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Safety and efficacy of a feed additive consisting of lasalocid A sodium (Avatec® 150G) for chickens for fattening and chickens reared for laying (Zoetis Belgium SA)

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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 the coccidiostat lasalocid A sodium (Avatec® 150G) for chickens for fattening and chickens reared for laying. Taking into account the results of all tolerance studies submitted for the re-evaluation of the additive, the FEEDAP Panel considers that lasalocid A sodium from Avatec® 150G is safe at a maximum content of 90 mg/kg complete feed for chickens for fattening. A margin of safety cannot be established. No conclusion on the safety of lasalocid for chickens reared for laying can be made. Three new floor pen studies showed efficacy of 90 mg lasalocid A sodium/kg complete feed reducing the adverse clinical consequences of an *Eimeria* infection in chickens for fattening. Considering also the previously reported positive floor pen study and the three positive anticoccidial sensitivity tests, the FEEDAP Panel can conclude on an efficient coccidiostatic level of 90 mg lasalocid A sodium/kg complete feed for chickens for fattening. This conclusion is extended to chickens reared for laying.

Keywords: Avatec® 150G, lasalocid, coccidiostats, chickens for fattening, chickens reared for laying, safety, efficacy

Requestor: European Commission

Question number: EFSA-Q-2022-00425

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1. Introduction

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

Regulation (EC) No 1831/2003 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, Zoetis Belgium SA, is seeking a Community authorisation of Lasalocid A sodium (Avatec® 150G) as a feed additive to be used as coccidiostats/histomonostat for chickens for fattening and chickens reared for laying. (Table 1).

Table 1: Description of the additive

Category of additive	Coccidiostats and histomonostats
Functional group of additive	Coccidiostat
Description	Lasalocid A sodium (Avatec 150G)
Target animal category	Chickens for fattening and chickens reared for laying
Applicant	Zoetis Belgium SA
Type of request	New opinion

On 16 May 2017 and 1 July 2020, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA), in its opinion on the safety and efficacy of the product, was not in a position to conclude on the safety and on the anticoccidial efficacy of Lasalocid A sodium (Avatec® 150G) in chickens for fattening/reared for laying.

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 the 29 June 2022.

2. Data and methodologies

2.1. Data

The present assessment is based on the data submitted by the applicant in the form of supplementary information[1] to a previous application on the same product (Avatec® 150G).[2]

In accordance with Article 38 of the Regulation (EC) No 178/2002[3] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39 e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[4] a non-confidential version of the supplementary information has been published on Open.EFSA.[5]

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 lasalocid A sodium (Avatec® 150G) is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

[1] Dossier reference: EFSA-Q-2022-00425.
[2] Dossier reference: FAD-2019-0046.
[3] 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.
[4] Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements
[5] Available at: https://open.efsa.europa.eu/questions/EFSA-Q-2022-00425
[6] 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.

3. Assessment

The additive Avatec® 150G is a preparation of the polyether ionophore lasalocid A sodium produced by fermentation. The additive is intended to be used for the control of coccidiosis in chickens for fattening and chickens reared for laying.

In 2017, the FEEDAP Panel issued an opinion on the safety and efficacy of the coccidiostat Avatec® 150G (lasalocid A sodium) for chickens for fattening and chickens reared for laying. (EFSA FEEDAP Panel, 2017b). In that opinion, since lasalocid dose at and above 125 mg/kg was not tolerated, no safe dose of lasalocid could be established for chickens for fattening/reared for laying. Additionally, no conclusion was drawn on the anticoccidial efficacy of the additive at the lowest proposed used level (75 mg lasalocid A sodium/kg complete feed) in chickens for fattening/reared for laying.

In 2020, the Panel issued a second opinion (EFSA FEEDAP Panel, 2020) based on new tolerance and efficacy studies in chickens for fattening submitted by the applicant to address the limitations identified by the Panel in its former opinion (EFSA FEEDAP Panel, 2017b). In that submission, the applicant proposed to reduce the maximum proposed concentration from 125 mg to 100 mg lasalocid A sodium/kg complete feed. No safe level was identified of lasalocid A sodium in feed for chickens for fattening, thus the Panel was not in the position to conclude on the safety of the newly proposed maximum concentration of 100 mg lasalocid A sodium/kg complete feed. The Panel could not either conclude on the coccidiostatic efficacy of the additive at the lowest use level (75 mg lasalocid A sodium/kg complete feed) in chickens for fattening and chickens reared for laying.

The authorisation of this additive is currently suspended for use in feed for chickens for fattening and chickens reared for laying (OJ L 204, 10.06.2021.p13).[7]

In the current application, the applicant proposed a unique concentration of 90 mg lasalocid A sodium/kg complete feed and submitted a new tolerance study and five new efficacy studies in chickens for fattening using Avatec® 150G at the newly proposed concentration.

For the purpose of this assessment, lasalocid A sodium will be referred as lasalocid.

3.1. Safety

3.1.1. Safety for the target species

In the current assessment the tolerance studies evaluated in the previous opinions were re-considered in order to allow a comprehensive assessment of the safety of lasalocid in chickens for fattening.

Previous studies

In the tolerance study (study 1) submitted in 2017 (EFSA FEEDAP Panel, 2017b), performance results indicated that oral administration of lasalocid in feed at all the concentrations tested (125 mg to 312.5 mg/kg complete feed) were not tolerated in chickens for fattening. Consequently, no safe concentration of lasalocid could be established.

In 2020, the FEEDAP Panel assessed two new tolerance studies (studies 2 and 3) with Avatec® 150G in chickens for fattening (EFSA FEEDAP Panel, 2020). These studies were designed to establish a margin of safety using a new maximum level of 100 mg lasalocid/kg complete feed. Results from study 2 showed adverse effects on growth and feed consumption at the proposed maximum level of 100 mg lasalocid/kg complete feed and below (90 mg/kg). Similar zootechnical results were found in study 3 although differences were only numerical. The lack of consistency among studies was assumed to be related to the diet used with study 2 being more representative of a commercial diet. The FEEDAP Panel concluded that the negative effects on zootechnical performance with Avatec® 150G could occur under practical conditions and consequently were considered adverse. Histopathology analyses identified minimal to mild hepatocellular hypertrophy. Due to lack of clinical pathology, morphologic evidence of degeneration and/or necrosis, and the likely reversibility of the findings (study 3), the liver findings were not considered adverse. Nevertheless, the FEEDAP Panel could not identify a safe level of lasalocid from Avatec® 150G in feed for chickens for fattening.

[7] Commission Implementing Regulation (EU) 2021/932 of 9 June 2021 suspending the authorisation of lasalocid A sodium (Avatec 15% cc) and lasalocid A sodium (Avatec 150 G) as feed additives for chickens for fattening and chickens reared for laying (holder of authorisation Zoetis Belgium S.A.) OJ L 204, 10.6.2021, p. 13.

The applicant provided a new tolerance study with Avatec® 150G (study 4) in chickens for fattening to establish a margin of safety of the newly proposed level of use of 90 mg lasalocid/kg complete feed.

Current application: study 4

The study was carried out according to the relevant EFSA Guidance (EFSA FEEDAP Panel, 2017a). Before the completion of the study, the protocol was reviewed and approved by the company's ethical committee. The personnel involved in the observations and measurements were blind to the treatments. The study with chickens for fattening was performed with Avatec® 150G containing by analysis 15.6% lasalocid.

A total of 384 male chickens (one-day old; Ross 308; Aviagen) were randomly allocated to four treatment groups each of them replicated in 8 pens (12 birds/pen; 96 birds/treatment[8]). Birds followed a three-phase feeding program (Starter: 0–14 days; Grower: 14–28 days; Finisher: 28–35 days). Diets were either, not supplemented (control), or supplemented with 90 (1×), 112.5 (1.25×) and 135 (1.5×) mg of lasalocid/kg complete feed. After 35 days, all birds received a control diet until day 56 to consider a potential recovery phase. Zootechnical performance from days 35 to 56 was not considered further in the assessment. Basal diets for each feeding phase consisted mainly of wheat, soybean meal and maize supplemented with methionine, lysine, phytase and vitamin-mineral premix. Diets contained 21.5% crude protein (CP; analysed 22.0%), 0.71% methionine and 14.7 MJ metabolisable energy (ME)/kg feed for the starter phase, 19.4% CP (analysed 20.0% CP), 0.61% methionine and 14.8 MJ ME kcal/kg feed for the grower phase and 18.6% CP (analysed 19.9% CP), 0.59% methionine and 14.8 MJ ME/kg feed for the finisher phase. Feed and water were offered *ad libitum*, with starter diet fed as crumbles and grower and finisher diets fed as pellets.

Bird health, mortality and litter quality were recorded daily. Birds were weighed at weekly intervals on pen basis. Average daily weight gain, daily feed intake and feed to gain ratio were calculated for the following periods 0–14 days, 14–28 days, 28–35 days, 35–56 days and 0–35 days. Blood samples for haematology[9] and clinical biochemistry[10] were taken from two birds per pen on days 21, 35/36 and 56/57. The chickens used for blood analyses on days 35/36 and 56/57 were weighed, killed and necropsied (pre-selected at the start of the trial) and a full set of organs subjected to gross pathology and histopathology (including optic nerve).[11]

The pen represented the experimental unit for statistical purposes. Statistical significance was determined at $p \leq 0.05$. Continuous variables measured repeatedly were analysed using a general linear mixed model for repeated measures. For variables where the treatment × time interaction reached 5% level of significance the least squares mean of the treatment effect were calculated for each time point and pairwise comparisons between the control and treatments were performed (at the unadjusted 5% level of significance). Continuous variables measured just once (i.e. organ weight and organ weight relative to body weight) were analysed using a general linear mixed model. For variables where treatment was significant, treatment least square means were calculated and pairwise comparisons between control and treatment groups performed (at the unadjusted 10% level of significance).

[8] Number of replicates and birds derived after power statistical calculation defining a significant difference of ±8% daily weight gain.

[9] Haemoglobin, red blood cell count (RBC), haematocrit (HCT), thrombocyte estimate, white blood cell count (WBC), differential white blood cell count (heterophils, lymphocytes, monocytes, eosinophils, basophils), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), fibrinogen.

[10] Haptoglobin, A/G ratio, albumin, amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bile acids, cholesterol, creatine, creatine kinase (CK), electrolytes (Na, Ca, Cl, K), gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), globulin, glucose, lactate dehydrogenase (LDH), Mg, P, total protein, uric acid, Protein electrophoresis

[11] Thymus, brain, trachea oesophagus, ventriculus, ileum, cloaca, heart, kidneys, spleen, breast muscle, ovary, testes, Pituitary gland, thyroid gland, larynx, crop, duodenum, ceca, bursa of Fabricius, liver, adrenal glands, peripheral nerves, skin, uterus, epididymides, eyes (with optic nerve), parathyroid gland, tongue, proventriculus, jejunum, colon, lung, gallbladder, pancreas, spinal cord, bone and marrow (distal femur), oviduct, gross lesions (female organs when the bird originally expected to be male turn out to be a female).

Table 2: Effect of lasalocid A sodium (Avatec® 150G) on zootechnical performance of chickens for fattening on day 35

	Control group	Use level 1×	Overdose 1.25×	Overdose 1.5×
Lasalocid A sodium (mg/kg complete feed)				
Intended	0	90	112.5	135
Analysed, starter	ND	85.6	112	127
Analysed, grower	ND	94.6	116	131
Analysed, finisher	ND	91.3	108	131
Mortality (n out of 96)	1	3	2	1
Final body weight (g)	2,512	2,473	2,458	2,449
Average daily feed intake (g)	110	105	110	105
Feed to gain ratio	1.57	1.54	1.63	1.54

ND: Not determined.

Zootechnical performance was in line with the objectives of Ross 308 (Aviagen 2019).[12] No statistical differences were observed at day 35 in body weight, feed intake and feed to gain ratio between the control group and the lasalocid treated groups (Table 2). Mortality rates were low and unaffected by the treatments.

Lasalocid-treated groups showed no statistical differences compared to the control for most of the haematology, chemistry or coagulation blood parameters (fibrinogen) under evaluation. Eosinophils counts were higher in the 1× group relative to control. Additionally, glucose showed a significant dose dependent decrease with a similar response for potassium but only numerical.

There were no lasalocid-related macroscopic observations or organ weight alterations at study termination (35/36 days).

Histopathology did not reveal treatment-related lesions for any of the organs under evaluation, including birds' brain and optical nerve. A histopathological analysis was double checked (peer reviewed), limited to 25% of the control birds and 75% of the birds of group 1.5× (both selected randomly), to verify the absence of hyperplasia and neoplasia.

Synopsis on the safety of chickens for fattening based on four recent studies

The data provided in study 1 (EFSA FEEDAP Panel, 2017b), studies 2 and 3 (EFSA FEEDAP Panel, 2020) together with the study 4 reported in the current opinion should allow a comprehensive assessment of the safety of lasalocid in chickens for fattening. These studies reported mortality, zootechnical performance, haematology (except study 3), clinical chemistry (except study 3), necropsy (including organ weight, liver only in study 3), and histopathology (for liver in studies 2 and 3 and for the complete organ set including brain and optical nerve in study 4).

The FEEDAP Panel checked the test items used in all studies. Four different batches were used; based on the results reported in the certificates of analysis, all batches were within the specifications.

The concentration range tested varied among the different tolerance studies as follows; from 125 to 312.5 mg lasalocid/kg complete feed in study 1, from 90 to 125 mg/kg in studies 2 and 3, and from 90 to 135 mg/kg complete feed in study 4. The assessment below is focused on the concentration range of 90–135 mg/kg.

Table 3: Experimental design of tolerance studies in chickens for fattening with lasalocid from Avatec® 150G

	Replicates × treatment (birds × replicate)	Breed sex (duration)	Composition feed (form)	Feeding phases	Dietary energy level (ME;MJ/kg)
Study 1	6 (10)	Ross 708 ♂ + ♀ (35 days)	Corn-soybean meal (Pellet)	Starter Grower	12.9 13.1

[12] https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/RossxRoss308-BroilerPerformanceObjectives2022-EN.pdf

	Replicates × treatment (birds × replicate)	Breed sex (duration)	Composition feed (form)	Feeding phases	Dietary energy level (ME;MJ/kg)
Study 2	6 (10)	Ross 708 ♂ (35 days)	Corn-soybean meal (Pellet)	Starter Grower	13.8 14.1
Study 3	6 (10)	Ross 308 ♂ (35 days)	Corn-soybean meal (Pellet; Avistart)	Starter Grower	13.8 14.1
Study 4	6 (10)	Ross 308 ♂ (35 days)	Wheat-soybean meal-corn (Pellet)	Starter Grower Finisher	14.7 14.8 14.8

The four tolerance studies reported relatively low mortality rates and only sporadic and non-dose-related differences in haematology, clinical blood chemistry and necropsy. Thus, zootechnical performance seems to be the most sensitive endpoint to evaluate lasalocid tolerance. A summary from the performance of the four tolerance studies is provided in Table 3 while Table 4 shows some details on the experimental design.

Table 4: Zootechnical performance(1) of chickens for fattening in four tolerance studies with lasalocid from Avatec® 150G

		Lasalocid (mg/kg complete feed)					
		0	**90**	**100**	**112.5**	**125**	**135**
Study 1(2)	BW (g)	1,831 (100)	–	–	–	1,745* (95)	–
	ADFI (g)	78 (100)	–	–	–	74 (95)	–
	F/G	1.49 (100)	–	–	–	1.50 (101)	–
Study 2	BW (g)	2,029 (100)	1,895* (93)	1,876* (93)	–	1,569* (77)	–
	ADFI (g)	76 (100)	72* (95)	71* (93)	–	64* (84)	–
	F/G	1.43 (100)	1.46 (102)	1.44 (101)	–	1.55* (108)	–
Study 3	BW (g)	2,504 (100)	2,458 (98)	2,416 (96)	–	2,361 (94)	–
	ADFI (g)	92 (100)	92 (100)	86 (93)	–	88 (96)	–
	F/G	1.42 (100)	1.46 (103)	1.43 (101)	–	1.44 (101)	–
Study 4	BW (g)	2,512 (100)	2,473 (98)	–	2,458 (98)	–	2,449 (97)
	ADFI (g)	110 (100)	105 (95)	–	110 (100)	–	105 (95)
	F/G	1.57 (100)	1.54 (98)	–	1.63 (104)	–	1.54 (98)

BW: body weight; ADFI: average daily feed intake; F/G: feed to gain ratio.
*: Means significantly differed from the control treatment in the same row ($p < 0.05$).
(1): Absolute values for body weight, daily feed intake and feed to gain ratio are provided; values relative to the control values are provided in brackets. The final body weight of the control group was compared to the performance objectives of the breed, and it resulted in 86, 90, 104 and 104% in study 1, 2, 3 and 4, respectively.
(2): In study 1, ADFI and F/G refer to average of males and females, since the statistical analysis was performed separately for each gender no comparison tests are provided in this table. For full details please check EFSA FEEDAP Panel, (2017b).

A significant reduction in growth rate was found at 125 mg lasalocid/complete feed in studies 1 and 2 (Table 4). In study 3, despite the relative growth reduction was to a similar extent as that seen in study 1, these differences were not significant. In study 4, no relevant growth impairment when 112.5 or 135 mg lasalocid/kg complete feed were supplemented. Furthermore, it was noted that in the 90 mg lasalocid/kg complete feed groups, birds reached 93–98% of the weight observed in the control group, but the difference was only significant in study 2.

The reduced final body weight in studies 1 and 2 reaching ≤ 95% of that of the control group was in accordance with a significantly lower feed intake. Feed to gain ratio was not significantly affected by lasalocid treatment except in study 2 where the lowest performance was observed.

Overall, the results lack consistency due to the growth depression observed in study 2 already at the newly proposed maximum concentration of 90 mg lasalocid/kg complete feed. This does not allow to derive an unequivocal conclusion solely based on the performance data as such.

The FEEDAP Panel assessed the conditions that could influence the outcome of these types of studies, such as (i) performance relative to breed objectives and the (ii) composition and nutrient profile of the basal diet.

A noticeable difference between the studies is the growth rate in relation to the performance objectives published by the breeder company for Ross 708 and Ross 308 (Aviagen, 2019). Final body weight of the control group amounted in study 1 to 85% of the performance objective value (1831/2155), in study 2 to 90% (2029/2255), in study 3 to 105% (2,504/2376) and in study 4 to 106% (2,512/2376). The studies with the superior growth (studies 3 and 4) developed a higher tolerance to lasalocid than those which failed to reach the level of the performance objectives (studies 1 and 2). The FEEDAP Panel expects that birds showing maximum performance requiring well balanced nutrient supply in the absence of metabolic disorders would be most sensitive against any toxic inhibition. This consideration is the basis of the FEEDAP guidance on safety of feed additives for the target animal (EFSA FEEDAP Panel, 2017b). Since the most recent study (study 4) was carried out according to the guidance and did not show safety concerns, the Panel gives more weight to this study.

Diet composition and nutrient profile used across the different studies might also be a source of variation. In its previous opinion (EFSA FEEDAP Panel, 2020), the Panel did not exclude an interaction between diet composition and lasalocid tolerance since a larger tolerance of lasalocid was observed when a diet with reduced content of antinutritional factors of soybean was used (study 3 vs. study 2). Thus, study 3 was given less weight as diet composition was not representative of European conditions. Dietary electrolyte balance (DEB), as suggested by the applicant,[13] is a relevant factor influencing lasalocid tolerance in chickens for fattening. The applicant recommends a range of DEB of 172–240 mEq/kg. In study 1, DEB amounted to 241–262 mEq/kg whereas in study 4, DEB was 195–239 mEq/kg. The latter may partially explain the poor performance observed in study 1 compared to study 4, where birds seemed to tolerate lasalocid better.

Taking into account the results of all the tolerance studies, the FEEDAP Panel considers 90 mg lasalocid A sodium/kg complete feed as the maximum level safe for chickens for fattening. A margin of safety cannot be established. Considering the absence of a margin of safety and the negative effects observed on performances of animals with low growth rate, the FEEDAP Panel considers that an extrapolation to chickens reared for laying is not possible. In addition, the FEEDAP Panel notes that feeding lasalocid to chickens reared for breeding would potentially have a negative effect on the breeding period (Perelman et al., 1993).

Conclusions on safety for the target species

The FEEDAP Panel considers that lasalocid A sodium from Avatec® 150G is safe at a maximum concentration of 90 mg/kg complete feed for chickens for fattening. A margin of safety cannot be established. No conclusion on the safety of lasalocid A sodium for chickens reared for laying can be made.

3.2. Efficacy

3.2.1. Efficacy for chickens for fattening

In the previous opinion, the FEEDAP Panel could not conclude on the efficacy of 75 mg lasalocid/kg complete feed for chickens for fattening owing to the lack of a sufficient number of efficacy studies (EFSA FEEDAP Panel, 2017b, 2020).

In the current application, the applicant submitted five floor pen studies (FP) conducted with the newly proposed unique concentration of 90 mg lasalocid/kg complete feed to support the efficacy of the additive in chickens for fattening.[14]

The studies followed a similar design (Table 5);[15] in each study, 1-day-old chickens (Ross 308, males and females; in FP5 only males) were penned and allocated into the following experimental groups: an uninfected untreated control group (UUC), an infected untreated control group (IUC) and an infected Avatec®-treated group (IT). The Avatec®-treated group received feed containing 90 mg lasalocid/kg complete feed. The intended dietary concentrations were analytically confirmed (see Table 5). Study duration was 35 days. The basal diet for all trials was composed mainly of corn, wheat and soybean meal and was provided in pellet form for *ad libitum* access. In the infected groups, all

[13] https://poultryhealthtoday.com/lasalocid-resurgence-in-broilers/
[14] Technical dossier/Section IV/Sect_IV_Efficacy.
[15] Technical dossier/Section IV/Annex_IV_1, 2, 3, 4 and 5.

birds were inoculated orally on day 14 via syringe with recent field isolates of pathogenic *Eimeria* species.[16] Birds of the UUC group were inoculated with a sham inoculum. Animal health and mortality were monitored at least twice daily. Feed intake and body weight of the animals were measured at study start, one day before inoculation and subsequently in weekly intervals, feed to gain ratio was calculated accordingly. Samples of excreta were analysed for oocyst excretion (pooled samples from days 20–21 – 22, days 27–28 – 29, and day 35). Intestinal lesions were scored on 5 birds/pen on days 21, 28, 35 in FP1, FP2 and FP3. In FP4 and FP5, lesions were scored on days 19, 20, 21 (5 birds/pen) and on days 28 and 35 (3 birds/pen). Scoring was conducted following the method by Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

The data were analysed using a general linear mixed model considering the fixed effects of sex, treatment and sex-by-treatment interaction (FP1-FP4) or the fixed effect of treatment (FP5). The pen was the experimental unit. In trials FP1-FP4, comparisons were made between treatments across sexes if the overall treatment effect was significant and if treatment by sex interaction was not significant; comparisons were made between treatments at the 5% level of significance ($p \leq 0.05$) by sex if the treatment-by-sex interaction was significant. In FP5, comparisons were made between treatments at the 5% level of significance ($p \leq 0.05$).

Table 5: Experimental design of floor pens with chickens for fattening fed Avatec®

Trial No (Date of conduct)	Replicates per treatment (Birds per replicate)	Inoculum characteristics			Lasalocid analysed in feed(1) (mg/kg complete feed)
		Country, date of isolation	Dose (number of oocysts) and strain per bird		
FP1 (6/2021)	12 (30)	Denmark 2/2021	*E. acervulina*	59,408	88.7/86.6
			E. tenella	42,112	
			E. mitis	376	
FP2 (11/2021)	10 (30)	Germany 2/2021	*E. acervulina*	112,520	97.3/98.7
			E. maxima	19,400	
			E. tenella	75,660	
FP3 (3/2022)	10 (30)	The Netherlands 10/2021	*E. acervulina*	202,500	91.7/91.1
			E. maxima	112,500	
			E. tenella	78,750	
FP4 (3/2022)	10 (30)	Hungary 11/2021	*E. acervulina*	90,000	90.5/95.8
			E. maxima	50,000	
			E. tenella	35,000	
FP5 (4/2022)	10 (30)	Poland 5/2021	*E. maxima*	76,200	99.0/98.1

(1): Birds received starter diet from day 1 to 12 and grower diet from day 13 to 35. The diets (starter/grower) contained by analysis 21.1/20.8% crude protein (CP) in FP1, 20.8/20.0% CP in FP2, 22.1/20.4% CP in FP3 and FP4, 21.1/20.0% CP in FP5. The calculated levels of total methionine (starter/grower) were 5.5/5.1 g/kg in FP1 and FP2, 5.5/5.0 in trials FP3 and FP4, 5.7/5.0 in FP5. The calculated levels of nitrogen corrected apparent metabolisable energy (AMEn) (starter/grower) were 12.3/13.0 MJ AMEn/kg in all the trials.

Coccidiosis-related mortality was not different between IT and IUC in FP1, FP2 and FP5. In FP3, lasalocid reduced mortality significantly from 47% to 23%, in FP4 from 23% to 16%.

The results of intestinal lesion scores (ILS) are listed in detail in Appendix A. In FP1, ILS due to *E. tenella* was higher in IT than in IUC on day 21. A positive result was found in FP2 on day 28 where the ILS due to *E. tenella* was significantly reduced from 0.99 to 0.36 in the IT group. In FP3, lasalocid

[16] The inocula used were tested for their virulence in a dose-titration study.In FP1, the dose selected resulted in lesion scores of 2.7 (*E. acervulina*) and 3.0 (*E. tenella*) and in a weight gain reduction of 56% at day 7 post-inoculation (PI), coccidiosis-related mortality was 7%. In FP2, the dose selected resulted in lesion scores of 2.8 (*E. acervulina*), 2.0 (*E. maxima*) and 1.5 (*E. tenella*) and in a weight gain reduction 53% at day 7 PI, coccidiosis-related mortality was 33%. In FP3, the dose selected resulted in lesion scores of 3.0 (*E. acervulina*), 3.0 (*E. maxima*) and 2.3 (*E. tenella*) and in a weight gain reduction of 58% at day 7 PI, coccidiosis-related mortality was 27%. In FP4, the dose selected resulted in lesion scores of 3.0 (*E. acervulina*), 2.6 (*E. maxima*) and 2.6 (*E. tenella*) and in a weight gain reduction of 53% at day day 7 PI, coccidiosis-related mortality was 7%. In FP5, the dose selected resulted in lesion scores of 2.6, 3.4 and 1.6 (*E. maxima*) at days 5, 6 and 7 PI, respectively; in a weight gain reduction of 20%, 49% and 52% at days 5, 6 and 7 PI, respectively; no coccidiosis-related mortality was recorded.

reduced the severity of intestinal lesions due to *E. maxima* at study end, lesions due to *E. tenella* were not influenced. In FP5, in which *E. maxima* inoculum was used, significantly lower ILS were found on days 20 and 21 in the IT group compared to IUC (1.72 vs 0.36 and 0.52 vs 0.16, respectively).

Findings on oocyst excretion are less indicative for the coccidiostatic action of lasalocid in these floor pen studies (Appendix B); no significant differences were found in FP2, FP3 and FP4. In FP1, a significant reduction by lasalocid was seen at days 27/28/29 and at study end. OPGs on days 20/21/22 were lower in FP5 in the IT groups compared to IUC.

Table 6 summarises the zootechnical performance, the data given refer to the measurements made at the end of the trials. Average daily gain was significantly higher in IT groups compared to IUC groups in FP1, FP2 and FP5; feed to gain ratio was improved by the lasalocid treatment in FP2, FP4 and FP5. No such effect was found in FP3.

Table 6: Zootechnical parameters of chickens for fattening fed Avatec® in floor pen studies

Groups	FP1			FP2			FP3			FP4			FP5		
	ADG (g)	AFI (g/d)	F/G	ADG (g)	AFI (g/d)	F/G	ADG (g)	AFI (g/d)	F/G	ADG (g)	AFI (g/d)	F/G	ADG (g)	AFI (g/d)	F/G
UUC	63.1^{a}	85.4^{a}	1.46	66.1^{a}	89.8^{a}	1.44^{b}	F: 63.8^{a} M: 69.2	81.8^{a}	1.41^{b}	68.5^{a}	81.9^{a}	1.39^{c}	71.3^{a}	79.7^{a}	1.33^{b}
IUC	58.7^{b}	76.8^{b}	1.50	59.0^{b}	75.1^{b}	1.56^{a}	F: 54.7^{b} M: 69.7	54.7^{b}	1.55^{a}	60.2^{b}	64.0^{b}	1.56^{a}	66.9^{b}	74.7^{b}	1.40^{a}
IT	61.8^{a}	78.9^{b}	1.46	64.8^{a}	78.9^{b}	1.46^{b}	F: 57.7^{b} M: 67.2	66.0^{b}	1.49^{a}	63.5^{b}	68.3^{b}	1.45^{b}	72.8^{a}	78.7^{b}	1.34^{b}

ADG: average daily gain; AFI: feed intake; F/G: feed to gain ratio; F: females; M: males.
a, b, c: Means with different letters within the same trial are significantly different ($p \leq 0.05$).

In summary, three floor pen studies demonstrate the coccidiostatic efficacy by reducing coccidiosis-related mortality (FP3 and FP4), and the severity of intestinal lesions (FP5). These findings are supported by improved performance data in FP4 and FP5.

Conclusions on efficacy for chickens for fattening

Three new floor pen studies showed efficacy of 90 mg lasalocid A sodium/kg complete feed reducing the adverse clinical consequences of an *Eimeria* infection in chickens for fattening.

Considering also the previously reported positive floor pen study and the three positive anticoccidial sensitivity tests at the concentration level of 75 mg lasalocid A sodium/kg complete feed (EFSA FEEDAP Panel, 2020), the FEEDAP Panel can conclude on an efficacious coccidistatic level of 90 mg lasalocid A sodium/kg complete feed for chickens for fattening. This conclusion is extended to chickens reared for laying.

4. Conclusions

The FEEDAP Panel considers that lasalocid A sodium from Avatec® 150G is safe at a maximum content of 90 mg/kg complete feed for chickens for fattening. A margin of safety cannot be established. No conclusion on the safety of lasalocid A sodium for chickens reared for laying can be made.

Three new floor pen studies showed efficacy of 90 mg lasalocid A sodium/kg complete feed reducing the adverse clinical consequences of an *Eimeria* infection in chickens for fattening.

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5. Documentation provided to EFSA/Chronology

Date	Event
29/06/2022	Dossier received by EFSA. Lasalocid A sodium (Avatec®). Submitted by Zoetis Belgium SA
12/07/2022	Reception mandate from the European Commission
04/08/2022	Acceptance mandate from the European Commission by EFSA – Start of the scientific assessment
23/11/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017a. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Halle I, van Beelen P, Holczknecht O, Vettori MV and Gropp J, 2017b. Scientific Opinion on the safety and efficacy of Avatec® 150G (lasalocid A sodium) for chickens for fattening and chickens reared for laying, and modification of the terms of authorisation for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, Guinea fowl, quails and partridges) except laying birds. EFSA Journal 2017;15(8):4857, 32 pp. https://doi.org/10.2903/j.efsa.2017.4857

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16 (5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2017.4857

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Gropp J, Rychen G, Holczknecht O and Vettori MV, 2020. Scientific Opinion on the safety and efficacy of Avatec® 150G (lasalocid A sodium) as a feed additive for chickens for fattening and chickens reared for laying. EFSA Journal 2020;18 (8):6202, 17 pp. https://doi.org/10.2903/j.efsa.2020.6202

Johnson J and Reid WM, 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor pen experiments with chickens. Experimental Parasitology, 28, 30–36.

Perelman B, Pirak M and Smith B, 1993. Effects of the accidental feeding of lasalocid sodium to broiler breeder chickens. The Veterinary Records, 132(11), 271–273.

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ANOVA	analysis of variance
AST	anticoccidial sensitivity test
bw	body weight
CP	crude protein
DEB	dietary electrolyte balance
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
F/G	feed to gain ratio
FP	floor pen
ILS	intestinal lesion score
IT	infected treated group
IUC	infected untreated group
LOD	limit of detection
MCHC	mean corpuscular haemoglobin concentration
OPG	oocyst counts per gram of excreta
UUC	uninfected untreated group

Appendix A – Lesion scores for different *Eimeria* species at different study days in floor pen trials

	Day 20	Day 21			Day 28			Day 35		
	E. max	*E. acer*	*E. ten*	*E. max*	*E. acer*	*E. ten*	*E. max*	*E. acer*	*E. ten*	*E. max*
FP1										
UUC	–	0.15^{b}	0.17^{c}	–	0.90^{a}	0.41^{b}	–	0.82^{a}	0.28	–
IUC	–	1.90^{a}	1.87^{b}	–	0.07^{b}	1.20^{a}	–	0.07^{b}	0.28	–
IT	–	1.78^{a}	2.37^{a}	–	0.10^{b}	0.91^{a}	–	0.08^{b}	0.32	–
FP2										
UUC	–	0.22^{b}	0.04^{b}	0.42	0.28^{a}	0.08^{c}	0.60	1.40^{a}	0.26^{b}	0.62
IUC	–	2.50^{a}	2.58^{a}	0.70	0.04^{b}	0.99^{a}	0.88	0.19^{b}	0.59^{a}	0.46
IT	–	2.38^{a}	2.52^{a}	0.66	0.08^{b}	0.36^{b}	0.54	0.42^{b}	0.46^{ab}	0.30
FP3										
UUC	–	0.02^{c}	0.00^{b}	0.16^{b}	0.30	0.13	0.47	1.23^{a}	0.07	0.87^{a}
IUC	–	2.35^{b}	2.53^{a}	0.52^{a}	0.38	0.20	0.98	0.50^{ab}	0.20	1.56^{a}
IT	–	2.80^{a}	2.31^{a}	0.32^{ab}	0.19	0.30	0.58	0.03^{b}	0.20	0.30^{b}
FP4										
UUC	–	0.00^{c}	0.02^{b}	0.02^{b}	0.47^{a}	0.03^{b}	0.23^{b}	1.43^{a}	0.17	0.70
IUC	–	1.93^{b}	2.33^{a}	0.44^{a}	0.07^{b}	0.24^{a}	0.65^{a}	0.07^{b}	0.19	0.71
IT	–	2.72^{a}	2.66^{a}	0.30^{a}	0.00^{b}	0.40^{a}	0.57^{ab}	0.00^{b}	0.33	0.57
FP5										
UUC	0.10^{b}	–	–	0.42^{a}	–	–	0.20	–	–	0.07
IUC	1.72^{a}	–	–	0.52^{a}	–	–	0.10	–	–	0.00
IT	0.36^{b}	–	–	0.16^{b}	–	–	0.27	–	–	0.07

E. acer: E. acervulina; *E. max*: *E. maxima*; *E. ten*: *E. tenella*.
a, b, c: means with different letters within a trial are significantly different ($p < 0.05$).

Appendix B – Results of oocyst excretion per gram per excreta (OPG) at different study days in floor pen trials

	Days 20-21-22	Days 27-28-29	Day 35
FP1			
UUC	6.37^{b}	$30,942^{ab}$	$93,004^{a}$
IUC	$254,421^{a}$	$58,316^{a}$	$5,521^{b}$
IT	$326,239^{a}$	$12,219^{b}$	328^{c}
FP2			
UUC	2^{b}	16,527	$66,360^{a}$
IUC	$255,764^{a}$	20,001	$1,357^{b}$
IT	$366,486^{a}$	14,584	$1,115^{b}$
FP3			
UUC	F: 0^{b} M: 0^{b}	1,739	$77,636^{a}$
IUC	F: $478,388^{a}$ M: $263,058^{a}$	4,596	625^{b}
IT	F: $825,418^{a}$ M: $176,277^{a}$	11,268	$2,543^{b}$
FP4			
UUC	4^{b}	1,656	$109,331^{a}$
IUC	$447,023^{a}$	11,706	4^{b}
IT	$381,180^{a}$	8,083	23^{b}
FP5			
UUC	1^{c}	208	76^{a}
IUC	$20,764^{a}$	108	7^{b}
IT	$6,993^{b}$	17	5^{b}

F: females; M: males.
a, b, c: means with different letters in a trial are significantly different ($p < 0.05$).