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Safety and efficacy of a feed additive consisting of 25-hydroxycholecalciferol monohydrate produced with *Saccharomyces cerevisiae* CBS 146008 for all ruminants (DSM Nutritional Products Sp. z.o.o.)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of 25-hydroxycholecalciferol monohydrate produced with *Saccharomyces cerevisiae* CBS 146008 as a nutritional feed additive for all ruminants. The additive is already authorised for use with chickens for fattening, turkeys for fattening, other poultry and pigs. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the additive does not give raise to any safety concern regarding the production strain. The additive is safe for cattle for fattening and dairy cows at the maximum recommended use level of 0.1 mg 25-OH-D_3/kg complete feed. This conclusion can be extended to other cattle categories and extrapolated to all ruminant species. The use of 25-OH-D_3 in all ruminants under the proposed conditions of use is considered safe for the consumer. The additive is not irritant to the skin or eyes. No conclusion on its potential to be a skin sensitiser or on its effects on the respiratory system can be reached due to absence of data. The use of the additive under assessment at the recommended conditions of use is considered safe for the environment. 25-OH-D_3 is an efficient source of vitamin D_3 for all ruminants when used according to the proposed conditions of use.

Keywords: nutritional additive, 25-hydroxycholecalciferol, vitamin D, *Saccharomyces cerevisiae* CBS 146008, safety, efficacy

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1. Introduction

1.1. Background and terms of reference

Regulation (EC) No 1831/2003[1] 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 authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from DSM Nutritional Products Ltd[2] (represented by DSM Nutritional Products Sp. z.o.o.) for the authorisation of the additive consisting of 25-hydroxycholecalciferol monohydrate produced with *Saccharomyces cerevisiae* CBS 146008, when used as a feed additive for ruminants (category: nutritional additives; functional group: vitamins, pro-vitamins and chemically well-defined substances having a similar effect).

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. The particulars and documents in support of the application were considered valid by EFSA as of 15 October 2021.

According to Article 8 of Regulation (EC) No 1831/2003, 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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of 25-hydroxycholecalciferol produced by *Saccharomyces cerevisiae* CBS 146008, when used under the proposed conditions of use (see Section 3.1.2).

1.2. Additional information

The additive 25-hydroxycholecalciferol (25-OH-D_3, synonyms calcidiol or calcifediol) is [redacted].

EFSA published four opinions on the use of 25-OH-D_3 as a feed additive, one on the safety and efficacy for chickens and turkeys for fattening and laying hens (EFSA, 2005); another on the safety and efficacy for poultry and pigs (EFSA, 2009); one on the safety and efficacy for all pigs, all poultry for fattening and ornamental birds and other poultry species (EFSA FEEDAP Panel, 2023a), and a last one on the renewal of the authorisation for poultry and pigs (EFSA FEEDAP Panel, 2023b). The EFSA Panel on Nutrition, Novel Foods and Food Allergens (EFSA NDA Panel) of EFSA has recently adopted an opinion on the safety of calcidiol (25-OH-D_3) monohydrate as a novel food (EFSA NDA Panel, 2021).

The additive 25-hydyroxycholecalciferol is currently authorised in a stabilised form for use in feed for chickens for fattening, turkeys for fattening, other poultry and pigs (Commission Regulation (EC) No 887/2009).[3]

The European Pharmacopoeia (2023) has monograph 01/2019:1295 dedicated to calcifediol monohydrate (synonym of 25-hydroxycholecalciferol monohydrate).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of 25-hydroxycholecalciferol monohydrate

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] DSM Nutritional Products Ltd, Wurmingsweg 576, 4303 Kaiseraugst, Switzerland. Represented in the EU by DSM Nutritional Products Sp. z.o.o., Tarczynska 113, 96–320 Mszczonow, Poland.
[3] Commission Regulation (EC) No 887/2009 of 25 September 2009 concerning the authorisation of a stabilised form of 25-hydroxycholecalciferol as a feed additive for chickens for fattening, turkeys for fattening, other poultry and pigs. OJ L 254/68, 26.09.2009.
[4] FEED dossier reference: FAD-2021-0034.

produced by *Saccharomyces cerevisiae* CBS 146008 as a feed additive. The dossier was received on 16/03/2021 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00341.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the 25-hydroxycholecalciferol in animal feed/marker residue in tissues are valid and applicable for the current application.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of 25-hydroxycholecalciferol is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel 2012a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The subject of the assessment, the 25-hydroxycholecalciferol monohydrate (25-OH-D_3), is classified a nutritional additive, functional group vitamins, pro-vitamins and chemically well-defined substances having a similar effect (EU register code 3a670a) and applied for use in feed for ruminants. The additive is currently authorised for pigs and poultry as a stabilised form of the active substance 25-OH-D_3. The term 'additive' in this opinion will be used to refer to the stabilised form of 25-OH-D_3 as described by the applicant (see EFSA FEEDAP Panel, 2023b).

To become effective, vitamin D_3 (cholecalciferol) requires as a first transformation step its hydroxylation by microsomal liver enzymes at C25. Further hydroxylation of 25-OH-D_3 to the most potent vitamin D_3 hormone 1,25-dihydroxycholecalciferol occurs mainly in the kidney.

3.1. Characterisation

The additive was fully characterised in the previous opinion of the FEEDAP Panel, which included the characterisation of the production strain, the characterisation of the active substance, including the evaluation of the presence of small/nanoparticles (EFSA FEEDAP Panel, 2023b).

3.1.1. Characterisation of the active substance

The active substance 25-hydroxycholecalciferol monohydrate ((3*S*,5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-triene-3,25-diol monohydrate; IUPAC name: (1*S*,3*Z*)-3-[(2*E*)-2-[(1*R*,3a*S*,7a*R*)-1-[(2*R*)-6-hydroxy-6-methylheptan-2-yl]-7a-methyl-2,3,3a,5,6,7-hexahydro-1*H*-inden-4-ylidene]ethylidene]-4-methylidenecyclohexan-1-ol;hydrate; synonyms: calcidiol, calcifediol; 25-hydroxy vitamin D_3) is identified with the Chemical Abstracts Service (CAS) number 63283-36-3 and the European Inventory of Existing Chemical Substances (EINECS) number 621-370-5.10 The molecular formula is $C_{27}H_{44}O_2 \cdot H_2O$ and its molecular weight is 418.66 Da. The structural formula is represented in Figure 1.

[5] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2018-0074_en

[6] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

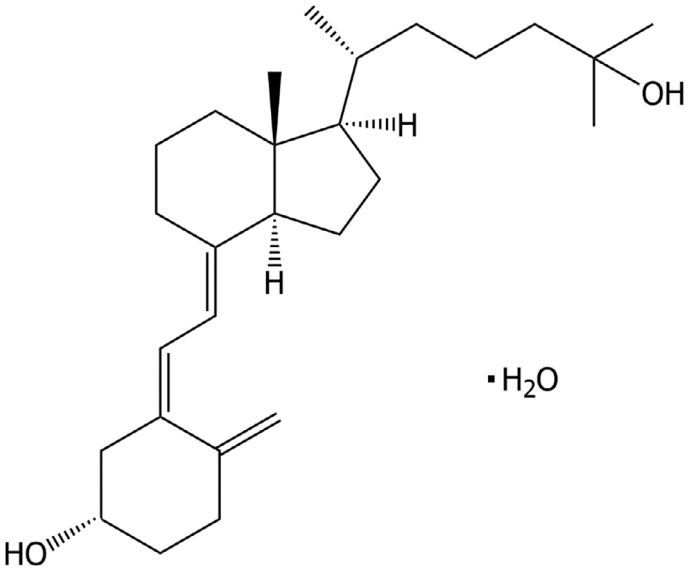

Figure 1: Structural formula of 25-hydroxycholecalciferol monohydrate

The authorisation by Commission Regulation (EC) No 887/2009 followed the previous specification and characterises the active substance with 25-hydroxycholecalciferol > 94%, other related sterols < 1% each and erythrosine < 5 mg/kg.

The applicant is proposing to keep the same specifications with regards the minimum content of the active substance and the limit for erythrosine, but to change the specifications for other related sterols from '< 1% each' to '≤ 1% each'. The applicant justifies this change with the fact that the values measured by the analytical method are whole numbers, either 0 or 1. The applicant also set specifications for water ≤ 5% and for the sum of other sterols ≤ 6%.

Analytical data on batch-to-batch variation (five batches analysed) showed average concentrations of 25-OH-D_3 of 104% (range 103–105%),[7] other related sterols ranged 0–1% and erythrosine was below the limit of detection (LOD) of 0.08 mg/kg.[8] Water was 4% in all five batches and the sum of other sterols averaged 1.3% (range 0.9–1.7%).

25-OH-D_3 is insoluble in water, soluble in acetone, ethanol, DMSO and other lipophilic solvents. Its melting point is 100–120°C.

Apart from erythrosine and other related sterols, the applicant set specifications for aluminium and lead (≤ 20 mg/kg) and for total organic solvents used in the manufacturing process (≤ 1%). Data from five batches showed compliance with the specifications.[9]

3.1.2. Conditions of use

The stabilised form of 25-OH-D_3 is intended to be used as a feed additive in feed for all ruminants at maximum use level of 0.1 mg/kg complete feed, corresponding to 4,000 IU vitamin D_3/kg feed. The formulated additive is intended to be used in the form of premixtures which are then added to the final feed.[10]

3.2. Safety

3.2.1. Safety of the production microorganism

The safety of the production organism *Saccharomyces cerevisiae* CBS 146008 has recently been assessed in the opinion for the renewal of the authorisation for pigs and poultry (EFSA FEEDAP Panel, 2023b). Absence of viable cells and recombinant DNA of the production strain in the additive was demonstrated. Therefore, the additive does not give raise to any safety concern regarding the genetically modified production strain.

[7] Technical dossier/Annex II.1.2. Analytical method HPLC with UV detection.
[8] Technical dossier/Supplementary information January 2023/Reply to question 4.
[9] Technical dossier/Annex II.1.2.
[10] Technical dossier/Supplementary information April 2023/2022-04-27 letter reply to EFSA Sin EFSA-Q-2021-00341.

3.2.2. Safety for the target species

For dairy cattle nutrition, the goal is to supply the animal with an amount of vitamin D_3 that achieves a serum 25-OH-D (representing 25-OH-D_2 [originating from plants] and 25-OH-D_3 metabolites combined) concentration that supports the multiple outcomes of vitamin D (Nelson and Merriman, 2014). Under normal circumstances in calves and lactating cows, serum 25-OH-D concentrations of 20 to 100 ng/mL support a normal calcium and phosphate balance in dairy cows. This is confirmed by a survey of Nelson et al. (2016) who analysed 25-OH-D_3 in 702 serum samples collected from cows across various stages of lactation, housing systems and locations in the United States. Average concentration was 68 ng/mL (standard deviation [SD] 22 ng/mL), with most of the samples ranging between 40 and 100 ng/mL. Most of the 12 herds surveyed supplemented cows with 30,000 to 50,000 IU of vitamin D_3/day and the average serum 25-OH-D levels of cows was near or above 70 ng/mL regardless of season or housing. In contrast, average serum 25-OH-D concentration of a herd supplementing with 20,000 IU/d was 42 ng/mL (SD 15 ng/mL), with 22% of the animals below 30 ng 25-OH-D/mL.

Serum concentrations of 25-OH-D_3 over 100 ng/mL do not seem to provide the transition cow much benefit compared to concentrations between 20 and 50 ng/mL as regards blood calcium (Nelson and Merriman, 2014).

Optimal serum 25-OH-D_3 concentrations to support immune function, lactation and reproduction in cattle are not yet determined. Levels indicating intolerance are also not known.

Consequently, indications of safety of 25-OH-D_3 for ruminants can only be derived from studies with oral vitamin D_3. In the EU, the maximum (also safe) contents of vitamin D_3 are, established by Regulation (EU) 2017/1492, 4,000 IU/kg complete feed (88% DM) for bovines and ovine, 10,000 IU/kg milk replacer for calves and 2,000 IU/kg complete feed for others (including in this case other ruminants, e.g. caprine).

According to the FEEDAP Guidance on the assessment of the safety of feed additives for the target species (2017c), safety for the target animals can be presumed without the need for additional studies for nutritional additives assessed and authorised following the provisions of Regulation (EC) No 1831/2003. This is the case for 25-OH-D_3.[11] The additive can therefore be considered safe for ruminants.

However, the applicant made an extensive literature search (completed in December 2020).[12] Search terms were 25-OH-D_3 (and synonyms) and ruminants (including species belonging to ruminants). The databases used were Scopus and PubMed. A total of 238 citations were examined for their relevance to safety and efficacy in ruminant species when 25-OH-D_3 was given via feed or water for drinking. Following the ad hoc established inclusion criteria, the applicant finally identified 18 publications. One study was considered relevant for safety and efficacy, nine others for safety (and metabolism) in target animals and the remaining eight studies for efficacy only.

In the studies of Wilkens et al. (2012, 2013) and Guo et al. (2018), 25-OH-D_3 was given to pregnant cows about 8 to 14 days (before parturition). These publications were not considered further under a safety aspect. Also, another paper of Wilkens et al. (2016), who studied the influence of 25-OH-D_3 and 1,25-dihydroxyvitamin D_3 on expression of P-glycoprotein and cytochrome P450 3A in sheep after a 10 days application is not considered relevant for the safety.

Celi et al. (2018) fed calves for fattening (3.5 months old, 106 kg body weight (BW), 10 animals/group) for 90 days increasing doses of 25-OH-D_3: 1.7 μg, 5.1 μg and 8.5 μg/kg BW (corresponding to 56, 169 and 249 μg/kg feed, respectively). The control group received 0.75 μg vitamin D_3/kg BW. Serum 25-OH-D_3 increased from 46 (control group without 25-OH-D_3) to 107, 188 and 217 ng/mL for the 25-OH-D_3 supplemented groups, respectively. All calves in the four groups gained weight continually: no growth depression was observed. No adverse effects of 25-OH-D3 were observed for any of the haematology[13] and serum chemistry parameters[14] measured monthly or during the routine clinical examinations (on a monthly basis also). In the post-mortem evaluation, no adverse effects of the different 25-OH-D_3 doses were observed, neither during the gross pathology nor in the histological

[11] Commission Regulation (EC) No 887/2009 of 25 September 2009 concerning the authorisation of a stabilised form of 25-hydroxycholecalciferol as a feed additive for chickens for fattening, turkeys for fattening, other poultry and pigs. OJ L 254, 26.9.2009, p. 68–70.

[12] Technical dossier/Section III/Annex III.1.4.

[13] WBC, RBC, Haemoglobin, HTC, MCV, MCH, MCHC, platelets, neutrophils, Neu % of WBC, Lymphocytes, Lym % of WBC, monocytes, Mon % of WBC, eosinophils, Eos % of WBC, basophils, Bas % of WBC, large unstained cells, Luc % of WBC, protrombine time, activated partial thromboplastine time.

[14] Urea, creatinine, urea/creatinine, glucose, sodium, potassium, chloride, ALP, ALT, AST, LDH, CPK, CGT, total protein, albumin, globulin, A/G, calcium, phosphate, magnesium, cholesterol, amylase, total bile acids.

examination. The data allow the conclusion that about 10,000 IU vitamin D from 25-OH-D_3/kg feed were well tolerated by growing cattle.

Tomkins et al. (2020) concluded from their studies in heifers administering among others a slow release 25-OH-D_3 bolus (HyD) for 188 days, that a target plasma concentration of 25-OH-D_3 for increasing P absorption in beef cattle is between 200 and 300 ng/mL.

Rodney et al. (2018a) supplemented feed for dairy cows during late gestation with 3 mg cholecalciferol or 25-OH-D_3/11 kg DM for about 30 days and analysed blood samples until 30 days post-partum for vitamin D metabolites, minerals and bone-related hormones. The results provided evidence that dietary manipulations can induce metabolic adaptations that improve mineral homeostasis with the onset of lactation that might explain some of the improvements observed in health and production when cows are fed with negative dietary cation-anion difference diet (DCAD) or supplemented with 25-OH-D_3. 3 mg 25-OH-D_3/11 kg DM would correspond to 0.24 mg/kg complete feed and to about 9.600 IU/kg complete feed. In another study of the same research group (Rodney et al., 2018b) with as similar design, dairy cows were fed diets with 25-OH-D_3 or cholecalciferol. Feeding 25-OH-D_3 compared with cholecalciferol increased plasma concentrations of 25-OH-D_3 pre- (264.2 [SD 8.0] vs. 61.3 [SD 8.0] ng/mL) and postpartum (170.8 [SD 6.2] vs. 51.3 [SD 6.2] ng/mL). In both studies, no adverse effects were seen. In summary, about 9.600 IU vitamin D form 25-OH-D_3 were tolerated by dairy cows when fed for about 30 days ante partum. This complies with the information provided by the NRC (1981) that up to 25,000 IU vitamin D from calciferol/kg (DM) feed would be safe for a period of < 60 days.

McGrath et al. (2013) administered 3 mg 25-OH-D_3/day to steers (18 months old and about 315 kg BW) fed diets with DCAD of 50 and 150 mEq/kg for 14 days. The 25-OH-D_3 increased urinary Ca excretion. No significant differences in the apparent digestibility of either Ca (37.2 [SD 2.7] %) or P (47.5 [SD 5.8] %) attributable to dietary treatment were recorded.

Oehlschlaeger et al. (2014) investigated the effects of 25-OH-D_3 (6 mg per cow and day) in combination with DCAD values of about −70 mEq/kg DM on pre-duodenal and overall gastrointestinal Ca absorption in a group of six rumen fistulated lactating cows (606 kg initial BW). The study lasted for 5 weeks (including an adaptation period of 2 weeks). The authors concluded that anionic salts in combination with 25-OH-D_3 positively influence the overall net Ca and phosphorus absorption, which is obviously associated with a reduced mobilisation of bone minerals.

In total, one study allows an additional conclusion (to the ones already made in previous FEEDAP opinions on the additive) on the safety of 25-OH-D_3 in a ruminant animal category. In this study (Celi et al., 2018) no adverse effects (zootechnical performance, haematology, blood routine chemistry, gross pathology and histopathology) were found in growing cattle given 246 μg 25-OH-D_3/kg feed, which corresponds to about the 2.5-fold of the maximum content applied (100 μg/kg complete feed). Therefore, there is additional evidence that 100 μg 25-OH-D_3/kg complete feed is safe for cattle for fattening. This conclusion can – in the light of the other available data previously assessed – be extended to all bovines, ovine and caprine.

In its previous scientific opinions on the safety and efficacy of vitamin D_3 (2012a,b, 2013, 2014), the FEEDAP Panel noted that it was not able to draw final conclusions on the upper safe levels of vitamin D in animal nutrition, based on a data collection of the National Research Council (NRC, 1987). The FEEDAP Panel considered the current maximum contents in EU feed legislation to be temporarily acceptable for the target animals. The FEEDAP Panel reiterates and confirms its previous position.

Considering serum levels of 25-OH-D_3 as endpoint, the 25-hydroxylated cholecalciferol is more efficient (about five times) than cholecalciferol (EFSA, 2005; EFSA NDA Panel, 2021). Serum 25-OH-D_3 serves as the pool from which 25-OH-D_3 is taken for the synthesis of the active metabolite, the vitamin-hormone 1,25-$(OH)_2$-D_3. Consequently, the serum level of 25-OH-D_3 may not only serve as a biomarker for the vitamin D status, indicating deficient or sufficient supply, but also as biomarker of the start of a vitamin D intoxication. No data are available, however, which would allow to establish a safe upper limit of 25-OH-D_3 in blood or serum for the different animals as such, or to evaluate potential interactions with season and housing (sunlight exposure), with other exogenous sources of vitamin D, and the vitamins A and K.

3.2.2.1. Conclusions on safety for the target species

The additive is safe for cattle for fattening and dairy cows at the maximum recommended use level of 0.1 mg 25-OH-D_3/kg complete feed. This conclusion can be extended to other cattle categories and extrapolated to all ruminant species.

3.2.3. Safety for the consumer

3.2.3.1. Mode of action, absorption, distribution, metabolism and excretion

Vitamin D is essential for life in higher animals, it is one of the primary biological regulators of calcium homeostasis. The supply of animals can occur by nutrition or endogenous synthesis of 7-dehydrocholesterol, which is converted in a first step to cholecalciferol in the skin after exposure to UV light. The vitamin is transported in the blood stream bound on a vitamin D binding protein. Cholecalciferol is hydroxylated at the C25 position in the liver (25-OH-D_3), followed by additional hydroxylation at C1 mainly in the kidney (by the 25-hydroxyvitamin D 1α-hydroxylase, a mitochondrial cytochrome P450 enzyme), resulting in the main active 1,25-$(OH)_2D_3$ and C24 (by 24-hydroxylase, a cytochrome P450 enzyme), resulting in mainly inactive 24,25-$(OH)_2D_3$ and 1,24,25-$(OH)_3D_3$. The active vitamin D hormone regulates calcium metabolism and bone formation. It also contributes to immune, reproductive and mammary physiology in cattle.

Serum levels of 25-OH-D_3 serve as a suitable marker of the vitamin D status of (men and) animals. The 25-OH-D_3 concentration also affects levels of 1α-hydroxylase and 24-hydroxylase in the kidneys. If the 25-OH-D_3 concentration is low, the body compensates by producing more parathyroid-hormone, thereby stimulating 1α-hydroxylase and depressing 24-hydroxylase. Conversely, as 25-OH-D_3 concentrations rise, less 1α-hydroxylase and more 24-hydroxylase are required to keep circulating 1,25 $(OH)_2D_3$ in the correct balance. Consequently, circulating 1,25-$(OH)_2D_3$ does not correlate with the 25-OH-D_3 concentration (Nelson and Merriman, 2014).

It should be noted that cattle, goats and sheep will be supplied with vitamin D_2 (ergocalciferol) when grazing or feed plant derivates. Upon consumption of dietary vitamin D by cattle, a small fraction is degraded to inactive vitamin D metabolites in the rumen by bacteria (Sommerfeld et al., 1983), the rest is absorbed in the intestinal tract in association with lipids and in the presence of bile salts (NRC, 2000).

In cattle there is an apparent discrimination against the vitamin D_2 form, presumably due to the reduced binding of vitamin D_2 metabolites to vitamin D-binding proteins in the blood, resulting in a quicker clearance from plasma (Sommerfeld et al., 1983). Ruminants do not maintain relevant body stores of vitamin D.

3.2.3.2. Residue studies

From the calves for fattening used in the study of Celi et al. (2018) described in Section 3.2.1 (safety for the target species), serum, fat, muscle, kidney and liver samples (10 per treatment group) were collected to evaluate the concentration of 25-OH-D_3. The concentrations of 25-OH-D_3 at day 90 of treatment in liver, muscle, kidney and fat were significantly increased in comparison to the control group (Table 1).

Table 1: Tissue content of 25-OH-D3 (μg/kg) after 90 days supplementation in calves, 10 samples per treatment

Tissue	Treatment groups			
	Control	56.4 μg 25OH/kg feed	168.7 μg 25OH/kg feed	249.1 μg 25OH/kg feed
Liver	4.5c	14.5b	27.4a	31.2a
Kidney	7.2c	23.1b	44.0a	39.7a
Muscle	1.8c	5.7b	10.8a	12.3a
Fat	4.1c	13.2b	20.7a	26.4a

Different letters within a row indicate statistical significance ($p < 0.05$).

The concentrations of 25-OH-D_3 in the study did not match the highest dietary level proposed in the conditions of use (100 μg/kg feed). For calculating consumer exposure, an average between the groups supplemented with 56.4 and 168.7 μg 25-OH-D_3/kg feed was used.

In a study of Rodney et al. (2018c), a total of 25 mid-lactation Holstein dairy cows (blocked by age and milk production) were randomly distributed to five treatment groups. The diet of the control group was left unsupplemented while the diet of the other four was supplemented with 0.5, 1, 2 and 4 mg 25-OH-D_3/day and cow for 30 days. Data on feed intake were not provided. Corresponding levels in a complete feed could be calculated based on the default values for daily feed intake of dairy cows of DM content of 88% and 20 kg DM/day (EFSA FEEDAP Panel, 2017a) and were 0.022, 0.044, 0.088

and 0.176 mg/kg complete feed. Milk volume was measured, and milk samples taken every 2 weeks to analyse milk levels of protein and fat, as well as protein and fat yield, somatic cell count and 25-OH-D_3 concentrations. The 25-OH-D_3 concentration in milk of the group with 0.088 mg 25-OH-D_3/kg was taken to indicate a value expected from the use of the highest proposed dose (0.1 mg 25-OH-D_3/kg complete feed). Following the guidance, two times the standard deviation was added to the mean resulting in 0.465 (0.4145 + 2 × 0.0254) μg 25-OH-D_3/kg.

3.2.3.3. Toxicological studies

The toxicological profile including genotoxicity of the additive was assessed by the FEEDAP Panel in 2005, 2009 (EFSA, 2005, 2009). New data on genotoxicity was assessed in the 2023 opinion (EFSA FEEDAP Panel, 2023b) and confirmed that the additive was not genotoxic. The FEEDAP Panel concludes that the additive is not genotoxic.

3.2.3.4. Assessment of consumer exposure

In its first opinion (EFSA, 2005), the FEEDAP Panel proposed a provisional upper tolerable limit (UL) for 25-OH-D_3 of 10 μg/day in adults and adolescents (11–17 years) and 5 μg/day in children (0–10 years). This was based on the UL for vitamin D_3 (50 μg/day in adults and 25 μg/day in children up to 11 years) and a relative biological activity factor of 5.

The FEEDAP Panel notes that the NDA Panel of EFSA has revised the UL levels for Vitamin D_3 for all age groups (EFSA NDA Panel, 2012, 2018): 25 μg/day for children up to 6 months, 35 μg/day for children 6–12 months, 50 μg/day for children 1–10 years and 100 μg/day for adolescents (11–17 years) and adults, including pregnant women.

In its previous opinion of 2009, the FEEDAP Panel conducted a 'worst-case scenario' exposure assessment for the consumer, based on the consumption model described in Regulation (EC) No 429/2008 and on data from studies done with the additive at the maximum use levels for pigs and poultry species. The results indicated that exposure of adults was below the provisional UL for 25-OH-D_3 set for adults (69%) but that that of children would be exceeded (138%). A refined calculation of exposure based on more realistic data (Scientific Cooperation of Member States [SCOOP]) indicated that exposure for both adults and children would be below the provisional UL (24% and 49%, respectively). Based on that, the Panel concluded that the total exposure resulting from the use of 25-OH-D_3 in all poultry and pig categories at the maximum doses would not represent a risk for the consumer.

The FEEDAP Panel would like to withdraw the provisional UL for 25-OH-D_3, since also cholecalciferol from other food and body's own synthesis would enter the body's store of 25-OH-D_3. It is therefore considered reasonable to apply the UL for vitamin D_3 and to introduce the 25-OH-D_3 intake multiplied with a conversion factor of 5.

In reassessing consumer exposure, the FEEDAP Panel is aware of the ongoing evaluation by the NDA Panel of setting a conversion factor for 25-OH-D_3 into Vitamin D_3. At the time of the adoption of the current FEEDAP Panel's scientific opinion, the work of the NDA Panel has not been completed. Therefore, as a pragmatic approach, the Panel considered that the residues of 25-OH-D_3 deposited in edible tissues and products should be expressed in terms of vitamin D_3 activity, and therefore, multiplied by 5 to consider the relative biological activity of the different compounds. This was then compared with the UL established by the NDA Panel for vitamin D_3.

New data have been made available regarding the deposition of 25-OH-D_3 in tissues or products of calves and dairy cows (see Section 3.2.3.2). In addition, the FEEDAP Panel is aware that 25-OH-D_3 is currently authorised for pigs and poultry. The Panel considers that the consumer exposure assessment should cover the uses of the ongoing application and the currently authorised uses (pigs and poultry). In that regard, the FEEDAP Panel recently adopted an opinion on the use of 25-OH-D_3 produced by a different production strain (*Pseudonocardia autotrophica* DSM 32858) in pigs and poultry. In that opinion, the FEEDAP Panel conducted a comprehensive consumer exposure calculation based on more recent data on the deposition of 25-OH-D_3 in food products from chickens for fattening, including food products from ruminants (EFSA FEEDAP Panel, 2023a). The FEEDAP Panel considers that the exposure assessment performed in that opinion applies to the current assessment as it also included the exposure from ruminants.

Based on the updated consumer exposure calculation and considering the updated UL for vitamin D_3, the Panel concludes that the contribution to consumer exposure to Vitamin D_3 from products of animals fed with 25-OH-D_3 is well below the UL (11.2% to 38.58%). Therefore, the use of 25-OH-D_3 in all ruminants under the proposed conditions of use is considered safe for the consumer.

3.2.4. Safety for the user

The dusting potential of the additive is up to 2.6 g/m^3 (EFSA FEEDAP Panel, 2023b),[15] therefore users may be exposed by inhalation.

In a previous opinion the FEEDAP Panel assessed a skin irritation study and an eye irritation study performed with the additive (Rovimix Hy·D 1.25%) and concluded that it is not irritant to skin or eyes (EFSA, 2005, 2009).

No new specific information was submitted to support the safety for the user. The additive is not irritant to the skin or eyes. No conclusion on its potential to be a skin sensitiser or on its effects on the respiratory system can be reached due to absence of data.

3.2.5. Safety for the environment

The production strain is genetically modified. No cells and no DNA of the production strain are present in the additive (EFSA FEEDAP Panel, 2023b). Therefore, no safety concerns for the environment are derived from the use of the production strain for production purposes.

The 25-OH-D_3 is a physiological metabolite in animals. It derives from vitamin D3, either from endogenous synthesis or exogenous supply. It is excreted only at low amounts. The use of 25-OH-D_3 in animal nutrition up to the maximum recommended use level does not represent a risk for the environment.

3.3. Efficacy

According to Regulation (EC) No 429/2008, efficacy studies are not required for vitamins provitamins and chemically defined substances having similar effects that are already authorised as feed additives under Directive 70/524/EEC.

Since 25-OH-D_3 is already authorised for chickens for fattening, turkeys for fattening, other poultry and pigs,[16] and EFSA concluded that its use is efficacious as a substitute of vitamin D3 (EFSA, 2005, 2009), no new demonstration of efficacy for ruminants would be necessary.

The applicant, however, made an extensive literature search (see Section 3.2.2) and identified eight studies (plus one *corrigendum* note) as relevant for supporting the efficacy of 25-OH-D_3 in ruminant species. No one of these studies is a typical vitamin efficacy study, in which the effect of the vitamin on classical endpoints is examined compared to a group with an insufficient or at least marginal supply of that vitamin. The main objective of the submitted efficacy trials with 25-OH-D_3 was to establish the equivalence of dietary 25-OH-D_3 to vitamin D_3 and to find out potential beneficial extra-effects, which are not necessarily associated with vitamin D_3.

- Several of those studies have observed increase in blood 25-OH-D_3 after supplementing dairy cows (de Oliveira et al., 2018 [unpublished];[17] Rodney et al., 2018c; Poindexter et al., 2020; Weiss et al., 2015; Guo et al., 2018; Taylor et al., 2008) or growing calves (Celi et al., 2018). This increase was higher compared to weight-equivalent doses of vitamin D3 (Poindexter et al., 2020) and followed the dose administered in a curvilinear manner (Rodney et al., 2018c).
- An increased urinary excretion of calcium was reported in dairy cows given 25-OH-D_3 (Weiss et al., 2015; de Oliveira et al., 2018 [unpublished]), but also higher blood calcium (de Oliveira et al., 2018 [unpublished], Poindexter et al., 2020).
- Martinez et al. (2018a,b) concluded in two publications that feeding 3 mg 25-OH-D_3/11 kg complete feed in DM to prepartum cows (21 or 30 last days of gestation) would have beneficial effects on health and lactation performance in the postpartum period. Also, in a study by Rodney et al. (2018c), mid-lactation cows were fed either 0 or 0.5 mg 25-OH-D3/ day for 27 days and the authors concluded on a positive effect of 25-OH-D_3 treatment on dairy cow metabolism based on associations identified between blood 25-OH-D_3 and concentrations of other metabolites, including calcium, osteocalcin, glucose, insulin, non-esterified fatty acids, β-hydroxybutyrate, cholesterol, magnesium, phosphorus and total protein.

[15] Technical dossier/Section II/Annex II.1.11 and supplementary information January 2023/Appendix 3.
[16] Commission Regulation (EC) No 887/2009 of 25 September 2009.
[17] Technical dossier/Section IV/Annex IV.1.14

– [redacted]. However, most publications did not identify an extra effect of 25-OH-D_3 on zootechnical parameters (Poindexter et al. (2020), Celi et al. (2018), Weiss et al. (2015), Guo et al. (2018)).

In all studies in which the blood level of 25-OH-D_3 was measured after oral administration, a significant response in blood level was found. It can be concluded that 25-OH-D_3 when ingested is absorbed unchanged in the gastro-intestinal tract of cows and calves. No differences exist between food-producing and companion animals concerning the metabolism of cholecalciferol, its conversion pathway by hydroxylation at C25 in the liver and subsequently by hydroxylation at C1 mainly in the kidney, resulting the 1,25-$(OH)_2$-D_3 as the main active vitamin-hormone regulating calcium metabolism. Blood concentrations of 25-OH-D_3 are therefore considered as supporting evidence for the bioavailability of oral 25-OH-D_3 in cows and growing cattle, which can also be extrapolated to all ruminants.

The studies submitted do not provide any information on the relative potency of 25-OH-D_3, on relation of weight to units as defined for vitamin D_3 with weight equivalence of 0.025 μg to one (1) unit.

3.3.1. Conclusions on efficacy

The additive 25-OH-D_3 is an efficient source of vitamin D_3 for all ruminants when used according to the proposed conditions of use.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[18] and Good Manufacturing Practice.

4. Conclusions

The production strain is genetically modified. No cells and no DNA of the production strain are present in the additive. Therefore, the use of the production strain CBS 146008 in the production of 25-OH-D_3 does not raise safety concerns.

The additive is safe for cattle for fattening and dairy cows at the maximum recommended use level of 0.1 mg 25-OH-D_3/kg complete feed. This conclusion can be extended to other cattle categories and extrapolated to all ruminant species.

The use of 25-OH-D3 in all ruminants under the proposed conditions of use is considered safe for the consumer.

The additive is not irritant to the skin or eyes. No conclusion on its potential to be a skin sensitiser or on its effects on the respiratory system can be reached due to absence of data.

The use of the additive under assessment at the recommended conditions of use is considered safe for the environment.

25-OH-D_3 is an efficient source of vitamin D_3 for all ruminants when used according to the proposed conditions of use.

5. Recommendations and/or remarks

The maximum content should also be valid for the combinations of 25-OH-D_3 with vitamin D_3.

The Panel considers that in the description of the additive the term 'other related sterols' should be substituted by 'other sterol derivatives.'

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Abbreviations

BW	body weight
CAS	Chemical Abstracts Service
DCAD	dietary cation-anion difference diet
DM	dry matter
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
SCOOP	Scientific Cooperation of Member States
SD	standard deviation