shown in Table 4.

Table 4: Meta-analysis of five efficacy trials with Danisco Xylanase 40000 on the performance of laying hens

	Intended dose (U/kg complete feed)	
Parameters	0	625
Feed intake (g/hen/day)	124	124
Laying rate (%)	90.7[a]	92.1[b]
Egg mass (g/hen/day)	56.9[a]	58.0[b]
Feed to egg mass ratio (g feed/g egg)	1.99[a]	1.96[b]

[a,b]: Means with different superscripts are significantly different ($P < 0.05$).

The results of the meta-analysis showed a statistically significant positive effect on the laying rate, egg mass and feed to egg mass ratio of laying hens receiving diets supplemented with Danisco Xylanase at 625 U/kg complete feed. Therefore, the FEEDAP Panel considers that there is evidence to support efficacy of this product in laying hens at the minimum recommended dose of 625 U/kg complete feed.

4.2. Efficacy for minor poultry species

In the previous opinions, the FEEDAP Panel concluded that Danisco Xylanase 40000 has the potential to be efficacious for chickens for fattening, turkeys for fattening and ducks for fattening at the dose of 625 U/kg complete feed (EFSA, 2007, 2008). The data submitted in the current application support the efficacy in laying hens at the same dose. Since the mode of action of xylanases can be reasonably assumed to be the same in all poultry species, the conclusions on efficacy for major poultry species can be extrapolated to include all minor poultry species at the dose of 625 U/kg complete feed.

5. 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[14] and Good Manufacturing Practice.

CONCLUSIONS

The solid and liquid forms of the product are considered to be equivalent in terms of efficacy and safety for the target animal.

Based on the data from three tolerance studies with major poultry species, the FEEDAP Panel concludes that Danisco Xylanase is safe for all minor poultry species (fattening and laying) at the maximum recommended dose (2 500 U/kg complete feed).

The FEEDAP Panel considers, based on the results of a meta-analysis of five trials, that Danisco Xylanase at the new proposed minimum dose of 625 U/kg complete feed has the potential to be efficacious in laying hens.

[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.

Since the additive has shown to be effective at the minimum dose of 625 U/kg in the major poultry species, and considering that the mode of action of xylanases can be reasonably assumed to be the same in all poultry species, the conclusions on efficacy for major poultry species can be extrapolated to all minor poultry species (fattening and laying) at the dose of 625 U/kg complete feed.

DOCUMENTATION PROVIDED TO EFSA

1. Danisco Xylanase 40000G/40000L. August 2011. Submitted by Danisco (UK) Ltd.

2. Danisco Xylanase 40000G/40000L. Supplementary information March 2012. Submitted by Danisco (UK) Ltd.

3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Danisco Xylanase 40000G/40000L.

4. Comments from Member States received through the ScienceNet.

REFERENCES

EFSA, 2007. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) and of the Scientific Panel on Genetically Modified Organisms (GMO) on a request from the European Commission on the safety and efficacy of Danisco Xylanase G/L (endo-1,4-beta-xylanase) as a feed additive for chickens for fattening, laying hens and ducks for fattening. The EFSA Journal, 548, 1–18.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2008. Scientific Opinion on the safety and efficacy of Danisco Xylanase G/L (endo-1,4-beta-xylanase) as a feed additive for turkeys for fattening. The EFSA Journal, 586, 1–12.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2011. Scientific Opinion on the safety and efficacy of Danisco Xylanase G/L (endo-1,4-beta-xylanase) for weaned piglets and pigs for fattening. EFSA Journal, 9(2):2008. 13 pp.

APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Danisco Xylanase 4000 G/L[15]

In the current application authorisation is sought under article 4(1) and 13(3) for *Danisco Xylanase 40000 G/L*, under the category/functional group 4(a) "zootechnical additives"/"digestibility enhancers", according to the classification system of Annex I of Regulation (EC) No 1831/2003. According to the Applicant, the *feed additive* contains *endo-1,4-β-xylanase* (EC 3.2.1.8) as the active agent, produced by the strain *Trichoderma reesei* (ATCC PTA 5588). The additive is intended to be marketed as different formulations: a solid (*Danisco Xylanase G*) and a liquid (*Danisco Xylanase L*) formulation with a target *endo-1,4-β-xylanase* activity of 40000 U/g.

The activity of *endo-1,4-β-xylanase* is expressed in xylanase units (U). According to the Applicant, one U unit is defined as the amount of enzyme which liberates 0.5 µmol of reducing sugar (expressed as xylose equivalents) from a cross-linked oat spelt xylan substrate per minute at pH 5.3 and 50 ^{o}C.

Specifically, authorisation is sought for the use of *Danisco Xylanase 40000 G/L* for laying hens and all poultry minor species. The *feed additive* is intended to be used in compound feed rich in non-starch polysaccharides (mainly arabinoxylans and beta-glucans). It is intended to be used in *premixtures* and/or complete *feedingstuffs*, with *endo-1,4-β-xylanase* activity ranging from 625 to 2500 U/kg in complete *feedingstuffs*.

For the determination of *endo-1,4-β-xylanase* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted colorimetric method, based on the quantification of water soluble dyed fragments produced by the action of *endo-1,4-β-xylanase* on commercially available azurine cross-linked arabinoxylan at pH 4.2 and 50 °C. The enzymatic activity of the sample is calculated using a reference enzyme standard with certified enzyme activity, available from the Applicant upon request. The validation and verification studies were performed using samples containing xylanase activities of 15500 U/g, 15500 U/kg and 1800 U/kg. Furthermore, the Applicant applied the abovementioned method to investigate the stability of feed samples containing 400 U/kg. The following method performance characteristics were reported:

- for the *feed additive*: - a relative standard deviation for *repeatability* (RSD_r) ranging from 6.6 to 9.5 %; - a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 7.2 to 11 % and – a *recovery rate* (R_{Rec}) ranging from 103 to 112 %;
- for *premixtures*: - RSD_r ranging from 2.3 to 8.0 %; - RSD_{ip} ranging from 6.6 to 6.9 %; and - R_{Rec} ranging from 93 to 95 %;
- for *feedingstuffs*: - RSD_r ranging from 2.3 to 5.8 %; - RSD_{ip} ranging from 4.0 to 6.9 %; - R_{Rec} ranging from 93 to 97 %; and limits of detection (LOD) and quantification (LOQ) of 40 and 133 U/kg, respectively.

Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified colorimetric method, based on the quantification of water soluble dyed fragments produced by the action of *endo-1,4-β-xylanase* on azurine cross-linked wheat arabinoxylan at pH 4.2 and 50 °C, to determine the *endo-1,4-β-xylanase* in *feed additive*, *premixtures* and *feedingstuffs*, within the concentration range covered by the experimental data.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

[15] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2011-0030.pdf