SCIENTIFIC OPINION

Scientific Opinion on the efficacy of Ronozyme® Rumistar (alpha-amylase) as a feed additive for dairy cows[1]

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

European Food Safety Authority (EFSA), Parma, Italy

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ABSTRACT

Ronozyme® Rumistar is a preparation of alpha-amylase produced by a genetically modified strain of *Bacillus licheniformis* which is intended for use as a zootechnical feed additive for dairy cows. The FEEDAP Panel issued an opinion on the safety and efficacy of this product as a feed additive for dairy cows. The assessment performed considered the characterisation of the product, the safety aspects for the consumer, user and environment, as well as for the genetic modification of the production strain and the efficacy of the product for the target species. However, the Panel could not draw conclusions on the efficacy of the product. The additive is intended to be used in lactating dairy cows fed a plant-based diet of "medium starch content". The applicant provided additional data on one study already evaluated. In a reduced set of data, the results showed a significant increase in cows fed Ronozyme® Rumistar compared with control cows when fed a total mixed ration diet with a normal starch content. Additionally, one digestibility study was provided but cannot be considered because of the parameters measured. Considering all the data made available by the applicant, no conclusions can be drawn on the efficacy of Ronozyme® Rumistar in dairy cows.

KEY WORDS

zootechnical additives, digestibility enhancers, alpha-amylase, *Bacillus licheniformis*, dairy cows, efficacy

[1] On request from European Commission, Question No EFSA-Q-2013-00331, adopted on 9 October 2013.

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[4] Revision 1: This scientific opinion is published following the clarifications received on the confidentiality claims made by the applicant.

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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 the efficacy of Ronozyme® Rumistar as a feed additive for dairy cows. This additive is a preparation of alpha-amylase produced by a genetically modified strain of *Bacillus licheniformis*. It is intended for use as a zootechnical feed additive (functional group: digestibility enhancers) for dairy cows and has not been previously authorised in the European Union. The FEEDAP Panel issued an opinion in 2012 on the safety and efficacy of this product as a feed additive for dairy cows. The assessment performed considered the characterisation of the product, the safety aspects for the consumer, user and environment, as well as for the genetic modification of the production strain and the efficacy of the product for the target species. However, the Panel could not draw conclusions on the efficacy of the product.

The additive is intended to be used in lactating dairy cows fed a plant-based diet of "medium starch content". The dose range is 300–400 KNU/kg dry matter (DM) complete feed (total mixed ration (TMR), including concentrates and forages). The additive can be either added to the concentrate or sprayed onto the TMR. The applicant further states that the supplementation with Ronozyme® Rumistar should start in early lactation if a maximum increase in milk yield is sought.

The applicant provided additional data on one study already evaluated. In a reduced set of data, the results showed a significant increase in milk production in cows fed Ronozyme® Rumistar compared with control cows (38.7 vs 36.5 kg/day) when fed a TMR diet with a normal starch content. Additionally, one digestibility study was provided but cannot be considered because of the parameters measured.

Considering all the data made available by the applicant, no conclusions can be drawn on the efficacy of Ronozyme® Rumistar in dairy cows.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and in particular, Article 9 defines the terms of the authorisation by the Commission.

The company DSM is seeking a Community authorisation of its *alpha-amylase* for use in dairy cows, produced by DSM. (Table 1)

Table 1: Description of the substance

Category of additive	Zootechnical additives
Functional group	Digestibility enhancers
Trade name	-
Description	*Alpha-amylase*
Target animal category	Dairy cows
Applicant	DSM
Type of request	Update opinion

On 15th June 2012, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority") in its opinion on "Safety and efficacy of Ronozyme Rumistar (*alpha-amylase*) for use in dairy cows" was not able to give a conclusive opinion on the efficacy of the product because of lack of data provided by the company.

Therefore, the Commission gave the possibility to the company to submit complementary information to complete the assessment on efficacy to allow a revision of that opinion.

The Commission has now received an additional dossier form the applicant, DSM, on *alpha-amylase* for use in dairy cows with supplementary information, concerning the efficacy for dairy cows and the mode of action of the additive.

The Commission, in order to give the appropriate follow-up to the application, asks the European Food Safety Authority to issue an updated opinion on the efficacy of this product under the terms of reference specified in the Annex. The data generated by the company and compiled in the above-mentioned supplementary report are enclosed with this letter.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In view of the above, the Commission asks to the European Food Safety Authority to deliver an opinion on the efficacy of this *alpha-amylase* for use in dairy cows, as zootechnical additive taking into account the new information submitted.

ASSESSMENT

1. Introduction

The additive Ronozyme® Rumistar is a preparation of alpha-amylase produced by a genetically modified strain of *Bacillus licheniformis* (DSM 21564). It is intended for use as a zootechnical feed additive (functional group: digestibility enhancer) for dairy cows. It has not been previously authorised in the European Union. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion in 2012 on the safety and efficacy of this product as a feed additive for dairy cows (EFSA FEEDAP Panel, 2012). This opinion considered the characterisation of the product, the safety aspects for the consumer, user and environment, as well as for the genetic modification of the production strain and the efficacy of the product for the target species. However, the Panel could not draw conclusions on the efficacy of the product.

The additive under application is intended to be used in lactating dairy cows fed a plant-based diet of "medium starch content". The dose range is 300–400 KNU/kg dry matter (DM) complete feed (total mixed ration (TMR), including concentrates and forages). The additive can be either added to the concentrate or sprayed onto the TMR. The applicant further states that the supplementation with Ronozyme® Rumistar should start in early lactation if a maximum increase in milk yield is sought.

1.1. Starch digestion in the rumen; potential benefits and risks for use of exogenous alpha-amylase

According to the applicant, alpha-amylase hydrolyses starch in the rumen into mixed oligosaccharides, which are normally fermented to volatile fatty acids by the rumen microflora. It is well known that complete hydrolysis of starch requires amylase, maltase and isomaltase or amyloglucosidase activity. The main site of starch digestion in ruminants is the rumen, but it can vary considerably between starch sources, even at similar levels of total tract digestibility. *In vivo* rumen degradable starch ranges from 60 % for maize and sorghum, to 85 % for oats and up to 90 % for rye, wheat and barley (Owens et al., 1986; Nocek and Tamminga, 1991). Starch digested in the rumen is used only about 70 % as efficiently as starch that is digested in the small intestine (Owens et al., 1986). However, some literature data suggest that there may be limitations in starch digestion, potentially because of inadequate pancreatic amylase secretion (Harmon et al., 2004). Further, according to the applicant, alpha-amylase shifts the site of starch digestion in cattle towards the rumen. At dietary starch levels up to 30 %, the faster release of oligosaccharides in the rumen can stimulate adhesion and proliferation of rumen micro-organisms in the amylolytic but especially the fibrolytic microbiota. Such stimulation of fibrolytic micro-organisms may increase ruminal fibre (neutral detergent fibre (NDF)) digestion.[5]

Several authors (e.g. Owens et al., 1998) have warned that rapid digestion of excessive amounts of starch in the rumen may lead to subacute ruminal acidosis (SARA), representing a potential concern for inclusion of exogenous amylases in ruminant diets. EFSA (2008) issued an opinion on an inhibitor of alpha-amylase intended to be used in the feed of ruminants to maintain optimal rumen pH levels (> 5.5) by slowing down starch fermentation in the rumen and, as a consequence, reducing the risk of induction of SARA. SARA occurs when ruminal pH is depressed for prolonged periods each day, for example < 5.6 for > 3 hours/day (Kleen et al., 2003) or < 5.8 for > 3 hours/day (Steele et al., 2011), mainly seen in high-yielding dairy cows during early and mid-lactation. The physiological significance and prevalence of SARA were fully described in that opinion.

The applicant provided some short-term studies aimed at demonstrating that the use of alpha-amylase would not result in decreases in ruminal pH. In these experiments, no negative effects on ruminal pH were observed when cows were fed TMR with a starch content of 20–30 % and a NDF content between 32.5 and 35.0 %.[6]

[5] Technical dossier/Annex 2.
[6] Technical dossier/Supplementary information August 2013.

2. Efficacy for dairy cows

In its previous assessment (EFSA FEEDAP Panel, 2012), the Panel concluded that "*From five efficacy studies provided by the applicant, significant positive effects were observed in only one trial in which an energy-deficient diet was used. Thus, no conclusions can be drawn on the efficacy of Ronozyme Rumistar in dairy cows*".

The applicant provided further data on one of the studies assessed in the previous opinion and a new short-term trial.

The applicant provided new data regarding the last study evaluated in the previous opinion (EFSA FEEDAP Panel, 2012). In that study, supplementation with Ronozyme® Rumistar significantly increased milk yield in cows fed diets with a normal starch content that were under study for 12 weeks and that presented < 150 days in milking when entering into the study (46.0 kg vs. 42.9 kg). However, the data for the whole period were not provided. This information has now been provided and data were subjected to further statistical analysis.[7] The applicant considered the production for the whole lactation period (330 days) only for cows complying with the following conditions: < 100 days in milking at the beginning of the study and > 77 days within trial and with a complete data set (seven records (in days) was the maximum of incompleteness allowed). This resulted in a reduced data set (control, $n = 65$; Ronozyme® Rumistar, $n = 47$) compared with the original data (100 in each treatment group). Milk production was significantly increased in cows fed Ronozyme® Rumistar as compared to the control from 36.5 to 38.7 kg/day, when fed a TMR diet with a normal starch content.

The applicant also submitted a faecal digestibility study (4 × 4 Latin square design),[8] carried out with 28 dairy cows. The digestibility of dry and organic matter, crude protein, acid detergent fibre, NDF and starch was studied. However, this study is not considered appropriate for the evaluation of the efficacy of alpha-amylase.

The applicant provided further literature data on starch and fibre digestion in the rumen as affected by starch source, level and technological treatments, including the use of exogenous fibre and starch degrading enzymes and their consequences on ruminal and total tract digestibility of nutrients. Although a favourable shift in starch digestion towards the rumen, accompanied with an increase in fibre digestion, by different feed interventions is possible, a net effect on the energy balance and the milk output of dairy cows over long periods (whole lactation period) remains unclear and needs further substantiation.

2.1. Conclusions on efficacy

Considering all the data made available by the applicant, the FEEDAP Panel concludes that the efficacy of Ronozyme® Rumistar in dairy cows fed total mixed ration diets containing 'a medium starch level' is insufficiently evaluated with appropriate studies and parameters (e.g. long-term experiments with measurement of milk production, considering the whole lactation period; short-term experiments, considering balances). Therefore, no conclusions can be drawn on the efficacy of Ronozyme® Rumistar in dairy cows.

Furthermore, the FEEDAP Panel considers that the use of amylase in dairy cows' feed could, in certain circumstances (e.g. lower dietary NDF content than that used in the studies provided), increase the risk of SARA or acidosis.

CONCLUSIONS

The FEEDAP Panel concludes that the efficacy of Ronozyme® Rumistar has not been demonstrated in dairy cows.

[7] Technical dossier/Annex 12.
[8] Technical dossier/Annex 8.

DOCUMENTATION PROVIDED TO EFSA

1. Ronozyme® Rumistar for dairy cows. January 2013. Submitted by DSM.
2. Ronozyme® Rumistar for dairy cows. Supplementary information. August 2013. Submitted by DSM.

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