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Safety and efficacy of a feed additive consisting of monensin sodium (Elancoban® G200) for chickens for fattening, chickens reared for laying and turkeys (Elanco GmbH)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a new scientific opinion on the coccidiostat monensin sodium (Elancoban® G200) when used as a feed additive for chickens for fattening and turkeys. Based on the new data provided, the Panel updates its previous conclusions as follows: monensin sodium is produced by fermentation by a non-genetically modified strain of *Streptomyces* sp. NRRL B-67924. Genome analysis suggests the production strain may belong to a new species within the genus *Streptomyces*. The production strain and its DNA were not detected in the final additive. The product is free of antimicrobial activity other than monensin. The FEEDAP Panel cannot conclude on the safety of monensin sodium from Elancoban® G200 in feed for chickens for fattening and chickens reared for laying at the proposed maximum use level due to a dose-related reduction of the final body weight. The toxicological profile of monensin sodium was evaluated in studies made with the product obtained from the parental strain ATCC 15413. Based on a comparison of the genomes of the two strains, the FEEDAP Panel concludes that toxicological equivalence has been established, thus the conclusions already drawn on Elancoban® G200 are valid for the product obtained with the new production strain concluding that the additive is safe for the consumer and the environment; the production strain does not represent an additional risk when safety for the user is considered. Monensin sodium from Elancoban® G200 is safe for turkeys up to 16 weeks of age at the concentration of 100 mg monensin sodium/kg feed and has the potential to control coccidiosis at the minimum concentration of 60 mg/kg complete feed.

Keywords: coccidiostats, monensin sodium, chickens for fattening, chickens reared for laying, turkeys, safety and efficacy

Requestor: European Commission

Question number: EFSA-Q-2021-00537

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1. Introduction

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

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Elanco GmbH, is seeking a Community authorisation of ELANCOBAN® G200 200 g/kg monensin sodium produced by *Streptomyces cinnamonensis* or mutants as a feed additive to be used as a coccidiostat and histomonostats for chickens for fattening, chickens reared for laying and turkeys (Table 1).

Table 1: Description of the additive

Category of additive	Coccidiostat and histomonostats
Functional group of additive	Coccidiostat and histomonostats
Description	ELANCOBAN® G200 200 g/kg monensin sodium produced by *Streptomyces cinnamonensis* or mutants
Target animal category	Chickens for fattening, chickens reared for laying and turkeys
Applicant	Elanco GmbH
Type of request	New opinion

On 13 November 2019, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product,[2] could not conclude on the safety of ELANCOBAN® G200 200 g/kg monensin sodium produced by *Streptomyces cinnamonensis* or mutants in chickens for fattening, chickens reared for laying and turkeys.

During the discussions with the Member States at a meeting of the Standing Committee on Plants, Animals, Food and Feed (Animal Nutrition section), it was suggested to check for the possibility to demonstrate the safety of the production strain for animals, humans and environment, the maximum dose for chicken for fattening and the efficacy as a coccidiostat for turkeys for fattening.

The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. The new data have been received on 13 January 2021 and the applicant has been requested to transmit them to EFSA as well.

In view of the above, the Commission asks the Authority to deliver a new opinion on ELANCOBAN® G200 200 g/kg monensin sodium produced by *Streptomyces cinnamonensis* or mutants as a feed additive for chickens for fattening, chickens reared for laying and turkeys based on the additional data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information[3] to a previous application on the same product.[4] The dossier was received on 10/12/2020 and the general information and supporting documentation are available on Open.EFSA at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00537.

In accordance with Article 38 of the Regulation (EC) No 178/2002[5] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2019.5891
[3] Dossier reference: EFSA-Q-2021-00537.
[4] Dossier reference: FAD-2013-0037, EFSA-Q-2013-00752.
[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[6] a non-confidential version of the supplementary information has been published on Open.EFSA.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA to deliver the present output.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of monensin sodium (Elancoban® G200) is in line with the principles laid down in Regulation (EC) No 429/2008[7] 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 assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019a).

3. Assessment

Elancoban® G200 is intended to be used for the prevention of coccidiosis in chickens for fattening at a concentration of 100–125 mg monensin sodium/kg complete feed, in chickens reared for laying at a concentration of 100–120 mg monensin sodium/kg complete feed up to a maximum of 16 weeks of age and in turkeys at a concentration of 60–100 mg monensin sodium/kg complete feed up to a maximum of 16 weeks of age. The withdrawal period in chickens and turkeys is 1 day.

The FEEDAP Panel adopted its first opinion on the re-evaluation of this additive in 2019 (EFSA FEEDAP Panel, 2019b). In this opinion, the FEEDAP Panel could not conclude on the safety of the additive for the target species, consumer, user and environment with regard to the safety of the production strain. Based on the available tolerance studies, the safety of Elancoban® G200 for chickens for fattening could not be established. The FEEDAP Panel could not conclude on the efficacy of the additive for turkeys.

In the current opinion, the FEEDAP Panel assessed the information submitted by the applicant to address the above-mentioned data-gaps.

3.1. Characterisation

3.1.1. Characterisation of the production strain

In its former opinion (EFSA FEEDAP Panel, 2019b), the FEEDAP Panel noted that uncertainties remained on the identification and characterisation of the production strain, including the presence of antimicrobial resistance genes. Moreover, the data provided did not allow to exclude the presence of viable cells/spores of the production strain in the additive. In addition, no data were submitted on the presence of DNA of the production strain in the final additive. For the current assessment, the applicant submitted data to address the above-mentioned gaps.

The active substance monensin sodium is produced by fermentation by a non-genetically modified strain of *Streptomyces* sp. which is deposited in the Agricultural Research Culture collection with the deposit number NRRL B-67924 (in-house identifier 730.20).[8] This strain derives from the parental ATCC 15413, originally identified as *Streptomyces cinnamonensis,* using several rounds of mutagenesis.[9] The species *S. cinnamonensis* is a later heterotypic synonym of *S. virginiae,* which is considered the correct taxonomic name (Komaki and Tamura, 2021).

[6] Decision available online: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements
[7] 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.
[8] Annex II_1.0.
[9] Additional data January 2023/Annex II_2.01.

The whole genome of the production strain [redacted] These data suggest that the production strain NRRL B-67924 may belong to a new species within the genus *Streptomyces*.

The production strain was tested according to the Clinical and Laboratory Standards Institute (CLSI) against the nine antibiotics recommended for '*Corynebacterium* and other Gram-positive' in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b). The production strain was shown to be resistant to ampicillin, vancomycin, tetracycline, erythromycin and clindamycin.[10]

The whole genome sequence (WGS) of the strain was interrogated for the presence of antimicrobial resistance (AMR) genes in two databases, ResFinder and ARG.ANNOT[11] using the default values parameters. Genes coding for resistance to vancomycin, ampicillin, chloramphenicol, erythromycin and tetracycline were detected. The FEEDAP Panel considers the presence of AMR genes to be a hazard.

The WGS of the production strain was interrogated for the presence of genes coding for toxins or virulence factors [redacted] No genes of concern were identified.

The product is considered to be free of antimicrobial activity other than monensin based on a study from 2017 performed with the current production strain and assessed in the previous opinion (EFSA FEEDAP Panel, 2019b).

The presence of viable cells of the production strain was analysed in three batches of the active substance (intermediate after the recovery process), in triplicate. [redacted] No colonies were detected.[12]

The presence of DNA of the production strain was tested in three batches of the active substance (intermediate after the recovery process) in triplicate by polymerase chain reaction (PCR) analysis. [redacted] No DNA of the production strain was detected.[13]

The Panel concludes that the production strain *Streptomyces* sp. NRRL B-67924 harbours AMR genes. The interrogation of the WGS data did not identify genes encoding for toxins or virulence factors. Considering that viable cells and DNA of the production organism were not detected in the active substance, representative of the final additive, the use of Elancoban® G200 does not raise safety concerns as regards the production strain.

The toxicological profile of monensin sodium was evaluated in studies made with the product obtained from the parental strain ATCC 15413, from which the actual production strain *Streptomyces* sp. NRRL B-67924 was derived after rounds of conventional mutagenesis. To establish the equivalence from a toxicological viewpoint, the applicant provided a comparison of the genomes of the two strains.[14] [redacted] None of the identified mutations are considered of

[10] Annex II_1.0.
[11] This database is no longer maintained; last version available is May 2018.
[12] Annex II.1.0 and Annex II_1.03.
[13] Annex II.1.0 and Annex II_1.08.
[14] Additional data January 2023/Annex II_2.01.

concern. Therefore, the FEEDAP Panel considers that the results of the studies testing monensin sodium produced by the parental strain of *Streptomyces* sp. NRRL B-67924 can be used to support the safety for the consumer of the additive under assessment.

The FEEDAP Panel concludes that, based on the equivalence from the toxicological viewpoint of the two strains, the conclusions already drawn on Elancoban® G200 are valid for the product obtained with the new production strain.

3.1.2. Physical properties of the additive

The additive is constituted by 'monensin granulated' which contains the active substance mainly in its mycelial form (referred to as 'monensin sodium mycelial') but may also contain crystalline monensin sodium and pelleting aid clays. The additive is formulated with monensin granulated mixed with anti-dusting oil and rice hulls (granular limestone may be also added). The FEEDAP Panel noted that the information submitted to characterise the additive in 2019 (EFSA FEEDAP Panel, 2019b), did not include suitable data to exclude the presence of small/nano particles as foreseen in the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021). Therefore, the applicant was requested to provide information in the context of the present assessment, choosing any of the appraisal routes indicated by the aforementioned guidance document.

The applicant submitted particle size analysis data using scanning electron microscopy method (SEM).[15] The shortcomings identified in the submitted data performed with the additive Elancoban® G200 did not allow the FEEDAP Panel to conclude on the absence of (a fraction of) small particles including nanoparticles in the additive. In absence of adequate data on the decision criteria for particle size, the FEEDAP Panel considered the following elements.

The active substance is classified as very slightly soluble[16] in water (109 mg/L); the octanol/water partition coefficient (K_{OW}) varies from 2.8 to 4.2 depending on the pH (EFSA FEEDAP Panel, 2019b). The test items used in the ADME, residue and toxicological studies, including tolerance studies, were representative of the active substance (EFSA FEEDAP Panel, 2019b). The results from these studies are in general sufficient to cover nanoscale considerations since the administration mimics the actual use in animals (also in terms of the studied doses), and the histopathological examination in several studies covered the relevant organs including gastrointestinal tissues and lymph nodes.

Considering the above and in line with the appraisal route described in Section 4 of the Guidance on technical requirements (EFSA Scientific Committee, 2021), the Panel concludes that the safety of monensin sodium from Elancoban® G200 can be adequately covered by the conventional risk assessment and any risks from particles that are potentially in the nano/small range have already been covered by the existing data.

The FEEDAP Panel notes that carriers and processing aids such as clays are present in the additive and could contribute to the exposure to nanoparticles. The available data did not allow to evaluate this aspect.

3.2. Safety

3.2.1. Safety for the target species

3.2.1.1. Safety for chickens for fattening and chickens reared for laying

In its former opinion on the re-evaluation of Elancoban® G200 (EFSA FEEDAP Panel, 2019b), the FEEDAP Panel could not conclude on the safety of the highest proposed dietary concentration of monensin sodium (125 mg/kg) for chickens for fattening and to derive a margin of safety as none of the studies submitted showed full compliance with the requirements of Regulation (EC) No 429/2008.

The applicant provided a new study in chickens for fattening following the FEEDAP Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c).[17]

A total of 1,000 one-day old chickens for fattening (male Ross 308, initial body weight 38 g) was distributed in 40 pens. The birds were housed in a climate-controlled facility with four individual rooms with 10 pens each. Following allocation to five groups (one untreated control and four treated) on

[15] Additional data January 2023/Annex II_1.23.
[16] The value is below the threshold set in Section 2.3.1 of the Guidance on technical requirements (EFSA Scientific Committee, 2021).
[17] Annex III_1, Annex III_2, Annex III_3.

day 1, group size was 200 birds (8 pens with 25 birds each). Group size was derived from a power analysis.

Feed was either not supplemented (control) or supplemented with monensin sodium from Elancoban® G200 to provide 125 mg monensin sodium/kg complete feed (1.0× maximum proposed (use) level), 150 mg monensin sodium/kg complete feed (1.2× overdose group), 175 mg monensin sodium/kg complete feed (1.4× overdose group) and 200 mg monensin sodium/kg complete feed (1.6× overdose group). The concentrations of the feed additive in the diets were confirmed by analysis.

The diets consisted mainly of wheat, soybean meal and soybean oil. A two-phase feeding program was used: a starter diet (containing by analysis 23.1% crude protein (CP), 4.9% ether extract (EE), 1.4% crude fibre (CF) and by calculation 11.9 MJ metabolisable energy (ME)/kg) was provided between days 1–14 followed by a grower diet (containing by analysis 21.1% CP, 5.6% EE, 1.8% CF and by calculation 12.4 MJ ME/kg) until the end of the study (day 35). Analysed or intended levels of methionine or total sulphur-containing amino acids were not reported. Diets were offered in crumbled form (starter) or pelleted form (grower); the birds had free access to feed and water.

Health status and mortality of the animals were checked twice daily during the study. The cumulative feed intake was measured on days 14 and 35, body weight on days 1, 14 and 35. Feed to gain ratio was calculated including dead/culled animals for the corresponding intervals. Blood sampling for haematology, clinical blood chemistry[18] and necropsy[19] was made on a total of five birds/pen at study end (on days 35, 36 and 37).

Data were expressed as least square means. All statistical testing was two-sided and conducted at the 5% significance level. The pen was the experimental unit. For the mortality and scheduled necropsy gross-findings, a generalised linear mixed model was used. For the performance variables, blood variables and organ weights, a linear mixed model analysis was conducted with treatment as fixed effect, and as random effects room, block (room) and treatment × room. For each variable, if the main effect of treatment was significant, linear contrasts were constructed to compare groups.[20]

Mortality was 0.8% and no differences were found among supplemented groups and control.

The performance objectives for the chicken strain used indicate a 35-day body weight of 2411 g. The control group reached only 2,080 g (86% of the objectives[21]) (see Table 2). The FEEDAP Panel notes that this may have reduced the sensitivity of the tolerance study. Final body weight and average daily weight gain were significantly reduced by all monensin treatments. Cumulative feed intake was not affected at the monensin use level but reduced by all higher monensin levels tested in the diet.

Table 2: Results of the most relevant parameters from the tolerance study in chickens for fattening with monensin sodium from Elancoban® G200

	Control	1.0×	1.2×	1.4×	1.6×
Monensin sodium (mg/kg feed)					
Intended	0	125	150	175	200
Analysed, starter	< LOD	107.8	132.3	154.5	178.3
Analysed, grower	< LOD	112.5	126.6	156.9	165.0
Performance parameters 0–35 days					
Average feed intake (g/day)	89^{a}	87^{ab}	79^{bc}	76^{c}	67^{d}
Final body weight (kg)	2.08^{a}	1.90^{b}	1.83^{b}	1.70^{c}	1.53^{d}
Average daily weight gain (g)	58^{a}	53^{b}	51^{b}	48^{c}	43^{d}

[18] Red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, thrombocytes and white blood cells, heterophils, eosinophils, basophils, monocytes, and lymphocytes and fibrinogen; aspartate aminotransferase, alanine aminotransferase, amylase, creatine kinase, alkaline phosphatase, total protein, albumin, globulins, uric acid, cholesterol, glucose, phosphate, magnesium, calcium, total bilirubin, sodium, potassium, chloride.

[19] Lung (left lung), liver (left kidney), kidney (left kidney), heart (left ventricle), spleen, pancreas, adrenal gland (left), thymus, thyroid gland (left), testes, proventriculus, duodenum, jejunum, ileum, colon, caecum (cross section of each caeca), cloaca including bursa of Fabricius and skeletal muscle (left quadriceps femoris).

[20] Annex III_2 and Additional data January 2023/Annex III_1.04.

[21] Aviagen. Parent Stock Performance Objectives: Ross 308. 2020.

	Control	1.0×	1.2×	1.4×	1.6×
Average feed to gain ratio	1.53	1.63	1.55	1.60	1.56
Haematology					
Fibrinogen (g/L)	0.712^{a}	0.630^{b}	0.652^{ab}	0.587^{b}	0.605^{b}
Clinical chemistry					
Cholesterol (mmol/L)	3.19^{a}	3.22^{a}	3.41^{b}	3.50^{b}	3.51^{b}
Alkaline phosphatase (U/L)	4,178^{a}	3,148^{b}	3,160^{b}	2,984^{b}	3,183^{b}
Organ weight (g/relative to BW%)					
Liver	42.6^{a}/1.95	38.3^{b}/1.98	37.4^{b}/1.95	33.8^{c}/2.00	31.2^{c}/1.95
Kidney	11.5^{a}/0.53	10.0^{ab}/0.52	9.9^{b}/0.51	8.9^{c}/0.52	8.4^{c}/0.52
Heart	13.1^{a}/0.60^{a}	12.0^{ab}/0.62^{a}	11.6^{b}/0.60^{a}	10.6^{c}/0.62^{a}	10.5^{c}/0.66^{b}

LOD: Limit of detection < 7.5 mg/kg.
a, b, c: Means within a row/trial with different superscript letters are significantly different ($p < 0.05$).

Haematological parameters[17] were not affected by monensin except fibrinogen, which was lower in all treated groups compared to the control value. However, this depression was not clearly dose-related. Clinical chemistry endpoints showed no significant differences among treatments, except increased cholesterol and reduced (but not dose-related) alkaline phosphatase; both changes are not considered biologically relevant in this context.

Necropsy showed reduced absolute weights of liver, kidney and heart, but the relative weight of liver and kidney was not affected by the treatment. The increase of the relative heart weight observed with the highest dose is not considered adverse. Gross-pathology observations of organs and tissues did not indicate differences among the five groups; histopathology of organs was not performed.

3.2.1.2. Microbial studies in chickens for fattening

In 2019, the FEEDAP Panel concluded that the use of monensin sodium as a coccidiostat in chickens did not affect the colonisation or shedding of *Salmonella* spp. in the gastro-intestinal tract based on the results of studies performed in 2004 (EFSA FEEDAP Panel, 2019b). For the current assessment, the applicant submitted three recent experiments in which the effect of monensin supplementation in chickens for fattening on the shedding of *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Campylobacter jejuni* was studied.[22]

The three studies shared a similar experimental design; in each study a total of 120 one-day-old male Ross 308 chickens were randomly allocated to four groups, one control group, non-supplemented with cells of the enteropathogen and coccidiostat, and three groups given at day 14 by oral gavage 10^{6} CFU of one field isolate strain of the enteropathogen and fed diets supplemented with 0, 125 monensin sodium/kg feed (1.0× maximum use level) or 147 mg monensin sodium/kg feed (1.2× overdose group). Each treatment group was replicated 6 times (pens) with 5 birds per replicate and the experiment lasted for 35 days. The intended levels of monensin sodium and the dose of pathogenic bacteria were analytically confirmed.

Cloacal swabs were collected from each animal at day 12 to confirm the counts of enteropathogens presenting pre-challenge, and again on days 15, 19, 26 and 35 to evaluate faecal shedding. Enteropathogen counts were performed on appropriate selective media. The monensin supplementation did not affect the counts of *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Campylobacter jejuni* in the faecal samples at any time.

The FEEDAP Panel concludes that the use of monensin sodium as a feed additive in chickens for fattening is unlikely to increase shedding of *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Campylobacter jejuni*.

3.2.1.3. Safety for turkeys up to 16 weeks

No new information has been submitted. Based on the tolerance study in turkeys for fattening assessed in the former opinion (EFSA FEEDAP Panel 2019b), the Panel concludes that 100 mg monensin sodium/kg complete feed is safe for turkeys up to 16 weeks.

[22] Annex II_1.04, Annex II_1.05, Annex_II_1.06.

Conclusions on the safety for the target species

The data provided on the production strain (see Section 3.1.1) allows to conclude that the additive raised no concern for the target species in that regard.

Based on the results of the new tolerance study in chickens for fattening, no safe level of monensin sodium from Elancoban® G200 in feed for chickens for fattening and chickens reared for laying could be identified due to a dose-related reduction of the average daily gain and the final body weight starting at 125 mg/kg feed. The additive is safe for turkeys up to 16 weeks of age at the concentration of 100 mg monensin sodium/kg feed.

Based on recent shedding studies, the FEEDAP Panel can conclude that the use of monensin sodium as a feed additive in chickens for fattening is unlikely to increase shedding of *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Campylobacter jejuni*. This conclusion can be extended to chickens reared for laying.

3.2.2. Safety for the consumer, user and environment

In its former opinion on the re-evaluation of Elancoban® G200, due to uncertainties on the identity and characterisation of the production strain and the lack of demonstration of the absence of the production strain and its DNA on the final product, a final conclusion on the safety of the additive for the consumer, user and the environment could not be reached (EFSA FEEDAP Panel, 2019b).

The data submitted for the current assessment regarding the production strain and the test items used in the toxicological studies (see Section 3.1.1) is considered adequate to fill in the formerly identified data gaps. Consequently, the FEEDAP Panel can conclude that Elancoban® G200 is safe for the consumers of tissues obtained from chickens for fattening or reared for laying and turkeys fed the additive under the proposed conditions of use. The existing maximum residue limits (MRLs) for poultry tissues ensure consumer safety provided that the withdrawal period of 1 day is respected.

The use of monensin sodium from Elancoban® G200 in complete feed for chickens for fattening does not pose a risk for the terrestrial compartment, aquatic compartment and sediment. The bioaccumulation potential of monensin in the environment is low. These conclusions are extended to chickens reared for laying and turkeys.

The production strain does not represent an additional risk for the users to those identified in the former opinion, i.e. mycelial monensin and Elancoban® are irritant for the eye. Neither mycelial monensin nor Elancoban® cause skin irritancy but systemic toxicity may occur following skin exposure. Elancoban® should be regarded as a potential skin and respiratory sensitiser. On the basis of the available information, inhalation exposure is considered a risk to persons handling the additive.

3.3. Efficacy

In its former opinion, the FEEDAP Panel was not in the position to conclude on the coccidiostatic potential of monensin sodium from Elancoban® G200 when used in turkeys for fattening due to insufficient data (EFSA FEEDAP Panel, 2019b). The applicant submitted a new anticoccidial sensitivity test (AST).

3.3.1. Efficacy in turkeys for fattening

In the AST[23] performed in April 2020, two field isolates from different origins (Inoculum A and Inoculum B) were tested.[24] Five groups were included in the experimental design: an uninfected untreated control (UUC, 8 pens), two infected untreated control groups (IUC_A and IUC_B, 12 pens each) and two infected Elancoban®-treated groups (IT_A and IT_B, 12 pens each); each pen contained 11 birds. The IT groups received feed containing 60 mg monensin sodium/kg complete feed; the intended dietary monensin concentrations were analytically confirmed. The turkeys (B.U.T. males) were randomly allocated to the groups after an acclimatisation period of 12 days; the experimental diets were fed for 16 days. Feed and water were offered for *ad libitum* access during the whole study. Two

[23] Annex IV_1, Annex IV_2, Annex IV_3.

[24] Inoculum A: isolated in the UK in November 2019, containing 295,400 *E. meleagrimitis*, 42,200 *E. meleagridis*, 84,400 *E. gallopavonis* (dose per bird). Inoculum B: isolated in Poland in May 2019, containing 2,260 *E. meleagrimitis*, 45,200 *E. meleagridis*, 11,300 *E. gallopavonis*, 167,240 *E. dispersa* (dose per bird).

days after allocation, each IUC and IT group of birds was infected orally via gavage with either inoculum A or B; the pathogenicity of both inoculums was assessed in a virulence study.[25]

Animal health and mortality were monitored daily. Feed intake and body weight of the birds were measured, feed to gain ratio was calculated. Endpoints relevant for coccidiostatic infection were coccidiosis-related mortality, coccidiosis-related morbidity[26] and faecal scoring,[27] lesion scoring[28] and oocyst excretion.[29] The data were analysed using linear regression models considering the treatment as the fixed effect. Group means were compared to the IUC group as reference. Statistical significance was assessed at $p \leq 0.05$. For each variable, calculations were done on a per pen basis since the pen was the experimental unit.

Results of the newly submitted AST (Table 3) demonstrates that monensin-treated birds had lower mortality rates and lower ILS compared to the infected untreated birds with both inoculums. A similar effect was seen on oocyst excretion: OPGs in the infected treated groups were lower at 5 days PI (Inoculum A and B) and 6 days PI (Inoculum B) compared to the infected untreated groups. Relevant improvements on zootechnical parameters were also seen in the monensin-treated birds.

Table 3: Results of AST in turkeys for fattening fed monensin sodium from Elancoban® G200

Trial	Groups	Coccidiosis related Mortality(1) (n)	Oocyst excretion (OPG)	Lesion scores(2)	Final body weight (g)	Daily/Total feed intake (g)	Average daily weight gain (g)	Feed to gain ratio
	Days	**1–13 PI**	**6 PI**	**6 PI**	**13 PI**	**Overall period (16 days)**		
	UUC	0	1,021	0.1/2.45	961	64	43	1.49
Inoc. A	IUC_A	16	176,933	0.38/3.38	840	60	29	2.08
	IT_A	6*	51,749*	0.30/1.93*	847	60	34*	1.76*
Inoc. B	IUC_B	53	159,432	2.70/3.22	739	54	22	2.57*
	IT_B	0*	156,626	1.10*/0.38*	915*	67*	38*	1.79

*: IT mean significantly different from IUC mean ($p \leq 0.05$).
(1): Out of 132.
(2): upper-middle intestinal region/lower intestinal-caecal region.

Conclusions on efficacy in turkeys up to 16 weeks

Based on the results of the floor pen trials and ASTs described and assessed in the previous FEEDAP opinion (EFSA FEEDAP Panel, 2019b) and those in the current opinion, the FEEDAP Panel concludes that monensin sodium from Elancoban® G200 has the potential to control coccidiosis in turkeys up to 16 weeks of age at the minimum level of 60 mg/kg complete feed.

4. Conclusions

The active substance monensin sodium is produced by fermentation by a non-genetically modified strain of *Streptomyces* sp. (NRRL B-67924). Data obtained by genome analysis suggest that the production strain NRRL B-67924 may belong to a new species within the genus *Streptomyces*. The production strain and its DNA were not detected in the final additive. The product is considered to be free of antimicrobial activity other than monensin.

The FEEDAP Panel cannot conclude on the safety of monensin sodium from Elancoban® G200 in feed for chickens for fattening and chickens reared for laying at the proposed maximum use level due to a dose-related reduction of the final body weight in the exposed animals starting at 125 mg/kg. Based on recent shedding studies, the FEEDAP Panel can conclude that the use of monensin sodium as a feed additive in chickens for fattening is unlikely to increase shedding of *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Campylobacter jejuni*. This conclusion can be extended to chickens

[25] On day 6 PI, both inocula resulted in a weight gain depression of > 25% and an increase of the intestinal lesion scores of two or more units in the lower intestinal/caecal region.
[26] Clinical signs relevant to coccidiosis included: diarrhoea without/with blood; faeces with mucus; huddling/acting chilled; unresponsive/listless; paleness combs, wattles, and/or legs; ruffled or unthrifty feathers.
[27] A faecal score based on a five-point scale (0 to 4) was given to each pen; 0 no diarrhoea and 4 most severe diarrhoea.
[28] On day 6 PI, five turkeys in each pen were evaluated for intestinal lesion scores. The scoring system followed the one described by Repérant and colleagues and reported in Gadde et al., 2020 (five points scale of 0–4).
[29] Faecal samples were collected from each pen on days 5, 6, 7 and 13 PI and further analysed for oocyst content.

reared for laying. The additive is safe for turkeys up to 16 weeks of age at the maximum inclusion level of 100 mg monensin sodium/kg complete feed. The data provided on the production strain allows to conclude that the additive raised no concern for the target species in that regard.

The FEEDAP Panel notes that the toxicological profile of monensin sodium was evaluated in studies made with the product obtained from the parental strain ATCC 15413, from which the actual production strain *Streptomyces* sp. NRRL B-67924 was derived. Based on a comparison of the genomes of the two strains, the FEEDAP Panel concludes that toxicological equivalence has been established, thus the conclusions already drawn on Elancoban® G200 are valid for the product obtained with the new production strain and the FEEDAP Panel can conclude that the additive is safe for the consumer and the environment under the proposed conditions of use; the production strain does not represent an additional risk when safety for the user is considered.

The FEEDAP Panel concludes that monensin sodium from Elancoban® G200 has the potential to control coccidiosis in turkeys up to 16 weeks of age at the minimum level of 60 mg/kg complete feed.

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Abbreviations

AMR	antimicrobial resistance
BW	body weight
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
Log K_{ow}	logarithm of octanol–water partition coefficient
MIC	minimum inhibitory concentration
MRL	maxiumum residue limit