REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of 25-Hydroxycholecalciferol (25-OH-D_3) Produced by a Genetically Modified Strain of *Saccharomyces Cerevisiae* SC0639 as a Feed Additive for All Ruminants (RP1317)

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FSA Research and Evidence

An application was submitted to the Food Standards Agency (FSA) in October 2021 from DSM Nutritional Products Ltd ('the applicant') for the new use (extension of species) of an additive containing 25-hydroxycholecalciferol (25-OH-D_3) produced by a genetically modified strain of *Saccharomyces cerevisiae* SC0639, under the category 'nutritional additive', functional group 'vitamins, pro-vitamins and chemically well-defined substances having a similar effect'. The applicant seeks an extension of use from chickens for fattening, turkeys for fattening, other poultry and pigs to include all ruminants. The additive is proposed to be used at an inclusion rate of 100 µg/kg of feed (corresponding to 4000 IU vitamin D_3/kg feed).

To support the FSA and Food Standards Scotland (FSS) in evaluating the dossier, the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.

The FSA/FSS concluded, based on the ACAF's advice, that the additive was correctly identified and characterised using whole genome sequencing, and upon receiving additional information, members were satisfied with the manufacturing process. Stability was determined to depend on the manufacturer and the composition of the stabilised form. The additive was also concluded to be homogeneously distributed in complete feed for turkeys. No further concerns were raised relating to identity. The ACAF concluded that the additive is safe for the target species, the consumer and the environment. The additive is not an irritant to skin or eyes but should be treated as potentially harmful if inhaled and as a potential skin sensitiser. Upon receipt of further information relating to the inclusion

rate of the additive, the ACAF concluded that the inclusion of the additive demonstrates similar effects on performance parameters when compared to vitamin D_3. The Committee therefore concluded that the additive is effective in raising serum/plasma 25-OH-D_3 levels in comparison with vitamin D_3 for ruminants when used according to the proposed conditions of use.

The views of ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

This is a joint FSA and FSS publication.

1. Introduction

The FSA and FSS have undertaken an assessment of a feed additive containing 25-hydroxycholecalciferol (25-OH-D_3) produced by a genetically modified strain of *Saccharomyces cerevisiae* SC0639 (DSM Nutritional Products Ltd in Switzerland, Wurmisweg 576, 4303, Kaiseraugst, Switzerland) under Assimilated Regulation (EC) No 1831/2003 (EC 2003). The application sought a new use (extension of use from chickens for fattening, turkeys for fattening, other poultry and pigs to include all ruminants). The additive falls under the category 'nutritional additive', functional group 'vitamins, pro-vitamins and chemically well-defined substances having a similar effect'.

To support the safety assessment by FSA and FSS, the ACAF provided advice to the FSA and FSS outlined in this document.

In line with Article 8 of 1831/2003, the assessment has considered and concluded that the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

The dossier was evaluated by the Advisory Committee on Animal Feedingstuffs (ACAF) at its December 2023 meeting, after which a request for further information was communicated to the applicant. The applicant's response to this request was evaluated by ACAF at its June 2024 meeting.

The views of ACAF have been taken into account in this safety assessment which represents the opinion of the FSA/FSS.

Table 1. Table showing products included in this assessment

Title	Product type	Intended use/s	Intended dose/intake
25-hydroxycholecalciferol (25-OH-D_3) produced by a genetically modified strain of *Saccharomyces cerevisiae* SC0639	Feed additive	Nutritional additive	100 µg/kg of feed (corresponding to 4000 IU vitamin D_3/kg feed)

2. Assessment

2.1. Section II: Identity, characterisation and conditions of use

25-OH-D_3 is made from the raw material 5,7,24-cholestatrienol, which is produced by a fermentation process using a genetically modified strain of *Saccharomyces cerevisiae* (SC0639). The additive is an off-white to yellowish powder consisting of > 94% 25-OH-D_3, ≤ 1% each of other related sterols and < 5 mg/kg erythrosine.

One example of a formulated product containing 25-OH-D_3 is ROVIMIX® Hy-D® 1.25%, which is manufactured by the applicant to contain a minimum of 12.5 g/kg of the active substance. The composition of Hy-D® 1.25% is given in Table 2.

Table 2. Composition of ROVIMIX® Hy-D® 1.25%

Composition	1 g contains (in mg)
25-OH-D_3	12.5
Authorised antioxidants (e.g. BHT)	37.5
Sodium ascorbate	25
Vegetable oil	50
Modified food starch	715
Maltodextrin	150
Silicon dioxide (E551)	10

The applicant provided data from five batches for the formulated product ROVIMIX® Hy-D® 1.25% supporting the identification values outlined below (Table 3):

Table 3. Identity table ROVIMIX® Hy-D® 1.25%.

Composition	
25-OH-D_3	> 94%
Other related sterols	≤ 1% each
Erythrosine	< 5 mg/kg
Water	≤ 5%
Organic solvents	≤ 1%
Sum of other sterols	≤ 6%
Appearance	
Off-white to yellowish powder	
Chemical-physical specifications	
Dusting potential	2.4 g/m^3
Particle size distribution	> 1000 µm – 5% 500 µm – 13% 100 µm – 55% 50 µm – 22% < 10 µm – 5%
Impurities	
Arsenic	≤ 3 ppm
Lead	≤ 2 ppm
Cadmium	≤ 1 ppm
Mercury	≤ 0.1 ppm
Enterobacteria	Absent in 25 g
Salmonella	Absent in 25 g
Yeast and moulds	Absent in 10 g
Methanol	168 – 204 ppm
Ethanol	< 10 ppm
Isopropanol	< 10 ppm
Acetone	< 10 ppm
Ethyl acetate	< 10 ppm
PCDD/PCDF	0.000567 ng WHO_{2005}-TEQ/kg (LB) 0.101 ng WHO_{2005}-TEQ/kg (UB)
Aflatoxin B1	< 0.5 ppb
Aflatoxin B2	< 0.5 ppb
Aflatoxin G1	< 0.5 ppb
Aflatoxin G2	< 0.5 ppb

The Committee evaluated the identity and characterisation of the additive, requesting information relating to microbiological contamination and data to quantify the growth media within the final additive. In their response, the applicant stated that the product placed on the market is spray-dried and is therefore subjected to very high temperatures, so microbial testing is not necessary. The Committee commented that these temperatures are insufficient for sterilisation and hence the applicant provided microbial

testing. Members agreed that the strain had been fully characterised using whole genome sequencing and were therefore able to conclude on the identity and characterisation of the additive.

Members noted that both HACCP (Hazard Analysis and Critical Control Points) and relevant assurance certification were not provided by the applicant, as well as only a single control point. Following a response from the applicant in addition to an updated manufacturing process and composition list, the Committee agreed that the HACCP documentation and relevant processing information provided were satisfactory.

The formulation prepared with butylated hydroxytoluene (BHT) was shown to be stable for 24 months when stored at 15 ± 2°C, 18 months when stored at 25 ± 2°C and 60% relative humidity (RH), and 6 months when stored at 40°C and 75% RH. Stability of the additive depends on the manufacturer and the composition of the stabilised form, such as the inclusion of antioxidants, e.g. BHT. Regarding stability in premixtures and feedingstuffs, the applicant demonstrated that the formulation ROVIMIX® Hy-D® 1.25% is stable during the manufacture of premixtures and can be stored up to 6 months in premixtures not containing minerals. The formulation is stable for 3 months in complete premixtures when stored at room temperature. Stability during pelleting at 70 - 90°C for 2.5 mins was demonstrated with the formulation remaining stable for 3 months after pelleting. Homogeneity of the additive in complete feed for turkeys was previously assessed.

The proposed conditions of use of the additive are described in Table 4.

Table 4. Conditions of use of 25-hydroxycholecalciferol proposed by the applicant

Species or category of animal	Min. content in feed	Max. content in feed	Withdrawal period
All ruminants	-	100 µg/kg of feed (corresponding to 4000 IU vitamin D_3/kg feed)	-
Other provisions and additional requirements for the labelling			
Specific conditions or restrictions for use	Stable to pelleting at 70 - 90°C for 2.5 minutes. Not intended for use in drinking water.		
Specific conditions or restrictions for handling.	For user safety – potentially harmful if inhaled. Wear personal protective equipment as the additive is a potential skin sensitiser.		

The ACAF accept the applicant's proposed conditions of use for the additive.

2.1.1. Conclusions on Section II

The ACAF concluded that the additive was fully identified and characterised using whole genome sequencing. Upon receipt of further information, members were satisfied with the manufacturing process. Members were

able to conclude positively on the stability of the formulation provided and homogeneity of the additive. No further concerns were raised for Section II of the dossier.

2.2. Section III: Safety

2.2.1. Safety for the target species

The Committee reviewed a literature search conducted by the applicant to identify information relating to the safety of 25-OH-D_3 for ruminants. No negative effects of dietary supplementation with the additive were reported; therefore the Committee concluded that the additive can be considered safe for the target species.

2.2.2. Safety for the consumer

A number of toxicological tests were available for 25-OH-D_3 and considered previously by the EFSA (European Food Safety Authority) FEEDAP Panel (The Panel on Additives and Products or Substances used in Animal Feed) in 2005 (EFSA, 2005). These studies included: acute toxicity in mice and rats, repeated dose sub-chronic toxicity in rats, two mutagenicity studies (a bacterial reverse mutation test and an *in vitro* mammalian cell micronucleus test) and reproduction studies in both rats and rabbits. A further chromosome aberration test in 2009 (EFSA, 2009) confirmed that the additive is not considered to be genotoxic, as the results did not show any clastogenic activity of the additive when tested up to cytotoxic concentrations. Taking account of the results of all the mutagenicity tests, the ACAF concluded that the additive is not genotoxic.

The ACAF agreed that the dietary exposure assessment performed using the FACE (Feed Additive Consumer Exposure) model was appropriate and the tolerable upper intake levels (ULs) used in the consumer risk assessment were in line with EFSA's previous evaluations of vitamin D_3 and 25-OH-D_3. The Committee concluded the additive can be considered safe for the consumer.

2.2.3. Safety for the user

The applicant had originally not provided any study data for safety for the user, hence the Committee requested reports relating to skin and eye irritancy. The following tests were presented, wherein the substance tested was ROVIMIX® Hy-D® 1.25%:

- Eye irritancy, following OECD protocol 405;
- Skin irritancy/corrosion, following OECD protocol 404.

For eye irritancy, treatment caused reversible redness and discharge which had resolved at 24 hours post-treatment; therefore the additive is not considered an eye irritant. For skin irritancy and corrosion, treatment caused mild erythema and oedema which resolved after 7 days, therefore the material is not corrosive or irritant to skin.

Dusting potential and particle size distribution data indicate that normal handling of 25-OH-D_3 could generate a respirable dust. Studies assessing inhalation toxicity and dermal sensitisation were not provided; therefore the additive should be considered potentially harmful if inhaled and as a potential skin sensitiser.

2.2.4. Safety for the environment

25-OH-D_3 is a natural substance that is extensively metabolised in the target animal and excreted in small amounts. Additionally, the additive predominantly substitutes for supplemental vitamin D_3 at the relevant maximum content; hence the Committee concluded that environmental studies are not necessary as the use of the additive in feedingstuffs does not represent a risk for the environment.

2.2.5. Conclusions on safety

The ACAF concluded that the additive is safe for the target animals, consumers, and the environment.

The additive is not irritant to skin or eyes but should be treated as potentially harmful if inhaled and as a potential skin sensitiser.

2.3. Section IV: Efficacy

The applicant provided four published studies evaluating the additive as a source of vitamin D for ruminants, focusing on the equivalence of dietary 25-OH-D_3 to vitamin D_3. Members raised concerns relating to the inclusion rate for the use of the additive in ruminants as over-supplementation was a potential risk. Upon receiving this additional information, the Committee concluded that the inclusion of the additive in ruminant diets has similar effects on performance parameters as vitamin D_3 and could therefore conclude the additive is an efficient source of vitamin D_3.

2.3.1. Conclusions on efficacy

The ACAF concluded that the inclusion of the additive has similar effects on performance parameters as vitamin D_3 when added to ruminant diets. The Committee, therefore, concluded that the additive is an efficient source of vitamin D_3 for ruminants when used according to the proposed conditions of use.

3. Analytical methods evaluation

The FSA/FSS consider the EURL to be a competent and reliable body for the evaluation of analytical methods for the authorisation of feed additives. The EURL evaluation standards were agreed when the UK was part of the EU and they have remained relevant for the standards expected at UK level. Based on these premises, the conclusions on the evaluation of the analytical methods for this application have been received and accepted to be applicable to the UK authorisation process.

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for 25-hydroxycholecalciferol (25-OH-D_3) (EURL, 2009):

> "For the determination of 25-OH-D_3 in stabilised forms of the feed additive the applicant proposed a single-laboratory validated and further verified method based on reversed-phase Ultra Performance Liquid Chromatography (UPLC) coupled to spectrophotometric (UV) detection. For the determination of 25-OH-D_3 in premixtures the applicant proposed a single-laboratory validated and further verified method based on reverse-phased High Performance Liquid Chromatography (HPLC) coupled to spectrophotometric (UV) detection. The method is applicable for samples containing 25-OH-D_3 at a minimum of 1500 µg/kg of the feed additive. For the determination of 25-OH-D_3 in low concentrated premixtures and feedingstuffs the applicant proposed a single-laboratory validated and further verified method based on HPLC coupled to tandem mass spectrometry (MS/MS). The method is applicable for samples containing 25-OH-D_3 between 10 and 3000 µg/kg of the feed additive. The following performance characteristics were reported by the applicant from the validation and verification studies for the quantification of 25-OH-D_3 in the feed additive (stabilised products), premixtures, low concentrated premixtures and feedingstuffs:
>
> - In the feed additive: a relative standard deviation for repeatability (RSD_r) and a relative standard deviation for intermediate precision (RSD_{ip}) of 1.2%, a recovery rate (R_{rec}) of 101%;
> - In premixtures (minimum 1500 µg/kg): a RSD_r ranging from 1.5 to 4.4%; a RSD_{ip} ranging from 1.6 to 5.0%; a R_{rec} of 102%; and

> - In low concentrated premixtures and feedingstuffs (10 to 3000 µg/kg): a RSD_r ranging from 3.0 to 5.3%; a RSD_{ip} ranging from 3.1 to 5.9%; a R_{rec} of 92%; and a limit of quantification (LOQ) of 2 µg of 25-OH-D_3/kg feedingstuffs.
>
> Based on the performance characteristics presented the EURL recommends for the official control the single-laboratory validated and further verified analytical method mentioned above based on UPLC-UV for the determination of 25-OH-D_3 in stabilised forms of the feed additive; the single-laboratory validated and further verified analytical method based on HPLC-UV for the determination of 25-OH-D_3 in premixtures: and the single-laboratory validated and further verified analytical method based on HPLC-MS/MS for the determination of 25-OH-D_3 in low concentrated premixtures and feedingstuffs.
>
> 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical methods proposed as appropriate for official controls for this feed additive.

4. Conclusions

The ACAF concluded that the additive was fully identified and characterised using whole genome sequencing. Members concluded positively on the stability of the formulation tested, noting that stability will depend on the manufacturer and composition of the stabilised form. Homogeneity of the additive was also demonstrated.

The additive is presumed safe for the target animals, the consumer and the environment. It is not irritant to skin or eyes but should be treated as potentially harmful if inhaled and as a potential skin sensitiser.

The ACAF concluded that the inclusion of the additive in ruminant diets has similar performance effects as vitamin D_3. The Committee therefore concluded that the additive is an efficient source of vitamin D_3 for ruminants when used according to the proposed conditions of use.

Abbreviations

Abbreviation	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
BHT	Butylated hydroxytoluene
EC	European Commission
EFSA	European Food Safety Authority
EURL-FA	European Reference Laboratory for Feed Additives
FACE	Feed Additive Consumer Exposure
FEEDAP	Panel on Additives and Products or Substances Used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland
HACCP	Hazard Analysis and Critical Control Point
HPLC	High performance liquid chromatography
IU	International unit
LOQ	Limit of quantification
MS	Mass spectrometry
RH	Relative humidity
R_{rec}	Recovery rate
RSD_{ip}	Relative standard deviation for intermediate precision
RSD_r	Relative standard deviation for repeatability
UL	Upper intake level
UPLC	Ultra performance liquid chromatography

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References

EC (European Commission). (2003). *Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2003/1831/contents

EURL-FA (European Reference Laboratory for Feed Additives). (2009). *Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. Bacillus subtilis ATCC PTA-6737*. https://joint-research-centre.ec.europa.eu/document/download/40c03568-24b6-4804-8ffc-bb9c5a6f1912_en?filename=FinRep-FAD-2008-0039.pdf

Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission on the evaluation of safety and efficacy of "Hy·D" (calcifediol), based on 25-hydroxylcholecalciferol/25-hydroxy-pre-cholecalciferol, as feed additive in accordance with Council Directive 70/524/EEC. (2005). *The EFSA Journal*, *224*, 1–35. https://doi.org/10.2903/j.efsa.2005.224

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from European Commission on the safety and efficacy of 25-hydroxycholecalciferol as feed additive for poultry and pigs. (2009). *The EFSA Journal*, *969*, 1–32.