Assessment on the safety and efficacy of canthaxanthin (Carophyll Red® 10%) as a feed additive for poultry, breeder hens, ornamental birds and ornamental fish.

Reference number RP597-600

Regulated Products Risk Assessment Unit
Science, Evidence and Research Division, FSA

Risk Assessment Team
Science Division, FSS

Regulated Product Dossier Assessment
Assessment finalised: 04/12/2023

Contents

List of tables

Abbreviations

Acronym	Definition
ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Animal Feed and Feed Additives Joint Expert Group
ADI	Acceptable daily intake
ADME	Absorption, distribution, metabolism and excretion
BHT	Butylated hydroxytoluene
CAS	Chemical Abstracts Service
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FSA	Food Standards Agency
FSS	Food Standards Scotland
LOQ	Limit of quantification
NP-HPLC	Normal Phase High-Performance Liquid Chromatography
ppm	Parts-per million
QPS	Qualified presumption of safety
RSD_r	Standard deviation for repeatability
RDS_R	Standard deviation for reproducibility
R_{Rec}	Recovery rate

Summary

Two applications were submitted to the Food Standards Agency in November 2021 from DSM Nutritional Products ("the applicant") for the authorisation of an additive (canthaxanthin-Carophyll® Red 10%), under the categories of 'zootechnical and sensory-colourant'. The additive is proposed to be used in breeder hens, poultry, ornamental birds and ornamental fish at a range level of 6-100 mg/kg of complete feed with a moisture content of 12%, depending on target species.

Canthaxanthin synthesised chemically has previously been authorised. This modification of authorisation application concerns an additional production process, wherein canthaxanthin is produced through fermentation using *Yarrowia lipolytica*.

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

The AFFAJEG concluded that the fermented product has a similar high purity to the synthetic canthaxanthin, therefore there were no further concerns regarding Section II.

The applicant conducted a literature review to cover the period since the last authorisation and found no negative effects had occurred in this time. Members evaluated the safety for the target species, consumer and the environment, concluding that the additive should continue to be considered safe. The additive is not considered to be irritant to skin and eyes and not thought to be a skin sensitiser. It is potentially harmful by inhalation, and some batches have been shown to very dusty.

No demonstration of efficacy was required for the modification of authorisation. For the extension of use, the AFFAJEG concluded that previous data demonstrating efficacy could be extrapolated to breeder hens. Members also concluded that the new production method of fermentation is not expected to negatively affect the efficacy of the additive.

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

1. Introduction

The FSA and FSS have undertaken a risk assessment for a feed additive (canthaxanthin-Carophyll® Red 10%, DSM Nutritional Products Ltd., Heanor Gate Ind. Est., Heanor, Derbyshire, DE75 7SG, United Kingdom) under retained regulation No 1831/2003[1] under the category of 'zootechnical and sensory additives-colourants'. Two applications were received for authorisation:

Zootechnical

- Modification of authorisation for breeder hens.

Sensory - Colourant

- Modification of authorisation for chickens for fattening and minor poultry species for fattening, laying poultry and poultry reared for laying, ornamental fish and ornamental birds and ornamental breeder hens.

Strictly the same feed additive preparation is used in both applications, therefore the two applications were evaluated together. The only differences between the dossiers are the proposed conditions of use. To support the safety assessment by FSA and FSS, the AFFAJEG and the ACAF provided advice to the FSA and FSS outlined in this document.

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

With thanks to the members of the AFFAJEG and ACAF during the course of the assessment, who were: Professor John Wallace, Professor Nicholas Jonsson, Martin Briggs, Professor Katrina Campbell, Susan MacDonald, Professor Matthew Fisher, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Helen Warren and Dr. Nick Wheelhouse. Dr. Adam Smith declared a conflict of interest and did not participate in the assessment of the dossier.

The dossier was evaluated by the AFFAJEG at their May 2022 meeting and by the ACAF at their October 2022 meeting. Further information was provided by the applicant in October 2022, responding to queries by the FSA.

This document outlines the discussion and conclusions of the AFFAJEG's and ACAF's assessment on the safety and efficacy of Carophyll® Red 10% as a feed additive.

2. Assessment

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

The additive consists of a 10% preparation of canthaxanthin, as granulated powder, with authorised feed additives and feed materials of feed or food grade quality. The applicant provided an example of the current composition of the additive, as well as data from five batches supporting the specification values. These are outlined below (Table 1). In the previous authorisation, the antioxidant compound used was ethoxyquin, however in anticipation of the suspension of ethoxyquin in the EU, the applicant replaced it with butylated hydroxytoluene (BHT):

Table 1. Identity table: CAROPHYLL® Red 10%

CAROPHYLL® Red 10% composition	
Ingredient	**Concentration**
Canthaxanthin	10%
Corn starch	15%
Dextrin yellow	10%
Lignosulfonate	60.6%
BHT	4.4%
Specification of crystalline canthaxanthin (from *Y. lipolytica*)	
Total colouring matter (as canthaxanthin)	Min. 96% / Max. 101%
Carotenoids other than canthaxanthin	Max. 5%
Appearance	
Crystalline powder, violet to violet-brown	
Impurities	
Sulphated ash	Max. 0.1%
Pb	Max. 2 ppm
Cd	Max. 1 ppm
Hg	Max. 1 ppm
As	Max. 2 ppm

The Committee evaluated the identity and characterisation of the additive, comparing the differences between the previous authorisation which had a synthetic production process, and the new application seeking authorisation for a fermentation production process. The new production process was considered to be well described after a flowchart of the steps involved was provided upon request. The microorganism used for the production of canthaxanthin, *Yarrowia lipolytica*, was identified as a QPS organism[2]. No live cells remained in the final product and levels of impurities were not a cause for concern for committee members.

The dusting potential was deemed to be low to medium, with tests demonstrating a three-fold difference between batches, but members felt the potential risks were sufficiently handled through the recommendation of use of protective equipment to limit exposure. The active substance canthaxanthin comprises at least 96% of the colourant component, with other carotenoids making up a maximum of 5%. These remaining carotenoids were not deemed to be a concern, as they are similar to other known, safe carotenoids.

Each application has different proposed conditions of use, one to be used as a zootechnical additive and the other as a colourant. The proposed conditions of use for its zootechnical function are described in Table 2.

Table 2: Proposed conditions of use of canthaxanthin as described in the zootechnical and sensory additive/colourant applications

Proposed mode of use in animal nutrition				
Additive	Canthaxanthin			
CAS No	514-78-3			
Category(-ies) of additive	Zootechnical and sensory additive/colourant			
Description				
Composition, description				
Crystalline powder consisting mainly of all-trans canthaxanthin, with minor amounts of cis-isomers of canthaxanthin, and max. 5% of other carotenoids.				
Trade name (if appropriate)	CAROPHYLL® Red 10%			
Name of the holder of authorisation (if appropriate)	--			
Conditions of use (zootechnical)				
Target species	**Min. content**	**Max content**	**Other provisions**	**Maximum residue levels**
	mg of active substance/kg of			

	complete feedingstuffs with a moisture content of 12%			
Breeder hens	6	6	1. In the directions for use of the additive and premixture, indicate the storage conditions and stability to heat processing. 2. The mixture of different sources of canthaxanthin shall not exceed 6 mg canthaxanthin/kg of complete feedingstuffs. 3. The mixture of this preparation with canthaxanthin and other carotenoids is allowed provided that the total concentration of the mixture does not exceed 80 mg/kg of complete feedingstuffs. 4. For user safety: breathing protection, safety glasses and gloves should be worn during handling.	15 mg canthaxanthin/kg liver (wet tissue) and 2.5 mg canthaxanthin/kg skin/fat (wet tissue)
Conditions of use (sensory additive/colourant)				
Target species	**Min. content**	**Max. content**	**Other provisions**	**Maximum residue levels**
	mg of active substance/kg of complete feedingstuffs with a moisture content of 12%			
Chickens for fattening and minor poultry species for fattening	-	25	1.Canthaxanthin may be placed on the market and used as an additive consisting of a preparation 2. The mixture of canthaxanthin with other carotenoids and xanthophylls shall not exceed 80 mg/kg of complete feed 3.For safety: breathing protection, safety glasses and gloves should be worn during handling	Poultry 15 mg canthaxanthin/kg liver (wet tissue) and 2.5 mg canthaxanthin/kg skin/fat (wet tissue) Laying poultry 30 mg canthaxanthin/kg egg yolk (wet tissue)
Laying poultry and poultry reared for laying	-	8		

Ornamental fish and ornamental birds except ornamental breeder hens	-	100	1.Canthaxanthin may be placed on the market and used as an additive consisting of a preparation 2. The mixture of canthaxanthin with other carotenoids and xanthophylls shall not exceed 100 mg/kg of complete feed 3.For safety: breathing protection, safety glasses and gloves should be worn during handling	
Ornamental breeder hens		8		

2.1.1. Conclusions on Section II

The AFFAJEG concluded that the dusting potential was low to medium and that the recommended use of protective equipment provided reassurance about the additive, as did the expansion on handling measures for safety. Given that the fermentation product has a similar high purity to the synthetic canthaxanthin, members had no issue with using the additive at the levels previously approved for the relevant target species.

No further concerns were raised for Section II of these dossiers.

2.2. Section III: Safety

The applicant conducted a literature review to cover the period 2014-2020 (since the last EFSA opinion on canthaxanthin[3-4]) and found no reported negative effects.

2.2.1. Safety for the target species

A pigmentation trial with laying hens was presented to demonstrate bioequivalence, showing that the canthaxanthin produced from *Y. lipolytica* has the same high purity as synthetic canthaxanthin, which has a large safety margin for the target species.

As *Y. lipolytica* is a QPS organism, no additional studies are required to demonstrate safety for target animals. Based on the information provided, members concluded that

the additive remains safe for the target species and extended these conclusions to the additional production process.

2.2.2. Safety for the consumer

ADME studies were not repeated as the new production process leads to the same chemical entity with the same high purity, however, genotoxicity studies, namely the bacterial reverse mutation assay and the in vitro chromosome aberration assay, were performed to support the new production process, which the Committee evaluated to conclude that this particular form of the additive is not genotoxic.

Toxicology experts in the Committee indicated that canthaxanthin has the potential to accumulate in the retina, resulting in an ADI (acceptable daily intake) of 0.03 mg/kg being established in previous authorisations. Members concluded that exposure to canthaxanthin as shown in this application remains well below the ADI and so the additive remains safe for the consumer.

2.2.3. Safety for the user/worker

Members assessed studies provided previously and concluded that canthaxanthin from *Y. lipolytica* is not likely to be an irritant to skin and eyes and unlikely to be a skin sensitiser. The additive has not been tested for inhalation toxicity. One of three batches had dusting potentials greater than 1 g/m^3, and as such was regarded as dusty. Workers should be protected from exposure to potentially hazardous dust.

2.2.4. Safety for the environment

The Committee concluded that canthaxanthin occurs naturally, biodegrading in water and soil, therefore the previous conclusion from the 2014 EFSA opinion[3] was to be maintained. This conclusion was that the oxidative susceptibility of carotenoids will not result in a substantial increase in canthaxanthin concentration in the environment and consequently not pose a risk to the environment.

2.2.5. Conclusions on safety

The AFFAJEG concluded that the additive can be considered safe for the target species, the consumer and the environment. The product is not likely to be an irritant to skin and eyes and unlikely to be a skin sensitiser. The additive has not been tested for

inhalation toxicity. One of three batches had dusting potentials greater than 1 g/m^3, and as such was regarded as dusty. Workers should be protected from exposure to potentially hazardous dust.

2.3. Section IV: Efficacy

The AFFAJEG evaluated Section IV of the dossier, for which no new efficacy studies were presented, for the potential of extrapolating existing conclusions from previous authorisations of the additive to the updated authorisation concerning an additional production process. One of the applications also seeks to extend its authorisation to breeder hens. The applicant demonstrated equivalence between the synthetic canthaxanthin and the fermentation product. Members concluded that the modification to the production process is not expected to negatively affect the efficacy of the additive when used at the proposed level of 6 mg/kg of complete feed for breeder hens. The same outcome was determined with the second application so that the efficacy conclusions could be extrapolated to all laying poultry and poultry reared for laying, chickens for fattening and minor poultry species for fattening, ornamental fish and ornamental birds when used at the proposed level of 8-100 mg/kg of complete feed depending on the species.

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for CAROPHYLL® Red 10%[5]:

“For the determination of the active substance, canthaxanthin, in the feed additive, the applicant submitted a ring trial validated method based on spectrophotometry. The following performance characteristics were reported:

- A standard deviation for repeatability (RSD_r) ranging from 0.2% to 0.8%;
- A standard deviation for reproducibility (RDS_R) ranging from 1.3 to 4.0%, and
- A recovery rate (R_{Rec}) ranging from 96.2 to 105%

Based on the performance characteristics presented the EURL recommends for official control the ring trial validated spectrophotometric method, submitted by the Applicant, to determine canthaxanthin, in the feed additive.

For the determination of canthaxanthin in premixtures and feedingstuffs the Applicant submitted a single laboratory validated and further verified method based on Normal Phase High-Performance Liquid Chromatography coupled to VIS detection (NP-HPLC-VIS). The following performance characteristics were reported:

- RSD_r ranging from 1.4 to 15%;
- A standard deviation for intermediate precision (RSD_{ip}) ranging from 2.1 to 14.8%
- R_{Rec} ranging from 85.5 to 107%, and
- A limit of quantification (LOQ) of 1mg/kg in feedingstuffs.

Based on the performance characteristics presented the EURL recommends for official control the single laboratory validated and further verified NP-HPLC-VIS method, submitted by the Applicant, to determine canthaxanthin in premixtures and feedingstuffs.

For the determination of canthaxanthin in water, the Applicant submitted the above mentioned NP-HPLC-VIS method. However no experimental data have been provided. Therefore, the EURL could not evaluate nor recommend a method for official control to determine canthaxanthin in water.

For the quantification of canthaxanthin in poultry tissues (liver, skin, fat) and egg yolk the Applicant proposed a single laboratory validated and further verified method, based on NP-HPLC coupled to VIS detection measuring at 466 nm. For the quantification of canthaxanthin in fish flesh (salmon and trout) the Applicant proposed the CEN/TS 162330-1:2011 analytical method (further ring trial validated by six laboratories in a collaborative study organised by the University of Trondheim – HIST of on behalf of the Norwegian Seafood Federation – FHL), based on NP-HPLC coupled to VIS detection measuring 470 nm. These methods were validated and verified at the content of canthaxanthin ranging from 0.7 to 114 mg/kg in poultry tissues, 2.5 to 40 mg/kg for egg yolk and applicable for a range above 0.02 mg/kg for fish flesh. The following performance characteristics were reported:

- For liver, skin, fat and egg yolk: RSD_r and RSD_{ip} ranging from 1 to 9.5%; R_{Rec} ranging from 93 to 110%, LOQ of 0.3 mg/kg and for egg yolk of 0.5 mg/kg;

- For fish flesh: RSD_r and RSD_R ranging from 4.1 to 9.2%; R_{Rec} of 95.8% and LOQ of 0.01 mg/kg.

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified methods, based on NP-HPLC-VIS submitted by the Applicant, to determine canthaxanthin in poultry tissues, egg yolk and the CEN/TS 16233-1:2011 method, based on NP-HPLC-VIS to determine canthaxanthin in fish flesh.

Further testing or validation of the methods is not considered necessary."

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

4. Conclusions

The additive CAROPHYLL® Red 10% was fully characterised and no major causes for concern were identified by the AFFAJEG in the identity, production, characterisation or conditions of use sections.

Members concluded that the additive can continue to be considered safe for the target species, the consumer and the environment. The product is not likely to be an irritant to skin and eyes and unlikely to be a skin sensitiser. One of three batches had dusting potentials greater than 1 g/m^3, and as such was regarded as dusty. Workers should be protected from exposure to potentially hazardous dust.

AFFAJEG concluded that introducing the new production process of fermentation is not expected to negatively affect the efficacy of the additive. There was also no issue with extrapolating the use of the additive to breeder hens.

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

5. References

1. EC (European Commission), 2003. Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition.

2. EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Sid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernandez Escamez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Hempen M and Herman L, 2020. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 12: suitability of taxonomic units notified to EFSA until March 2020. EFSA Journal 2020;18(7):6174. https://doi.org/10.2903/j.efsa.2020.6174

3. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific opinion on the safety and efficacy of canthaxanthin as a feed additive for poultry and for ornamental birds and ornamental fish. EFSA Journal 2014;12(1):3527. https://doi.org/10.2903/j.efsa.2014.3527

4. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific opinion on the safety and efficacy of CAROPHYLL® Red 10% (preparation of canthaxanthin) for all poultry for breeding purposes (chickens, turkeys and other poultry). EFSA Journal 2013;11(1):3047. https://doi.org/10.2903/j.efsa.2013.3047.

5. EURL-FA(European Reference Laboratory for Feed Additives), 2016. Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. Canthaxanthin. Available at: FAD-2010-0407 (europa.eu)

For enquiries about this publication, contact the Food Standards Agency.

Follow us on Twitter: @foodgov

Find us on Facebook: facebook.com/FoodStandardsAgency