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Safety and efficacy of a feed additive consisting of astaxanthin-rich *Phaffia rhodozyma* for salmon and trout (Igene Biotechnology, Inc.)

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Abstract

Following a request from the European Commission, the FEEDAP Panel was asked to deliver a scientific opinion on the safety and efficacy of astaxanthin (ATX)-rich *Phaffia rhodozyma*. The additive, belonging to the category 'sensory additives' and the functional group 'substances which, when fed to animals, add colours to food of animal origin' is intended to be used in feed for salmon and trout from an age of six months onwards up to a maximum content of 100 mg ATX/kg complete feed. The product is produced by the telemorph of *Phaffia rhodozyma*, *Xanthophyllomyces dendrorhous,* and it is declared to contain 995 g dried inactivated biomass and 5 g ascorbic acid per kg additive. The main active principle of the additive is ATX; however, the FEEDAP Panel noted that some other carotenoids are also present in lower quantities. The minimum ATX concentration is specified to be 5,000 mg per kg additive. The yeast *Xanthophyllomyces dendrorhous* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment; therefore, the use of the production strain in the production of the additive would not raise any safety concern for the target species, the consumers of products from animals fed the additive and the environment. In the absence of a tolerance study with the additive, the FEEDAP Panel cannot conclude on the safety for the target species. In the absence of residue and toxicity data of ATX, no final conclusions on the safety for the consumer can be drawn. The FEEDAP Panel concluded that the additive is irritant to skin and eyes, and a skin and respiratory sensitiser, although exposure by inhalation is likely low. The FEEDAP Panel considers that ATX from the biomass does not pose a significant additional risk to the environment compared with other natural sources of ATX. In absence of adequate evidence, no conclusion can be made on the efficacy of the additive.

Keywords: sensory additive, colourant, *Phaffia rhodozyma*, astaxanthin, salmon, trout, safety, efficacy

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 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 70/524/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 a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from ADM Specialty Ingredients (Europe) B.V., as part of Naturxan Joint Venture, for re-evaluation of the product astaxanthin-rich *Phaffia rhodozyma* (ATCC SD 5340), when used as a feed additive for salmon and trout (category: sensory; functional group: substances which, when fed to animals, add colours to food of animal origin). On 24 December 2015, the EC sent a withdrawal of the initial request and successively on 4 May 2016 a void of the withdrawal in which the EC also informed that the applicant was Igene Biotechnology, Inc.[2] The EU representative of the company is Intertek Deutschland GmbH.[3]

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 authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 3 January 2017.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product astaxanthin-rich *Phaffia rhodozyma* (ATCC SD 5340) when used under the proposed conditions of use (see Section 3.1.6).

1.2. Additional information

The safety and efficacy of this additive was assessed by the FEEDAP Panel in 2006 (EFSA, 2006). Astaxanthin (ATX)-rich *Phaffia rhodozyma* (ATCC SD-5340) was provisionally authorised for 4 years for use in salmon and trout until 3 August 2011.[4]

Synthetic astaxanthin[5] and its derivative astaxanthin dimethyldisuccinate[6] are also authorised in the EU as feed additives for fish and crustaceans while the red carotenoid-rich bacterium *Paracoccus carotinifaciens*,[7] a natural source of ATX, is authorised for salmon and trout.

The FEEDAP Panel adopted a series of opinions on ATX of synthetic and of natural origin (EFSA, 2004,2005,2007a,b, EFSA FEEDAP Panel, 2010, 2014a,b, 2019a,b).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Igene Biotechnology, Inc, 21045-2024, Columbia, MD, United States of America.
[3] Intertek Deutschland GmbH, Stangenstrasse 1, 70771 Leinfelden-Echterdingen Germany.
[4] Commission Regulation (EC) No 828/2007 of 13 July 2007 concerning the permanent and provisional authorisation of certain additives in feedingstuffs. OJ L 184, 16.7.2007, p. 12. and corrigendum CS, OJ L 235 CS of 7.9.07 p. 8.
[5] COMMISSION IMPLEMENTING REGULATION (EU) 2015/1415 of 20 August 2015 concerning the authorisation of astaxanthin as a feed additive for fish, crustaceans and ornamental fish, OJ L 220, 21.8.2015, p. 7.
[6] COMMISSION IMPLEMENTING REGULATION (EU) 2020/998 of 9 July 2020 concerning the renewal of the authorisation of astaxanthin-dimethyldisuccinate as a feed additive for fish and crustaceans and repealing Regulation (EC) No 393/2008. OJ L 221, 10.7.2020, p. 96.
[7] Commission Regulation (EC) No 721/2008 of 25 July 2008 concerning the authoriation of a preparation of red carotenoid-rich bacterium *Paracoccus carotinifaciens* as feed additives. OJ L 193, 26.7.2008, p.23 amended by COMMISSION REGULATION (EU) No 334/2010 of 22 April 2010 amending Regulation (EC) No 721/2008 as regards the composition of feed additives. OJ L 102, 23.4.2010, p. 21.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[8] in support of the authorisation request for the use of ATX-rich *Phaffia rhodozyma* (ATCC SD 5340) as a feed additive.

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

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of ATX-rich *Phaffia rhodozyma* (ATCC SD 5340) is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c), Guidance on the assessment of microbial biomasses for use in animal nutrition (EFSA FEEDAP Panel, 2011b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019b).

3. Assessment

The additive ATX-rich *Phaffia rhodozyma* (ATCC SD-5340) is intended to be used as a sensory additive (functional group: colourants) in feed for salmon and trout. The applicant is seeking the re-evaluation of the use of the product in salmon and trout and proposes the maximum content of 100 mg ATX/kg complete feed. The trade name of the product is Aquasta®.

It is noted that the product is produced by the telemorph of the yeast *Phaffia rhodozyma*, i.e. *Xanthophyllomyces dendrorhous* (see Section 3.1.1), and therefore in this opinion, the feed additive under assessment will be referred as astaxanthin-rich *Xanthophyllomyces dendrorhous* (ATCC SD-5340) (ATX-rich XD).

3.1. Characterisation

3.1.1. Characterisation of the production organism

The additive under assessment is produced by fermentation using the yeast *Xanthophyllomyces dendrorhous* ATCC SD-5340, which is considered the telemorph of *Phaffia rhodozyma*.

The strain under assessment is declared to be deposited at the American Type Culture Collection with SD number 5340,[11] but the deposition certificate was not provided. The production strain was

[8] FEED dossier reference: FAD-2010-0060.
[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2010-0060_aquasta.pdf
[10] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[11] Technical dossier/Spontaneous submission July 2019/Annex (10).

derived from strain ATCC 96594 by classical mutagenesis and using an antimycin A treatment, followed by visual selection for pigment production.[12]

The parental strain ATCC 96594 (CBS 6938) was identified as *Xanthophyllomyces dendrorhous* based on internal transcribed spacer (ITS) sequence analysis published by Fell and Blatt (1999). This species is considered as the teleomorph of *Phaffia rhodozyma*. However, the Panel notes that the methods used to characterise the strain are not up to date and that the parental strain was identified instead of the production strain under assessment.

3.1.2. Characterisation of the additive

The additive is declared to contain 995 g dried inactivated ATX-rich XD and 5 g ascorbic acid per kg. The FEEDAP Panel noted that in the manufacturing process it is indicated that also citric acid is added to achieve a concentration of 2% in the final product and according to the manufacturing process ascorbic acid is present not at 0.5% (as declared above), but at 5%.

Analytical data on the content of protein, fat, water and ash was provided from three recent batches of the biomass with mean values (single values in brackets) of protein 37.4% (38.1, 38.0, 36.1%), fat 6.63% (6.5, 5.6, 7.8%), water 6.2% (5.8, 6.4, 6.4%) and ash 3.3% (3.4, 3.2, 3.2%).[13]

In the current dossier, ATX-rich XD is specified to contain at least 5,000 mg/kg of total ATXs (i.e. the sum of the *all-E*, *9Z*-, *13Z*- and *15Z*-isomers), while the specification according to the provisional authorisation ending on 3 August 2011[4] was 10 g ATX/kg. Results from (i) three batches manufactured in 2018[14] showed values of 8,981, 12,478 and 9,322 mg ATX/kg and (ii) five batches manufactured in 2019, showed values of 7,235 8,520, 8,594, 7,954, 8,567 mg ATX/kg.[15] The applicant also quantified the ATX-isomers by high pressure liquid chromatography. All-*E*-isomers were in the range of 43.1-50.7% of total chromatographic area; 9*Z*-, 13*Z*- and 15*Z*-isomers were in the range of 1.0–1.5, 4.2–6.0 and 1.2–1.5 area%, respectively.

Other carotenoids present in the additive are beta-carotene, adonirubin, echinenone, 3-hydroxyechinenone and 3-hydroxy-3′-4′-didehydro-beta-psi-caroten-4-one (HDCO). Their quantities were measured in five batches manufactured in 2019 (see Table 1).[16] It is noted that the measured ATX concentrations in the different batches is in the range of 67-72% of the total carotenoids.

Table 1: Carotenoid content in astaxanthin-rich *Xanthophyllomyces dendrorhous* (ATCC SD-5340) from batches manufactured in 2019

	Range (mg/kg)	Average (mg/kg)
Astaxanthin (all isomers)	7,235–8,594	8,174
beta-Carotene	1,242–1,930	1,605
Adonirubin	464–619	574
Echinenone	256–365	298
3-Hydroxyechinenone	631–832	751
HDCO	252–427	350

The applicant states that in addition there are traces of vitamins but no analytical data were provided.

Three batches of the additive manufactured in 2018 were analysed for arsenic and heavy metals. Arsenic, aluminium, cadmium, lead and mercury were not detected, but the limit of detection (LOD) of the analytical method used were not provided (EPA methods).[17] Chromium was measured in two of the above-mentioned three batches (2.03 and 1.20 mg/kg).

The same three batches and additional five batches manufactured in 2019 were analysed for microbiological impurities. The batches from 2018 were free of mould and yeasts, *Salmonella* spp. (in 25 g) and coliforms were not detected, aerobic plate count was 5×10^5, 1×10^5 and 7×10^5 CFU/g. In the batches from 2019, *Salmonella* spp. (in 25 g) and *E. coli* (in 1 g) were absent; fungi, yeast and total CFU were in the range of $<$ 10–53 CFU/g, $<$ 10 CFU/g and 227–9,967 CFU/g, respectively.

[12] Technical dossier/Spontaneous submission July 2019/Annex (8).
[13] Technical dossier/Spontaneous submission August 2019/Annex (2).
[14] Technical dossier/Spontaneous submission July 2019/Annex_II_1_02.
[15] Technical dossier/Spontaneous submission August 2019/Annex_(3).
[16] Technical dossier/Spontaneous submission August 2019/Annex (3).
[17] Technical dossier/Spontaneous submission July 2019 (2) CoA Aquasta.pdf

The FEEDAP Panel notes that the total counts are high and would deserve attention/monitoring during the production process.

Aflatoxins B1, B2, G1, and G2 were not detected in three recent batches of the additive; LODs of the methods used were not indicated (AOAC methods).[18] Dioxins (polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)), dioxin-like polychlorinated biphenyls (PCBs) and non-dioxin-like PCBs were measured in three recent batches of the additive and amounted to 0.166, 0.163 and 0.087 ng WHO-PCDD/F-TEQ/kg, 0.175, 0,172 and 0.094 ng WHO-PCDD/F-PCB-TEQ/kg and 0.020, 0.025 and 0.019 μg/kg, respectively.[19]

The detected amounts of the above-described impurities do not raise safety concerns.

No data on the presence of viable cells of the production strain in the additive were provided.

The product is a free-flowing, dark red, granular powder with a bulk density of approximately 500 kg/m^3. The dusting potential of three batches was determined using the Stauber–Heubach method[20] and showed values of 3, 10 and 19 mg/m^3 (average 10.6 mg/m^3). Particle size of three batches of the additive was measured by laser light scattering method.[21] The percentage (V/V) of particles with diameter < 50 μm was 54.2, 85.0 and 99.6 and < 10 μm was 5.31, 20.5 and 64.4%.

3.1.3. Characterisation of the active substances

The applicant provided information on the characterisation of ATX as the main carotenoid in the additive; however, it is noted that other carotenoids are present in considerable amounts for which no information was given.

ATX (3,3'-dihydroxy-beta, beta-carotene-4,4'-dione, Chemical Abstracts Service (CAS) number 472-61-7), has the molecular formula $C_{40}H_{52}O_4$ and the molecular weight 596.85 g/mol. Its structural formula is given in Figure 1.

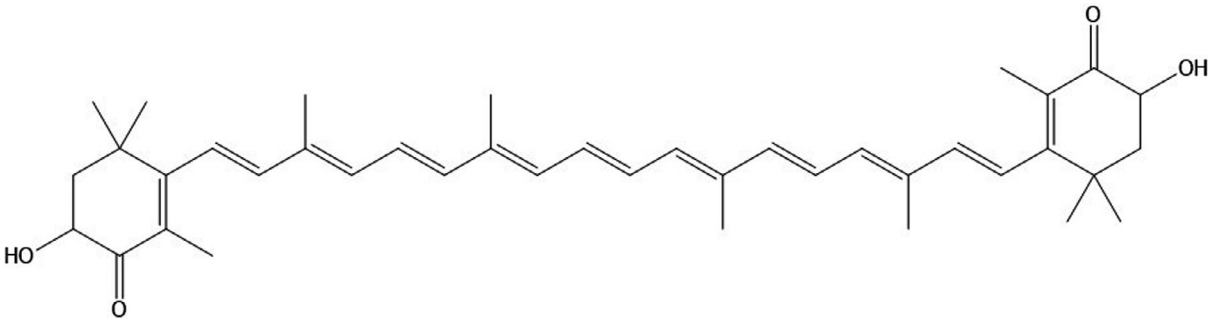

Figure 1: Structural formula of astaxanthin

3.1.4. Manufacturing process

The additive is produced by fermentation using *Xanthophyllomyces dendrorhous* ATCC SD-5340 as a production organism. After fermentation, the biomass is concentrated by centrifugation including washing with reverse-osmosis water in order to remove dissolved solids. The resulting yeast cell paste is milled by glass beads to break cell walls. After this step, citric and ascorbic acids are added to reach 2 and 5%, respectively, in the final dried product. The cell paste is spray dried, blended to assure homogeneity, sifted and packaged.[22]

3.1.5. Stability and homogeneity

The applicant submitted the same studies that were assessed by the FEEDAP Panel in 2006 (EFSA, 2006).

The applicant submitted a paper on the effect of storage on oxidative quality and stability of extruded ATX-coated fish feed pellets that was considered not relevant as it was done with a different product (Dethlefsen et al., 2016).

[18] Technical dossier/Spontaneous submission August 2019/Annex (4).
[19] Technical dossier/Spontaneous submission August 2019/Annex (5).
[20] Technical dossier/Spontaneous submission August 2019/Annex (7).
[21] Technical dossier/Spontaneous submission August 2019/Annex (6).
[22] Technical dossier/spontaneous submission July 2019/Annex (4) and (5).

The FEEDAP Panel notes that no studies covering range of feedingstuffs commonly used in fish production and of an adequate duration were submitted for the current assessment.

3.1.6. Conditions of use

ATX-rich XD is intended to be used in feed for salmon and trout from the age of six months onwards at a maximum content of 100 mg ATX/kg complete feed.

3.2. Safety

No new studies were submitted for the re-evaluation of the safety of the additive or the active substance ATX other than those already evaluated by the FEEDAP Panel in 2006.

It is noted that some of the safety studies evaluated by the FEEDAP Panel in 2006 were carried out using a formulation of the additive containing ethoxyquin instead of ascorbic acid.[23] Although these differences were considered by the applicant as unlikely to significantly affect the toxicity, the FEEDAP Panel noted that no evidence were provided to support this. The safety of the additive for the target species and the consumer was assumed based on studies not in line with current standards and based on data from the literature, evaluated in an EFSA opinion in 2005 (EFSA, 2005).

Considering the above uncertainties and the lack of information on the characterisation of the production strain, the applicant was requested to provide data on the taxonomic identification of the production strain, a new tolerance study in target species performed with the additive under assessment, data on residues (including the carotenoids present in the product), and studies to define the toxicological profile of the active substance ATX.

To address the above requests, the applicant submitted limited data on the taxonomic identification of the production strain (see Section 3.1.1), some published papers on the safety of the active substance ATX for the target species (Zhao et al., 2016, Noori and Razi, 2017, Rahman et al., 2017)[24] and on the deposition and toxicity of ATX (Brizio et al., 2013, Tago at al., 2014, Vega et al., 2015, Edwards et al., 2016, Schneider et al., 2016), which are reviewed below.

3.2.1. Safety of the production strain

The yeast *Xanthophyllomyces dendrorhous* is a teleomorph of *Phaffia rhodozyma*, which is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA BIOHAZ Panel, 2021). The identity of the parental strain has been conclusively established, although the identification of the production strain should be preferably provided using whole genome sequence data.

The Panel concluded that the use of *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*) ATCC SD-5340 in the production of the additive would not raise any safety concerns for the target species, the consumers of products from animals fed the additive, and the environment.

3.2.2. Safety for the target species

In 2006, the FEEDAP Panel concluded that the ATX-rich *Phaffia rhodozyma* (formulation not given), when used at the proposed condition of use, was considered safe for salmonids, based on the results of a tolerance study in Atlantic salmon. In the same opinion, it was reported that, based on data from literature described in an EFSA opinion adopted in 2005 (EFSA, 2005), ATX was tolerated in salmon and trout with a margin of three in salmon and of two in trout (EFSA, 2006).

The applicant did not submit any new data on the safety of the product ATX-rich XD for the target species. The only data submitted was the same tolerance study which was assessed in the previous opinion in 2006. The Panel re-assessed this study and considered that it did not fulfil the current requirements to conclude on the safety for the target animals (i.e. several blood chemistry parameters were not measured, and the test item was not characterised). Therefore, in order to allow the Panel to conclude on the safety for the target species, the applicant was requested to provide a new tolerance study. The applicant did not perform a new study but re-submitted the same papers that were evaluated in the opinion in 2006 (EFSA, 2006) and more recent published papers reporting studies in trout and salmon[24] (Zaho et al., 2016, Noori and Razi, 2017, Rahman et al., 2017). The papers were evaluated by the FEEDAP Panel and they were not considered relevant for the assessment of target

[23] Acute, subchronic toxicity, mutagenicity, eye and skin irritation and skin sensitisation.
[24] Technical dossier/Supplementary information May 2018 and spontaneous submission July 2019.

animal safety for the following reasons: no overdose group was included in the study (Zaho et al., 2016, Rahman et al., 2017) and no haematology was performed in a study with only two times the use level (Noori and Razi, 2017).

3.2.2.1. Conclusions on safety for the target species

In the absence of a tolerance study with the additive under assessment, the FEEDAP Panel cannot conclude on the safety of the additive ATX-rich XD for the target species.

3.2.3. Safety for the consumer

3.2.3.1. Absorption, distribution, metabolism, excretion and residues

No new studies were submitted on the absorption, distribution, metabolism and excretion (ADME) of ATX. In the former opinion (EFSA, 2006), the ADME of ATX was summarised as follows:

> 'Astaxanthin is a vitamin A precursor for fish, it is important for performance, specific functions in reproduction and metabolism, and health in salmonids.' 'It is used to produce flesh with the desired red colour equal to flesh from wild salmonids. The absorption capacity is limited. Atlantic salmon plateau was of at about 10 mg astaxanthin/kg flesh and trout at a higher level of about 20–25 mg astaxanthin/kg flesh. Based on the literature the FEEDAP Panel concludes that the astaxanthin level in farmed and wild salmonids is comparable. Absorption is determined by several factors, the occurrence of astaxanthin in free or esterified form, and dietary factors (lipid level), excretion of undigested astaxanthin amounts to about approximately 50%. Astaxanthin is metabolised in salmonids through reductive pathways, leading to idoxanthin, adonixanthin and zeaxanthin. No cleavage of the polyen chain is observed. Epimerization from 3S to 3R and vice versa occurs. Metabolites are mainly excreted via the bile. After astaxanthin application, the pigment deposited in flesh of trout and Chinook salmons is predominantly astaxanthin, in the arctic charr also idoxanthin. Preliminary results indicate a metabolic pathway in rats (including cleavage of the polyen chain) very different from fish, but essentially similar to that seen in humans'.

No information on the identification and quantification of the residues in edible tissues of fish fed with the additive were provided in the dossier. Therefore, it is not possible to assess the exposure of the consumer to residues of ATX or other carotenoids from the consumption of fish products.

3.2.3.2. Toxicological studies

In 2006 the FEEDAP Panel concluded that the additive showed no evidence of genotoxicity (in a bacterial reverse mutation assay and in a mouse lymphoma assay) or toxicological effects of concern in mice either following an acute dose or after 90 days based on studies performed with a formulation of the additive containing ethoxyquin instead of ascorbic acid. The FEEDAP Panel at that time saw no evidence for adverse effects but could not finally conclude on the consumer safety of the additive due to the inadequacies of the 90-day study.

No new toxicological studies were provided with the additive ATX-rich XD under assessment.

In the 2006 opinion, the toxicological profile of the active substance ATX was not evaluated and no information on the genotoxic potential, subchronic and chronic toxicity, carcinogenicity and reproductive toxicity of pure ATX was provided for the current assessment.

Following EFSA's request, the applicant submitted a series of published papers,[25] which were either not relevant for the assessment (not performed with pure ATX (Tago et al., 2014) or performed at the dietary level of the active ingredient below the currently authorised maximum level (Brizio et al., 2013)) or could not be used due to data protection provisions.[26]

Due to the lack of toxicity data, the Panel is not in the position to derive a safe dose for ATX from ATX-rich XD and therefore to establish a health-based guidance value (i.e. acceptable daily intake (ADI)).

[25] Technical dossier/Supplementary information May 2018.

[26] The information included in the published papers submitted was formerly submitted and evaluated in the context of the assessment of the dossier FAD-2017-0029 (EFSA-Q-2015-00754), and therefore, in line with Article 20 of Regulation (EC) No 1831/2003, are currently under data protection.

3.2.3.3. Conclusions on safety for the consumer

In the absence of adequate toxicity and residue data, the Panel cannot conclude on the safety of ATX-rich XD for the consumer.

3.2.4. Safety for the user

No studies with the additive on the effects on the respiratory system were provided. Considering the low dusting potential of the additive (in the range of 0.010 g/m^3), its inhalation exposure is presumed to be limited. However, considering the proteinaceous nature of the product, it should be considered a respiratory sensitiser.

In 2006, the FEEDAP Panel evaluated Good Laboratory Practice (GLP)-compliant eye and skin irritation studies (OECD guideline 405 and 404) and a GLP-compliant dermal sensitisation study (OECD guideline 406) performed with a formulation of the additive containing ethoxyquin instead of ascorbic acid. The product was found to be non-irritant to skin and slightly irritant to the eyes (causing slight redness but no other effect). It is noted that, since the specific irritant potential of a product may be modified by a change in formulation (ascorbic acid used instead of ethoxyquin), the relevance of these data to the product under assessment is unknown. However, considering the irritancy potential of ascorbic acid (EFSA FEEDAP Panel, 2013), it is assumed that the additive is an irritant to skin and eyes.

Based on the results of a dermal sensitisation study, it was concluded that the additive was a moderate skin sensitiser in guinea-pigs (EFSA, 2006). The possibility that the product under assessment might be also a skin sensitiser cannot be excluded.

The FEEDAP Panel concludes that the additive is irritant to skin and eyes, is a skin and respiratory sensitiser, although exposure by inhalation is likely low.

3.2.5. Safety for the environment

The production strain *Xanthophyllomyces dendrorhous* qualifies for the QPS approach to safety assessment and is therefore considered safe for the environment.

The FEEDAP Panel considers that ATX from the biomass does not pose a significant additional risk to the environment compared with other natural sources of ATX (e.g. present in seawater, bacteria, algae and shrimp).

3.3. Efficacy

In the opinion adopted in 2006 (EFSA, 2006), it was noted that efficacy of the active substance ATX was widely demonstrated in the literature. In the same opinion, the FEEDAP Panel concluded on the efficacy of the additive based on a study performed in rainbow trout testing the bioavailability of ATX from *Phaffia rhodozyma* cultures, and two efficacy trials, one in rainbow trout and one in Atlantic salmon, both comparing the colouring potential of the product (formulated with ethoxyquin) and synthetic ATX.

No new studies were submitted in the dossier.

The FEEDAP Panel re-evaluated the data already assessed in 2006. Some limitations/uncertainties were identified in the studies, in particular on the test item used, therefore, the applicant was requested to provide additional data in the form of a bioavailability study. The applicant did not provide the data requested.

In absence of such information, the FEEDAP Panel is not in the position to conclude on the efficacy of the product ATX-rich XD when used in feed for salmonids and trout.

4. Conclusions

The additive under assessment, consisting of ATX-rich *Phaffia rhodozyma*, is produced by the telemorph of *Phaffia rhodozyma*, *Xanthophyllomyces dendrorhous* (ATCC SD-5340), which is considered by EFSA to be suitable for the QPS approach to safety assessment; therefore, the use of the production strain in the production of the additive would not raise any safety concern for the target species, the consumers of products from animals fed the additive and the environment. The main active principle of the additive is ATX, however, the FEEDAP Panel noted that some other carotenoids are also present in lower quantities.

In the absence of a tolerance study with the additive under assessment, the FEEDAP Panel cannot conclude on the safety for the target species.

No information on the identification and quantification of the residues of the additive in edible tissues was provided. No conclusion can be made on the toxicity of ATX since no information were provided in the dossier. Overall, no conclusions on the safety for the consumer can be drawn.

The FEEDAP Panel concludes that the additive is irritant to skin and eyes, is a skin and respiratory sensitiser, although exposure by inhalation is likely low.

The FEEDAP Panel considers that ATX from the biomass does not pose a significant additional risk to the environment compared with other natural sources of ATX.

In the absence of adequate evidence, no conclusions can be made on the efficacy of the additive.

5. Documentation provided to EFSA/Chronology

	Event
13/07/2010	Dossier received by EFSA. Reception of spontaneous submission. Submitted by Igene Biotechnology, Inc
04/05/2016	Reception mandate from the European Commission
03/01/2017	Application validated by EFSA – Start of the scientific assessment
14/02/2017	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: method of analysis (EURL)*
10/03/2017	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation of, safety for the target species, consumer safety, user safety, efficacy*
13/05/2018	Reception of supplementary information from the applicant
04/10/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives - Scientific assessment re-started
29/06/2019	Reception of spontaneous submission Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
23/08/2019	Reception of spontaneous submission Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: efficacy*
27/01/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ATX	Astaxanthin
ATX-rich XD	astaxanthin-rich *Xanthophyllomyces dendrorhous*
CAS	Chemical Abstracts Service
CFU	colony forming unit
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
LOD	limit of detection
NP-HPLC	normal-phase high-performance liquid chromatography
OECD	Organisation for Economic Co-operation and Development
QPS	Qualified Presumption of Safety
UV/VIS	ultraviolet-visible

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method of the Analysis for

In the current application authorisation is sought under Article 10(2) of Regulation (EC) No 1831/2003 for *Astaxanthin-rich Phaffia rhodozyma*. The authorisation as *feed additive* is sought under the category/functional group 2(a) "sensory additives"/"colourants: substances which, when fed to animals, add colours to food of animal origin", according to the classification system of Annex I of Regulation (EC) No 1831/2003. In the current application submitted according to Article 10(2) of Regulation (EC) No 1831/2003, the authorisation for salmon and trout is requested.

The active substance in the feed additive is *astaxanthin*, produced by the red yeast *Phaffia rhodozyma* (ATCC SD 5340). The *feed additive* is a dark purple powder containing as active substance at least 5000 mg/kg of total *astaxanthin*, i.e. the sum of the all-E, 9Z-, 13Z and 15Z- isomers. The feed additive is intended to be incorporated directly into *feedingstuffs* with a proposed maximum content (expressed as total astaxanthin) of 100 mg/kg complete *feedingstuffs*.

For the quantification of astaxanthin in the feed additive and in feedingstuffs the Applicant submitted a single-laboratory validated and further verified method based on Normal Phase High Performance Liquid Chromatography with UV/VIS detection (NP-HPLC-UV/VIS).

In addition, the EURL has developed and single-laboratory validated a multi-analyte method based on reversed phase HPLC coupled to spectrophotometric detection (RP-HPLC-UV/VIS) for the determination of all carotenoids currently authorised as *feed additive* within the EU, including thus the one of the current application. This method has also been subjected to ring-trial validation.

Based on the performance characteristics presented, the EURL recommends for official control the validated and further verified NP-HPLC-UV/VIS method for the quantification of astaxanthin in the feed additive and in *feedingstuffs* and the ring-trial validated RP-HPLCUV/VIS for the quantification of *astaxanthin* in *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.