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Safety and efficacy of a feed additive consisting of agar for pets and non-food-producing animals (Hispanagar)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of agar as a feed additive for pets and non-food-producing animals. Owing to the lack of information, the FEEDAP Panel is not in the position to conclude on the safety of agar 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.

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Keywords: technological additive, binder, thickened, gelling agent, agar, safety, efficacy

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Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.2. Additional information.....	4
2. Data and methodologies.....	4
2.1. Data.....	4
2.2. Methodologies.....	5
3. Assessment.....	5
3.1. Characterisation.....	5
3.1.1. Characterisation of the additive.....	5
3.1.2. Manufacturing process.....	6
3.1.3. Stability and homogeneity.....	6
3.1.4. Conditions of use.....	6
3.2. Safety.....	6
3.2.1. Safety for the user.....	7
3.3. Efficacy.....	7
4. Conclusions.....	8
5. Documentation as provided to EFSA/Chronology.....	8
References.....	8
Abbreviations.....	9
Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for agar.....	10

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003¹ 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^{2,3} for the re-evaluation of the product agar, when used as a feed additive for pets and non-food-producing animals (category: technological additives; functional group: stabilizers, thickeners, gelling agents 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 agar, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive under assessment is agar, to be used as a technological additive in feed for all pets. Agar is currently authorised as a feed additive⁴ and as a food additive in accordance with Annex II to Regulation (EC) No 1333/2008⁵. Specific purity criteria on agar (E 406) have been defined in Commission Regulation (EU) No 231/2012⁶. Agar has not been previously assessed by EFSA as feed additive but has been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1974) and by the Scientific Committee for food (SCF, 1989) and was considered safe for use in food. The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) delivered an opinion on the safety of agar, reaching similar conclusions (EFSA ANS Panel, 2016).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁷ in support of the authorisation request for the use of agar 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, other scientific reports, to deliver the present output.

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 Marinalg International, Avenue Jules, Bordetlaan 142, Brussels, Belgium.

³ On 29/10/2014, EFSA was informed by the applicant that the applicant company changed to Hispanagar; C/ López Bravo, 98; Spain.

⁴ Commission Directive of 8 July 1985 amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs, <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1985:245:0001:0032:EN:PDF>

⁵ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

⁶ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

⁷ FEED dossier reference: FAD-2010-0208.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the agar in animal feed. The Executive Summary of the EURL report can be found in Annex A.⁸

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of agar is in line with the principles laid down in Regulation (EC) No 429/2008⁹ 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 additive under assessment, agar, is intended to be used as a technological feed additive (functional groups: stabilisers, thickeners, gelling agents and binders) in feedingstuffs for pets and other non-food-producing animals.

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive consists of pure agar. Agar (Chemical Abstracts Service (CAS) number 9002-18-0, European Inventory of Existing Commercial Chemical Substances (EINECS) number 232-658-1) is a solid additive, marketed in the form of powder, agglutinated strips or flakes. The additive could vary from colourless to light yellowish-orange, yellowish-grey to pale yellow. It has a gel point of $36 \pm 1.5^\circ\text{C}$, melting point of $87 \pm 1.5^\circ\text{C}$, a pH of 6.8–7.0 and density of 1,090 kg/m³.

Agar is a polysaccharide and is mainly constituted by agarose (a linear polymer composed of (1-3)-linked agarobiose with units of β -D-galactopyranose and (1-4)-linked to 3,6-anhydro- α -L-galactopyranose) and agarpectin, a charged polysaccharide containing galactopyranose residues with sulfate up to 8% and methyl and pyruvic acid acetyl groups (Imeson, 2010).

Agar is described in Regulation (EC) No 231/2012¹⁰, which lays down the specification for food additives, as 'a hydrophilic colloidal polysaccharide consisting mainly of galactose units with a regular alternation of L and D isomeric forms. These hexoses are alternately linked with alpha-1,3 and beta-1,4 bonds in the copolymer. On about every 10th D-galactopyranose unit one of the hydroxyl groups is esterified with sulfuric acid which is neutralised by calcium, magnesium, potassium or sodium. It is extracted from certain strains of marine algae of the families Gelidiaceae and Gracilariaceae and relevant red algae of the class Rhodophyceae'.

The additive is manufactured to meet the specification set for its use as a food additive: soluble in boiling water (insoluble in cold water); loss on drying at 105°C for 5 h: $\leq 22\%$; total ash at 550°C on the anhydrous basis: $\leq 6.5\%$ (dried basis); acid insoluble ash at 550°C on the anhydrous basis: $\leq 0.5\%$; insoluble matter, in hot water: $\leq 1.0\%$; starch, gelatine and other proteins: not detectable; water absorption: ≥ 25 mL by 5 g agar diluted in 100 mL water. The analysis of five batches of the feed additive showed compliance with the specifications set for agar used as a food additive (loss on drying: 9.6–10.6%; total ash: 2.8–3.8%; acid insoluble ash: 0.11–0.25%; insoluble matter: 0.03–0.15%; water absorption: 48–65 mL/100 mL water.¹¹ The results of the analysis of five additional batches¹² showed concentrations of agar of 87.5–92.7% and of water of 7.3–12.5%.

⁸ The full report is available on the EURL website: <https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>

⁹ 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.

¹⁰ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council.

¹¹ Technical dossier/Section II/ Annex II_2_CoA_Agar.

¹² Technical dossier/Supplementary Information June 2019/ 3-Agar E406 Feed additive additional information – Hispanagar/2-Quantitative analysis of agar 06112015.

The analysis of five batches indicated a content of lead, mercury and cadmium, below the respective limits of detection (LODs)¹³ and a content of arsenic < 0.08–< 0.14 mg/kg.¹¹ The results are in compliance with the specification set for the use of agar as a food additive.¹⁴

No analytical evidence was provided to support the compliance with the specification for dioxins and dioxins-like polychlorinated biphenyls (PCBs), pesticides, botanical impurities, mycotoxins and microbial purity, which includes total plate counts (< 5,000 colony forming unit (CFU)/g), yeast and moulds (< 500 CFU/g), *Escherichia coli* and *Salmonella* spp. absent in 5 and 10 g, respectively.

The results of the laser diffraction analysis of one batch of the additive showed that 90% of the particles have diameter < 305 µm, 50% of the particles was < 166 µm and 10% of the particles was < 74 µm.¹⁵ No information on the dusting potential of the additive was provided.

3.1.2. Manufacturing process

The manufacture of agar starts with the harvesting, cleaning, washing and drying of the relevant seaweeds. Depending on the seaweed, the extraction from the dried algae follows different steps: Seaweeds are washed and treated with NaOH at 85–90°C for 1 h (*Gracilaria*) or pretreated with acids (*Gelidium*). The pretreated seaweeds are chopped and processed with hot water (from 95°C to 110°C depending on the species) and pressure, in the case of *Gelidium*. The product obtained is a 1–2% agar solution in water, which undergoes a coarse filtration to remove seaweed particle matter. The thick extract is then mixed with filter aid and pumped through a filter press. Subsequently, the extract is cooled to form a gel, which may be bleached to reduce any colour and soaked in water to reduce the salt content. The gel is then concentrated following two processes, the freeze-thaw process or the gel press process. The concentrated gel is then shredded and dried and reduced to strips or flakes, which are then ground to the commercial size (~ 100–150 µm).

3.1.3. Stability and homogeneity

For technological additives, stability can be demonstrated by persistence of the effect, and no demonstration of homogenous distribution is considered necessary if the efficacy of the additive is demonstrated. The applicant has provided two studies showing the effects of agar on the gel strength of feedingstuffs, described in Section 3.3.

3.1.4. Conditions of use

The additive is intended to be used in feedingstuffs for all pets and non-food-producing animals. No minimum and maximum content are proposed; however, the applicant suggested the following use in feed: intended to be used in dry, moist and liquid feedingstuffs and also in water according to good manufacturing practice; typical use level may be up to 20,000 mg/kg feed of the wet final product.

3.2. Safety

No specific studies or information on the safety of the product under assessment for the target species were submitted.

Agar was evaluated by the SCF (1989, 2001) and by JECFA (1974, 2006), which did not propose an acceptable daily intake (ADI), owing the large tolerability showed in the studies available.

In 2016, the EFSA ANS Panel delivered an opinion on the 'Re-evaluation of agar (E 406) as a food additive', in which it was concluded that 'agar is unlikely to be absorbed unchanged and slightly fermented by intestinal microbiota; sufficient toxicity data were available; there was no concern with respect to the genotoxicity of agar; no carcinogenic effects were reported in carcinogenicity studies in mice and rats at the doses of 4,500 mg/kg body weight (bw) per day and 2,500 mg/kg bw per day, respectively, the highest doses tested; oral intake of agar (4,500 mg/person corresponding to 64 mg/kg bw per day) was tolerated in humans for 12 weeks without noticeable side effects. Therefore, the Panel concluded that there is no need for a numerical ADI for agar and that there is no safety concern for the general population at the refined exposure assessment for the reported uses of agar as a food additive'.

¹³ Limit of detection (LOD): 3.0 mg/kg lead, 0.04 mg/kg mercury and 1.0 mg/kg cadmium.

¹⁴ Lead < 5 mg/kg, Mercury < 1 mg/kg, Cadmium < 1 mg/kg and Arsenic < 3 mg/kg.

¹⁵ Supplementary Information June 2019/3-Agar E406 Feed additive additional information – Hispanagar/ 4-Dusting potential of agar and carrageenan.

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

3.2.1. 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 agar for the user.

3.3. Efficacy

The applicant has provided two studies to support the efficacy of agar as a gelling agent, stabiliser and thickener. No evidence was provided on the effects of the additive as a binder.

In the first study,¹⁶ five batches of agar from different geographical origin and species¹⁷ were used to prepare samples of gel (one sample each) formed from agar diluted in water at 0.5, 1.0 or 1.5%. The gel samples were formed diluting agar in water heated and left to boil for 15 min. Then the solution was left to cool for 18 h at 20°C. The samples were tested for gel strength 24 h after preparation and after 3 months of storage at 20°C. The results showed that the strength of the gel (ranges at 24 h: 0.5% inclusion level: 49–189 g/cm²; 1.0% inclusion level: 296–704 g/cm²; 1.5% inclusion level: 659–1,202 g/cm²) remained constant or increased after three months of storage in all samples.

In a second study,¹⁸ aimed to demonstrate the efficacy of the additive in feedingstuffs, the gel strength of a jelly prepared with 0 or 700 mg agar/kg jelly in three canned chunk feeds (two feeds for cats and one for dogs) prepared with the unsupplemented or supplemented jelly was measured. The two feeds for cats had moisture content of 88.4% and 88.1%, the feed for dogs 88.5%. The gel strength was tested in feed with and without chunks and measured with a penetrometer, measuring the breaking force as g force, using probes of 6 or 12 mm depending on the feed. Ten cans for each jelly preparation and for each feed were measured, immediately after preparation and after three 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.

Table 1: Gel strength of the jelly and canned chunks feeds

Type of feed	Chunks	Agar (mg/kg)	Force (g)	
			Average (±SD)	
			Time (months)	
			0	3
Jelly steam oven feed for cat	No	0	10.7 (±2.5)	13.6 (±1.3)
	No	700	52.6 (±9.7)	53.7 (±9.1)
	Yes	0	14.3 (±4.3)	8.5 (±1)
	Yes	700	52.9 (±6.4)	64.0 (±4.4)
Jelly chunk feed for cat	No	0	12.8 (±1.8)	12.2 (±2.1)
	No	700	44.8 (±9.9)	48.3 (±3.7)
	Yes	0	13.4 (±1.7)	19.3 (±1.8)
	Yes	700	61.9 (±5.6)	74.8 (±3.6)
Jelly steam oven feed for dog	No	0	4.6 (±1)	8.3 (±0.7)
	No	700	29.4 (±9.4)	34.5 (±3.8)
	Yes	0	8.9 (±2.5)	12.0 (±0.8)
	Yes	700	53.8 (±5.1)	64.0 (±7.2)

¹⁶ Supplementary Information June 2019/3-Agar E406 Feed additive additional information – Hispanagar/5-Agar stability study EFSA 21122015.pdf.

¹⁷ Origin of the products: Agar Type 1 (H-2412) Species *Gracilaria*, origin South East Asia; Agar Type 2 (H-2413) Species *Gracilaria*, origin South America; Agar Type 3 (H-2414) Species *Gelidium*, origin North America; Agar Type 4 (H-2415) Species *Gelidium*, origin Europe; Agar Type 5 (H-2416) Species *Gelidium*, origin North Africa.

¹⁸ Supplementary Information June 2019/ 3-Agar E406 Feed additive additional information – Hispanagar/6-Agar stability in feedstuff EFSA 15122015 Nestle.pdf.

Results of the study showed that feed supplemented with 700 mg agar/kg feed had a higher gel strength than the control feed, this effect is maintained for at least 3 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 efficacy 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 a gelling agent, thickener and contributes to stabilise canned pet feed. In the absence of data, the Panel cannot conclude on the efficacy of the agar as a binder.

5. Documentation as provided to EFSA/Chronology

Date	Event
26/10/2010	Dossier received by EFSA. Agar. Submitted by Marinalg International.
29/11/2013	Reception mandate from the European Commission
24/04/2014	Application validated by EFSA – Start of the scientific assessment
10/07/2014	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
24/07/2014	Comments received from Member States
08/10/2014	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation</i>
29/10/2014	EFSA was informed by the applicant that the applicant company changed to Hispanagar
14/06/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
18/02/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: safety for the target species and user</i>
23/03/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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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
FAO	Food Agricultural Organization
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
NOAEL	no observed adverse effect level
PCB	polychlorinated biphenyl
SD	standard deviation
SCF	Scientific Committee on Food
WHO	World Health Organization

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

In the current application authorisation is sought under article 10(2) for Agar under the category/ functional groups 1(c), 1(d), 1(e) and 1(f) 'technological additives'/'emulsifiers' 'stabilisers', 'thickeners', 'gelling agents' 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. The feed additive is a solid preparation in the form of powder, agglutinated strips, cut, flaked or granulated. It is intended to be incorporated directly into feedingstuffs and in water. The applicant did not specify minimum or maximum levels but states that the typical level of use may be up to 2% by weight of the wet final product. For the characterisation of the feed additive (Agar) the Applicant submitted the internationally recognised FAO JECFA monographs for food additives, recommended by Commission Directive 2008/84/EC, which is based on: - solubility; - gel formation with water; - microscopic examination; and - two precipitate formation tests: with ammonium sulfate and with lead acetate solutions and the determination of the threshold gel concentration, which should not be higher than 0.25%. Furthermore, purity is determined applying the following tests: - water absorption; - loss on drying; - total ash; - acid-insoluble ash; and - foreign insoluble matter. Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned methods described in the FAO JECFA monographs – as recommended by Commission Directive 2008/84/EC - to characterise the feed additive (Agar). The Applicant provided no experimental data or any analytical methods for the quantification of Agar in feedingstuffs or water as the accurate determination of Agar in those matrices is not achievable experimentally. Therefore the EURL cannot evaluate nor recommend any method for official control to quantify Agar in feedingstuffs or water. 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.