European Food Safety Authority

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Safety and efficacy of Danisco Xylanase G/L (endo-1,4-beta-xylanase) as a feed additive for turkeys for fattening [1]

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

(Question No EFSA-Q-2007-109)

Adopted on 22 November 2007

PANEL MEMBERS*

Georges Bories, Paul Brantom, Joaquim Brufau de Barberà, Andrew Chesson, Pier Sandro Cocconcelli, Bogdan Debski, Noël Dierick, Anders Franklin, Jürgen Gropp, Ingrid Halle, Christer Hogstrand, Joop de Knecht, Lubomir Leng, Anne-Katrine Lundebye Haldorsen, Alberto Mantovani, Miklós Mézes, Carlo Nebbia, Walter Rambeck, Guido Rychen, Atte von Wright and Pieter Wester

SUMMARY

Danisco Xylanase G/L is an enzyme product (dry and liquid forms, respectively) of endo 1,4 beta-xylanase produced by submerged fermentation of the genetically modified micro-organism *Trichoderma reesei*. The additive is intended for use in turkeys for fattening at a dose range of 1250-2500 U kg^{-1} complete feedingstuffs.

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver an opinion on the efficacy and safety for the target animals, the consumer, user and the environment of the product Danisco Xylanase G/L. The FEEDAP Panel considers that the safety aspects other than those related to the new target species are covered in the previous opinion and would not be affected by this extension of use. Therefore, the present opinion focuses only on the safety and efficacy of this enzyme preparation for the target species turkeys for fattening.

Four experiments have been provided by the applicant to support the efficacy of the product. In two of them, significant improvements in weight gain and feed conversion were observed at the minimum recommended dose (1250 U kg^{-1}). The meta-analysis of the data from the four experiments supports the efficacy of the product at the minimum recommended dose. Therefore, the FEEDAP Panel considers that there is evidence to support efficacy of this product at the minimum recommended dose of 1250 U kg^{-1} in turkeys for fattening.

[1] For citation purposes: Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 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 turkeys for fattening. *The EFSA Journal* (2007) 586, 1-12

* One member of the Panel did not participate in the discussion on the subject referred to above.

Based on the data from the tolerance study presented and the tolerance shown by other poultry species (chickens for fattening, laying hens and ducks), the FEEDAP Panel concludes that this product is safe for turkeys for fattening at the recommended use level.

Key words: zootechnical additive, digestibility enhancer, enzyme, Danisco Xylanase, turkeys for fattening, safety, efficacy

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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 4(1) of that Regulation lays down that any person seeking an 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 the company Danisco Animal Nutrition[3] for authorisation of the product Danisco Xylanase to be used as a feed additive for turkeys for fattening (category: zootechnical additives; functional group: digestibility enhancers) under the conditions described 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). EFSA received directly from the applicant the technical dossier in support of this application. 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 9 August 2007.

The additive Danisco Xylanase G/L is a preparation of endo-1,4 beta-xylanase produced by the genetically modified micro-organism *Trichoderma reesei* (ATCC PTA 5588). This product has not been previously authorised in the Community.

EFSA delivered an opinion on the safety and efficacy of this product when used as feed additive for chickens for fattening, laying hens and ducks for fattening in September 2007 (EFSA, 2007).

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. Therefore, EFSA shall deliver an opinion on the efficacy and the safety for the target animals, the consumer, user and the environment of the product Danisco Xylanase G/L which is a preparation of endo-1,4 beta-xylanase produced by *Trichoderma reesei* (ATCC PTA 5588) when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Enzymes as well as Friedrich Schöne for the preparation of this opinion.

[2] OJ L 268, 18.10.2003, p.29

[3] Danisco Animal Nutrition, legal entity Finnfeeds International Limited, PO Box 777, Marlborough, Wiltshire, SN8 1XN, United Kingdom

Table 1. **Register entry as proposed by the applicant**

Additive	DANISCO XYLANASE
Registration number/EC No/No (if appropriate)	EC 3.2.1.8
Category of additive	Zootechnical additives
Functional group of additive	Digestibility enhancer

Description			
Composition, description	**Chemical formula**	**Purity criteria (if appropriate)**	**Method of analysis (if appropriate)**
Preparation of Endo-1,4 betaxylanase (EC 3.2.1.8) produced by *Trichoderma reesei* (ATCC PTA 5588) with a minimum activity of: Liquid form: 40000 U g^{-1} Dry form: 40000 U g^{-1}	N/A	Guaranteed minimum activity of 40000 U g^{-1}	1 U is the amount of enzyme which liberates 0.5 µmol of reducing sugar (expressed as xylose equivalents) from a cross-linked oat spelt arabino-xylan substrate at pH 5.3 and 50°C in one minute.

Trade name (if appropriate)	DANISCO XYLANASE G and DANISCO XYLANASE L
Name of the holder of authorisation (if appropriate)	Danisco Animal Nutrition (legal entity Finnfeeds International Limited)

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period (if appropriate)**
		Units of activity kg^{-1} of complete feedingstuffs		
Turkeys for fattening	-	1250	2500	-

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), e.g. containing more than 40% wheat or 60% maize.
Specific conditions or restrictions for handling (if appropriate)	Harmful. May cause sensitization by inhalation. Do not breathe dust (or spray). Avoid contact with skin. Wear suitable protective clothing and gloves.
Post-market monitoring (if appropriate)	All batches of Danisco Xylanase 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)	Not applicable

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

Danisco Xylanase G and Danisco Xylanase L are granulated and liquid forms, respectively, of endo 1,4 beta-xylanase (EC 3.2.1.8) produced by submerged fermentation of the genetically modified micro-organism *Trichoderma reesei* (ATCC PTA 5588). The additive is intended for use in turkeys for fattening given feeds with high contents of non-starch-polysaccharides such as arabinoxylans, at a dose range of 1250-2500 U kg^{-1} complete feedingstuffs.

The efficacy and safety of this product for chickens for fattening, laying hens 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 were assessed by EFSA (EFSA, 2007). The applicant is now asking for an extension of use of this product for turkeys for fattening.

The FEEDAP Panel considers that the safety aspects other than those related to the new target species are covered in the previous opinion and would not be affected by the extension of use. Therefore, the present opinion focuses only on the safety and efficacy of this enzyme preparation for the target species turkeys 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

A total of four floor pen experiments were performed to support evidence of efficacy in turkeys for fattening. In all four experiments the solid form of the product was used.

Each trial consisted of a negative control with one or more treatment groups. The length of the study in all trials was 16 weeks, which were divided in four phases of four weeks each. The diets were offered *ad libitum* in form of mash/crumbs in the first phase and in form of pellets for the rest of the trial. The enzyme was added pre-pelleting to the diet and the enzyme activity in feed was confirmed by analysis. Feed intake and body weight of the animals were measured every four weeks. The health status of the animals was monitored daily.

Since the experiment design is similar in all the trials, the treatments and main results are summarised in Table 2.

Mortality was not affected by treatment in any of the trials. Weight gain was significantly increased ($P \leq 0.05$) in trials 1 and 2, in both males and females, by the inclusion of Danisco Xylanase at 1250 U kg^{-1} feed. Feed conversion was improved in both trials for males and only trial 1 for females. A significant improvement in feed conversion was observed in trial 4 when 2500 U kg^{-1} were added to the basal diet. No significant effects were seen in the performance parameters measured in trial 3.

Table 2. **Summary of the design and results of the four efficacy trials with Danisco Xylanase G in turkeys for fattening (0-16 weeks)**

Trial number		Total number of animals (replicates/ treatment x birds/ replicate)	Trial duration (weeks)	Diet	Dose (U kg^{-1})	Weight gain (kg bird^{-1})	Feed intake (kg)	Feed/gain	Mortality$^{(n)}$
1[4]	♂	160 (5 x 16)	16	Hard wheat soybean meal	0	13.77^{b}	42.78	3.11^{a}	5
					1250	14.91^{a}	42.80	2.87^{b}	5
	♀	160 (5 x 16)			0	10.18^{b}	32.68	3.21^{a}	1
					1250	10.82^{a}	31.35	2.90^{b}	2
2[5]	♂	160 (5 x 16)	16	Soft wheat soybean meal	0	13.68^{b}	42.39	3.10^{a}	5
					1250	14.86^{a}	42.97	2.89^{b}	7
	♀	160 (5 x 16)			0	10.36^{b}	31.40	3.03	4
					1250	11.05^{a}	31.47	2.85	2
3[6]	♂	384 (8 x 12)	16	?	0	12.41	31.90	2.57	5
					625	12.36	32.52	2.63	10
					1250	12.55	31.05	2.47	6
					2500	12.62	32.45	2.56	6
4[7]	♂	240 (6 x 10)	16	Soft wheat soybean meal	0	13.74	37.17	2.58^{a}	5
					625	13.90	34.68	2.53^{a}	0
					1250	14.10	35.63	2.52^{a}	0
					2500	14.09	34.36	2.49^{b}	3

$^{a, b}$: Means in a column within a given trial (and sex) with different superscripts are statistically different (P<0.05)

Statistical meta-analysis of data from the four efficacy studies

Since the four studies followed a similar design, the applicant subjected the data from the four individual studies to meta-analysis. [8]

From the meta-analysis, it could be concluded that supplementation with Danisco Xylanase at the minimum recommended dose significantly improved body weight gain and feed/gain ratio (Table 3).

Table 3. **Meta-analysis on the performance of turkeys for fattening, as influenced by Danisco Xylanase G addition (0-16 weeks)**

Danisco xylanase(U kg^{-1})	Feed intake (kg bird^{-1})	Weight gain (kg bird^{-1})	Feed/gain
0	33.06	11.62^{b}	2.88^{a}
625	32.64	11.97^{ab}	2.81^{ab}
1250	32.50	12.08^{a}	2.69^{c}
2500	32.46	11.94^{ab}	2.77^{bc}

$^{a, b, c}$: Means in a column with different superscripts are statistically different (P<0.05)

Conclusions on efficacy

A meta-analysis done on four efficacy trials showed a statistically significant positive effect on the performance of turkeys for fattening of a supplementation with 1250 U of Danisco Xylanase kg^{-1} complete feed. Therefore, the FEEDAP Panel considers that there is evidence to

[4] Technical dossier/Section III/ReferencesC1 & C3
[5] Technical dossier/Section III/References C2 & C4
[6] Supplementary information September 2007/Appendix 2
[7] Supplementary information September 2007/Appendix 3
[8] Supplementary information September 2007/Appendix 1

support efficacy of this product in turkeys for fattening at the minimum recommended dose of 1250 U kg^{-1} complete feed.

4. Safety for the target species

A total of 240 male turkeys were distributed at seven days of age into three treatments (eight replicates of ten turkeys per treatment). [9] The treatments comprised a control diet, based on soft wheat, rye and soybean meal supplemented with Danisco Xylanase G at 0, 2500 (1X) or 37500 (15X) mg kg^{-1} complete feed (enzyme activity confirmed by analysis). The trial lasted for 112 days. Individual body weight and feed consumption were measured at 7, 28, 56, 84 and 112 days, and the birds were monitored twice daily for health status. Haematology and blood chemistry were not performed.

Mortality was not affected by the treatment (7.5, 8.8 and 8.8 %). Supplementation with Danisco Xylanase at 15X overdose did not affect the performance of the turkeys. Body weight gain of the birds was over 14 kg and feed/gain ratio about 2.5 at the end of the trial (112 days).

Based on the data from the tolerance study presented and the tolerance shown by other poultry species (chickens for fattening, laying hens and ducks), the FEEDAP Panel concludes that this product is safe for turkeys for fattening at the recommended levels.

5. Post-market monitoring

No risks associated with the use of the product are foreseen. It is considered that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation (Regulation (EC) No 183/2005) and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

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

The FEEDAP Panel considers that there is evidence from a meta-analysis based on four trials to support the efficacy of Danisco Xylanase in turkeys for fattening at the minimum dose of 1250 U kg^{-1} complete feed.

Based on the data from the tolerance study presented and the tolerance shown by other poultry species (chickens for fattening, laying hens and ducks), the FEEDAP Panel concludes that this product is safe for turkeys for fattening at the recommended levels.

RECOMMENDATIONS

The Register entry, under 'specific conditions or restrictions of use' should read: '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 non-starch polysaccharides (mainly arabinoxylans).'

[9] Technical dossier/Section IV/Reference D1

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Danisco Xylanase G and Danisco Xylanase L. Single species: turkeys for fattening. February 2007. Submitted by Danisco Animal Nutrition.
2. Dossier on Danisco Xylanase G and Danisco Xylanase L. Single species: turkeys for fattening. Reply to issues raised in letter from EFSA dated 21 August 2007. September 2007. Submitted by Danisco Animal Nutrition.
3. CRL evaluation report on the analytical methods of Danisco Xylanase G/L for turkeys for fattening.
4. Comments from the Member States received through the EFSAnet.

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. <http://www.efsa.europa.eu/EFSA/Scientific_Opinion/feedap_op_ej548_danisco_xylanase_cff_ly_dff_en,7.pdf>

APPENDICES

APPENDIX A

Executive Summary of the Evaluation Report of the Community Reference Laboratory Feed Additives Authorisation on the Method(s) of Analysis for Danisco Xylanase for turkeys for fattening.

In the current application authorisation is sought for *Danisco Xylanase* under the category 'zootechnical additives', group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use *Danisco Xylanase* as a digestibility enhancer for turkeys for fattening. The product is intended to be marketed as a powder (*Danisco Xylanase G*) and as liquid formulation (*Danisco Xylanase L*).

The active agent of *Danisco Xylanase* is endo-1,4-β-xylanase produced by a strain of *Trichoderma reesei* (ATCC PTA 5588). The enzymatic activity is expressed in units (U). One U is the amount of endo-1,4-β-xylanase that liberates 0.5 µmol of reducing sugar (xylose equivalents) per minute from a cross-linked oat spelt xylan at pH 5.3 and 50°C. The product has a target activity of 40000 U/g. *Danisco Xylanase G* is intended to be mixed into *premixtures* and/or *feedingstuffs*, whereas *Danisco Xylanase L* is sprayed directly onto feed to obtain an enzyme activity level of 1250 to 2500 U/kg in *feedingstuffs*.

For the determination of the activity of endo-1,4-β-xylanase in the *feed additive*, *premixtures* and *feedingstuffs*, the applicant proposes a colorimetric method based on the quantification of water soluble dyed fragments produced by the action of endo-1,4-β-xylanase on commercially available cross-linked xylan substrates. Enzymatic activity of the sample is calculated using a reference enzyme standard. The applicant introduced some adaptations to the protocol. The modified methods have been single laboratory validated.

For the determination of the activity of endo-1,4-β-xylanase in the *feed additive,* the applicant proposes a method which measures the enzyme-catalysed formation of water soluble dyed fragments released from cross-linked wheat arabinoxylan. The rate of release is measured on a spectrophotometer at 590 nm and quantified against a reference enzyme standard, available from the applicant upon request. The analysis – however – is carried out at *different* conditions (pH 4.0 and 40°C on a cross-linked wheat arabinoxylan) compared to those given in the proposed register entry (pH 5.3 and 50°C on a cross-linked oat spelt xylan) and the enzymatic activity is calibrated against a reference enzyme of which the activity is obtained applying the conditions of the proposed register entry. Method performance characteristics include a limit of detection (LOD) of 1.2 U/g, limit of quantification (LOQ) of 1.5 U/g products and a relative standard deviation for repeatability (RSD_r) of 4.4%.

For the determination of the activity of endo-1,4-β-xylanase in *premixtures,* the applicant proposes a method based on the same principle as described above, but employing a different extraction procedure. The measurements are carried out at pH 5.3 and 40°C on a cross-linked wheat arabinoxylan. Method performance characteristics include a LOD of 13.0 U/g, LOQ of 19.3 U/g, an RSD_r of 3.5 % and recovery rates of 96.4 %.

For the quantification of the activity of endo-1,4-β-xylanase in *feedingstuffs,* the applicant proposes a method, based on the same principle as described above, measuring enzymatic activity on a cross-linked wheat arabinoxylan at pH 4.2 and 50°C. Calibration is performed on standards prepared from identical blank feed samples fortified with exact amounts of the reference enzyme, available from the applicant. Method performance characteristics include a

LOD of 285 U/kg, a LOQ of 530 U/kg, a RSD_r of 7.5% and a recovery rate of 97%. In the case that identical blank feed samples are *not* available, a standard addition technique is employed.

Though the methods proposed by the applicant are based on well known principles and show acceptable performance characteristics, the CRL is concerned that the suggested approach of measuring the enzymatic activity at *different* conditions compared to the conditions of the proposed register entry and to the conditions of the determination of the activity of a reference enzyme, introduces additional uncertainty into the measurements. Therefore, for consistent analytical results, the CRL recommends:

- that the enzymatic activity in the *feed additive*, in *premixtures* and in *feedingstuffs* is determined at identical conditions;
- that the harmonised analytical conditions are identical with conditions specified in the register entry.

In the case that the analytical conditions remain *different* for determination of enzymatic activity in various matrices and *different* from those as proposed in the Register entry, the CRL cannot evaluate the proposed methods for their suitability for official controls.

Further testing or validation is not considered necessary.