European Food Safety Authority

EFSA Journal 2009; 7(10):1353

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of MycoCell (*Saccharomyces cerevisiae*) 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

ABSTRACT

The microbial additive MycoCell is a preparation of viable cells of *Saccharomyces cerevisiae*. It is intended to be applied daily in dairy cows as a top dressing, or incorporated into a total mixed ration to provide 1 x 10^{10} CFU/head/day. The active agent, *Saccharomyces cerevisiae*, is considered by EFSA to have QPS status and, as such, can be presumed as safe for the target species, the consumers and the environment. The additional experimental data provided in support of safety for dairy cows and the genotoxicity data do not conflict with the conclusions reached on safety using the QPS approach. The additive has been unexpectedly shown to be a skin sensitiser. Given this result and in the absence of information on dusting potential, the product should also be treated as a respiratory sensitiser. In addition, it should be noted that the product is intended for top dressing at farm level. There is insufficient evidence that MycoCell at 1 x 10^{10} CFU/head/day improves production parameters in dairy cows.

KEY WORDS

Zootechnical additive, *Saccharomyces cerevisiae*, dairy cows, efficacy, safety, QPS

[1] On request from the European Commission, Question No EFSA-Q-2007-165, adopted on 15 October 2009.

[2] Panel members**:** Gabriele Aquilina, Georges Bories, Paul Brantom, Francesca Caloni, Andrew Chesson, Pier Sandro Cocconcelli, Joop de Knecht, Noël Albert Dierick, Mikolaj Antoni Gralak, Jürgen Gropp, Ingrid Halle, Nils-Gunnar Ilbäck, Reinhard Kroker, Lubomir Leng, Sven Lindgren, Anne-Katrine Lundebye Haldorsen, Alberto Mantovani, Miklós Mézes, Derek Renshaw and Maria Saarela. Correspondence: FEEDAP@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Micro-organisms including Joaquim Brufau, Guido Rychen and Atte von Wright for the preparation of this opinion.

Suggested citation: EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on the safety and efficacy of MycoCell (*Saccharomyces cerevisiae*) for dairy cows. EFSA Journal 2009; 7(10):1353. [12 pp.]. doi:10.2903/j.efsa.2009.1353. Available online: www.efsa.europa.eu

SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety and efficacy of the product MycoCell as feed additive for dairy cows.

The microbial additive MycoCell is a live preparation of *Saccharomyces cerevisiae* yeast culture. It is intended to be applied daily as a top dressing, or incorporated into a total mixed ration (1×10^{10} CFU/head/day).

The active agent, *Saccharomyces cerevisiae*, is considered by EFSA to have QPS status and, as such, can be presumed as safe for the target species, the consumers and the environment. The additional experimental data provided in support of safety for dairy cows and the genotoxicity data do not conflict with the conclusions reached on safety using the QPS approach.

The additive has been unexpectedly shown to be a skin sensitiser. If this proves to be the case, in the absence of information on dusting potential, the product should also be treated as a respiratory sensitiser. In addition, it should be noted that the product is intended for top dressing at farm level.

There is insufficient evidence that MycoCell at 1×10^{10} CFU/head/day produces beneficial effects in dairy cows.

The *in vitro* studies provided do not support the proposal for the functional group 'other zootechnical additives' (reduction of mycotoxin concentration in the gastrointestinal tract).

In addition the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) recommended a number of modifications to the description and conditions of use of the additive proposed by the applicant.

TABLE OF CONTENTS

Table of Contents 3
Background 4
Terms of reference....4
Acknowledgements 4
Assessment 6
1. Introduction 6
2. Characterisation 6
2.1. Characterisation of the additive 6
2.2. Characterisation of the active agent 6
2.3. Stability and homogeneity 6
2.4. Conditions of use 7
2.5. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)....7
3. Safety 7
3.1. Safety for the user 7
3.1.1. Skin and eye irritancy 7
3.1.2. Sensitisation....7
4. Efficacy....8
4.1. Efficacy for dairy cows 8
4.1.1. Effects of MycoCell on the rumen bacteria 9
4.1.2. Effect of MycoCell on mycotoxins 9
4.1.3. Conclusions on efficacy....9
5. Post-market monitoring 10
Conclusions and Recommendations....10
Documentation provided to EFSA 10
References 11
Appendices 12

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 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 Micron BioSystems Ltd[5] for authorisation of the product MycoCell to be used as a feed additive for dairy cows (category: zootechnical additives; functional groups: digestibility enhancers, gut flora stabilisers and other zootechnical additives: reduces mycotoxin concentrations in the GIT) 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.[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 March 2008.

The additive MycoCell is a preparation of *Saccharomyces cerevisiae* NYCC R404. This product has not been previously authorised in the Community.

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 efficacy and the safety for the target animals, the consumer, user and the environment of the product MycoCell which is a preparation of *Saccharomyces cerevisiae* (NYCC R404), 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 Micro-organisms, including Joaquim Brufau de Barberà, Guido Rychen and Atte von Wright for the preparation of this opinion.

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

[5] Micron BioSystems Ltd, Unit 2 Abersychan Industrial Estate, Pontypool, Torffaen, NP4 7BA, United Kingdom.

[6] EFSA dossier reference: FAD-2007-0022

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

Additive	MycoCell
Registration number/EC No/No (if appropriate)	
Category of additive	Zootechnical additives
Functional group of additive	4a Digestibility enhancers; (increases fibre digestion). 4b Gut flora stabilisers; (stabilises rumen flora especially fibre digesting organisms). 4d Other zootechnical additives; (reduces mycotoxin concentrations in the GIT).

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Saccharomyces cerevisiae	-	As per cell count below	Current Mycological techniques

Trade name (if appropriate)	MycoCell
Name of the holder of authorisation (if appropriate)	Micron Bio-systems Ltd

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		CFU per day		
Dairy cows	-	1×10^{10}	-	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	None
Specific conditions or restrictions for handling (if appropriate)	Good Farm manufacturing practice is sufficient and keep stored cool
Post-market monitoring (if appropriate)	None
Specific conditions for use in complementary feedingstuffs (if appropriate)	Not for use in pelleting processes

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 microbial additive MycoCell is a preparation of viable cells of *Saccharomyces cerevisiae* NYCC R404. The applicant has requested an authorisation under the category of zootechnical additives (functional groups: digestibility enhancers, gut flora stabilisers and other zootechnical additives) for its use in dairy cows. This product has not been previously authorised in the Community.

Saccharomyces cerevisiae is considered by the European Food Safety Authority (EFSA) to be suitable for a QPS approach for safety assessment (EFSA, 2008).

2. Characterisation

2.1. Characterisation of the additive

MycoCell contains washed, dried cells of *Saccharomyces cerevisiae* blended with dextrose as carrier and containing sodium aluminosilicate as a flow agent, and silicon dioxide as an anti-caking agent. The additive contains *Saccharomyces cerevisae* NYCC R404 at minimum concentration of 2.0 x 10^9 CFU/g.

Five batches have been analysed to establish the composition, batch to batch variation and purity of the final product.[7] The mean concentration was found to be 2.2 x 10^9 CFU/g. The particle size distribution showed that 24 % of particles had a particle size lower than 125 µm. No analyses of lower particle size or dusting potential of the formulated product were provided. Microbial analyses (contaminating yeasts and moulds, aerobic bacteria, *Escherichia coli*, *Salmonella* spp.*)* showed no microbial contamination of concern. Chemical impurities (lead, mercury, cadmium and arsenic) were analysed and the data provided showed that the observed results fall below concentrations that could give cause of concern. The applicant states that the product is monitored for mycotoxin contamination (aflatoxins, deoxynivalenol, zearalenone, T-2 toxin, ochratoxins and fumonisins) arising form the growth medium. However, no analytical data have been provided.

2.2. Characterisation of the active agent

The *Saccharomyces cerevisiae* R404 was registered and deposited in the National Yeast Culture Collection, Institute of Food Research, Norwich Laboratory.[8] It has not been genetically modified. Identity was established on the basis of biochemical and morphological characteristics. Although no molecular methods were applied for the species and strain identification, the phenotypic characteristics were typical of *Saccharomyces* species, and the FEEDAP Panel concludes that a QPS approach is appropriate. No data on genetic stability were provided.

2.3. Stability and homogeneity

Six individual batches were studied aiming at determining the shelf-life of MycoCell at 4°C, 15°C, 20°C, 30°C and 40ºC.[9] The product remains stable at temperatures up to 30ºC for 12 months. An approximate 10% loss was seen at 40ºC over the same period.

Due to the conditions of use (2.4) no data on stability in premixtures or feed is considered necessary.

[7] Technical dossier/Supplementary information September 2009

[8] Technical dossier/Annexes/Appendix A2

[9] Technical dossier/Section II and Supplementary information September 2009

Homogeneity of MycoCell when delivered via a total mixed ration was studied.[10] MycoCell was added to the initial ingredient blend in the TMR mixer wagon prior to adding the forages. Ten samples were analysed within two hours of the completion of the blend. This procedure was repeated the next day without the addition of MycoCell to the ration to determine the background level of contaminating yeasts in the ration that would principally come from the silage. The coefficient of variation of TMR samples was on average 11%.

2.4. Conditions of use

MycoCell is intended to be applied daily as a top dressing to feed, or incorporated into TMR (1×10^{10} CFU/head/day).

2.5. 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 agent in animal feed. The Executive Summary of the CRL report can be found in Appendix A.

3. Safety

Saccharomyces species are considered by EFSA to have QPS status and not to require any specific demonstration of safety. In the view of the FEEDAP Panel, the identity of the production strain is established as a *Saccharomyces* and therefore no further assessment of safety for the target species, consumer and the environment is required. However, the applicant included details of a tolerance study made with dairy cows, and two genotoxicity studies.[11] None of the results from these studies would conflict with the QPS approach.

3.1. Safety for the user

3.1.1. Skin and eye irritancy

A study compliant with the OECD guideline 404 was performed to assess the irritancy potential of MycoCell applied to the skin of three New Zealand rabbits.[12] The test material produced a primary irritation index of zero and was classified as non-irritant.

A second study compliant to the relevant OECD guideline 405 was performed to assess the irritancy potential of MycoCell to the eye of three New Zealand White rabbits.[13] The test material was classified as a minimal irritant product.

3.1.2. Sensitisation

A study compliant to the relevant OECD guideline 429 was performed to assess the skin sensitisation potential of MycoCell using the murine local lymph node assay.[14] This study revealed that MycoCell should be considered as a potential skin sensitiser. Given the fact that there is a positive response in skin and given the absence of information on dusting potential, the product also should be treated as a respiratory sensitiser.

[10] Technical dossier/Supplementary information September 2009

[11] Technical dossier/Sections IV.1.1.1 and IV.2.1

[12] Technical dossier/Section IV.3.1.1

[13] Technical dossier/Section IV.3.1.2

[14] Technical dossier/Section IV.3.2.1

4. Efficacy

4.1. Efficacy for dairy cows

Six efficacy studies were aimed at evaluating the effects of MycoCell on performance of dairy cows. Four of them were carried out in one Member State, in two different locations.

Experiment 1

A total of 58 lactating cows (ranging from 14 days post-partum to 155 days post-partum) was allocated to two experimental groups (control, MycoCell) in pairs according to previous milk yield, parity, body condition score and live weight.[15] The animals were offered *ad libitum* access to the same total mixed ration (based on maize silage, grass silage, molasses, sugar beet pulp, wheat, maize, soya) either with the addition of MycoCell at 1 x 10^{10} CFU/head/day or without the microbial additive for a period of 114 days.

The following parameters were measured: milk yield, milk fat, protein, lactose and somatic cells counts (twice weekly basis), body condition score and live weight (monthly basis), feed intake of each group (daily basis), blood parameters (non-esterified fatty acids, beta-hydroxy-butyrate (BHB), glucose, urea, albumin, globulin, total protein, GLDH, AST, at six weekly intervals), conception rate after first service, general health of the cows. All the data were tested for normality and data found to be normally distributed were analysed using GLM ANOVA. The other data were analysed by Chi-square.

No significant results were observed other than an increase in milk lactose content in MycoCell treated animals (46 g/kg vs. 45.1 g/kg, $P < 0.001$) and a higher glucose and a lower beta-hydroxy-butyrate in the blood samples taken at the end of the experiment.

Experiment 2

A total of 42 lactating cows were allocated to two experimental groups (control, MycoCell) in pairs according to previous milk yield, parity, body condition score and live weight.[16] The animals were offered *ad libitum* access to the same total mixed ration (based on maize and grass silage) either with the addition of MycoCell at 1 x 10^{10} CFU/head/day or without the microbial additive for a period which lasted 114 days.

Studied parameters included milk yield, milk fat, protein, lactose and milk somatic cells counts (weekly basis), body condition score, live weight (two weekly intervals) and foot lameness, blood parameters (4, 8 and 16 weeks post-partum), conception rate after first service and general health of the cows. All the data were tested for normality and data found to be normally distributed were analysed using GLM ANOVA. The other data were analysed by Chi-square.

Supplementation with MycoCell significantly increased mean milk yield (27.1 vs. 29.5 kg/head/day, $P<0.001$), total fat (0.95 vs. 1.04 kg/head/day, $P<0.001$) and protein yield (0.86 vs. 0.94 kg/head/day, $P<0.001$) in animals treated with MycoCell.

[15] Technical dossier/Annex 3.2.1

[16] Technical dossier/Annex 3.2.2

Experiment 3

A total of 62 lactating cows were allocated to two experimental groups (control, MycoCell) in pairs according to previous milk yield, parity, body condition score and live weight.[17] The animals were offered *ad libitum* access to the same total mixed ration either with the addition of MycoCell at 1 x 10^{10} CFU/head/day or without the microbial additive for a period which lasted 101 days (dose confirmed by analysis).

Studied parameters included milk yield (weekly), milk composition (twice-weekly basis), body condition score (monthly), feed intake levels, blood chemistry (6 weekly intervals), conception rate, general health of the cows. All the data were tested for normality and data found to be normally distributed were analysed using an analysis of variance (ANOVA).

Supplementation with MycoCell significantly increased feed intake (0.2%, $P<0.01$) and body scores (2.79 in control animals and 2.88 in treated animals, $P<0.01$). No significant effects on milk yield and composition were observed.

Other zootechnical trials

Three other trials were described with a common design based on successive periods of supplementation and absence of supplementation in the same animals.[18] The FEEDAP Panel considers that these experiments are not acceptable since the animals were in different physiological status for the different periods and thus were not comparable. In addition the duration of the trials is not considered adequate.

4.1.1. Effects of MycoCell on the rumen bacteria

Two studies aiming at testing the effect of MycoCell on rumen bacteria were provided.[19] These studies were performed with three strains of bacteria grown in isolation in a laboratory medium. Although some stimulation of growth was seen in the presence of MycoCell, the relevance of these observations to the functioning rumen cannot be established.

4.1.2. Effect of MycoCell on mycotoxins

In order to test the effects of MycoCell on mycotoxins, the additive was suspended in malt extract broth and maintained either at 4°C (control) or at 30°C (growing yeast). Toxins (deoxynivanol, zearalenone and a fumonisin) were added to a concentration of 200 µg/L. Samples for analysis were taken immediately and at two-hour intervals over an eight hour period. Samples were centrifuged and the supernatants assayed for toxins.[20]

The limited data provided suggest that there was an immediate non-specific adsorption to the additive; however the results do not allow any conclusions to be drawn on the benefits this may have in animals.

4.1.3. Conclusions on efficacy

There is insufficient evidence that the additive produces beneficial effects in dairy cows. Published results indicate that yeast given to ruminants increase fibre digestion by increasing the number of cell

[17] Technical dossier/Supplementary information June 09

[18] Technical dossier/Annexes 3.2.3, 3.2.4 and 3.2.5

[19] Technical dossier/Sections IV.1.2.1 and IV.1.2.2

[20] Technical dossier/Section IV.1.2.3

wall degrading bacteria. However, the limited *in vitro* studies submitted would not allow this conclusion to be reached. The *in vitro* studies provided do not support the proposal for the functional group 'other zootechnical additives' (reduction of mycotoxin concentration in the GIT).

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[21] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The active agent, *Saccharomyces cerevisiae*, is considered by EFSA to have QPS status and, as such, can be presumed as safe for the target species, the consumers and the environment. The additional experimental data provided in support of safety for dairy cows and the genotoxicity data do not conflict with the conclusions reached on safety using the QPS approach.

The additive has been unexpectedly shown to be a skin sensitiser. If this proves to be the case, in the absence of information on dusting potential, the product should also be treated as a respiratory sensitiser. In addition, it should be noted that the product is intended for top-dressing at farm level.

There is insufficient evidence that MycoCell at 1 x 10^{10} CFU/head/day produces beneficial effects in dairy cows.

The *in vitro* studies provided do not support the proposal for the functional group 'other zootechnical additives' (reduction of mycotoxin concentration in the gastro intestinal tract).

RECOMMENDATIONS

The FEEDAP Panel recommends the following modifications in the description and conditions of use of the additive (Table 1):

- Inclusion of the strain accession number
- Inclusion of the minimum declared content of yeast cells in the additive (2 x 10^9 CFU/g)
- Inclusion of appropriate purity criteria
- Use levels should be expressed as CFU/kg of feed and the equivalent in CFU/head/day given under "other provisions"
- Method of analysis should reflect the CRL Report

DOCUMENTATION PROVIDED TO EFSA

1. Registration dossier for the product MycoCell (*Saccharomyces cerevisiae* NYCC R404). May 2007. Submitted by Micron Bio-Systems Ltd.
2. Supplementary information on the product MycoCell (*Saccharomyces cerevisiae* NYCC R404). June 2009. Submitted by Micron Bio-Systems Ltd.

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

3. Supplementary information on the product MycoCell (*Saccharomyces cerevisiae* NYCC R404). September 2009. Submitted by Micron Bio-Systems Ltd.

4. Evaluation report of the Community Reference Laboratory feed additives authorisation on the methods(s) of analysis for MycoCell for dairy cows.

5. Comments from Member States received through the EFSAnet.

REFERENCES

EFSA (European Food Safety Authority), 2008. The maintenance of the list of QPS microorganisms added to food or feed. Scientific Opinion of the Panel on Biological Hazards. The EFSA Journal (2008) 923, 1-48

http://www.efsa.europa.eu/en/scdocs/scdoc/923.htm

Appendices

APPENDIX A

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for Mycocell for dairy cows

In the current application authorisation is sought for MycoCell under the category 'zootechnical additives', functional groups 'digestibility enhancers', 'gut flora stabilisers', 'other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of MycoCell for dairy cows is requested. MycoCell is provided in two forms, MycoCell concentrate and MycoCell farm packs which contain at least 5×10^9 and 2×10^8 to 2×10^9 c.f.u. viable cells of the yeast strain *Saccharomyces cerevisiae* NCYC R404 as the active agent per gram, respectively. The feed additive may be effectively used in any feed for dairy cows at a recommended minimum dose of 1×10^{10} c.f.u. per day.

For the determination of the active agent (*Saccharomyces cerevisiae* NCYC R404) in the MycoCell concentrate and farm packs, a pour plate method for enumeration is proposed which is considered appropriate for the intended purpose.

For the quantification of the active agent *S. cerevisiae* NCYC R404 in premixtures and feedingstuffs, the CRL-FA proposes a ring-trial validated method. The method's performance characteristics are standard deviations for repeatability (s_r) and reproducibility (s_R) of around 0.17 – 0.36 $\log_{10}$ and 0.55 – 0.60 $\log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively [System. Appl. Microbiol. 2003, 26, 147-153]. The method has a limit of quantification (LOQ) of 10×10^5 c.f.u./kg. The CRL considers the method suitable for official control purposes, if the target level is expressed in terms of c.f.u. per kg feedingstuffs not in terms of c.f.u. per day as specified in the proposed register entry.

A PCR method for strain identification which performed appropriately in a ring-trial validation study [System. Appl. Microbiol. 2004, 27, 492-500] is recommended for official controls for the field of application sought.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required by the CRL.