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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of inositol as a feed additive for fish, dogs and cats[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 12 December 2014, replaces the earlier version published on 6 May 2014.[4]

ABSTRACT

Inositol is an essential micronutrient for salmon, carp, tilapia and shrimps, particularly juveniles. This conclusion is extended to all fish and crustaceans. The requirements differ among species and may be in the range of 250–500 mg/kg complete feed for fish. The use of inositol in feed for fish and crustaceans is considered safe at the recommended levels; setting a maximum content is not considered necessary. Based on the study provided in dogs, it was not possible to (i) demonstrate the essentiality of dietary inositol for dogs and (ii) conclude on the safety of dietary inositol at the recommended use levels of between 500 and 3 000 mg/kg. The FEEDAP Panel is not in the position to conclude on the efficacy and safety of inositol for cats. Supplementing feed for fish and crustaceans will not substantially increase the exposure of the consumer to inositol. Considering dietary exposure and endogenous synthesis of inositol, the additional exposure from fish fed an inositol-supplemented diet is of no concern for consumer safety. Considering the potential inhalation exposure upon handling the additive and the absence of data on inhalation toxicity, the FEEDAP Panel concludes that inhalation exposure from handling inositol could be hazardous. In the absence of data, inositol should be considered as irritant to skin and eyes and as a skin sensitiser. Inositol occurs naturally in the environment, particularly in plants. The use of inositol in fish nutrition is not expected to substantially increase the concentration in the environment. Consequently, the supplementation of fish feed with inositol does not pose a risk to the environment.

KEY WORDS

nutritional additive, vitamins and provitamins, inositol, safety, efficacy

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1 On request from the European Commission, Question No EFSA-Q-2010-01322, adopted on 9 April 2014.

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4 This scientific opinion has been amended following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text. To avoid confusion, the original version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of inositol as a nutritional additive to feed and water for drinking for all animal species.

Inositol, a carbocyclic polyol, is a sugar-like carbohydrate synthesised by most plants and animals. Inositol contributes to biological processes in almost all tissues, and is a precursor of phosphatidylinositol, an important component of biological membranes and lung surfactant. Inositol plays an important role as the structural basis for a number of secondary messengers in eukaryotic cells (e.g. inositol 1,4,5,-triphosphate).

Inositol is an essential micronutrient for salmon, carp, tilapia and shrimps, particularly juveniles. This conclusion is extended to all fish and crustaceans. The requirements differ among species and may be in the range of 250–500 mg/kg complete feed for fish. The use of inositol in feed for fish and crustaceans is considered safe at the recommended levels; setting a maximum content is not considered necessary.

Based on the study provided in dogs, it was not possible to (i) demonstrate the essentiality of dietary inositol for dogs and (ii) conclude on the safety of dietary inositol at the recommended use levels of between 500 and 3 000 mg/kg. The FEEDAP Panel is not in the position to conclude on the efficacy and safety of inositol for cats.

Supplementing feed for fish and crustaceans will not substantially increase the exposure of the consumer to inositol. Considering dietary exposure and endogenous synthesis of inositol, the additional exposure from fish fed an inositol-supplemented diet is of no concern for consumer safety.

Considering the potential inhalation exposure upon handling the additive and the absence of data on inhalation toxicity, the FEEDAP Panel concludes that inhalation exposure from handling inositol could be hazardous. In the absence of data, inositol should be considered as irritant to skin and eyes and as a skin sensitiser.

Inositol occurs naturally in the environment, particularly in plants. The use of inositol in fish nutrition is not expected to substantially increase the concentration in the environment. Consequently, the supplementation of feed with inositol does not pose a risk to the environment.

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BACKGROUND

Regulation (EC) No 1831/2003[5] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7; in addition 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 time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the VITAC EEIG Vitamins Authorisation Consortium[6]for authorisation of a new use (i.e. use in water for drinking) and re-evaluation of authorisation of inositol, when used as a feed additive for all animal species (category: nutritional additive; functional group: vitamins, provitamins and chemically well-defined substances having similar effect) under the conditions mentioned in Table 1.[7]

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 4(1) (authorisation of a feed additive or new use of a feed additive) and 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.[8] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 18 November 2011.

Inositol has been authorised without time limit under Council Directive 70/524/EEC[9] for its use for all animal species as a nutritional additive.

The Panel on Food Additives and Nutrient Sources added to food (ANS) issued an opinion on the use of Inositol hexanicotinate (inositol hexaniacinate) as a source of niacin (vitamin B_3) added for nutritional purposes in food supplements (EFSA, 2009). The NDA Panel expressed an opinion on the substantiation of health claims related to inositol and cognitive function (ID 1588) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 (EFSA NDA Panel, 2009).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of inositol, when used under the conditions described in Table 1.

[5] Regulation (EC) 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.

[6] On 13 March 2013, EFSA was informed by the applicant that VITAC EEIG was liquidated on 19 December 2012 and their rights as applicant were transferred to FEFANA asbl (EU Association of Specialty Feed Ingredients and their Mixtures), representing notably the following company: Trouw Nutritional International B.V. Avenue Louise, 130A, Box 1, 1050 Brussels, Belgium.

[7] On 31 July 2013, EFSA was informed by the applicant of the following: target species changed from 'all animal species' to 'fish, cats and dogs'.

[8] EFSA Dossier reference: FAD-2010-0196.

[9] Commission list of the authorised additives in feedingstuffs published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01). OJ C 50, 25.2.2004, p. 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Inositol			
Registration number/EC No/No (if appropriate)	Not appropriate			
Category(ies) of additive	3. Nutritional additives			
Functional group(s) of additive	(a) Vitamins, pro-vitamins and chemically well defined substances having a similar effect			
Description				
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)	
Inositol	IUPAC name: cis-1,2,3,5-trans-4,6-Cyclohexanehexol Chemical formula: $C_6H_{12}O_6$ CAS number: 87-89-8	Minimum 97 % of inositol	Microbiological activity analysis	
Trade name	Not appropriate			
Name of the holder of authorisation	Not appropriate			
Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		mg /kg of complete feedingstuffs		
Fish Cats Dogs	No restrictions - during all life cycle	Not applicable	Not applicable	Not appropriate
Other provisions and additional requirements for the labelling				
Specific conditions or restrictions for use	Not appropriate			
Specific conditions or restrictions for handling	Handle in accordance with good industrial hygiene and safety practices.			
Post-market monitoring	Not appropriate			
Specific conditions for use in complementary feedingstuffs	Not appropriate			
Maximum Residue Limit (MRL)				
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues	
Not appropriate	Not appropriate	Not appropriate	Not appropriate	

ASSESSMENT

This opinion is based in part on data provided by a consortium representing a single company involved in the production and distribution of inositol. It should be recognised that these data cover only a fraction of existing additives containing inositol. The application is for the active substance and the composition of the additive formulations is not the subject of the application. The Panel has sought to use the data provided, together with data from other sources, to deliver an opinion.

1. Introduction

Inositol, a carbocyclic polyol, is a sugar-like carbohydrate synthesised by most plants and animals. It exists in nine possible stereoisomers, four of which are physiologically active. The predominant form in plants and animals is *myo*-inositol. Other naturally occurring isomers (though in minimal quantities) are scyllo-, muco-, D-chiro- and neo-inositol, and possible isomers are L-chiro-, allo-, epi- and cis-inositol. In plants, phosphorus is stored mainly as hexa- and penta-inositol phosphate (phytate).

Inositol contributes to biological processes in almost all tissues, as a precursor of phosphatidylinositol, an important component of biological membranes and lung surfactant. Inositol plays an important role as the structural basis for a number of secondary messengers in eukaryotic cells (e.g. inositol 1,4,5-triphosphate).

Inositol is included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 and is foreseen for re-evaluation. Inositol is applied for re-evaluation as a nutritional additive, functional group vitamins, pro-vitamins and chemically well-defined substances having similar effects. It is intended to be added to feed for fish, cats and dogs.

myo-Inositol is described in the European Pharmacopoeia (PhEur), monograph (MG) 1805.

Inositol is listed as a pharmacologically active substance in veterinary medicinal products[10] and is not subject to maximum residue levels when used in food-producing animals. Inositol is listed as an ingredient in cosmetic products as an antistatic, humectant and hair-conditioning agent.[11]

Inositol hexanicotinate is authorised for addition for specific nutritional purposes in to foods for particular nutritional uses as a source of niacin.[12] Inositol can be added to processed cereal-based foods and baby foods for infants and young children[13] and to infant formulae and follow-on formulae when reconstituted as instructed by the manufacturer.[14]

2. Characterisation

2.1. Characterisation of the active substance

The active substance is identical to the additive.

myo-Inositol, with the generic name inositol, is defined by the International Union of Pure and Applied Chemistry (IUPAC) as cis-1,2,3,5-trans-4,6-cyclohexanehexol (synonyms: (1r,2R,3S,4s,5R,6S)-cyclohexane-1,2,3,4,5,6-hexol, cyclohexanehexol) with the Chemical Abstracts

[10] Commission Regulation (EU) 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1.
[11] Commission Decision 2006/257/EC of 9 February 2009 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, p. 1.
[12] Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, p. 9.
[13] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 6.12.2006, p. 16.
[14] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p. 1.

Service (CAS) number 87-89-8 and the European Inventory of Existing Chemical Substances (EINECS) number 201-781-2. The structural formula of *myo*-inositol is shown in Figure 1.

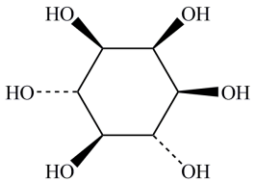

Figure 1: Structural formula of *myo*-inositol

The molecular formula of inositol is $C_6H_{12}O_6$ and its molecular weight is 180.16. It has a melting point of 225–227 °C, a bulk density of 0.45 g/cm^3 and a density of 1.752 g/cm^3. It is very soluble in water (14 g dissolves in 100 mL). The pH of an aqueous solution is approximately neutral.

Inositol is a white or almost white crystalline odourless powder with a very slight sweet taste.

By specification, the additive contains ≥ 97.0 % *myo*-inositol (anhydrous substance) in compliance with PhEur (97.0–102.0 %, anhydrous substance). Analysis of five batches of inositol under application, each by a separate laboratory (method not given and producer(s) unknown) showed an average of 99.5 % anhydrous inositol (range 99.3–99.9 %).[15] The product (three batches) also complied with the thresholds of PhEur for substance-related impurities (impurity A (D-mannitol) and impurity B (propane-1,2,3-triol or glycerol), < 0.3 % each; total impurities including unspecified related substances, < 1.0 %) and for lead (< 0.5 mg/kg).[16] In three batches of the product, the arsenic content was < 0.1 mg/kg, the lead content was < 0.05 mg/kg, the cadmium and mercury contents were < 0.01 mg/kg and the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs) was < 0.128ng WHO-PCCD/F-PCB-TEQ (World Health Organization PCDD, PCDF and PCB toxic equivalents)/kg.[17]

In total, four batches of inositol were analysed for particle size distribution by laser diffraction. The amount (v/v) of the particle fractions < 50 µm and < 10 µm diameter in three batches was 69 to 72 % and 22 to 29 %, respectively, and in the fourth batch was 16 % and 1.5 %, respectively.[18] The dusting potential measured according to the Stauber–Heubach method in three batches ranged between 0.28 and 1.14 g/m^3.[19]

2.2. Manufacturing process

Hydrolysis of natural phytic acid (phytate) at 8 Pa and 150 °C leads to crude inositol in a water solution. Phosphorous (P) is then precipitated as calcium phosphate by the addition of calcium hydroxide. Calcium phosphate is removed by filtration and water is evaporated under vacuum. Cooling down to room temperature makes the inositol crystallise. Crystallisation is repeated and the product discolours with active carbon. The moist finished product is dried in a hot air oven at 120 °C.

The applicant provided a flow chart and detailed description of the manufacturing process, and critical control points are identified and monitored.

[15] Technical dossier/Section II/Annex_II_1.
[16] Technical dossier/Supplementary information/February 2013/Annex A.
[17] Technical dossier/Section II/Annex_II_2.
[18] Technical dossier/Section II/Annex_II_3.
[19] Technical dossier/Supplementary information February 2013/Annexes B and C.

2.3. Stability and homogeneity

2.3.1. Shelf life

Inositol (three batches of one producer, stored in double polyethylene bags in a fibre drum) was demonstrated to have a shelf life of 48 months at 25 °C.[20] The additive (four batches) was shown to be stable at 40 °C for three months.[21]

2.3.2. Stability in premixtures and feed

Inositol (three batches) was added to a vitamin–mineral premixture (without choline chloride) for pigs at a concentration of 15 g/kg. Samples were stored in polyethylene bottles at 25 °C and 40 °C for up to six months. Results showed a 4 % and 8.5 % reduction in the content of inositol after six months at 25 °C and 40 °C, respectively.[22]

The stability of inositol (one batch) was further investigated when added to a premixture containing trace elements and choline chloride (20 g/kg) at the inclusion level of 15 g/kg. The measured initial value was lower than intended (13 g/kg). Final recovery after six months was 11 g/kg.[23]

Inositol (three batches) was incorporated in a complete feed for pigs at a level of 150 mg/kg. Samples were stored for three months at 25 °C. The inositol was analysed after hydrolysis of the samples. The initial analytical values were 10 times higher than the supplemental level, indicating high levels of background inositol. During storage, variable results (decrease as well as increase of the total inositol content) were observed.[18]

The effect of feed processing (extrusion) and storage was studied in fish feed. The basal feed for salmon contained high levels of fish meal and fish oil but also considerable amounts of wheat, soybean and rapeseed meal. The basal diet (background about 1 200 mg inositol/kg) was supplemented with 0, 250 and 500 mg inositol/kg. The supplemented inositol was not fully recovered (about 70–80 %). Total inositol was reduced by extrusion by about 200 mg/kg in all feeds. It remained essentially unchanged during a six-month storage period.[24]

2.3.3. Homogeneity

The capacity of inositol to homogeneously distribute in fish feed was calculated on the basis of 10 sub-samples, each from both meal and extruded feed, each unsupplemented and supplemented (250 mg/kg). In all cases, the coefficient of variation was < 9 %.[23] The experimental set-up is unsuitable to measure the homogeneous distribution, since the added quantity was only 20–30 % of the background concentration.

2.4. Physico-chemical incompatibilities in feed

No physico-chemical incompatibilities or interactions have been reported between inositol and feed materials or carriers when the additive was added to premixtures and feed. No such incompatibilities or interactions are expected.

2.5. Conditions of use

Inositol is intended for use in feed for fish, cats and dogs without a maximum limit and withdrawal period. The recommended use level for salmonids is 350–500 mg inositol/kg feed, the typical supplementation level in pet food ranges from 500 mg/kg to 3 000 mg/kg. The additive can be administered to feed via premixtures or directly to complete or complementary feed.

[20] Technical dossier/Section II/Annex_II_7.
[21] Technical dossier/Section II/ Annex_II_8.
[22] Technical dossier/Section II/Annex_II_9.
[23] Technical dossier/Supplementary information/February 2013/Annex E.
[24] Technical dossier/Supplementary information/February 2013/Annex F.

2.6. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of inositol in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

3. Absorption, distribution, metabolism and excretion (ADME)

Inositol occurs in nature in both free and bound (inositol phosphates and phosphatidylinositol) forms. Free inositol is extensively absorbed (> 90 %, according to Nahapetian and Young, 1980) by active transport in the small intestine (Caspary and Crane, 1970). Inositol from dietary phosphoinositol, a minor component of phospholipids, is similarly absorbed extensively as lysophosphatidylinositol after removal of the fatty acid at the syn-2 position of glycerol by pancreatic phospholipases (Cohn et al., 2010). The bioavailability of *myo*-inositol from inositol hexaphosphate (phytic acid) is very limited and depends on a variety of factors, for example phytate solubility, minerals, plant phytases, intestinal microbial phytases, supplemented phytases, and food processing (Nolan et al., 1987; Sandberg et al., 1989; Sakamoto et al., 1993; Sandberg, 2002; Sandberg and Andlid, 2002; Abebe et al., 2007; Kumar et al., 2012).

Inositol is widely distributed in the organism. Inositol is incorporated in the phospholipid pool, in particular as phosphatidylinositol, a component of cell membranes (Holub, 1986). In male rats, injected radiolabelled inositol is concentrated mainly in the liver, whereas small amounts of accumulation do occur in muscle tissue and not at all in adipose tissue (Lewin et al., 1976). In young rats, dietary concentrations of 1 500 mg/kg had no significant influence on liver deposition compared with counterparts fed a purified *myo*-inositol-free diet (Burton et al., 1976), whereas 5 000 mg *myo*-inositol/kg diet increased the levels of *myo*-inositol in the liver and kidney (Burton and Wells, 1976).

Endogenous inositol is synthesised mainly in the kidney, and concentrations of free inositol in the renal medullary cells are 1 000-fold higher than in blood (García-Pérez and Burg, 1991); in humans, about 4 g/day are estimated to be produced (Clements and Diethelm, 1979). Plasma levels are regulated by the kidneys through glomerular filtration, reabsorption in the renal medulla and catabolism or excretion (Holub, 1986; García-Pérez and Burg, 1991). Studies with radiolabelled inositol indicate that the capacity of the kidneys to catabolise inositol is much higher than the capacity to excrete unchanged inositol (García-Pérez and Burg, 1991). In tissues of mammals, including humans, *myo*-inositol can be converted to either L- or D-chiro-inositol by epimerases (see Carlomagno and Unfer, 2011). Inositol is catabolised to D-glucuronic acid; through subsequent metabolic steps, *O*-xylulose 5-phosphate is produced and enters the pentose phosphate cycle.

4. Efficacy and safety of inositol in fish, dogs and cats

Efficacy and safety are based on the same datasets; therefore, they are assessed in the same section.

4.1. Fish

In fish, deficiency of *myo*-inositol results in anorexia, lethargy, dark skin coloration, erosion, fin degeneration, loss of skin mucosa, oedema, anaemia, reduced growth and inefficient feed conversion, as well as a decreased activity of cholinesterase and certain aminotransferases, increased neutral lipids and reduced phospholipids in liver (Halver, 1982; Holub et al., 1982; NRC, 2011). Requirements are not well established and may vary among species. According to NRC (2011), the general requirements of inositol for fish seem to be in the range of 250–500 mg/kg diet based on studies with purified diets. Recommendations for salmonids are 350–500 mg supplemental inositol/kg complete feed (AWT, 2002), considering the variability in the background inositol content and its availability in feed materials.

Shiau and Su (2005) studied the effect of increased supplementation of inositol up to two-fold the recommended dose (0, 150, 250, 350, 450, 600 and 1 200 mg/kg semi-purified diet without inositol) in juvenile hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*, 0.51 g initial body weight (bw))

for eight weeks. The inositol content was confirmed by analysis. Three aquaria with 15 fish each were used per treatment. Feed was offered at a rate 5 % bw/day divided in two meals. There was no significant effect on mortality. Broken-line analysis of body weight gain suggested a requirement of about 400 mg inositol/kg. Liver lipids decreased from 6.0 g/100g wet tissue without inositol supplementation to 4.4 g at 350 mg supplemental inositol/kg without further reduction at higher inositol concentrations.

Jiang et al. (2009a) studied the effect of supplemental *myo*-inositol in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian) on growth, digestion and antioxidant potential in a 60-day trial. The diets were calculated to contain a total inositol background level of 171 mg/kg, increasing to 250, 400, 550, 700, 850 and 1 000 mg/kg by addition of inositol. The content of inositol in experimental diets was analytically confirmed. Group size was 150 carps (22 g initial bw) in three glass aquaria with 50 carps in each. Feed was given eight times per day to apparent satiation for 60 days. Survival was not reported. There was a linear increase of growth and feed intake up to 550 mg inositol/kg. The final body weight of this group (92 g) was not different from that of the highest total inositol content in the diet. Another study by the same authors (Jiang et al., 2009b) did not contribute to identifying different requirements or safety levels.

Shiau and Su (2004) studied the effect of increasing supplemental inositol (up to 8 000 mg/kg semi-purified diet, background 8 mg inositol/kg) on grass shrimps (*Penaeus monodon*, giant tiger prawn) for eight weeks. The group size was 45 individuals (3 replicates with 15 shrimps in each, initial bw 0.44 g). Feeding rate was 8 % of body weight. Weight gain analysis (broken-line model) would indicate that the requirement for dietary *myo*-inositol might be about 3 400 mg/kg. Survival was low and was related to dose (41, 44, 52 and 57 % at 250, 500, 1 000 and 2 000 mg supplemented inositol/kg, respectively) and reached the highest level (70 %) at 4 000 mg supplemental inositol. However, considerable leaching of inositol was observed (43, 62 and 79 % after 30, 60 and 120 minutes, respectively) and feed consumption data were not provided. It can be concluded that inositol is required for survival and optimal growth of prawns. However, owing to leaching, any conclusion on the quantities required or considered safe is not possible.

Atlantic salmon (*Salmo salar* L.) fry (0.19 g bw) were fed diets consisting mainly of fish meal, soy concentrate and fish oil and containing 296 mg inositol/kg, supplemented with 0, 100, 200, 400, 800 or 1 600 mg inositol/kg for 28 weeks (Waagbø et al., 1998). Intended values were analytically confirmed. Initially, there were two tanks with 1 000 fry in each, but this reduced to 600 after 16 weeks. At the end of the study, supplemental inositol did not affect body weight (about 24 g final bw), mortality (2.7–5.4 %) or body composition. No data, including liver lipids after eight-week feeding, demonstrated an inositol requirement of salmon above 300 mg/kg feed. At the end of the experiment, haemoglobin concentration increased with dietary levels of inositol, but there were no significant differences between the supplemented and the control groups. At the same time, triacylglycerol plasma levels were negatively correlated with dietary inositol supplementation ($P < 0.01$). On the other hand, the data show that levels up to 2 200 mg analytically determined inositol/kg (corresponding to 1 600 mg supplemented inositol/kg) did not result in adverse effects. This corresponds to four times the highest recommended supplementation level.

4.2. Dogs and cats[25]

No deficiencies of inositol have been documented in either dogs or cats (NRC, 2006). Consequently, no requirement has been established by the National Research Council (NRC).

Watson et al. (2006) studied the influence of inositol on canine epidermal barrier function *in vivo* as measured by transepidermal water loss (TEWL). Thirty-two Labrador retrievers (3–12 years old) were divided into two sex- and age-matched groups and fed a basal diet containing 80 mg inositol/400 kcal and an experimental diet containing 230 mg inositol/400 kcal for 12 weeks. These values correspond to 720 and 2 070 mg/kg assuming that a typical dry dog food contains 3 600 kcal/kg. However,

[25] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

inositol was not the only variable in the study design; inositol was added together with choline, pantothenate, nicotinamide and histidine. The composition of the basal diet was not reported. Barrier function was measured using TEWL. It was found that a combination of pantothenate, choline, nicotinamide, histidine and inositol, when fed at supplemented concentrations, was able to significantly reduce TEWL in dogs at the earliest after nine weeks. However, *in vitro* data on ceramide and total lipid synthesis and transepidermal diffusion rate did not indicate any effect of inositol alone but in combination with choline. In the view of the FEEDAP Panel, these data are not sufficient to support that inositol has a role in the epidermal barrier function in dogs and can, therefore, not be taken as a demonstration of essentiality of inositol.

The applicant provided additional studies on Labrador retrievers[26] and on cats[27]. However, the data were not considered by the FEEDAP Panel because of the inherent weakness of the study design and reporting.

4.3. Conclusions on efficacy and safety of inositol in target animals

Inositol is an essential micronutrient (a chemically defined substance having similar effects as a vitamin) for salmon, carp, tilapia and shrimps, particularly juveniles. This conclusion is extended to all fish and crustaceans. The requirements differ among species and may be in the range of 250–500 mg/kg complete feed for fish. The use levels recommended by the applicant are in the same range.

A level four times the highest recommended supplementation level was found to be safe for salmon. A level twice the highest recommended level was shown to be safe for tilapia and carp. No specific endpoint indicating intolerance was identified. Therefore, the use of inositol in feed in fish and crustaceans is considered safe at the recommended levels; setting a maximum content is not considered necessary.

Based on the study provided in dogs, it was not possible to (i) demonstrate the essentiality of dietary inositol for dogs and (ii) conclude on the safety of dietary inositol at the recommended use levels between 500 and 3 000 mg/kg, since the highest level tested was 2 070 mg/kg. The FEEDAP Panel is not in the position to conclude on the efficacy and safety of inositol for cats.

5. Safety for the consumer

5.1. Toxicological studies and observations in humans

No conventional toxicological studies are available, but the results of studies in rodent models of chronic diseases (including diabetes and cancer) suggest that the toxicity of inositol is low. The only adverse effects observed were thickening of basement membranes of capillaries of the retina and glomeruli of non-diabetic rats treated for nine months with 2 % *myo*-inositol in the diet (equivalent to 1 800 mg/kg bw per day, using the EFSA default conversion factor of 0.09 for sub-chronic exposure of rats) and worsening of the capillary thickening in glomeruli along with an increase in the amount of pericyte-containing capillaries in the retina of diabetic and non-diabetic rats treated for five or nine months with 2 % *myo*-inositol in the diet (Tilton et al., 1993). A no observed adverse effect level (NOAEL) could not be determined, as only one dose level was used. Other studies comparing effects of inositol in diabetic and non-diabetic rats (Pugliese et al., 1990; Coppey et al., 2002) showed that dietary levels of 0.5 to 2 % inositol (equivalent to 450 to 1 800 mg/kg bw per day) reduced some of the metabolic, neurological and circulatory changes of diabetes, but the treatments either had no effect on these parameters in non-diabetic rats (Pugliese et al., 1990) or were not tested in non-diabetic rats (Coppey et al., 2002).

The effect of inositol on cancers induced by other chemicals has been investigated in mice. Liao et al. (2007) used long-term exposure in drinking water to cyclic dextran sulphate sodium (DSS) in

[26] Technical dossier/Supplementary information/January 2014/Annex A and Appendix A.
[27] Technical dossier/Supplementary information/January 2014/Annex B, Appendix B1 and Appendix B2.

combination with an iron-enriched diet to induce ulcerative colitis (which is associated with colon cancer) in female C57BL/6 mice. *myo*-Inositol or hexaphosphate inositol was given at 1 % (equivalent to 9 000 mg/kg bw per day) in the drinking water of groups of colitis-induced or non-induced mice for 255 days. None of the treatments affected mortality, body weight gain or feed consumption. Both forms of inositol decreased the number and size of colo-rectal tumours in the colitis-induced groups. No colorectal tumours were found in untreated controls or non-induced groups given *myo*-inositol or hexaphosphate inositol.

Another group of studies (Kassie et al., 2008, 2010a, b) was designed to investigate the effect of 1 % dietary *myo*-inositol (equivalent to 2 000 mg/kg bw per day) in combination with various doses of dietary N-acetyl-S-(N-2-phenethylthiocarbamoyl)-L-cysteine (APTCC) on the production of cancer by tobacco smoke carcinogens given by gavage to female A/J mice. Animals were killed after 27 or 44 weeks of treatment. Body weights and weights of liver and kidneys were not affected by the *myo*-inositol/APTCC treatment in either carcinogen-exposed or unexposed groups. *myo*-Inositol reduced the total number of lung tumours and the number of lung adenocarcinomas produced by the treatment with tobacco carcinogens, inhibiting cancer cell proliferation and Akt phosphorylation (part of the insulin signalling pathway) and inducing apoptosis. Histopathology of the major organs showed no further changes in any groups other than an increase in the frequency of eosinophilic bodies in the cytoplasm of urinary bladder epithelial cells that was proportional to the dose of APTCC and thus unlikely to be a result of the inositol.

A review of 12 controlled clinical trials in a total of 250 adults given oral doses of 4 to 30 g inositol/person per day (equal to 67 and 500 mg/kg bw per day for a 60 kg person) over 1 to 12 months, found that the most frequently reported and dose-related adverse effects were flatulence, loose stools and diarrhoea (Carlomagno and Unfer, 2011).

Hallman et al. (1986) compared a test group of 37 pre-term infants given an intragastric dosage of 160 mg inositol/kg bw per day for 10 days (or 75 % of this dose intravenously on occasions when enteral dosing was not possible) with a placebo control given glucose in place of inositol. Compared with the placebo control group, the test group had lower mortality, required less mechanical ventilation (for their respiratory distress syndrome), had fewer cases of bronchopulmonary dysplasia and had less failures of indomethacin-induced closure of the ductus arteriosus. Friedman et al. (2000) compared groups of 24 pre-term infants given, by full enteral feeding, infant formulae containing 242, 710 or 2 500 µmol inositol/L at a rate of 150 mL/kg bw per day (i.e. 36, 107 or 375 µmol/infant per day; equivalent to 3.3, 9.5 or 34 mg/kg bw per day for a 2 kg bw pre-term infant) for 30 days. Infants given the high dose had a lower incidence of severe retinopathy of prematurity than those given the low dose. Other parameters, including body weight gain and the need for mechanical ventilation, were unaffected by the treatment. Although the studies in pre-term infants were not designed to identify adverse effects, the data provide some assurance that inositol is well tolerated in humans.

5.1.1. Conclusions on toxicology

Rodent studies of effects of inositol on a limited range of parameters mostly showed no adverse effects over an oral dose range of 450–9 000 mg/kg bw per day. Only one study showed any adverse effects (at 1 800 mg/kg bw per day) but a NOAEL could not be identified. Investigations of effects in adult humans and premature babies also considered only a limited range of parameters. Only minor adverse effects were reported when oral doses of 67–500 mg/kg bw per day were given to adults. Limited data on premature babies treated with inositol also suggest that inositol is well tolerated in humans.

Although the available studies suggest a low toxicity of *myo*-inositol in both rodents and humans, the data are too limited to allow an upper tolerable intake level (UL) to be defined.

5.2. Inositol in tissues and carry over to animal products

The current analytical methods available for *myo*-inositol analysis in biological materials quantitate either the ‘free’ (unbound) amount or the total amount. One of the major drawbacks with total

methods is the uncertainty on the completion of the hydrolysis of the phosphate forms of *myo*-inositol. Acid hydrolysis (6N HCl) at 120 °C (autoclave) has been found to liberate more than 90 % of *myo*-inositol from phosphoinositides, including phytates (Ellingson et al., 2012). In a survey performed in the USA on 487 foods (Clements and Darnell, 1980), total *myo*-inositol was measured using the analytical method described above. It was observed that the greatest amounts of *myo*-inositol were present in fruit, beans, grains and nuts (range 0.6–11.5 g/kg), the highest concentrations reflecting the high content of phytates. In contrast, lower concentrations of total *myo*-inositol were found in animal products (meat, 0.06–0.40 mg/kg, with the exception of liver, 1.0–1.7 g/kg; fish, 0.02–0.15 g/kg; eggs, 0.09 g/kg whole, 0.34 g/kg yolk). A summary of the available data in the literature from Europe (Souci et al., 2008), without reference to the analytical methods used, are in line with these results. These values are consistent with the fact that free inositol is a highly hydrophilic compound with no affinity for lipids. Additional dietary exposure (400, 800 and 1 600 mg inositol/kg feed) of fish for 28 weeks resulted in a mean inositol concentration of 0.163 g inositol/kg whole body (mean of all levels), 0.125 g being measured in the unsupplemented control group (Waagbø et al., 1998). These data show that feed supplementation with inositol does not lead to considerable bioaccumulation.

Inositol is also normally present in the milk of mammals, including humans; in cows' milk, levels are between 0.027 and 0.049 g/L (Ogasa et al., 1975; Byun and Jenness, 1982; Souci et al., 2008). In cows' milk, phospholipids represent 0.25–0.96 % of the fat, of which 7.7 % corresponds to phosphoinositol, representing 0.006–0.026 g/L (Contarini and Povolo, 2013).

5.3. Assessment of consumer safety

myo-Inositol is found in mixed diets (animal and plant food sources) of humans, under its three main forms already described. Data from the 1980s estimated the normal dietary intake of total inositol in the USA to be in the range 0.235–1.5 g/person per day (Clements and Darnell, 1980; as cited by EFSA, 2009).

The use of inositol in feedingstuffs for food-producing animals is restricted to fish. The contribution of fish to consumer exposure, calculated from background levels of total inositol measured in fish samples (maximum concentration reported, 0.15 g/kg; Clements and Darnell, 1980) and based on maximised exposure (300 g fish/person per day), indicate a value of 0.045 g inositol/person per day. Based on the EFSA database (EFSA, 2011) (165 g fish and sea food per day) the contribution would represent about 0.025 g inositol/person per day. Considering that the supplementation of fish feed with 1 600 mg inositol/kg feed for 28 days increases inositol deposition in the flesh by 30 % (see section 4.1.2), the consumer exposure would become 0.059 g inositol/person per day. That very limited increase of consumer exposure (0.034 g inositol/day) should be put into perspective by comparing it with the endogenous inositol production in humans (about 4 g inositol/person per day) and the background contribution of the diet (0.235–1.5 g/person per day). The results of the limited toxicological studies further support the safety of these levels of consumer exposure to inositol.

5.4. Conclusions on the safety for consumers

Inositol is a natural constituent of the human diet and has low oral toxicity. Supplementing feed for fish and crustaceans will not substantially increase the exposure of the consumer to inositol. Considering dietary exposure and endogenous synthesis of inositol, the additional exposure from fish fed inositol-supplemented diets does not give rise to concern for consumer safety.

6. Safety for the user

6.1. Effects on the respiratory system

The dusting potential of three batches of inositol was determined to be up to 1.14 g/m^3. Therefore, handling the additive may lead to inhalation exposure of users.

No acute inhalation toxicity study was provided. It would be prudent to assume that inhalation of dust from the additives presents a health hazard to workers and measures should be taken to minimise inhalation exposure.

6.2. Effects on the eyes and skin

Inositol is used as an ingredient in cosmetic products. Notwithstanding the large number of data on the skin and hair care effects of inositol upon topical use, no studies to identify its potential for skin and eye irritation and skin sensitisation were provided. In the absence of data, the FEEDAP Panel considers it prudent to assume that inositol is irritant to skin and eyes and is a skin sensitiser.

6.3. Conclusions on user safety

Considering the potential inhalation exposure upon handling the additive and the absence of data on inhalation toxicity, the FEEDAP Panel concludes that inhalation exposure from handling inositol could be hazardous.

In the absence of data, inositol should be considered as irritant to skin and eyes and as a skin sensitiser.

7. Safety for the environment

Inositol is widely present in plants as a natural component. The use of inositol in fish nutrition is not expected to substantially increase the concentration in the environment. Consequently, the supplementation of fish feed with inositol does not pose a risk to the environment.

8. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[28] and Good Manufacturing Practice.

CONCLUSIONS

Inositol is an essential micronutrient for salmon, carp, tilapia and shrimps, particularly juveniles. This conclusion is extended to all fish and crustaceans. The requirements differ among species and may be in the range of 250–500 mg/kg complete feed for fish. The use of inositol in feed for fish and crustaceans is considered safe at the recommended levels; setting a maximum content is not considered necessary.

Based on the study provided in dogs, it was not possible to (i) demonstrate the essentiality of dietary inositol for dogs and (ii) conclude on the safety of dietary inositol at the recommended use levels between 500 and 3 000 mg/kg. The FEEDAP Panel is not in the position to conclude on the efficacy and safety of inositol for cats.

Supplementing feed for fish and crustaceans will not substantially increase the exposure of the consumer to inositol. Considering dietary exposure and endogenous synthesis of inositol, the additional exposure from fish fed inositol-supplemented diets is of no concern for consumer safety.

Considering the potential inhalation exposure upon handling the additive and the absence of data on inhalation toxicity, the FEEDAP Panel concludes that inhalation exposure from handling inositol could be hazardous. In the absence of data, inositol should be considered as irritant to skin and eyes and as a skin sensitiser.

[28] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Inositol occurs naturally in the environment, particularly in plants. The use of inositol in fish nutrition is not expected to substantially increase the concentration in the environment. Consequently, the supplementation of fish feed with inositol does not pose a risk to the environment.

DOCUMENTATION PROVIDED TO EFSA

1. Inositol as a feed additive for all animal species. November 2010. Submitted by VITAC EEIG Vitamins Authorisation Consortium.

2. Inositol. Supplementary information. February 2013. Submitted by VITAC EEIG Vitamins Authorisation Consortium.

3. Inositol. Supplementary information. September 2013. Submitted by VITAC EEIG Vitamins Authorisation Consortium.

4. Inositol. Supplementary information. January 2014. Submitted by VITAC EEIG Vitamins Authorisation Consortium.

5. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for inositol.

6. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for inositol[29]

In the current application authorisation is sought under articles 4(1) and 10(2) for *Inositol* under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories. According to the Applicant, *Inositol* is an odourless white crystalline powder with a minimum purity of 97 %. The *feed additive* is intended to be incorporated in *premixtures* and *feedingstuffs*. The Applicant did not specify any minimum or maximum concentrations of *Inositol* in *feedingstuffs*, however, the following doses were recommended: from 350–500 mg /kg for salmonids and a maximum of 1000 mg/kg for laying hens.

For the quantification of *Inositol* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed a single-laboratory validated and further verified method based on the microbiological activity analysis. The following performance characteristics were reported for *feed additive*, *premixtures* and *feedingstuffs*:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 1.9 to 9.5 %;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.2 to 16.2 %;
- a recovery rate (R_{rec}) ranging from 82 to 119 %; and
- a limit of detection and quantification (LOD and LOQ) of 4 and 8 mg/kg, respectively.

Additionally, for the identification of *Inositol*, the EURL identified the internationally recognised European Pharmacopoeia method (Ph. Eur. 01/2008:1805), based on liquid chromatography and infrared absorption spectrophotometry. Even though no performance characteristics are provided the EURL considers this method to be suitable within the frame of official control.

Based on these performance characteristics, the EURL recommends for official control the European Pharmacopeia method (monograph 1805) based on liquid chromatography for the identification of *Inositol* in *feed additive* and the single-laboratory validated and further verified method based on microbiological activity analysis for the quantification of *Inositol* in *feed additive*, *premixtures* and *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.

[29] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0196.pdf