European Food Safety Authority

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SCIENTIFIC OPINION

Safety and efficacy of Avizyme 1505 (endo-1,4-β-xylanase, α-amylase, subtilisin) 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-2007-112)

Adopted on 17 June 2009

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to issue a scientific opinion on the safety and efficacy of Avizyme 1505 as a feed additive for turkeys for fattening.

The additive Avizyme 1505 is a preparation of endo-1,4-β-xylanase produced by the genetically modified micro-organism (GMM) *Trichoderma reesei* (ATCC PTA 5588), α-amylase produced by the GMM *Bacillus amyloliquefaciens* (ATCC 3978) and subtilisin (protease) produced by the GMM *Bacillus subtilis* (ATCC 2107). In a previous opinion, the safety of this product for the consumer, the user and the environment, as well as the safety aspects of the genetic modification were established. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 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.

Avizyme 1505 is intended to be used in diets for turkeys for fattening at a dose of 200 mg kg^{-1} complete feed (endo-1,4-β-xylanase 300, subtilisin 4000, α-amylase 400 units of activity kg^{-1} of complete feed).

[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 Avizyme 1505 (endo-1,4-beta-xylanase, alpha-amylase, subtilisin) as feed additive for turkeys for fattening. *The EFSA Journal* (2009) 1154, 1-11

The tolerance trial provided showed that turkeys for fattening tolerated a 15-fold overdose of Avizyme 1505. Therefore, the FEEDAP Panel concludes that the use of the additive at the recommended conditions is safe for turkeys for fattening.

A significant effect of Avizyme 1505 at a dose of 200 mg kg^{-1} on the performance of turkeys for fattening was observed in three efficacy trials. Therefore, the FEEDAP Panel considers that there is evidence to support the efficacy of Avizyme 1505 at this dose in turkeys for fattening.

The FEEDAP Panel notes that the method of analysis for the xylanase present in Avizyme 1505 is not considered by the CRL as fit for the purpose of official control methods.

Key words: zootechnical additive, digestibility enhancer, enzyme, xylanase, amylase, protease, turkeys for fattening, efficacy, safety for the target species

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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 Avizyme 1505 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.[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 3 September 2007.

The additive Avizyme 1505 is a preparation of endo-1,4-β-xylanase produced by the genetically modified micro-organism (GMM) *Trichoderma reesei* (ATCC PTA 5588), α-amylase produced by the GMM *Bacillus amyloliquefaciens* (ATCC 3978) and subtilisin (protease) produced by the GMM *Bacillus subtilis* (ATCC 2107). This product has not been previously authorised in the European Community.

EFSA has delivered an opinion on the safety and efficacy of this product when used as feed additive for chickens and ducks for fattening (EFSA, 2009).

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 Avizyme 1505 which is a preparation of endo-1,4-β-xylanase produced by *Trichoderma reesei* (ATCC PTA 5588), α-amylase produced by *Bacillus amyloliquefaciens* (ATCC 3978) and subtilisin (protease) produced by *Bacillus subtilis* (ATCC 2107) 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 Joaquim Brufau de Barberà, Bogdan Debski, Christer Hogstrand, Carlo Nebbia, Walter Rambeck, Guido Rychen, Friedrich Schöne, Atte von Wright and Pieter Wester 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
[4] Dossier reference: FAD-2007-0017

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

Additive	Avizyme 1505
Registration number/EC No/No (if appropriate)	Endo-1,4-beta-xylanase EC 3.2.1.8 Subtilisin EC 3.4.21.62 Alpha-amylase E.C. 3.2.1.1
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-beta-xylanase EC 3.2.1.8 produced by *Trichoderma reesei* ATCC PTA 5588), alpha-amylase produced by *Bacillus amyloliquefaciens* (ATCC 3978) and subtilisin produced by *Bacillus subtilis* (ATCC 2107), with a minimum activity of: - Dry form : Endo-1,4- beta-xylanase: 1500 U g^{-1} Subtilisin: 20000 U g^{-1} Alpha-amylase: 2000 U g^{-1}	N/A	Guaranteed minimum activity of Endo-1,4- beta-xylanase: 1500 U g^{-1} Subtilisin: 20000 U g^{-1} Alpha-amylase: 2000 U g^{-1}	Xylanase: 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 xylan substrate at pH 5.3 and 50ºC in one minute. Subtilisin: 1 U is the amount of enzyme which liberates 1 µmol of phenolic compound (tyrosine equivalents) from a casein substrate per minute at pH 7.5 and 40ºC Amylase: 1 U is the amount of enzyme which liberates 1 µmol of glucosidic linkages from a water insoluble cross-linked starch polymer substrate per minute at pH 6.5 and 37ºC

Trade name (if appropriate)	AVIZYME 1505
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	-	Endo-1,4-beta-xylanase: 300 Subtilisin: 4000 Alpha-amylase: 400	Endo-1,4-beta-xylanase: 300 Subtilisin: 4000 Alpha-amylase: 400	-

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% maize.
Specific conditions or restrictions for handling (if appropriate)	Harmful. Irritating to respiratory system and skin. Risk of serious damage to the eyes. May cause sensitization by inhalation. Do not breathe dust. Avoid contact with skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable clothing gloves and eyes/face protection.
Post-market monitoring (if appropriate)	All batches of Avizyme 1505 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 costumers 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

Avizyme 1505 is a triple enzyme mixture containig endo-1,4-β-xylanase produced by a strain of the genetically modified micro-organism (GMM) *Trichoderma reesei* (ATCC PTA 5588), α-amylase produced by a strain of the GMM *Bacillus amyloliquefaciens* (ATCC 3978) and subtilisin produced by a strain of the GMM *Bacillus subtilis* (ATCC 2107). Their respective activity levels have been optimised for use in poultry diets that are based on corn and soybean meal. Avizyme 1505 is intended to be used in diets for turkeys for fattening, at a recommended dose of 300 U endo-1,4-β-xylanase kg^{-1}, 4000 U subtilisin kg^{-1} and 400 U α-amylase kg^{-1} of complete feed (equivalent to 200 mg Avizyme 1505 kg^{-1} complete feed).

The efficacy and safety of this product for chickens 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 has been assessed by EFSA (EFSA, 2009). 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 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.

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 substances in animal feeds. The Executive Summary of the CRL report can be found in the Appendix.

The FEEDAP Panel notes that the method of analysis for the xylanase present in Avizyme 1505 is not considered by the CRL as fit for the purpose of official control methods. The FEEDAP Panel recommends that a more sensitive method should be developed.

3. Safety for turkeys for fattening

A 43-day tolerance study[5] was carried out with 288 one-day-old female BUT10 turkeys divided into three treatment groups with eight replicates of 96 birds per treatment. A pelleted diet based on maize and soyabean meal was offered *ad libitum* and Avizyme 1505 was added at doses equivalent to 0, 200 (1X) and 3000 (15X) mg kg^{-1}. The enzyme activities in feed were confirmed by analyses.

During the tolerance study, mortality, feed intake and body weight of the birds were monitored and feed to gain ratio was calculated. On day 44, blood was taken from ten birds per treatment. Blood samples were analysed for haematology (total erythrocyte count, total leukocyte count, haemoglobin concentration, mean cell volume, platelet count and differential leukocyte count) and for biochemical parameters (fibrinogen, glucose, urea, total protein, albumin, ALKP, ALAT, LDH, ASAT, GGT, albumin/globulin ratio, creatinine, calcium, phosphorus, chloride, total bilirubin, sodium, and potassium).

[5] Supplementary information June 2009

Mortality and culling was generally low and not affected by the treatments (2.1 %, 3.1 % and 2.1 %). Final body weight (1638, 1695, 1738 g animal^{-1}) was improved in the 15X group compared to the control group and the feed to gain ratio (1.66, 1.62 and 1.62 g g^{-1}) was significantly higher ($P < 0.01$) in the control group compared to the treatment groups. Of all the blood parameters measured, only total protein concentration (36.5 g L^{-1}, 35.4 and 37.8) was modified by the treatments, but the modification was not dose-related.

3.1. Conclusions on the safety for turkeys for fattening

Based on the study presented, in which female turkeys tolerated a 15-fold overdose of Avizyme 1505, the FEEDAP Panel concludes that this product is safe for turkeys for fattening at the recommended dose.

4. Efficacy for turkeys for fattening

Three trials performed in two different locations have been provided by the applicant to support the efficacy of Avizyme 1505 for turkeys for fattening. The design and main results of the three trials are summarised in Table 2.

The first[6] and second[7] trials were done at the same location at different times and had the same experimental design. Trial 1 was done in BUT8 males while trial 2 involved BUT8 females. The animals received a basal diet based on maize (35–52%) and soybean meal, which was supplemented with Avizyme 1505 at 0 or 200 mg kg^{-1} complete feed. In the third trial,[8] male hybrid turkeys were used, which received a basal diet based on maize (45–70%) and soybean meal, which was supplemented with a 2.5 times diluted form of Avizyme 1505 at the intended doses of 0 and 200 mg kg^{-1} complete feed. Enzyme activity in feed was confirmed by separate analysis of the different enzymes.

Table 2. **Summary of the design and results of the three efficacy trials with Avizyme 1505 in turkeys for fattening (0-16 weeks)**

Trial number	Total number of animals (replicates/ treatment) *birds/replicate*	Trial duration (weeks)	Dose in equivalents to Avizyme 1505 (mg kg^{-1})	Weight gain (kg bird^{-1})	Feed intake (kg bird^{-1})	Feed/gain (kg kg^{-1})	Mortality (n)
1	160 (5) *16*	16	0 200	13.71^{b} 14.71^{a}	41.52 41.88	3.03^{a} 2.85^{b}	4/80 4/80
2	160 (5) *16*	16	0 200	10.29^{b} 10.75^{a}	31.48 32.22	3.06 3.00	0/80 3/80
3	120 (6) *10*	16	0 200	12.46^{b} 13.06^{a}	29.44^{b} 31.39^{a}	2.33^{a} 2.28^{b}	1/60 4/60

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

In all cases the trial lasted for a period of 16 weeks which was divided in four phases of four weeks each. Feed intake and body weight were measured and weight gain and feed conversion calculated every four weeks. Animals were daily monitored for health.

[6] Technical dossier/Section III/Reference C1
[7] Technical dossier/Section III/Reference C2
[8] Technical dossier/Section III/Reference C3

Weight gain was significantly ($P < 0.05$) improved in three trials and feed conversion improved in two trials (trial 1 and 3) by the supplementation with Avizyme 1505 at 200 mg kg^{-1} complete feed. In trial 3 an increase in feed intake was also observed.

4.1. Conclusions on the efficacy for turkeys for fattening

Supplementation with 200 mg Avizyme 1505 kg^{-1} complete feed resulted in an improvement on the performance of turkeys for fattening in three trials. Therefore, the FEEDAP Panel considers that there is evidence to support the efficacy at the dose of 200 mg Avizyme 1505 kg^{-1} feed in turkeys for fattening.

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[9] and Good Manufacturing Practice.

CONCLUSIONS

The tolerance trial provided showed that turkeys for fattening tolerated a 15-fold overdose of Avizyme 1505. Therefore, the FEEDAP Panel concludes that the use of the additive at the recommended conditions is safe for turkeys for fattening.

A significant effect of Avizyme 1505 at a dose of 200 mg kg^{-1} on the performance of turkeys for fattening was observed in three efficacy trials. Therefore, the FEEDAP Panel considers that there is evidence to support efficacy of Avizyme 1505 at this dose in turkeys for fattening.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Avizyme 1505. Single species: turkeys for fattening. February 2007. Submitted by Danisco Animal Nutrition.
2. Dossier on Avizyme 1505. Single species: turkeys for fattening. Reply to issues raised in letter from EFSA dated 11 September 2007. September 2007. Submitted by Danisco Animal Nutrition.
3. Dossier on Avizyme 1505. Single species: turkeys for fattening. Reply to issues raised in letter from EFSA dated 27 October 2008. June 2009. Submitted by Danisco Animal Nutrition.
4. Evaluation report of the Community Reference Laboratory for feed additives on the methods of analysis for Avizyme 1505 for turkeys for fattening.
5. Comments from Member States received through the ScienceNet.

REFERENCES

EFSA (European Food Safety Authority), 2009. Opinion of the Scientific Panel on Additives and Products or Substances on the efficacy of Avizyme 1505 (endo-1,4-β-xylanase, α-amylase, subtilisin) in chickens for fattening and ducks. *The EFSA Journal* (2009) 1156, 1-25.

[9] OJ L 35, 8.2.2005, p.1

APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for Avizyme 1505 for turkeys for fattening

In the current application authorisation is sought for *Avizyme 1505* 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 *Avizyme 1505* as a digestibility enhancer for turkeys for fattening. The product is intended to be marketed as a granular powder formulation.

The active agents of *Avizyme 1505* are 1) endo-1,4-β-xylanase, produced by a strain of *Trichoderma reesei* (ATCC PTA 5588), 2) α-amylase, produced by a strain of *Bacillus amyloliquefaciens* (ATCC 3978) and 3) subtilisin, produced by a strain of *Bacillus subtilis* (ATCC 2107). Enzymatic activity of the active agents is expressed in units (U):

- One U of endo-1,4-β-xylanase is the amount of enzyme 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;
- One U of α-amylase is the amount of enzyme that liberates 1 µmol of glucosidic linkages per minute from a water insoluble cross-linked starch polymer substrate at pH 6.5 and 37°C;
- One U of subtilisin is the amount of enzyme that liberates 1 µmol of phenolic compound (tyrosine equivalents) per minute from a casein substrate at pH 7.5 and 40°C.

The product has a target activity of 1500 U endo-1,4-β-xylanase/g, 2000 U α-amylase/g and 20000 U subtilisin/g. *Avizyme 1505* is intended to be mixed into *premixtures* and/or *feedingstuffs* to obtain an enzyme activity level of 300 U endo-1,4-β-xylanase/kg, 400 U α-amylase/kg and 4000 U subtilisin/kg in *feedingstuffs.*

In general, the methods proposed for the determination of the activity of the active agents in different matrices are based on quantification of dyed compounds produced by enzymatic action of commercially available substrates. Enzymatic activity of the samples is calculated using reference enzyme standards, available from the applicant upon request, of which the activity is obtained applying the conditions described by the definitions of units. When analysing *feedingstuffs,* calibration is performed on standards prepared from identical blank feed samples fortified with exact amounts of the reference enzymes. In the case that identical blank feed samples are *not* available, a standard addition technique is employed. The applicant introduced some adaptations to the protocols provided by the suppliers of substrates. All modified methods have been single-laboratory validated and showed acceptable performance characteristics such as limit of detection, limit of quantification and relative standard deviation for repeatability.

For the determination of the activity of endo-1,4-β-xylanase in the *feed additive*, *premixtures* and *feedingstuffs*, the applicant proposes a method based on the quantification (λ = 590 nm) of water soluble dyed fragments produced by the action of endo-1,4-β-xylanase on cross-linked wheat xylan substrates. Enzymatic activity is calculated using a reference enzyme standard, of which the activity is measured at pH 5.3 and 50°C on a cross-linked oat spelt xylan. Analyses are carried out at pH 4.0 and 40°C (*feed additive*), at pH 5.3 and 40°C (*premixtures*) and at pH 4.2 and 50°C (*feedingstuffs*).

For the determination of the activity of α-amylase in the *feed additive,* the applicant proposes a method based on the quantification (λ = 405 nm) of free *p*-nitrophenol produced by the action of α-amylase on blocked *p*-nitrophenyl maltoheptaoside at pH 5.6 and 37°C. Enzymatic activity is calculated using a reference enzyme standard, of which the activity is measured at pH 6.5 and 37°C. For the analysis of the activity of α-amylase in *premixtures* and *feedingstuffs,* quantification (λ = 620 nm) of dyed oligomers produced by the action of α-amylase on azurine-crosslinked starch at pH 6.4 and 37°C is proposed.

For the determination of the activity of subtilisin in the *feed additive, premixtures* and *feedingstuffs,* the applicant proposes a method based on the quantification (λ = 590 nm) of *dyed oligomers* produced by the action of subtilisin on azurine-cross linked casein. Enzymatic activity is calculated using a reference enzyme standard, of which the activity is measured by quantification of *phenolic compounds* released from casein at pH 7.5 and 40°C. Analyses are carried out at pH 10 and 50°C (*feed additive* and *feedingstuffs*) and at pH 8.0 and 40°C (*premixtures*).

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 in various matrices compared to the conditions described by the definitions of units and to the conditions of the determination of the activity of reference enzymes, 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 described by the definitions of units;
- that the minimum activity of endo-1,4-β-xylanase, specified in the register entry (300 U/kg) is replaced by the limit of quantification of the method, which is 500 U/kg.

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

Further testing or validation is not considered necessary.