European Food Safety Authority

The EFSA Journal (2009) 969, 1-32

SCIENTIFIC OPINION

Safety and efficacy of 25-hydroxycholecalciferol as a feed additive for poultry and pigs[1]

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2008-014)

Adopted on 5 February 2009

PANEL MEMBERS*

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SUMMARY

Following a request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of 25-hydroxycholecalciferol (E 670 a) as feed additive for poultry and pigs. This additive is already authorised for use without a time limit in chickens for fattening, turkeys and laying hens. The applicant is now asking for a re-evaluation of the existing authorisation and for an extension of the authorisation to all poultry and pigs.

The additive ROVIMIX® Hy•D® 1.25% contains 12.5 g 25-OH-D_3 kg^{-1} as active substance, which is a physiological precursor of the vitamin D_3 active hormone. 25-OH-D_3 from ROVIMIX® Hy•D® 1.25% is efficacious in all poultry species and pig categories when substituting for vitamin D_3 in feed.

ROVIMIX® Hy•D® 1.25% is considered safe for the target animals at maximum contents of 0.100 mg 25-OH-D_3 kg^{-1} complete feed for chickens and turkeys for fattening, of 0.080 mg 25-OH-D_3 kg^{-1} complete feed for all other poultry species and of 0.050 mg 25-OH-D_3 kg^{-1} for all pig categories.

After reassessing the *in vivo* toxicological studies on laboratory animals submitted, the FEEDAP Panel confirms its earlier conclusions that the 'effects seen in the toxicity studies

[1] For citation purposes: 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. *The EFSA Journal* (2009) 969, 1-32

* One member of the Panel did not participate in the discussion on the subject referred to above because of possible conflicts of interest.

which have been conducted are entirely consistent with a physiological overload of Vitamin D or its metabolites and are not indicative of any unexpected toxicity arising from the source or production method of the substance.'

There is no new information which would require a modification of the formerly proposed UL of 10 µg 25-OH-D_3 adult person^{-1} day^{-1} and 5 µg 25-OH-D_3 child^{-1} day^{-1}.

Exposure calculation of the consumer was based on former tissues and product deposition data in poultry and new data in pigs. The total intake was estimated to amount to 6.9 µg 25-OH-D_3 person^{-1} day^{-1}. This value complies with the provisional UL for adults (69 %) but is above that for children (138 %, based on the intake of adults). Refined calculation (more realistic consumption data) reduced the estimated exposure to 2.44 µg 25-OH-D_3 person^{-1} day^{-1}, which is below the provisional UL for both the adults (24 %) and the children (49 %). The FEEDAP Panel concludes that the total exposure resulting from the use of 25-OH-D_3 in all poultry species and pig categories, at the proposed maximum doses, would not present a risk for the consumer.

The additive ROVIMIX® Hy•D® 1.25% is not irritant to the skin or eye. Data on sensitisation as well as on respiratory toxicity were not provided. The most likely route of workplace exposure to 25-OH-D_3 is by inhalation. A worst case calculation scenario results in a potential daily inhalation of about 4 µg for workers preparing premixtures containing ROVIMIX® Hy•D® 1.25%. This figure is included in the part of the UL not covered by food consumption.

The use of 25-OH-D_3 in feedingstuffs does not represent a risk for the environment.

The FEEDAP Panel has made some recommendations concerning the specification of the additive, focusing on the safety of the 25-OH-D_3 formulation for the target animals and the user.

Key words: nutritional additive, vitamin D, 25-hydroxycholecalciferol, calcifediol, poultry, pigs, safety, efficacy

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BACKGROUND

Regulation (EC) No 1831/2003[2] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lay down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/525/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company DSM Nutritional Products Ltd., represented in EU by DSM Nutritional Products SP. z.oo[3] for the re-evaluation of 25-hydroxycholecalciferol (E 670 a) as a feed additive in chickens and turkeys for fattening and in laying hens, and for the authorisation of 25-hydrocholecalciferol (E 670 a) to be used as a feed additive in pigs and to all poultry (category: Nutritional additives; functional group: vitamins, pro-vitamins and chemically well-defined substances having similar effect) under the conditions mentioned 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 10(2) (re-evaluation of an existing feed additive), and under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[4] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 16 July 2008.

EFSA issued an opinion on the safety and efficacy of Hy•D (calcifediol) based on 25-hydroxylcholecalciferol/ 25-hydroxy-pre-cholecalciferol (EFSA, 2005). The additive 25-hydroxycholecalciferol (E 670 a) has been authorised for its use in laying hens, chickens and turkeys for fattening without a time limit under Council Directive 70/524/EEC.[5]

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, user and consumer and the environment of the product 25-hydroxycholecalciferol, when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank Gerhard Flachowsky and Anna Castoldi for the preparation of this opinion.

[2] OJ L 268, 18.10.2003, p.29
[3] DSM Nutritional Products SP. z.oo, Tarczynska 113, 496-320 Mszczonow, Poland
[4] Dossier reference: FAD-2007-0051
[5] OJ L 271, 30.09.2006, p.12

Table 1. **Register entry as proposed by the applicant**

Additive	25-hydroxycholecalciferol
Registration number/EC No/No (if appropriate)	E 670 a
Category of additive	Nutritional Additive
Functional group of additive	Vitamin, provitamin and chemically well defined substance having a similar effect

Description			
Composition, description	**Chemical formula**	**Purity criteria** (if appropriate)	**Method of analysis** (if appropriate)
25-hydroxycholecalciferol	$C_{27}H_{44}O_2.H_2O$	Min. 94 %	HPLC
Other sterols		Max. 1 % (each)	HPLC
Water	H_2O	3.8-5 %	Karl Fischer
Organic solvents		Max. 1 %	Loss on drying
Erythrosine	$C_{20}H_6I_4Na_2O_5$	Max. 5 ppm	UV Spectroscopy

Trade name (if appropriate)	Not applicable
Name of the holder of authorisation (if appropriate)	Not applicable

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period** (if appropriate)
		mg kg^{-1} of complete feedingstuffs		
Chickens for fattening	-	-	0.100 mg	-
Turkeys	-	-	0.100 mg	-
Laying hens	-	-	0.080 mg	-
All other categories of poultry for fattening and laying, including geese, ducks and game birds	-	-	0.080 mg	-
Piglets (suckling and weaned)	-	-	0.050 mg	-
Pigs for fattening	-	-	0.050 mg	-
Sows for reproduction	-	-	0.050 mg	-
Sows in order to have benefit in piglets	-	-	0.050 mg	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	25-hydroxycholecalciferol shall be placed on the market in a stabilised formulation. 25-hydroxycholecalciferol shall be incorporated in a premixture, as a stabilised formulation, before its incorporation into a feedingstuff. The combination of 25-hydroxycholecalciferol with vitamin D_3 (cholecalciferol) is allowed provided that the total of the mixture does not exceed 0.050 mg kg^{-1} of complete feedingstuff for all categories of pigs,

	0.125 mg/kg of complete feedingstuff for chickens for fattening and turkeys and 0.080 mg/kg of complete feedingstuffs for other poultry.
Specific conditions or restrictions for handling (if appropriate)	25-hydroxycholecalciferol shall be used for premixtures in a non dusting formulation. Formulations of 25-hydroxycholecalciferol shall be labelled in accordance with the rules of Directive 1999/45 on the labelling of preparations.
Post market monitoring (if appropriate)	There is a general traceability system and a complaint procedure in place. An emergency telephone number is printed on each label.
Specific conditions for use in complementary feedingstuffs (if appropriate)	**-**

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 25-hydroxycholecalciferol (E 670 a) has already been authorised for its use as feed additive in laying hens, chickens for fattening and turkeys without a time limit by Commission decision (EC) 1443/2006 (under Council Directive 70/524/EEC).[6] EFSA issued an opinion on the safety and efficacy of Hy•D (calcifediol) based on 25-hydroxylcholecalciferol/25-hydroxy-pre-cholecalciferol (EFSA, 2005). The applicant is now asking for a re-evaluation of the use of the product in chickens for fattening and turkeys and laying hens, and for an extension of use to all other categories of poultry and to pigs (all categories).

The product 25-hydroxycholecalciferol (25-OH-D_3) is proposed to be classified as a nutritional additive, under the functional group vitamins, pro-vitamins and chemically well-defined substances having similar effect.

The authorisation of 25-hydroxycholecalciferol is non-holder specific. Consequently, the new dossier does not contain the data package normally required for additives, only information on the active substance. According to Regulation (EC) No 1831/2003 Art. 8 (4), 'In the event of an opinion in favour of authorising the feed additive, the opinion shall also include the following elements: (b) the designation of the feed additive including its categorization and allocation within functional groups provided for in Article 6, its specification, including, where applicable, purity criteria and method of analysis.' Therefore, EFSA asked the applicant to provide additional information on the additive (the final formulation under which 25-OH-D_3 is intended to be marketed), in particular on all the physico-chemical characteristics of the additive, to assess stability, homogeneity and user safety. The applicant provided the required information for the additive ROVIMIX® Hy•D®1.25%.

The opinion is based on the technical dossier for 25-hydroxycholecalciferol and the additional information provided for the additive ROVIMIX® Hy•D®1.25%.

2. Characterisation

2.1. Characterisation of the additive

The additive ROVIMIX® Hy•D®1.25% is a beige to brown fine powder that contains 12.5 g 25-hydroxycholecalciferol kg^{-1} as active substance. One kg of ROVIMIX® Hy•D®1.25% contains also 37.5 g antioxidant (authorised for feed use), 25 g sodium ascorbate, 50 g vegetable oil, 715 g modified food starch, 150 g maltodextrin and 10 g silicon dioxide.

Five batches of ROVIMIX® Hy•D®1.25% were examined for the content of the active substance.[7] The lowest content was 1.30 %, all the other contents were 1.38 %.

2.1.1. Physical properties of the additive

The applicant submitted data on four batches[8] of ROVIMIX® Hy•D®1.25% showing particle size and dustiness, as well as the concentration of some heavy metals. On average, particles of respirable size (≤ 10 µm) amounted to 1.8 %, particles ≤ 50 µm to 29 %. Dustiness is given with 769 mg 25 g^{-1} sample, which corresponds to 3 % of the particles. The data show a

[6] OJ L 271, 30.09.2006, p.12
[7] Supplementary information January 2009/Annex 2
[8] Supplementary information January 2009/Annex 4

considerable reduction in particle size and dustiness compared to the data formerly submitted for the product called Hy•D beadlets and assessed by EFSA (EFSA, 2005).

The total content of heavy metals and As determined in three batches was below 10 mg kg^{-1}, values for Pb, Hg, and As were below 2 mg kg^{-1}.

2.1.2. Manufacturing process of the additive

The manufacturing process is described in detail in the supplementary information provided by the applicant.[9] The active substance dissolved in oil is mixed with the solids, homogenised and spray-dried.

2.2. Active substance

Details on the active substance 25-OH-D_3 (including production) are given *in extenso* in the former FEEDAP opinion (EFSA, 2005). The recent submission does not add substantial new information, particularly concerning purity of the active substance.

The specifications for the active substance as proposed by the applicant (at least 94 % 25-OH-D_3, not more than 1 % other sterols each, not more than 1 % solvents determined as loss by drying and not more than 5 mg erythrosine kg^{-1}) are supported by the analysis of nine batches.[10]

2.3. Stability and homogeneity

2.3.1. Stability

The applicant noted that 25-OH-D_3 is a substance that is adversely affected by oxygen, light and heat; therefore, a stabilised form is needed in order to be used as a nutritional additive, and ROVIMIX® Hy•D® 1.25% is such a formulated product.

The shelf life of ROVIMIX® Hy•D® 1.25% was examined in two studies. [11] In the first study, three batches of ROVIMIX® Hy•D® 1.25% were kept in sealed aluminium bags at a temperature below 15 ºC. After 12 months, the recovery of the initial values was of 99.5 %. In the second study, the stability of ROVIMIX® Hy•D® 1.25% was studied when kept in the commercial packaging (thick polyethylene bag with plastic ties, inside a sealed aluminium foil lined fibre board drum) at 11 ºC (room relative humidity, eight batches), and 25 ºC (60 % relative humidity, five batches). The recoveries of the initial values were > 90 % when kept at 11 ºC for 12 months or at 25 ºC for three months.

The stability of ROVIMIX® Hy•D® 1.25% was studied when diluted with $CaCO_3$ and when included in a vitamin premixture or in complete premixtures. The stability of ROVIMIX® Hy•D® 1.25% (one batch) when added at 122 mg kg^{-1} either in a vitamin premix or when diluted with $CaCO_3$ was studied[12] when kept in plastic bags for up to six months at two different temperatures, 25 ºC and 35 ºC. After one month, the recovery of the initial values was higher than 90 % in all the cases. After six months, the recovery was of 88 % when the samples were kept at 25 ºC (both when mixed with $CaCO_3$ and with the vitamin premix), and when kept at 35 ºC the recovery was of 78 % when mixed with the $CaCO_3$ and 84 % when mixed with a vitamin premix.

[9] Supplementary information January 2009/Annex 5
[10] Technical Dossier/Identity/Appendix 2-2-5-C
[11] Supplementary information January 2009/Annex 2
[12] Supplementary information January 2009/Annex 6

The stability of ROVIMIX® Hy•D® 1.25% when introduced to four different complete premixtures for poultry (containing trace elements, and three of them including choline chloride) was tested.[13] Supplementation of the premixtures ranged from 7.6 to 21.0 mg kg^{-1}, depending on the study. The samples were kept for up to three months at 25 ºC and 60 % relative humidity. The initial values were lower than the values obtained after one or three months, therefore the recovery was calculated as the concentration at a given point to the initial target concentration. The results showed a recovery of 92 % (mean value) after one month, and after three months the recovery in the premix not containing choline chloride was of 92 %, while in the premixes containing choline chloride the mean value was 80 %.

Finally, the stability of ROVIMIX® Hy•D® 1.25% in a complete diet for chickens for fattening was tested.[14] The mash diet, based on maize and soyabean meal, was supplemented with 4 g ROVIMIX® Hy•D® 1.25% kg^{-1} and pelleted at temperatures of 70 and 90 ºC, or with 20 g ROVIMIX® Hy•D® 1.25% kg^{-1} and pelleted at temperatures of 75 and 85 ºC. After pelleting, the recovery was 88 %, 95 %, 92 % and 85 % for 70, 75, 85 and 90 ºC, respectively. Samples of the pelleted feed were stored in paper bags at 25 ºC and 60 % relative humidity for up to three months. The recovery after storage was 91 %, 82 %, 79 % and 77 % for diets pelleted at 70, 75, 85 and 90 ºC, respectively.

2.3.2. Homogeneity

The homogeneity in complete feed for turkeys was tested[15] using three batches of ROVIMIX® Hy•D® 1.25%. The coefficient of variation of the 25-OH-D_3 concentration in six samples varied from 5 to 11 % for the different batches; the mean concentration (97 %) was near to the target value. ROVIMIX® Hy•D® 1.25% was therefore homogeneously distributed in pelleted feed.

2.3.3. Conclusions

The shelf life of the additive ROVIMIX® Hy•D® 1.25% is 12 months (storage condition ≤ 15 ºC). The stability of ROVIMIX® Hy•D® 1.25% in premixtures and complete feed (also pelleted feed) is considered satisfactory for practical purposes. ROVIMIX® Hy•D® 1.25% can also be homogeneously distributed in the feed.

2.4. Conditions of use

The ROVIMIX® Hy•D® 1.25% is intended for use as a feed additive (Table 1) at a maximum dose of 0.100 mg 25-OH-D_3 kg^{-1} feed for chickens for fattening and turkeys, at a dose of 0.080 mg 25-OH-D_3 kg^{-1} feed for laying hens (corresponding to the already approved maximum content), at a dose of 0.080 mg 25-OH-D_3 kg^{-1} feed for all the other categories of poultry and at a dose of 0.050 mg 25-OH-D_3 kg^{-1} feed for pigs (all categories). The applicant further proposes that the combination of 25-hydroxycholecalciferol with vitamin D_3 (cholecalciferol) may be allowed provided that the total mixture does not exceed 0.050 mg kg^{-1} complete feed for all categories of pigs, 0.125 mg kg^{-1} of complete feed for chickens for fattening and turkeys and 0.080 mg kg^{-1} of complete feed for laying hens and other poultry.

[13] Supplementary information January 2009/Annex 7
[14] Supplementary information January 2009/Annex 8
[15] Supplementary information January 2009/Annex 9

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 substance in animal feed. The Executive Summary of the CRL report can be found in the Appendix.

3. Efficacy for target species

3.1. Efficacy for poultry

The efficacy assessment on poultry for the target species chickens and turkeys for fattening and laying hens was already made and is part of the opinion of FEEDAP on 25-hydroxycholecalciferol /25-hydroxy-pre-cholecalciferol adopted in 2005 (EFSA, 2005). No recent data has been provided for chickens for fattening and laying hens.[16] One new study has been provided for chickens reared for laying, one for turkeys for fattening, and two trials on minor species (Barbary duck and Japanese quail).

3.1.1. Recommendations for vitamin D_3 in poultry

Vitamin D_3 recommendations for poultry were already summarised in the former EFSA opinion (2005). This information is now updated in Table 2. The values obtained from the National Research Council (NRC) recommendations correspond to the requirement to satisfy normal performance of the animals; those from the Gesellschaft für Ernährungsphysiologie (GfE) are allowances.

Table 2. **Recommendations/Requirements for Vitamin D_3 in poultry**

Species/Category	Age (weeks)	Recommendation/Requirement[1]		Remarks[2]	Reference[3]
		IU kg^{-1} diet	mg kg^{-1} diet		
Chickens for fattening	0-6	450	0.01125	On DM basis	GfE, 1999
	0-8	200	0.005	Requirement Diet at 90 % DM	NRC, 1994
Chickens reared for laying [4]	0-18	250	0.00625	On DM basis	GfE, 1999
	0-18	200 (190)	0.005 (0.047)	Requirement Diet at 90 % DM	NRC, 1994
	18 to first egg	300 (280)	0.0075 (0.007)	Requirement Diet at 90 % DM	NRC, 1994
Laying hens	>18	450	0.01125	On DM basis	GfE, 1999
	>18	300	0.0075	Requirement Diet at 90 % DM	NRC, 1994
Turkeys for fattening	1-2	1500	0.0375	On DM basis	GfE, 2004
	>2	1100	0.0275	On DM basis	GfE, 2004
	0-24	1100	0.0275	Requirement male and female	NRC, 1994
Geese	0-4	200	0.005	Requirement Diet at 90 % DM	NRC, 1994
Ducks[5]	0-7	400 (900)	0.010	Requirement Diet at 90 % DM	NRC, 1994

[1] 1 IU = 25 ng vitamin D_3, or 1 µg = 40 IU vitamin D_3.

[2] DM, dry matter.

[3] GfE, Gesellschaft für Ernährungsphysiologie (Germany); NRC, National Research Council (USA).

[4] Values correspond to the requirements for White Leghorn type chickens, within brackets for Brown Leghorn chickens.

[5] Values within brackets correspond to the requirements for ducks for breeding.

[16] Technical Dossier/Section III/Appendixs 3-2-3-1-A and 3-2-3-1-C

3.1.2. Efficacy trials in poultry

Chickens reared for laying

A total of 1600 female chickens reared for laying (ISA Brown) were distributed in groups of 40 animals in 40 pens (five replicates per treatment).[17] A basal diet (starter/grower and rearing diets) based on wheat, barley and soybean meal was supplemented either with graded levels of vitamin D_3 (0.005, 0.025, 0.050 and 0.075 mg kg^{-1}) or graded levels of 25-OH-D_3 (0.025, 0.050, 0.075 mg kg^{-1}, in the form of ROVIMIX® Hy•D® 1.25%); a diet combining both sources was also used (0.0375 mg each product kg^{-1}). The intended levels of vitamin D_3 and 25-OH-D_3 were confirmed by analysis, and the diets contained monensin sodium. The birds were fed the experimental diets from the first day of life up to day 112.

General status of the animals was monitored daily, feed intake was recorded weekly and body weight was controlled on days 0, 42, 84 and 112. On days 0, 42, 84 and 112, three birds per pen (n = 15) were slaughtered: blood samples were collected to perform analyses on the plasma concentration of 25-OH-D_3, calcium and phosphorus; the tibia were collected to measure the breaking strength, the stiffness, the weight and the ash content.

Table 3 summarises the results obtained. Mortality was below 1 % and not affected by the treatments. In the same way, the overall performance was not affected by the dietary treatments. Tibia ash content was influenced by the dietary treatments but the differences between the sources of vitamin D did not become significant. The breaking strength of tibia appeared not to be correlated to bone ash and did not reach significant differences (P = 0.375). Blood concentration of 25-OH-D_3 was correlated with increasing dose of either vitamin D_3 or 25-OH-D_3, the lowest values being found in animals fed the diet containing 0.005 mg kg^{-1} of vitamin D_3 and the highest in animals fed the 25-OH-D_3 at the highest level. Comparing the two sources of vitamin D at the same dosage, a significant difference was only found at the highest level of inclusion (day 112).

Table 3. **Results obtained at day 112 in the pullets (for plasma and tibia n=15)**

Treatment		Body Weight (g)	Total feed intake (g)	Feed gain ratio (kg kg^{-1})	Tibia Ash (%)	Plasma measurements		
Source	Dose (mg kg^{-1})					25-OH-D_3 (ng mL^{-1})	calcium (mg dL^{-1})	phosphorus (mg dL^{-1})
Vitamin D_3	0.005	1384	7113	4.24	35.2	0.6	11.3	6.4
	0.025	1422	7161	4.18	35.6	6.2	11.7	6.5
	0.050	1404	7241	4.24	33.3	12.9	11.2	6.5
	0.075	1407	7147	4.24	32.6	16.8	11.2	6.4
25-OH-D_3	0.025	1422	7245	4.20	36.9	8.0	11.2	6.4
	0.050	1422	7395	4.29	34.5	15.2	11.0	6.2
	0.075	1401	7175	4.23	33.0	28.1	11.5	6.5
Vitamin D_3 + 25-OH-D_3	0.0375 + 0.0375	1405	7201	4.23	33.1	25.6	11.5	6.5

Turkeys

One recent trial in turkeys was provided by the applicant.[18] However, due to the insufficient experimental design and the lack of substantial information, the FEEDAP Panel did not consider this study.

[17] Technical Dossier/Efficacy/Appendix 3-2-3-1
[18] Technical Dossier/Efficacy/Appendix 3-2-3-1-B

Barbary Duck and Laying Japanese Quail trials

The applicant reported two trials on minor species, one being carried out in growing ducks[19] and the other one in laying Japanese quails.[20] However, the trial carried out in growing ducks will not be considered by the FEEDAP Panel due to the deficiencies in the experimental design.

Two hundred and twenty laying Japanese quails (six weeks old) were randomised to 11 experimental groups (two replicates with ten birds each) and fed diets based on maize and soybean meal. The diets were fed for three weeks without supplementary vitamin D. In the following three weeks, the treatments included 0.0025, 0.005 and 0.010 mg vitamin D_3 kg^{-1}, and 0.00125, 0.0025, 0.005, 0.010 and 0.020 mg 25-OH-D_3 kg^{-1} (in the form of Hy•D® 1.25%); data on analytical confirmation of the intended concentration were not provided.

During the experiment, two losses in the 0.0025 mg vitamin D_3 group and one loss in the 0.020 mg 25-OH-D_3 kg^{-1} were recorded. The body weight of the birds and feed intake was controlled throughout the period, during the test period egg shell dry weight per day and animal was calculated, and at the end of the experiment all the birds were slaughtered in order to collect the right femur to determine ash and calcium content. Blood samples were also collected and calcium and bone-specific alkaline phosphatase were determined.

Results of the experiment are presented in Table 4. The body weight of the animals and feed intake were not influenced by the dietary treatments. Egg production and egg shell formation (expressed in mg $animal^{-1}$ day^{-1}) increased with increasing levels of both vitamin sources. Plasma alkaline phosphatase and bone-specific alkaline phosphatase decreased with increasing levels of vitamin D; no differences were found between the two sources of vitamin D.

Table 4. **Results obtained in the trial carried out with laying Japanese quails**

Treatment					Serum		Femur bone	
Source	Dose (mg kg^{-1})	Feed intake (g day^{-1})	Egg yield (%)	Egg shell dry weight (mg day^{-1})	calcium (mg dL^{-1})	Bone-specific alkaline phosphatase (IU L^{-1})	ash (%)	calcium (mg g^{-1} Ash)
Vitamin D_3	0.00250	24.7	41^{ab}	304^{a}	22.0^{a}	1292^{ab}	59.2^{b}	372.7
	0.00500	24.8	77^{b}	693^{b}	23.9^{a}	644^{b}	61.2^{ab}	366.1
	0.01000	26.8	80^{b}	755^{b}	27.5^{ab}	459^{b}	61.1^{ab}	379.2
25-OH-D_3	0.00125	23.0	33^{a}	221^{a}	24.6^{a}	1971^{a}	57.5^{b}	355.8
	0.00250	25.2	67^{ab}	565^{ab}	23.8^{a}	1210^{a}	57.7^{b}	372.4
	0.00500	26.0	85^{b}	774^{b}	28.9^{ab}	349^{b}	63.6^{ab}	366.6
	0.01000	25.6	75^{b}	696^{ab}	29.8^{b}	306^{b}	64.3^{ab}	360.5
	0.02000	25.4	85^{b}	772^{b}	27.8^{ab}	338^{b}	65.1^{ab}	376.2

$^{a, b,}$ values within one column with different superscript are different ($P < 0.05$).

3.2. Efficacy for pigs

A total of five trials are reported by the applicant in order to prove the efficacy of the product. Two of the trials were carried out in weaned piglets, two were performed in growing-fattening pigs and one in sows over two productive cycles. Those trials were carried out in three different countries between 2006 and 2007.

In general, the main objective of the trials was to prove that the efficacy of 25-OH-D_3, provided in all cases in the form of ROVIMIX® Hy•D® 1.25%, is equivalent to that of vitamin D_3. The

[19] Technical Dossier/Efficacy/Appendix 3-2-3-1-E
[20] Technical Dossier/Efficacy/Appendix 3-2-3-1-F

two forms of vitamin D were compared at the dietary levels used under practical conditions and, with the exception of two trials, a diet with a low content of vitamin D was included as a dietary treatment. However, no study was carried out in order to compare the biopotency of 25-OH-D_3 to that of vitamin D_3.

The parameters evaluated included zootechnical parameters of the animals, and in the case of the nutritional effects the assessment consisted in blood parameters (plasma calcium, phosphorus and 25-OH-D_3) and in bone parameters (physical and chemical characteristics).

Dietary analyses of the tested substances were provided for four trials and confirmed the intended dosages. Those data were not provided for one piglet trial which was not considered in the following assessment.

3.2.1. Recommendations for vitamin D_3 in pigs

Recommendations issued by two sources are reported in Table 5. The values obtained from the NRC recommendations correspond to the requirement to satisfy normal performance of the animals; those from the GfE are allowances.

Table 5. **Recommendations/Requirements for Vitamin D_3 in pigs**

Species/Category	kg	Recommendation/Requirement[1]		Remarks[2]	Reference[3]
		IU kg^{-1} diet	mg kg^{-1} diet		
Piglets	up to 25	500	0.0125	On DM basis	GfE, 2008
	3-10	220	0.0055	Requirement Diet at 90 % DM	NRC, 1998
	10-20	200	0.005	Requirement Diet at 90 % DM	NRC, 1998
Pigs for fattening	25-120	150-200	0.0038-0.005	On DM basis	GfE, 2008
	20-120	150	0.0037	Requirement Diet at 90 % DM	NRC, 1998
Breeding sows	-	200	0.005	On DM basis	GfE, 2008
	-	200	0.005	Requirement Diet at 90 % DM	NRC, 1998

[1] 1 IU = 25 ng vitamin D_3, or 1 µg = 40 IU vitamin D_3.

[2] DM, dry matter.

[3] GfE, Gesellschaft für Ernährungsphysiologie (Germany); NRC, National Research Council (USA).

3.2.2. Efficacy trials in pigs

Weaned piglets

Two trials are reported by the applicant in weaned piglets. However, the second study in piglets could not be considered because the basal diet already contained 2000 IU vitamin D_3 kg^{-1}.

In the trial considered,[21] a total of 72 crossbred piglets with an initial mean body weight of 10.7 kg were distributed in pens of two animals (one castrated male and one female). The animals were assigned to one of the six experimental diets, representing a total of six pens per treatment. The experimental diets were based on wheat, barley and soybean meal, and were provided to animals *ad libitum* for 35 days. Three of the experimental diets contained increasing doses of vitamin D_3: 0.005, 0.025, and 0.050 mg kg^{-1}, and the other three diets

[21] Technical Dossier/Efficacy/Appendix 3-2-3-2-A

presented increasing levels of 25-OH-D_3 (ROVIMIX® Hy•D® 1.25%): 0.025, 0.050 and 0.100 mg kg^{-1}.

One loss (0.05 mg 25-OH-D_3 kg^{-1}) was recorded in the first week. Performance of the piglets was evaluated throughout the experimental period; at the end of the period, blood samples from six animals per treatment were taken, and then the animals were slaughtered. Those samples were collected in order to be analysed for 25-OH-D_3, calcium and phosphorus content. After slaughtering, one metacarpal bone, left femur and tibia were collected. Ash content, calcium and phosphorus were measured in the metacarpal, in the epiphysis of femur and tibia and in the diaphysis of the femur. The femur was also submitted to physical characteristics evaluation (weight, length, mid-shaft diameter, bone breaking strength, wall thickness).

No modifications related to the dietary treatments were found in the performance of the animals (Table 6) and the concentrations of calcium and phosphorus in plasma were not different between the experimental treatments. On the contrary, and as expected, the plasma concentration of 25-OH-D_3 presented a significant dose-dependent increase. No modifications related to the experimental treatments were found when considering the traits measured in the femur, nor in the composition of the different bone samples (ash, Ca, and P).

Table 6. **Summary of the overall results obtained in the weaned piglet trial**

Treatment		**Body weight gain (g day^{-1})**	**FCR (kg kg^{-1})**	**Bone ash content (% DM^{-1})[1,2]**	**Plasma measurements[2]**		
Source	**dose (mg kg^{-1})**				**25-OH-D_3 (ng mL^{-1})**	**phosphorus (mg dL^{-1})**	**calcium (mg dL^{-1})**
Vitamin D_3	0.005	524	1.48	39.4	8.5[a]	12.2	11.4
	0.025	494	1.44	39.6	10.6[a]	11.0	10.0
	0.050	545	1.42	39.8	17.2[b]	12.0	11.4
25-OH-D_3	0.025	526	1.45	39.4	28.3[c]	10.3	11.7
	0.050	519	1.43	38.7	49.6[d]	10.2	11.5
	0.100	541	1.46	37.7	101.1[e]	12.1	10.8

[1] Ash content in the fourth metacarpal bone.

[2] Measurements carried out on the day 35 of experiment (n = 6).

[a,b,c,d,e,] values within the same column with different superscript are different ($P < 0.05$).

Pigs for fattening

In a first trial,[22] a total of forty-eight (LW x LD) castrated males with an initial body weight of 29 kg were alloted in groups of four animals and distributed to three dietary treatments, representing a total of four pens per treatment. The diets were based on maize, barley and soybean meal, and the treatments were as follows: one diet supplemented with vitamin D_3 at 0.030 mg kg^{-1}, the second supplemented with 0.030 mg 25-OH-D_3 (ROVIMIX® Hy•D® 1.25%) kg^{-1} , and the third diet supplemented with 0.015 mg from vitamin D_3 as well as from 25-OH-D_3 kg^{-1}. Diets were offered to the animals *ad libitum* for 90 days.

In addition to the zootechnical parameters, blood samples were taken at different stages of the experimental period and evaluated for plasma calcium, phosphorus, parathyroid hormone, creatinine and 25-OH-D_3. Bone parameters (strength and ash content of metacarpals and metatarsals) were measured after the end of the study.

Results (Table 7) showed no significant treatment-related modifications in the performance of the animals, although some numerical differences were found. As expected the plasma

[22] Technical Dossier/Efficacy/Appendix 3-2-3-2-C

concentration of 25-OH-D_3 was higher in the animals receiving 25-OH-D_3 alone, compared to the other treatments; the differences between treatments increased with time. However, no modifications were found in plasma concentration of calcium and phosphorus. Bone parameters were not modified by the treatments, with the exception of ash content in the metatarsal which showed a significant increase in the animals fed the diet with the combination of the two products compared to the other diets.

Table 7. **Summary of the results obtained in the first trial carried out in growing pigs**

Treatment		Body weight gain (g day^{-1})	FCR (kg kg^{-1})	Bone ash content (%)[1]		Plasma measurements[1]		
Source	Dose (mg kg^{-1})			Metacarpal	Metatarsal	25-OH-D_3 (ng mL^{-1})	phosphorus (mg dL^{-1})	calcium (mg dL^{-1})
Vitamin D_3	0.030	813	3.03	61.2	61.0[a]	25.7[a]	8.3	11.4
25-OH-D_3	0.030	833	3.02	61.0	61.9[ab]	57.6[b]	8.2	11.3
Vitamin D_3 + 25-OH-D_3	0.015 + 0.015	837	2.95	61.1	62.5[b]	42.4[b]	8.5	11.4

[1] Measurements carried out on the day 89 of experiment; dimension of bone ash content not explicitly given in the report.
[a,b,c,d,] values within the same column with different superscript are different ($P < 0.05$).

In the second trial,[23] a total of 48 crossbred pigs with an initial body weight of 24.5 kg were individually penned and distributed into six treatments. The basal diet based on wheat, barley and soybean meal with no vitamin D added was used as a control diet. The treatments were obtained by adding to the basal diet one of the two sources of vitamin D, resulting in two vitamin D_3 treatments (0.0125 and 0.0250 mg kg^{-1}) and three 25-OH-D_3 (ROVIMIX® Hy•D® 1.25%) treatments (0.0125, 0.0250, and 0.050 mg kg^{-1}). In the course of the experiment, animals were fed two different types of diets (grower/finisher). The experiment was completed when the animals reached slaughter weight (~108 kg).

In addition to the zootechnical parameters, blood sampling was carried out for determination of plasma 25-OH-D_3, calcium and phosphorus on day 35 and at slaughtering. Radius and metacarpal bone were collected at the end of the study and physical and chemical analyses were carried out; carcass characteristics (carcass weight and % lean) were also evaluated at the end of the study.

No mortality occurred during the study. The performance of the animals was not modified by the experimental treatments (Table 8). As expected, the presence of 25-OH-D_3 in the diet resulted in an increase in the concentration in plasma of this compound, the effect being dose-related. However, no modifications on calcium and phosphorus concentration in plasma were reported. The chemical composition of the bone was not modified, neither the physical characteristics of the radius.

[23] Technical Dossier/Efficacy/Appendix 3-2-3-2-D

Table 8. **Summary of the overall results of the second trial carried out in growing pigs**

Treatment		Body weight gain (g day^{-1})	FCR (kg kg^{-1})	Bone ash content (% DM^{-1})[1]	Plasma measurements[2]		
Source	Dose (mg kg^{-1})				25-OH-D_3 (ng mL^{-1})	calcium (mg dL^{-1})	phosphorus (mg dL^{-1})
Vitamin D_3	0	901	2.50	40.7	7.6^{a}	9.7	8.5
	0.0125	863	2.40	40.6	12.4^{ab}	10.1	9.8
	0.0250	914	2.40	41.1	19.3^{bc}	10.2	9.9
25-OH-D_3	0.0125	898	2.45	40.2	25.3^{c}	10.4	8.2
	0.0250	886	2.48	41.7	36.0^{d}	10.5	9.2
	0.0500	896	2.41	40.5	59.7^{e}	10.1	10.4

[1] Ash content in the fourth metacarpal bone.

[2] Measurements done at the end of the experimental period.

^{a,b,c,d,e,} values within the same column with different superscript are different ($P < 0.05$).

Sows

A total of 36 sows were distributed into one of the four treatments (nine sows per treatment).[24] Dietary treatments consisted in a supplementation of 0.005 mg vitamin D_3 kg^{-1}, 0.050 mg vitamin D_3 kg^{-1}, 0.050 mg 25-OH-D_3 (ROVIMIX® Hy•D® 1.25%) kg^{-1} and 0.025 mg from vitamin D_3 as well as from 25-OH-D_3 kg $^{-1}$. The performance of the sows and their litters was evaluated over two reproductive cycles (~15 months).

The sows were fed two different diets, according to the reproductive stages, gestation and lactation. The composition of the diets is not reported but analyses for the crude nutrients are provided. Restricted feeding regime was applied (daily feed amount during gestation 2.2-2.4 kg, and 2.4-5.6 kg during lactation).

The results of the study (Table 9) should be considered with some reservations because of some inadequacies in the experimental design and the reporting of data.

Results showed variations in the body weight of the sows at some stages of the trial which could not be attributed to treatment or feed intake. Plasma concentration of 25-OH-D_3 was higher in the sows receiving diets containing 25-OH-D_3. This increase in plasma concentration of 25-OH-D_3 was also partly found in the offspring. However, calcium and phosphorus plasma concentration were not modified. Some changes were found in the performance of the sows and their offspring, but there was no clear tendency to vitamin D_3 or 25-OH-D_3 supplementation.

3.3. Studies on the quality of animal produce

Recent studies on sensory properties of animal products from the target animals were not submitted. The FEEDAP Panel concluded in its former assessment (EFSA, 2005) that the quality of animal products from chickens and turkeys for fattening and laying hens was not significantly influenced by the source of vitamin D.

[24] Technical Dossier/Efficacy/Appendix 3-2-3-2-E

Table 9. **Summary of the results obtained in the trial with sows**

Treatment		Sow				Piglets weight at weaning (kg)	
		Body weight (kg)		Plasma 25-OH-D_3 (ng mL^{-1})			
Source	Dose (mg kg^{-1})	Initial[1]	Final[1]	Initial[1]	Final[1]	1st cycle	2nd cycle
Vitamin D_3	0.005	167	193	29.1	15.5[b]	5.8[b]	7.2
	0.050	162	234	32.1	26.5[b]	8.0[ab]	8.9
25-OH-D_3	0.050	163	201	39.8	70.0[a]	6.9[ab]	7.1
Vitamin D_3 + 25-OH-D_3	0.025 + 0.025	185	212	42.1	63.8[a]	8.6[a]	8.1

[1] Initial refers to the breeding in the first cycle, and final refers to the weaning in the second cycle.

[a,b,] values within the same column with different superscript are different ($P < 0.05$).

3.4. Conclusions on the efficacy

In its former assessment of the efficacy of 25-OH-D_3, the FEEDAP Panel concluded that 'The efficacy of 25-OH-D_3 concerning weight gain, feed conversion and bone mineralisation for chickens for fattening is at least equivalent to that of vitamin D_3 when supplemented at dietary levels of 30 to 69 µg kg^{-1}. At lower doses (2.5 and 25 µg kg^{-1}), the efficacy concerning bone and feed conversion of 25-OH-D_3 is doubled compared to that of the vitamin D_3. The bone ash data shows that efficacy of 25-OH-D_3 is even higher than that of vitamin D_3 (about two fold). Concerning laying hens, it has been demonstrated that 25-OH-D_3, in the dose range of 41 to 82 µg kg^{-1}, is at least equivalent to vitamin D_3 for optimizing hen performance and egg quality. In turkeys, it can be concluded that 25-OH-D_3 can be used as a substitute for vitamin D_3 in the range tested by the applicant (40 to 100 µg kg^{-1}).'

The submitted study for chickens reared for laying also allows the conclusion that 25-OH-D_3 from ROVIMIX® Hy•D® 1.25% is an efficacious source of dietary vitamin D.

The applicant applied for the use of the product also in 'all other categories of poultry for fattening and laying, including geese, ducks and game birds.' In laying Japanese quails, 25-OH-D_3 from Hy•D® 1.25% was shown to be so effective as vitamin D_3 as a source vitamin D.

Concerning other minor poultry categories, the FEEDAP Panel considers that where the mode of action is well-known in the major species and can be reasonably assumed to produce the same effect in the major and minor species, direct extrapolation of efficacy is accepted without further experimental evidence in the minor species. In principle, data can be extrapolated from major to minor species if they are physiologically similar. As a consequence, the FEEDAP Panel concludes to the efficacy of 25-OH-D_3 as a source of vitamin D for all other categories of poultry for fattening and laying, including geese, ducks and game birds.

The experimental data provided after the use of ROVIMIX® Hy•D® 1.25% in piglets (0.025 to 0.100 mg 25-OH-D_3 kg^{-1}), in pigs for fattening (0.0125 to 0.0500 mg 25-OH-D_3 kg^{-1}) and sows (0.050 mg 25-OH-D_3 kg^{-1}), support the efficacy of ROVIMIX® Hy•D® 1.25% as a source of vitamin D. Increased plasma levels after administration of 25-OH-D_3 demonstrate intestinal absorption of the active substance of the product. In piglets and pigs for fattening the zootechnical parameters as well as plasma Ca and P are not influenced by 25-OH-D_3 when compared to weight equivalent doses of vitamin D_3.

Studies which allowed conclusions on the relative biopotency of 25-OH-D_3 in comparison to vitamin D_3 in pigs were not submitted. The studies on pigs were performed with rather high dietary concentrations of both 25-OH-D_3 and vitamin D_3, where potential differences in the

biopotency can usually not be measured. It could be concluded that there was no evident requirement for dietary vitamin D supplementation from the comparison of the body weight gain data. Under these conditions, comparative biopotency measurements cannot be made.

The quality of products from animals treated with 25-OH-D_3 is not expected to be adversely affected.

4. Safety

4.1. Tolerance studies on target species

No new tolerance studies were provided for laying hens and turkeys for fattening.[25] To the knowledge of the FEEDAP Panel, no new information is available that justifies the need to modify its former conclusion on the safety of the product (EFSA, 2005). The applicant newly submitted one tolerance trial on chickens for fattening and one on piglets, which is considered the most sensitive category of pigs. The intended doses of 25-OH-D_3 and vitamin D_3 were analytically confirmed.

4.1.1. Chickens for fattening

Three hundred one-day-old chickens of both sexes were equally allotted in groups of 20 animals and distributed to one of the five dietary treatments, representing three pens per experimental treatment.[26] Diets were based on maize and soybean meal, and contained 0.125 mg vitamin D_3 kg^{-1}, 0.070, 0.280, 0.560 and 0.700 mg 25-OH-D_3 (ROVIMIX® Hy•D® 1.25%) kg^{-1}, respectively. Diets were offered to the animals *ad libitum* for 36 days (starter and grower diets).

A summary of the results can be found in Table 10. General health status was recorded daily and the animals that died during the experimental period were submitted to a post-mortem evaluation. The mortality during the experimental period (3-7/60) did not show any dose relationship.

Performance of the animals was controlled during the experimental period on day 22 and day 36. The birds fed the diets containing 0.560 and 0.700 mg 25-OH-D_3 kg^{-1} showed a lower weight gain during the whole experimental period (reduction by 20 % and by 38 % in the 0.560 and 0.700 mg 25-OH-D_3 kg^{-1} groups, respectively, compared to the animals fed vitamin D_3). In the case of the highest dose, feed to gain ratio was significantly increased compared to the other treatments.

At the end of the study, four animals per pen were slaughtered in order to perform a post-mortem evaluation (liver, kidney, lung, gut, heart); tibia and kidney from those four animals plus two more animals were collected to determine ash content and bone strength. A higher number of animals presenting anomalies were found in the birds fed the two highest doses of 25-OH-D_3. Ash content of the kidneys was higher in birds from the two higher doses of 25-OH-D_3 compared to animals fed the other three diets. However, only one animal of the 0.560 mg 25-OH-D_3 kg^{-1} group presented macroscopic signs of renal calcification.

[25] Technical Dossier/Safety/Appendixs 4-1-1-B and 4-1-1-C
[26] Technincal Dossier/Safety/Appendix 4-1-1-A

Table 10. **Results of the tolerance study carried out in chickens for fattening**

Treatment		Body weight gain (g)	FCR (kg kg^{-1})	Animals with abnormities at necropsy[1]	kidney ash (%)[2]
Source	Dose (mg kg^{-1})				
Vitamin D_3	0.125	2331^{a}	1.54^{b}	4	5.9^{b}
25-OH-D_3	0.070	2294^{a}	1.53^{b}	5	6.0^{b}
	0.280	2173^{a}	1.62^{b}	5	6.1^{b}
	0.560	1840^{b}	1.64^{b}	7	6.9^{a}
	0.700	1421^{c}	1.89^{a}	10	6.5^{ab}

[1] The total of animals evaluated per treatment were 12.

[2] The number of animals evaluated per treatment was 18.

^{a,b,c,d,e} values within the same column with different superscript are different ($P < 0.05$).

4.1.2. Piglets

A total of 48 forty-day-old crossbred weaned piglets, with an initial body weight of about 9 kg (24 castrated males, 24 females, six per pen), were fed for 42 days balanced diets (based on cereals, potato and soybean protein) containing 0.050 mg vitamin D_3 kg^{-1} feed, 0.050 mg 25-OH-D_3 kg^{-1}, 0.250 mg kg^{-1} and 0.500 mg 25-OH-D_3 kg^{-1}, respectively, from ROVIMIX Hy•D.[27] Feed (about 18 % CP, 0.79 % Ca and 0.56 % P) was fed restrictively for the first nine days, and was offered *ad libitum* afterwards. Feed intake and body weight was measured weekly. The animals were clinically examined daily (general status, faecal consistency) and weekly (clinical work-ups) by a veterinarian in the course of the study.

Due to a colibacillosis outbreak, all piglets were treated for nine days with tiamulin and colistin; one animal (mid dose 25-OH-D_3) died on day 18 of the study, the penmates were treated on the same day with enrofloxacin and dexamethasone.

At the end of the trial, faeces samples were taken from all pens to determine the occurrence of gastrointestinal parasites, and blood samples were collected from all animals by puncture of the vena jugularis, vena brachiocephalica and vena cava cranialis for haematology, glucose, urea, creatinine, total protein, albumin, total bilirubin, AST, GGT, GLDH, ALP, bone-specific alkaline phosphatase (BAP), electrolytes and 25-OH-D_3 in plasma. Urine samples were taken (partly by cystocentese) after euthanasia and analysed for creatinine, calcium and phosphorus, and semi-quantitatively for protein, glucose, ketones, blood, bilirubin, urobilinogen, leucocytes and nitrites. Half of the piglets were necropsied after 42 days, the other half after 49 days. The bone breaking index and the bone distension index were determined. Liver, muscle and skin were analysed for 25-OH-D_3. All data were statistically examined by analysis of variance (ANOVA).

No statistically significant differences between the groups were found for weight gain (mean 422 g day^{-1}), feed intake (mean 679 g day^{-1}) and feed conversion ratio (1.61 g feed g^{-1}gain). Haematology and serology did also not reveal significant differences between the groups. BAP (mean 103 IU L^{-1}) did not indicate alterations in bone turnover by 25-OH-D_3. The 25-OH-D_3 content in plasma, liver, muscle and skin expectedly reflected the oral supply. The plasmatic concentration of 25-OH-D_3 was 17 ng mL^{-1} in the vitamin D_3 group, and 64, 227 and 360 ng mL^{-1} in the 25-OH-D_3 groups, respectively. No urinary parameters differed significantly, however urinary calcium excretion as indicated by the Ca/Creatinine quotient decreased from 1.39 in the control group to 0.90, 1.07 and 1.00 in the groups with 0.050, 0.250 and 500 mg 25-OH-D_3 kg^{-1} feed, respectively.

[27] Technical Dossier/Safety/Appendix 4-1-1-D

Bone (femur) weight and length as well as bone breaking index, bone ash, Ca and P were not different among the groups. The weights of heart, lung, liver, kidneys and spleen did not reveal group differences. In kidney, dry matter, ash and Ca content did not differ significantly either, nor did histology.

4.1.3. Conclusion on safety for the target animals

Based on the newly provided data and on the former assessment of the product, the FEEDAP Panel concludes that the maximum content of 25-OH-D_3 in feed for chickens and turkeys for fattening (0.100 mg kg^{-1} feed), laying hens, chickens reared for laying and minor poultry species (0.08 mg kg^{-1} feed) is safe for the target animals.

The lowest margin of safety identified for chickens for fattening is 2.8, considering that the maximum content is 0.100 mg 25-OH-D_3 kg^{-1} feed. To the knowledge of the FEEDAP Panel, no new information is available that justifies the need to modify its former conclusion (EFSA, 2005) on the margin of safety for laying hens (< 5.5) and for turkeys for fattening (about 5).

The safety for the minor poultry species is extrapolated from the above studies on major poultry species.

The study in piglets did not show any adverse effect of 25-OH-D_3 when fed at the recommended dose and at its five- and tenfold overdose. Even a tendency for renal calcification, a sensitive indicator of hypervitaminosis D, could not be observed. The FEEDAP Panel considers therefore that 25-OH-D_3 used at a dose of 0.050 mg kg^{-1} feed is safe for all categories of pigs.

4.2. Metabolism and residue studies

4.2.1. Fate of 25-OH-D_3

In its former opinion, the FEEDAP Panel (EFSA, 2005) concluded that:

i) 25-OH-D_3 is the initial metabolite of cholecalciferol (resulting from hydroxylation in the liver) in mammals and birds, 1α,25-$(OH)_2$-D_3 (second hydroxylation step in the kidney) being the active metabolite,
ii) no retroconversion of 25-OH-D_3 to vitamin D_3 occurs,
iii) it is very likely that the metabolic fate of orally administered 25-OH-D_3 would be the same as that of the endogenously produced 25-OH-D_3.

4.2.2. Deposition

Data on the deposition of 25-OH-D_3 in chickens for fattening, turkeys for fattening[28] and laying hens[29] were previously submitted by the applicant and assessed by the FEEDAP Panel (EFSA, 2005). The corresponding studies were performed with levels of 0.069 to 0.69 mg kg^{-1} in chickens for fattening, of 0.041 to 0.825 mg kg^{-1} in laying hens and of 0.099 to 0.495 mg kg^{-1} in turkeys for fattening. Comparison of the deposition in tissues of 25-OH-D_3 was made with vitamin D_3 administered at the lower dose.

Additional data for chickens for fattening and new data for pigs have been submitted. In this study, 25-OH-D_3 was analysed using an HPLC-MS method with limits of quantification (LOQs) of 5 µg kg^{-1} muscle, 10 µg kg^{-1} liver and kidney and 20 µg kg^{-1} fat.

[28] Technical Dossier/Safety/ Appendix 4-1-1-B and 4-1-3-3-B
[29] Technical Dossier/Safety/Appendix 4-1-1-C and 4-1-3-3-C

4.2.2.1. Chickens for fattening

A 35-day dose-response study[30] was performed on one-day-old chicks allotted to groups of 20 animals (two pens of five animals of each sex) distributed into one of the six following experimental treatments: control diet with 0.125 mg vitamin D_3 kg^{-1} feed, two groups with 0.070 and 0.100 mg 25-OH-D_3 from ROVIMIX® Hy•D® 1.25% (confirmed) kg^{-1}, respectively, and three groups combining the two products, one with 0.0625 mg vitamin D_3 and 0.0625 mg 25-OH-D_3 kg^{-1}, one with 0.055 and 0.070 mg kg^{-1} and one with 0.025 and 0.100 mg kg^{-1}, respectively. The intended dosages were confirmed by analysis. At day 36, the animals were slaughtered and the tissues sampled (abdominal fat, liver, kidney and muscle); the birds' tissues were pooled per pen before analysis (four replicates per group). The results are given in Table 11.

Table 11. **25-OH-D_3 concentration in tissues (µg kg^{-1}) of chicken for fattening administered vitamin D_3 and/or 25-OH-D_3**

Vitamin D_3 (mg kg^{-1} feed)	**0.125**	**-**	**-**	**0.065**	**0.055**	**0.025**
25-OH-D_3 (mg kg^{-1} feed)	**-**	**0.070**	**0.100**	**0.065**	**0.070**	**0.100**
Liver	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Kidney	<LOQ	<LOQ	<LOQ	11.6[2]	<LOQ	13.0[2]
Breast muscle	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Thigh muscle	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.7[3]
Abdominal fat	<LOQ	<LOQ	<LOQ	<LOQ	26.7[2]	29.6[2]

[1] <LOQ: below limit of quantification, 5 µg kg^{-1} in muscle,10 µg kg^{-1} in liver and kidney and 20 µg kg^{-1} in fat.

[2] concentration determined in one pooled sample out of four.

[3] concentration determined in two pooled samples out of four.

The fact that the analytical method used had higher LOQs than those of the former study (HPLC-MS instead of HPLC-radioimmunoassay analysis) makes the comparison of the results of both studies difficult, all the more as the 25-OH-D_3 concentration in most tissues is below or near the LOQs. Moreover, data concern the abdominal fat instead of skin/fat.

4.2.2.2. Pigs

Two trials are reported by the applicant, one carried out in weaned piglets and one in growing fattening pigs.

The study carried out in weaned piglets[31] is that already presented in the tolerance section (see Section 4.1.2). At slaughter (after 42 and 49 days under study), tissue samples (liver and muscle, 12 samples per treatment) were collected and analysed for 25-OH-D_3 concentration using the method described previously (see Section 4.2.2.1, chickens for fattening). The administration of vitamin D_3 resulted in tissue levels of 25-OH-D_3 below the LOQ. Piglets fed with 0.050 mg 25-OH-D_3 kg^{-1} showed an average of 19.3 µg 25-OH-D_3 kg^{-1} in all liver samples (n = 12). In muscle, only four out of 12 samples showed detectable amount leading to a mean value of 6.0 µg 25-OH-D_3 kg^{-1}.

A second study with growing pigs[32] was carried on two groups of 20 mixed sex 42-day-old pigs (five replicates of four pigs per pen) administered either 0.050 mg vitamin D_3 or 0.050 mg 25-OH-D_3 from ROVIMIX® Hy•D® 1.25% kg^{-1} feed (dosages controlled analytically) for a 84-

[30] Technical Dossier/Safety/Appendix 4-1-3-3-A
[31] Technical Dossier/ Safety/Appendix 4-1-1-D
[32] Technical Dossier/Safety/Appendix 4-1-3-3-K

day period. The animals were slaughtered and tissues (liver, kidney, muscle, perirenal fat and sub-cutaneous fat) were sampled. The results are given in Table 12.

Table 12. **25-OH-D_3 concentration in tissues (µg kg^{-1}) in pigs for fattening fed vitamin D_3 or 25-OH-D_3 at 0.050 mg kg^{-1} feed**

	Vitamin D_3	25-OH-D_3
Liver	<LOQ[1]	12.3 ± 1.5 (2)[2]
Kidney	<LOQ	13.6 ± 1.4 (17)[2]
Muscle	<LOQ	5.7 ± 4.0 (6)[2]
Perirenal fat	<LOQ	<LOQ
Sub-cutaneous fat	<LOQ	<LOQ

[1] <LOQ = below the limit of quantification of 5 µg kg-1 in the muscle, 10 µg kg-1 in the liver and kidney, and 20 µg kg-1 in the fat

[2] Average value and standard deviation calculated from samples with detectable levels; within brackets the number of samples with detectable levels out of 20.

The same conclusions as those drawn from the study on piglets apply to the results obtained in pigs for fattening for muscle, kidney and liver, and extend to fat.

4.2.3. Conclusions on deposition

The FEEDAP Panel concluded in its former opinion (EFSA, 2005) that the 'deposition of 25-OH-D_3 in the laying hen egg or turkey tissues is not significantly different in the birds that received either vitamin D_3 or a similar quantity of 25-OH-D_3 corresponding to the dose proposed for use.'

The FEEDAP Panel concluded also that 'deposition of 25-OH-D_3 in chicken tissues resulting from 25-OH-D_3 supplementation of the diet at the dose' (69 µg kg^{-1}) 'is about three times higher than that found following the use of the same quantity of vitamin D_3.' The use of different supplementation levels and different analytical methods with different LOQ in the former and present deposition studies makes comparisons in chickens for fattening difficult.

25-OH-D_3 could not be detected in liver or muscle in piglets after administration of 0.05 mg vitamin D_3 kg^{-1} feed; 25-OH-D_3 was determined in all liver samples (average value: 19 µg kg^{-1} tissue) and in four muscle samples out of 12, with an average of 6 µg kg^{-1} after administration of the same dose of 25-OH-D_3. The study on pigs for fattening showed comparable results except in liver, for which 25-OH-D_3 could be detected in only two out of 20 samples (mean of two samples: 12 µg kg^{-1}) after a 25-OH-D_3 treatment.

4.3. Studies on laboratory animals

As repeatedly stated, the FEEDAP Panel considers conventional toxicology studies to be inappropriate for testing pure substances which are dietary nutrients (essential dietary nutrients), which is the case for 25-OH-D_3. Such substances have a physiological concentration which is optimum for health and performance. Dietary intakes of such substances that lead to amounts which are significantly below or above the optimum for health and performance will inevitably cause a physiological imbalance and consequent adverse effects.

In the particular case of 25-OH-D_3, some toxicological studies were available and considered by the FEEDAP Panel in 2005 (acute toxicity in mice and rats, repeat dose sub-chronic toxicity in rats, two mutagenicity studies and reproduction studies in both rat and rabbit).

The Panel concluded in 2005 that there was no reason to suspect 25-OH-D_3 of having a genotoxic effect. This conclusion was confirmed by more recently conducted chromosome aberration test[33] carried out according to OECD guideline 473. The results did not show any clastogenic activity of 25-OH-D_3 when tested up to cytotoxic concentration.

The FEEDAP Panel concluded also in 2005 that the 'effects seen in the toxicity studies which have been conducted are entirely consistent with a physiological overload of Vitamin D or its metabolites and are not indicative of any unexpected toxicity arising from the source or production method of the substance.' After reassessing the *in vivo* toxicological studies submitted with the current dossier, the FEEDAP Panel confirms this position.

4.4. Assessment of consumer safety

The human use of 25-OH-D_3 and the status of 25-OH-D_3 in humans have been reviewed in the former opinion of the FEEDAP Panel (EFSA, 2005). Based on former proposals for vitamin D_3 (EC, 2002; Institute of Medicine, 1997) the FEEDAP Panel suggested a provisional Tolerable Upper Limit (UL) for humans of 10 µg 25-OH-D_3 day^{-1} in adults and adolescents (11 to 17 years old) and 5 µg 25-OH-D_3 day^{-1} in children (0 to 10 years old).

4.4.1. Consumer exposure to 25-OH-D_3

In its former opinion (EFSA, 2005), the FEEDAP Panel identified the different sources of vitamin D for humans including that produced in the skin under the influence of UV-light and dietary vitamin D_3. The contribution of 25-OH-D_3 from some foodstuffs to vitamin D activity was also analysed. The contents of 25-OH-D_3 in milk and fish are typically low (< 1 µg kg^{-1}), range from 2 to 5 µg kg^{-1} in meat (e.g. 2.5 µg kg^{-1} in chicken meat) and offal and may reach 10 µg kg^{-1} in egg yolk.

The daily exposure of the consumer to 25-OH-D_3 has been calculated by the FEEDAP Panel considering:

i) the 25-OH-D_3 contents in tissues and eggs corresponding to the highest 25-OH-D_3 dosages recommended for use in poultry and pig feed. For chickens for fattening, the results from the new study (Table 11) have been retained for the liver, kidney and muscle. LOQ values were used where the measurements indicated values below LOQs. Where the individual values were not available, average values were taken instead. The value for skin/fat (18.7 µg kg^{-1}) was taken from the former study after interpolation of the available data, as already done in the former opinion. As the 25-OH-D_3 concentrations measured in tissues of turkeys for fattening were all below those obtained in chickens, the latter values were taken for the calculation. For the egg, values from the study on laying hens formerly assessed (EFSA, 2005) were taken. For the pig, values from Table 12 (pigs for fattening) were taken into consideration (the values of the liver are those obtained in the piglet trial).

ii) the theoretical worst case consumption figures as laid down under Regulation (EC) No 429/2008[34] fixing guidelines for assessment of additives in animal nutrition. The exposure resulting from the consumption of meat has been chosen as the most conservative. The sum of the exposures skin/fat plus kidney (chicken) appeared to be higher than that from fat plus kidney (pig) and was retained for the calculation.

The results are presented in Table 13.

[33] Technical Dossier/Safety/Appendix 4-2-2-A
[34] OJ L 133, 22.5.2008, p.1

Table 13. **25-OH-D_3 consumer theoretical intake based on consumption model from Regulation (EC) No 429/2008**

Amount consumed	25-OH-D_3 (µg kg^{-1} tissue/product)	25-OH-D_3 intake (µg $person^{-1}$ day^{-1})
300 g Muscle (from either chickens or pigs)	6	1.8
100 g Liver (from piglets)	19	1.9
10 g Kidney (from chickens)	13	0.1
90 g Skin/fat (from chickens)	19	1.7
100 g Egg	14	1.4
Total		**6.9**

The theoretical exposure of 6.9 µg 25-OH-D_3 day^{-1} is very close to that calculated formerly on the basis of the data available for chicken for fattening, turkeys and laying hens (6.4 µg 25-OH-D_3 day^{-1}). Therefore, the extension of the use of 25-OH-D_3 to pigs at the dose proposed does not increase consumer exposure which complies with the suggested provisional UL for adults but exceeds the suggested provisional UL for children.

Refined calculation (Table 14) based on more realistic data, i.e. SCOOP data (EC, 2004), was performed according to the former opinion (EFSA, 2005). Exposure was estimated to 2.44 µg $person^{-1}$ day^{-1}.

Table 14. **25-OH-D_3 human intake based on SCOOP data (EC, 2004) (Maximum meat intake: 175 g day^{-1}, maximum egg intake 36 g day^{-1})**

Amount consumed	Content of 25-OH-D_3 (µg kg^{-1})[1]	25-OH-D_3 intake (µg day^{-1})
105 g Muscle[2]	6	0.63
35 g Liver[2]	19	0.66
3.5 g Kidney[2]	13	0.05
31.5 g Skin/fat[2]	19	0.60
36 g Egg	14	0.50
Total		**2.44**

[1] See Table 13.

[2] Meat intake calculated with the same proportion as in Regulation (EC) No 429/2008, i.e. 60 % muscle, 20 % liver, 2 % kidney and 18 % skin/fat.

4.4.2. Conclusions on the safety for the consumer

Considering the conclusions of the former opinion of the FEEDAP Panel and the newly submitted data, the FEEDAP Panel constitutes as a worst case intake situation the consumption of muscle from the two sources, pig or poultry, of liver from piglets, kidney and skin/fat from chickens and eggs. Using the highest values, the estimation of the total intake would be 6.9 µg 25-OH-D_3 $person^{-1}$ day^{-1} and by this not essentially higher than the former estimate (6.4 µg 25-OH-D_3 $person^{-1}$ day^{-1}). This value complies with the provisional UL for adults (69 %) but is above that for children (138 % based on the intake of adults).

A refined calculation reduced the exposure to 2.44 µg 25-OH-D_3 $person^{-1}$ day^{-1} which is below the provisional UL for both the adults (24 %) and the children (49 %).

The FEEDAP Panel concludes that the total exposure resulting from the use of 25-OH-D_3 in all poultry and pig categories, at the proposed maximum doses, would not represent a risk for the consumer.

4.5. Safety for the user/worker

The user/worker, when handling the product, is exposed to the final form in which the additive is placed on the market. Therefore, the risk for the user/worker is assessed from studies conducted with ROVIMIX® Hy•D® 1.25%.

4.5.1. Effects on the respiratory system

No data on respiratory toxicity has been provided.

4.5.2. Effects on skin and eyes

4.5.2.1. Skin irritation

A GLP compliant study[35] on skin irritation of ROVIMIX® Hy•D® 1.25% was conducted in three rabbits according to OECD guideline 404. The treatment resulted in very slight or well-defined erythema and/or very slight oedema in the treated skin areas of the three rabbits. The skin irritation resolved within seven days after exposure in all animals. No skin corrosion was observed.

4.5.2.2. Eye irritation

A GLP compliant study[36] of eye irritation was carried out in three rabbits according to OECD guideline 405. The eye instillation of a 0.1 ml volume containing 51 mg of ROVIMIX® Hy•D® 1.25% caused some irritation to the conjunctiva which completely resolved within 24 hours.

4.5.3. Systemic toxicity

Evidence of systemic toxicity was provided by studies with 25-OH-D_3 on laboratory animals, as well as on humans.[37] Excessive and prolonged exposure to 25-OH-D_3 may lead to signs of hypervitaminosis D, including calcium deposition in internal organs and soft tissues.

The most likely route of workplace exposure to 25-OH-D_3 is by inhalation. The major criterion for assessing worker exposure is the UL for 25-OH-D_3, in particular the part of the UL which is not covered by food consumption (10.0 – 2.44 = 7.56 µg person^{-1} day^{-1}).

In its former report (EFSA, 2005), the FEEDAP Panel referred to a monitoring study[38] on workers preparing two batches of purified 25-OH-D_3. Atmospheric levels achieved during handling were 0.33 and 0.15 mg 25-OH-D_3 m^{-3}. Blood levels of 25-OH-D_3 and 1,25-diOH-D_3 were not affected. The potential risk for workers handling the additive, ROVIMIX® Hy•D® 1.25%, should be considerably lower than for those handling purified 25-OH-D_3.

Data on the airborne exposure of worker/user handling ROVIMIX® Hy•D® 1.25% were not provided; however; the applicant described a scenario[39] based on the assumptions that 100 % of

[35] Technical Dossier/Safety/Appendix 4-4-1-C
[36] Technical Dossier/Safety/Appendix 4-4-1-B
[37] Technical Dossier/Safety/Appendix 4-2-4-B
[38] Technical Dossier/Safety/Appendix 4-2-3-C
[39] Technical Dossier/Safety/Appendix 4-4-2-A

25-OH-D_3 from the inspired air are transferred to the blood and that dust particles from ROVIMIX® Hy•D® 1.25% uniformly contain 1.25 % 25-OH-D_3.

4.5.3.1. Preparation of premixtures containing ROVIMIX® Hy•D® 1.25%

The scenario is based on the following assumptions:

i) A premixture factory produces within one working day 40 batches, 30 % of which contain ROVIMIX® Hy•D® 1.25%.

ii) The preparation of one premixture needs a maximum of 20 seconds for handling the additive. The resulting four minutes of exposure are rounded up to ten minutes to increase the margin of safety.

iii) The inhaled air in ten minutes is 0.21 m^3 (ten m^3 per person in eight hours).

iv) Workers carry masks which reduce exposure to dust from atmospheric level (5 mg m^{-3}) to 10 %.

v) Worst case assumption is made by considering the inspired dust to fully consist of ROVIMIX® Hy•D® 1.25%.

From the above assumptions, 1.3 µg 25-OH-D_3 results as the maximum daily airborne exposure for the worker preparing premixtures.

In describing another worst case situation, the FEEDAP Panel assumes that all premixtures prepared during a working day would contain ROVIMIX® Hy•D® 1.25%. Daily exposure of the worker would then increase to 4.3 µg, which is also within the available part of the UL (7.56 µg).

Packing premixtures with a certain amount of ROVIMIX® Hy•D® 1.25% was also considered. Airborne exposure to 25-OH-D_3 fully depends on the 25-OH-D_3 content of the premixture and is therefore considerably lower (about two orders of magnitude) than the above estimated value.

Airborne exposure of users will consequently decrease with each further dilution step of ROVIMIX® Hy•D® 1.25% in the final feedingstuffs.

4.5.4. Conclusions on the safety for the worker/user

ROVIMIX® Hy•D® 1.25% is not irritant to the skin or the eye. Data on sensitisation as well as on respiratory toxicity were not provided.

The most likely route of workplace exposure to 25-OH-D_3 is by inhalation. A worst case calculation scenario based on assumptions results in a potential daily inhalation of about 4 µg for workers preparing premixtures containing ROVIMIX® Hy•D® 1.25%. This figure is within that amount of the UL not required for food intake.

4.6. Safety for the environment

The 25-OH-D_3 is a physiological metabolite in animals derived from vitamin D_3, either from endogenous synthesis or exogenous supply. It is excreted only at low amounts. The FEEDAP Panel considers studies on the environmental impact of 25-OH-D_3 not necessary, taking also into account that 25-OH-D_3 predominantly substitutes for supplemental vitamin D_3 at the relevant maximum content.

The FEEDAP Panel concludes that the use of 25-OH-D_3 in feedingstuffs does not represent a risk for the environment.

5. Post-market monitoring

No specific risks associated with the use of the product are expected. The FEEDAP Panel considers that there is no need for specific requirements of a post-market monitoring plan other than those already established in the Feed Hygiene Regulation[40] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

ROVIMIX® Hy•D® 1.25% contains 12.5 g 25-OH-D_3 kg^{-1} as active substance, which is a physiological precursor of the vitamin D_3 active hormone. 25-OH-D_3 from ROVIMIX® Hy•D® 1.25% is efficacious in all poultry species and pig categories when substituting for vitamin D_3 in feed.

ROVIMIX® Hy•D® 1.25% is considered safe for the target animals at maximum contents of 0.100 mg 25-OH-D_3 kg^{-1} complete feed for chickens and turkeys for fattening, of 0.080 mg 25-OH-D_3 kg^{-1} complete feed for all other poultry species and of 0.050 mg 25-OH-D_3 kg^{-1} for all pig categories.

After reassessing the *in vivo* toxicological studies on laboratory animals submitted, the FEEDAP Panel confirms its earlier conclusions (EFSA, 2005) that the 'effects seen in the toxicity studies which have been conducted are entirely consistent with a physiological overload of Vitamin D or its metabolites and are not indicative of any unexpected toxicity arising from the source or production method of the substance.'

There is no new information which would require a modification of the formerly proposed UL of 10 µg 25-OH-D_3 adult $person^{-1}$ day^{-1} and 5 µg 25-OH-D_3 $child^{-1}$ day^{-1}.

Exposure calculation of the consumer was based on former tissues and product deposition data in poultry and new data in pigs. The total intake was estimated to be 6.9 µg 25-OH-D_3 $person^{-1}$ day^{-1}. This value complies with the provisional UL for adults (69 %) but is above that for children (138 %, based on the intake of adults). Refined calculation (more realistic consumption data) reduced the estimated exposure to 2.44 µg 25-OH-D_3 $person^{-1}$ day^{-1}, which is below the provisional UL for both the adults (24 %) and the children (49 %). The FEEDAP Panel concludes that the total exposure resulting from the use of 25-OH-D_3 in all poultry species and pig categories, at the proposed maximum doses, would not present a risk for the consumer.

The additive ROVIMIX® Hy•D® 1.25% is not irritant to the skin or eye. Data on skin sensitisation as well as on respiratory toxicity were not provided. The most likely route of workplace exposure to 25-OH-D_3 is by inhalation. A worst case calculation scenario results in a potential maximum daily inhalation of about 4 µg for workers preparing premixtures containing ROVIMIX® Hy•D® 1.25%. This figure is included in the part of the UL not covered by food consumption.

The use of 25-OH-D_3 in feedingstuffs does not represent a risk for the environment.

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

RECOMMENDATIONS

As for vitamin D_3, the simultaneous use of 25-OH-D_3 and vitamin D_2 should not be allowed.

The conditions of use in the Register entry could be simplified. The list 'laying hens, all other categories of poultry for fattening and laying, including geese, ducks and game birds' as proposed by the applicant could be condensed to 'all other poultry categories'. Equally, the list of 'piglets (suckling and weaned), pigs for fattening, sows for reproduction, and sows in order to have benefit in piglets' could be shortened to 'all categories of pigs'.

According to Article 8(4) of Regulation (EC) No 1831/2003, the FEEDAP Panel proposes the following specification for the Register entry to maintain animal safety (by providing a stable formulation of 25-OH-D_3 and a homogeneous distribution of 25-OH-D_3 as the active substance in a feed additive similar to ROVIMIX® Hy•D® 1.25%) and user safety (dustiness should not exceed the figures measured for ROVIMIX® Hy•D® 1.25%):

- stabilised form of 25-OH-D_3,
- particles ≤ 10 µm not more than 2 %, particles ≤ 50 µm not more than 35 %,
- dustiness not higher than 4 % (Heubach method).

The specifications for the active substance (25-OH-D_3) should be the following:

- purity at least 94 %,
- not more than 1 % other sterols each,
- not more than 1 % solvents (determined as loss on drying),
- not more than 5 mg erythrosine kg^{-1}

FINAL REMARK

The current authorisation of 25-OH-D_3 for chickens for fattening and turkeys allows finally a higher supply of total vitamin D activity with the combined use of 25-OH-D_3 and vitamin D_3 than when using vitamin D_3 alone. In its former opinion (EFSA, 2005), the FEEDAP Panel recommended as a maximum content 0.100 mg 25-OH-D_3 kg^{-1} for chickens and turkeys for fattening. This was considered to be at least equivalent to the established maximum content of vitamin D_3 for these animal categories (5000 IU kg^{-1}). The current legislation increases indirectly the maximum content of 5000 IU kg^{-1} to higher values (activity) when using 25-OH-D_3 and vitamin D_3 together.

The FEEDAP Panel repeats and extends its former statement:

> The combination of 25-hydroxycholecalciferol with vitamin D_3 (cholecalciferol) is allowed provided that the total of the mixture does not exceed 0.050 mg kg^{-1} complete feedingstuffs for all categories of pigs, 0.100 mg kg^{-1} complete feedingstuffs for chickens for fattening and turkeys and 0.080 mg kg^{-1} complete feedingstuffs for other poultry.

DOCUMENTATION PROVIDED TO EFSA

1. 25-hydroxycholecalciferol as a Nutritional Additive for Poultry and Pigs. December 2007. Submitted by DSM Nutritional Products.
2. Supplementary information. 25-hydroxycholecalciferol as a Nutritional Additive for Poultry and Pigs. January 2009. Submitted by DSM Nutritional Products.
3. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for 25-hydroxycholecalciferol.
4. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for 25-hydroxycholecalciferol

25-hydroxycholecalciferol is a product already authorised as feed additive by Regulation (EC) No 1443/2005, under the category 'Nutritional Additive', functional group 'Vitamins, provitamins and chemically well defined substances, having a similar effect' according to the classification system of Annex I of Regulation (EC) No 1831/2003 for chickens for fattening, laying hens and turkeys.

The current application is for the re-evaluation according to Article 10(2) of 25-hydroxycholecalciferol and for its new use for poultry and pigs according to Article 4(1) of Regulation (EC) No 1831/2003

This authorization is sought to use 25-hydroxycholecalciferol for poultry and pigs and the proposed inclusion level of active substance ranges from 0.05 to 0.100 mg/kg complete feedingstuffs, depending on the target animal species. If the 25-hydroxycholecalciferol is combined with vitamin D_3 the proposed inclusion level of the active substance ranges from 0.05 to 0.125 mg/kg complete feedingstuffs.

The active substance shall have a minimum purity of 25-hydroxycholecalciferol of 94% measured by a Reverse Phase High Performance Liquid Chromatography (RF-HPLC) with Diode Array Detection (DAD) or Ultraviolet (UV) detection at 270 nm.

For the determination of the active substance (25-hydroxycholecalciferol) in the *feed additive* the applicant proposed a Normal Phase High Performance Liquid Chromatography (NP-HPLC) method equipped with UV detection at 260 nm.

The following acceptable performance characteristics obtained using a Rovimix formulation were reported: - a relative intermediate precision standard deviation (RSD_R) of 2.6 % and – a recovery rate close to 100 %. The method is therefore considered suitable for official control.

For the determination of 25-hydroxycholecalciferol in *premixtures* the applicant proposed a NP-HPLC method with DAD or UV detection at 265 nm. A 25-hydroxyergocalciferol internal standard is used for determination of 25-hydroxycholecalciferol in premixtures with a content of 25-hydroxycholecalciferol lower than 100 mg/kg of premixtures. The method was validated with respect to selectivity, linearity, range of application, recovery, accuracy and intermediate precision. The following acceptable performance characteristics were reported : - a limit of quantification (LOQ) of 2 mg/kg premixtures; - a recovery rate close to 100 % determined at different concentration levels; - a repeatability relative standard deviations (RSD_r) ranging from 1.0 to 3.25 % and a RSD_R ranging from 1.5 to 4.3 %. The method is considered suitable for official control.

For the determination of 25-hydroxycholecalciferol in *feedingstuffs* the applicant proposes a HPLC method connected with to a mass spectrometer (MS) using a 26,27-d_6-25-hydroxycholecalciferol internal standard.

The method has been single-laboratory validated with respect to selectivity, linearity, range of application, recovery, accuracy and intermediate precision and showing an acceptable performance profile. The following acceptable performance characteristics were reported: - a LOQ of 0.005 mg/kg feedingstuffs; - a recovery rate ranging from 100 to 110 %; - a RSD_r ranging from 8.5 to 13.2 %; and - RSD_R ranging from 8.8 to 17.5 %. The method is considered suitable for official control of the content of 25-hydroxycholecalciferol in feedingstuffs.

For the analytical determination of vitamin D_3, (included as additional requirement in Annex III), the applicant suggests the CEN method (EN 12821:2000). This method was originally validated for food and the applicant provided additional data demonstrating that the method is suitable for the analysis of vitamin D_3 in feedingstuffs. The following performance characteristics were reported: - a limit of detection (LOD) and a LOQ of 0.008 and 0.02 mg/kg feedingstuffs, respectively. From the information provided by the applicant the CRL calculated a RSD_R of 17 % for concentration level of vitamin D_3 around 0.020 mg/kg feedingstuffs. The method is considered suitable for official control of vitamin D_3 content in feedingstuffs at the concentration range covered in the validation.

Further testing or validation is not considered necessary.