Safety and efficacy of a feed additive consisting of carrageenan for pets and other non-food-producing animals (Marinalg International)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fasmon Durjava, Maryline Koubia, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Kettil Svensson, Rosella Brozzi, Jaume Galobart, Lucilla Gregoretti, Gloria López-Gálvez, Konstantinos Sofianidis, Maria Vittoria Vettori and Matteo Lorenzo Innocenti

Abstract
Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of carrageenan as a feed additive for pets and other non-food-producing animals. The additive is manufactured in two forms, refined and semi-refined carrageenan. Owing to the lack of information, the FEEDAP Panel is not in the position to conclude on safety of the additives for pets and other non-food-producing animals and for the user. The FEEDAP Panel concludes that the additive is efficacious as a gelling agent, thickener and contributes to stabilise canned pet feed. No conclusion can be drawn on the efficacy of the additive as a binder and emulsifier.

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Keywords: technological additive, emulsifiers, gelling agents, stabilisers, thickeners, binders, carrageenan, safety, efficacy

Requestor: European Commission
Question number: EFSA-Q 2013-00970
Correspondence: feedap@efs.europa.eu
Panel members: Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Mojca Fasmon Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

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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 Marinalg International\(^2\) for re-evaluation of the product carrageenan, when used as a feed additive for pets and other non-food-producing animals (category: technological additives; functional groups: gelling agents, stabilisers, thickeners and binders).

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 24 April 2014.

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 and user and on the efficacy of the product carrageenan, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

Carrageenan is currently authorised as a feed additive\(^3\) subject to re-evaluation according to Article (10) of Regulation (EC) 1831/2003. Carrageenan is authorised to be used as a feed additive in accordance with Annex II to Regulation (EC) No 1333/2008\(^4\) with specific purity criteria defined in Commission Regulation (EU) No 231/2012\(^5\).

Carrageenan has not been previously assessed by EFSA as a feed additive. It has been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1972, 1974, 1984, 1987, 1992, 1993, 1995, 1999, 2000, 2001, 2002, 2007a, b, 2008, 2014, 2015). The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) delivered an ‘opinion on the re-evaluation of carrageenan (E 407) and processed Eucheuma seaweed (PES; E 407a) as food additives’ (EFSA ANS Panel, 2018). In this opinion the ANS Panel concluded that the existing group acceptable daily intake (ADI) for carrageenan (E 407) and PES (E 407a) of 75 mg/kg body weight (bw) and day should be considered temporary, while the database should be improved within 5 years after publication of this opinion.

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\(^6\) in support of the authorisation request for the use of carrageenan as a feed additive.

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\(^2\) Marinalg International, Avenue Jules, Bordetlaan 142, Brussels, Belgium.


\(^6\) FEED dossier reference: FAD-2010-0205.
The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts’ knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the guar gum in animal feed. The Executive Summary of the EURL report can be found in Annex A.7

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of carrageenan is in line with the principles laid down in Regulation (EC) No 429/20088 and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

The product under assessment, carrageenan, is a polysaccharide extracted from seaweed of different families belonging to the class of Rhodophyceae (red seaweeds) intended to be used as a technological feed additive (functional groups: emulsifiers, stabilisers, thickeners, gelling agents and binders) in feedingstuffs for all pets.

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive consists of pure carrageenan. Carrageenan (Chemical Abstracts Service (CAS) number: 9000-07-1, European Inventory of Existing Commercial Chemical Substances (EINECS) number 232-524-2) is a polysaccharide extracted from seaweed of different families belonging to the class of Rhodophyceae (red seaweeds). The main components of carrageenan are the polysaccharides kappa-, iota- and lambda- carrageenan, consisting of the ammonium, calcium, magnesium, potassium and sodium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharides. The ratio of the different carrageenan components depends on the seaweed from which it is extracted. Depending on the manufacturing process, two different forms of the additive are available: carrageenan and PES.

Carrageenan (refined) (synonyms: Irish moss gelose; eucheuman (from Eucheuma spp.); iridophycan (from Iridae spp.); hypnean (from Hypnea spp.); furcellaran or Danish agar (from Furcellaria fastigiata); carrageenan (from Chondrus and Gigartina spp.)), is manufactured by an extraction process from several seaweeds of families belonging to the class of Rhodophyceae (red seaweeds), such as Furcellariaceae, Gigartinaceae, Hypnaceae, Phyllophoraceae and Solieriaceae. PES (synonyms: semi refined carrageenan and Philippine natural grade carrageenan (PNG-carrageenan), alternatively refined carrageenan (ARC)), is manufactured from the seaweed family of Solieriaceae, in particular species of Eucheuma.

7 The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports
The two forms of the additive are manufactured to meet the specifications set for the food additives carrageenan (E 407) and PES (E 407a).9 For carrageenan (refined), the specifications as food and feed additive are loss on drying < 12%, viscosity (1.5% solution) at 75°C ≥ 5 mPa·s; methanol, ethanol, 2-propanol (singly or in combination) ≤ 0.1%; sulfate (dried hydrocolloid basis, as SO₄) ≥ 15% and ≤ 40%; total ash ≥ 15% and ≤ 40%; acid insoluble ash (in 10% hydrochloric acid) ≤ 1%; acid-insoluble matter (in 1% v/v sulfuric acid) ≥ 2%. The analysis of 10 batches of the additive showed compliance with the specifications.

The analysis of the same batches of the additive showed concentrations of lead, mercury, and cadmium below the respective limits of detections (LODs). Arsenic was found in concentrations ≤ 1.3 mg/kg. Coliforms (in 5 g) and Salmonella spp. (in 25 g) were not detected in any of the batches; total plate count and filamentous fungi and yeasts were below the specifications of ≤ 5,000 colony forming unit (CFU)/g and ≤ 200 CFU/g, respectively, in all the batches. No analytical data were provided on possible presence of dioxins and dioxins-like polychlorinated biphenyls (PCBs), pesticides, botanical impurities and mycotoxins. The analysed impurities do not raise safety concerns.

The dusting potential (Stauber-Heubach) tested in one batch of the additive (four replicated analysis) was 9.35 g/m³.12 For PES, the specifications as food and feed additive are loss on drying < 12%, viscosity (1.5% solution) at 75°C ≥ 5 mPa·s; methanol, ethanol, 2-propanol (singly or in combination) ≤ 0.1%; sulfate (dried hydrocolloid basis, as SO₄) ≥ 15% and ≤ 40%; total ash ≥ 15% and ≤ 40%; acid insoluble ash (in 10% hydrochloric acid) ≤ 1%; acid-insoluble matter (in 1% v/v sulfuric acid) ≥ 8% and ≤ 15%. The analysis of 10 batches of the additive showed compliance with the specifications.

The analysis of the same batches of the additive showed concentrations of lead, mercury, and cadmium below the respective limits of detections. Arsenic was found in concentrations ≤ 1.7 mg/kg. Coliforms (in 5 g) and Salmonella spp. (in 25 g) were not detected in any of the batches; total plate counts and filamentous fungi and yeasts were below the specifications of ≤ 5,000 CFU/g and ≤ 200 CFU/g, respectively, in all the batches. No analytical data were provided on possible presence of dioxins and dioxins-like PCBs, pesticides, botanical impurities and mycotoxins. The analysed impurities do not raise safety concerns.

The dusting potential (Stauber-Heubach) tested in one batch of the additive (four replicated analysis) was 2.04 g/m³.14 When used as food additives, carrageenan and PES are further specified to contain ≤ 5% of low molecular weight carrageenan (molecular weight fraction below 50 kDa). According to the applicant, no method of analysis is suitable for such measurement, and therefore this was not included as a specification for the use of carrageenan and PES as a feed additive.

### 3.1.2. Stability and homogeneity

One batch of each form of the additive, carrageenan (refined) and PES, were stored for one year in the original package at 30°C and 65% RH. The samples were analysed for pH, loss on drying and gel strength (g/cm²). No major changes were observed during the trial, with the exception of an increase of the loss on drying of carrageenan (refined) (start of the trial 4.88%, after 12 months 7.36%).

For technological additives, stability can be demonstrated by the persistence of the effect; no demonstration of homogenous distribution is considered necessary if the efficacy of the additive is demonstrated. The applicant has provided one study showing the effects of carrageenan on the gel strength of feedingstuffs, described in Section 3.3.
3.1.3. Conditions of use

Both forms of the additive are intended to be used as a technological additive (functional groups: gelling agents, stabilisers, thickeners and binders) in feedingstuffs for pets and other non-food-producing animals, with no minimum or maximum content. The applicant proposed typical use levels of 20,000–30,000 mg/kg of carrageenan in the final wet feed.

3.2. Safety

3.2.1. Safety for the target species

No specific studies done with the additive under assessment in the target species have been submitted.


In 2018, the EFSA ANS Panel delivered an opinion on the re-evaluation of carrageenan (E 407) and PES (E 407a) as food additives. The ANS Panel concluded that ‘... the ADME database was sufficient to conclude that carrageenan was not absorbed intact; in a sub-chronic toxicity study performed with carrageenan almost complying with the EU specification for E 407 in rats, the no-observed-adverse-effect level (NOAEL) was 3,400–3,900 mg/kg bw per day, the highest dose tested; no adverse effects have been detected in chronic toxicity studies with carrageenan in rats up to 7,500 mg/kg bw per day, the highest dose tested; there was no concern with respect to the carcinogenicity of carrageenan; carrageenan and processed Eucheuma seaweed did not raise a concern with respect to genotoxicity; the NOAEL of sodium and calcium carrageenan for prenatal developmental dietary toxicity studies were the highest dose tested; the safety of processed Eucheuma seaweed was sufficiently covered by the toxicological evaluation of carrageenan; data were adequate for a refined exposure assessment for 41 out of 79 food categories. However, the Panel noted uncertainties as regards the chemistry, the exposure assessment and biological and toxicological data. Overall, taking into account the lack of adequate data to address these uncertainties, the Panel concluded that the existing group acceptable daily intake (ADI) for carrageenan (E 407) and processed Eucheuma seaweed (E 407a) of 75 mg/kg bw per day should be considered temporary, while the database should be improved within 5 years after publication of this opinion’.

Most of the studies on which the previous evaluation was based were not made available by the applicant, in addition the uncertainties highlighted in the ANS Panel opinion were not addressed. Therefore, in the absence of adequate data, the FEEDAP Panel is not in the position to conclude on safety of carrageenan for pets and other non-food-producing animals at the proposed conditions of use in feed.

3.2.2. Safety for the user

No specific information was provided by the applicant. In the absence of data, the FEEDAP Panel is not in the position to conclude on the safety of carrageenan for the user.

3.3. Efficacy

The applicant has provided one study to support the efficacy of carrageenan as a gelling agent, stabiliser and thickener. No evidence was provided on the effect of the additive as a binder.

The gel strength of a jelly of three canned chunk feed (two feeds for cats and one for dogs) prepared with the unsupplemented or supplemented jelly (3,200 mg carrageenan/kg jelly (both refined or PES)) was measured. The gel strength was tested in feed with and without chunks and measured with a texturometer, measuring gel strength by analysis of the breaking force (expressed in g force). Four samples for each jelly preparation and for each feed were measured, immediately after preparation and after 1, 2, 4 and 6 months of storage. No statistical analysis was reported. The results of the analysis of the jelly and of the feeds are reported in Table 1.

16 Technical dossier/Supplementary Information May 2016/STABILITY AND HOMOGENEITY OF GELLED PET FOOD PRODUCTS USING CARRAGEENAN AND PROCESSED EUCHEUMA SEAWEED.
Results of the study showed that unsupplemented gravies and feeds did not form any gel, whereas those supplemented with 3,200 mg carrageenan/kg formed a gel with a similar gel strength when considering both types of carrageenan used. This effect was maintained for at least 6 months in canned samples.

The FEEDAP Panel concludes that the additive is efficacious as a gelling agent, thickener and contributes to stabilise canned pet feed. No conclusion can be drawn on the effects of the additive as a binder.

4. Conclusions

Owing the lack of data, no conclusions could be drawn on the safety of the additive for the target species or the user.

The FEEDAP Panel concludes that the additive is efficacious as gelling agent, thickener and contributes to stabilise canned pet feed. No studies were provided to support the efficacy of the additive as a binder.

5. Documentation as provided to EFSA/Chronology

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<td>29/11/2013</td>
<td>Reception mandate from the European Commission</td>
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<td>24/04/2014</td>
<td>Application validated by EFSA – Start of the scientific assessment</td>
</tr>
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<td>25/07/2014</td>
<td>Comments received from Member States</td>
</tr>
<tr>
<td>18/09/2014</td>
<td>Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives</td>
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Table 1: Effect of the additive carrageenan (PES or refined) on the gel strength of three different feeds

<table>
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<tr>
<th>Type of feed</th>
<th>Carrageenan inclusion level</th>
<th>Chunks</th>
<th>Type of additive</th>
<th>Force (g) Average (±SD)</th>
<th>Time (months)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Canned feed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>for cat 1</td>
<td>0</td>
<td>No</td>
<td>--</td>
<td>No gel formation</td>
<td></td>
</tr>
<tr>
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<td>--</td>
<td>No gel formation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3,200</td>
<td>No</td>
<td>PES</td>
<td>310 (±18)</td>
<td>316 (±16)</td>
</tr>
<tr>
<td></td>
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<td>Yes</td>
<td>PES</td>
<td>314 (±38)</td>
<td>333 (±30)</td>
</tr>
<tr>
<td></td>
<td>3,200</td>
<td>No</td>
<td>Refined</td>
<td>325 (±23)</td>
<td>349 (±20)</td>
</tr>
<tr>
<td></td>
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<td>Yes</td>
<td>Refined</td>
<td>366 (±36)</td>
<td>369 (±34)</td>
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<td>0</td>
<td>No</td>
<td>--</td>
<td>No gel formation</td>
<td></td>
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<tr>
<td>for cat 2</td>
<td>0</td>
<td>Yes</td>
<td>--</td>
<td>No gel formation</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>No</td>
<td>0 (PES)</td>
<td>222 (±12)</td>
<td>203 (±9)</td>
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<td>270 (±22)</td>
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<td>337 (±28)</td>
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</tr>
<tr>
<td>for dog</td>
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<td>Yes</td>
<td>--</td>
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<td>3,200 (refined)</td>
<td>364 (±70)</td>
<td>358 (±43)</td>
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References


Abbreviations

ADI average daily intake
ADME absorption, distribution, metabolism and excretion
ANS EFSA Scientific Panel on Additives and Nutrient Sources added to Food
ARC alternatively refined carrageenan
BW body weight
CAS Chemical Abstracts Service
CFU colony forming unit
CV coefficient of variation
DM dry matter
EINECS European Inventory of Existing Chemical Substances
EURL European Union Reference Laboratory


www.efsa.europa.eu/efsajournal
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for carrageenan

In the current applications authorisation is sought under article 10(2) for Carrageenan under the 'category’/‘functional groups’ 1(c), 1(d), 1(e) and 1(f) ‘technological additives’/’emulsifiers',1, ‘stabilisers’1,2, ‘thickeners’1,2, ‘gelling agents’1,2 according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the feed additive for ‘pets and other non-food-producing animals’ 1, ‘cats and dogs’2. Carrageenan is colourless-to-tan powder produced from natural strains of red seaweeds Rhodophyceae. The feed additive mainly consists of carrageenan polysaccharides. According to the Applicants, two types of the products exist: refined Carragennan1 and semi-refined Carragennan1,2, which mainly differ in the acid insoluble matter (AIM) content derived from algal cellulose. Refined Carragennan contains less than 2% of AIM, while the AIM content in semi-refined Carragennan ranges from 8 to 15%. Furthermore, the Applicants suggested the following additional specifications to characterise the feed additive: - more than 5 mPa·s for viscosity; - less than 12% for loss on drying; - 15 to 40% for sulphate and total ash contents; and - less than 1% for acid insoluble ash content. The Applicants stated that the specific purity criteria set in the Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012 for the food additives are applicable for the feed additive. The feed additive is intended to be incorporated directly into feedingstuffs. No recommended minimum or maximum concentration levels were proposed by the Applicants. However, the typical inclusion levels range from 1 to 30 g feed additive /kg feedingstuffs.

For the characterisation of Carrageenan in the two forms of the feed additive (i.e. refined and semi-refined) the Applicants submitted two internationally recognised FAO JECFA monographs for food additives (‘Carrageenan’ and ‘Processed Eucheuma Seaweed’), recommended by Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012. Identification is based on: - solubility; - infrared spectroscopy; positive tests for - galactose & anhydrogalactose and - hydrocolloid & predominant type of copolymer. Additional characterisation is based on the following quantitative assays: - viscosity; - pH values; - loss on drying; - sulphate; - total ash; - acid insoluble ash; and - acid insoluble matter contents. Even though no performance characteristics are provided, the EURL recommends for official control the two FAO JECFA monographs mentioned above to characterise refined and semirefined Carrageenan in the feed additive - as recommended by Commission Directive 2009/10/EC and Commission Regulation (EU) 231/2012. The Applicants provided no experimental data or any analytical methods for the quantification of Carrageenan in feedingstuffs, as the accurate determination of Carrageenan in feedingstuffs is not achievable experimentally. Therefore the EURL cannot evaluate nor recommend any method for official control to quantify Carrageenan 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) is not considered necessary.