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Efficacy of Cygro® 10G (maduramicin ammonium-α) for turkeys

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Abstract

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 efficacy of Cygro® 10G. The active substance of Cygro® 10G is the polyether ionophore maduramicin ammonium-α, a coccidiostat intended to be used in feed for turkeys for fattening. In a former opinion, the FEEDAP Panel concluded that the efficacy of Cygro® 10G in turkeys for fattening had not been sufficiently demonstrated. In the present submission, new efficacy studies have been provided by the applicant. A positive effect of Cygro® 10G in preventing coccidiosis in turkeys was shown in three anticoccidial sensitivity tests (ASTs). However, owing to the lack of floor pen studies showing a positive effect, the FEEDAP Panel is not in the position to conclude on the efficacy of Cygro® 10G for turkeys for fattening.

Keywords: coccidiostats and histomonostats, Cygro® 10G, maduramicin ammonium-α, turkeys, efficacy

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1. Introduction

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

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for 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 maduramicin ammonium to be used as a coccidiostats and histomonostats in turkeys (Table 1).

Table 1: Description of the substances

Category of additive	Coccidiostats and histomonostats
Functional group of additive	Coccidiostats and histomonostats
Description	Maduramicin ammonium
Target animal category	turkeys
Applicant	Zoetis Belgium S.A.
Type of request	New opinion

On 28 January 2015, 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 (EFSA FEEDAP Panel, 2015), considered that based on the recent studies (floor pen studies, field studies and sensitivity studies), the efficacy of maduramicin ammonium in turkeys for fattening has not been sufficiently demonstrated.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data have been sent to EFSA and Commission on 19 December 2018.

In view of the above, the Commission asks the Authority to deliver a new opinion of maduramicin ammonium as a coccidiostat and histomonostat in turkeys based on the additional data submitted by the applicant.

1.2. Additional information

The FEEDAP Panel issued an opinion on the safety and efficacy of Cygro® 10G (maduramicin ammonium-α) when used as a coccidiostat in turkeys (EFSA FEEDAP Panel, 2015). In this opinion, the FEEDAP Panel was not able to conclude on the efficacy of the additive for turkeys for fattening based on the available studies.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information[1] to a previous application of the same product.[2]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the efficacy of Cygro® 10G (maduramicin ammonium-α) is in line with the principles laid down in Regulation (EC) No 429/2008[3] and the relevant guidance document: Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

[1] FEED dossier reference: FAD-2019-0003.
[2] FEED dossier reference: FAD-2010-0390.
[3] 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 Cygro® 10G is a preparation of the polyether ionophore maduramicin ammonium-α (Maα) produced by fermentation of *Actinomadura yumaensis* NRRL 12515. The additive is intended for the control of coccidiosis in turkeys for fattening (up to 16 weeks of age) at a concentration of 5 mg/kg complete feed with a withdrawal period of four days.

In the previous opinion, the FEEDAP Panel could not conclude on the efficacy of the additive because of a series of limitations in the floor pen trials, field studies and anticoccidial sensitivity tests (EFSA FEEDAP Panel, 2015). The applicant submitted additional data to address the limitations identified by the Panel.

3.1. Efficacy

3.1.1. Floor pen studies

The applicant re-submitted the four studies[4] assessed by the FEEDAP Panel in 2015 (EFSA FEEDAP Panel, 2015). Two out of the four studies assessed (trial 1 and trial 3) did not show improvement in any of the specific endpoints (e.g. lesion/faecal score, oocyst excretion, morbidity, coccidiosis-related mortality); therefore, these studies cannot be considered as positive for the demonstration of the coccidiostatic efficacy of Cygro® 10G. The remaining trials (2 and 4) were re-considered in light of the current requirements with regard the specific endpoints (Guidance on the assessment of the efficacy of feed additives, EFSA FEEDAP Panel, 2018). Both trials were described in detail in the former opinion (EFSA FEEDAP Panel, 2015).

With regard to the specific endpoints, in trial 2, the oocyst excretion showed a significant improvement in the infected treated (IT) group with respect to the infected untreated control (IUC) group (88,833 vs. 250,066) during the period of days 15–28. In absence of the raw data and the statistical output, this result could not be verified by the Panel.[5] Considering this limitation and also the fact that the study was conducted in 2009, Trial 2 cannot be used for the demonstration of efficacy.

In trial 4, the Panel also noted significant improvement by the treatment on the oocyst excretion (47,895 of IT vs. 121,127 of IUC) on day 21 and also at days 35, 42, 49, 56, 98 and 105. The results could be verified by checking the raw data and the statistical output which was made available.[6] However, the Panel noted a high mortality rate in the untreated uninfected (UUC) group (6 birds, corresponding to 10%) similar to the level of the IUC group (7 birds, corresponding to 11.9%) and the highest being in the IT group (11 birds corresponding to 18.3%). This high mortality was mainly due to non-coccidiosis related causes therefore the Panel considers that Trial 4 cannot be used for the demonstration of efficacy.

3.1.2. Anticoccidial sensitivity tests

The applicant submitted in total six anticoccidial sensitivity tests (ASTs), three of them performed in 2012[7] and three in 2018.[8] Since ASTs are primarily used to allow an assessment under recent field conditions (also considering resistance development), the FEEDAP Panel assessed only the three studies from 2018.

The three recent ASTs with a similar experimental design were made with the groups UUC, IUC and IT. In AST-3, a fourth group was used including birds infected and administered another coccidiostat. The IT group received feed supplemented with Cygro® 10G at an intended concentration of 5 mg Maα/kg feed (analysed concentrations: 4.9, 4.9 and 5.2 mg Maα/kg feed in the three tests, respectively). In each test, 1-day-old turkeys for fattening (Converter Hybrid, males and females) were used. The birds (sex separated) were randomly allocated to the experimental groups on day 14. Group size was 70 birds (10 replicates with 7 birds each). Birds were artificially infected on study day 16 with sporulated oocysts from

[4] Technical dossier/Trial 1 V3788 Page 410; Trial 2 CT012-08MAxxxx Page 607; Trial 3 CT013-08MAxxxx Page 768; Trial 4 5117R-03-11-223 Page 943 and clarifications received by email December 2019 and January 2020.
[5] Technical dossier/Clarification received by email February 2020.
[6] Technical dossier/Clarification received by email December 2019.
[7] Technical dossier/A112R-GB-12-032 Page 1201; A112R-DE-12-033 Page 1282; A112R-BE-12-034 Page 1362.
[8] Technical dossier/AST-1 A112C-BE-18-224 Page 35; AST-2 A112C-BE-18-225 Page 143; AST-3 A112C-BE-18-258 Page 242 and Supplementary information September 2019.

field isolates.[9] Animal health and mortality were monitored up to 22 days of life. Feed intake and weight gain of the animals were measured, feed to gain ratio was calculated. Samples of excreta were analysed for oocyst excretion. Intestinal lesions were scored from 0 (normal gut) to 4, following a lesion scoring system specific for *Eimeria meleagrimitis* and *Eimeria adenoeides*.

The data were analysed by a general linear mixed model with fixed effects (treatment, sex, and treatment by sex) and random effects (block within sex, block within sex by treatment and error). All hypothesis tests were conducted at the 0.05 level of significance using two-sided tests.

In AST-1, five birds died during the study. All mortalities occurred between days 21 and 22. Four out of five deaths were coccidiosis related and all were from the IUC group showing a significant difference between IUC and IT groups. In AST-2, six birds died or were culled during the study due to causes not related to coccidiosis. All mortalities occurred between day 19 and day 22 (2 UUC, 1 IUC, 3 IT). In AST-3, seven birds were culled during the study due to causes not related to coccidiosis (2 UUC, 3 IT, 2 ITP).

Table 2 summarises the results of the three ASTs.

Table 2: Results of anticoccidial sensitivity tests in turkeys

	Tr. group	Average daily feed intake (g)	Average daily weight gain (g)	Feed to gain ratio	Total OPG(2)	Lesion scores	
						E. adenoeides	*E. meleagrimitis*
		D16–22	D16–22	D16–22	D22	D22	
AST-1(1)	UUC	82*/77*	42*	1.85*/1.94*	9*	0.2*	0.3*
	IUC	65/64	24	2.71/2.78	750,948	1.6	2.6
	IT	65/79*	33*	1.92*/2.76	506,784	0.8*	2.3
AST-2	UUC	69	36	1.99	1*	0.2*	0.3*
	IUC	74	34	2.21	486,609	2.1	2.3
	IT	79	37	2.05	790,103	0.5*	2.0
AST-3	UUC	76	40*	1.94*	2*	0.5*	1.0
	IUC	80	34	2.34	318,915	1.7	1.4
	IT	77	37*	2.05*	123,872	0.7*	1.3

AST: anticoccidial sensitivity test.
*: Indicates a significant difference to IUC ($p \leq 0.05$).
(1): Average daily feed intake and average daily weight gain are reported for males/females.
(2): Oocyst count per gram of excreta.

In all tests, intestinal lesion scores due to *E. adenoeides* in the IT group showed significantly lower values than in the IUC group, while lesion scores due to *E. meleagrimitis* were not affected. No difference was seen in the oocyst excretion between IT and IUC groups. In AST-1 and AST-3, the average daily weight gain of the IT group was significantly higher as that of the IUC groups. In AST-3, the feed to gain ratio showed improved values in IT group compared to IUC group.

Synopsis of ASTs

All three AST tests showed in the *Eimeria* infected birds lower intestinal lesion scores due to *E. adenoeides* on study day 22 as a result of the treatment with the additive. No significant effects due to treatment were found for intestinal lesions due to *E. meleagrimitis* and for oocyst excretion. Improved body weight gain was also found in two of the three ASTs. Coccidiosis related mortality was also reduced in the IT group in one of the AST. The data together are considered indicative for an anticoccidial action of the additive.

3.1.3. Other short-term trials

The applicant submitted another short-term study (performed in 2019) in which 14-day-old turkeys (male and female Converter Hybrid) were penned and distributed into two treatment groups: IUC and IT. Both treatment groups included 140 animals housed in 20 floor pens (ten pens per sex) with seven birds per pen. The IT group received feed containing 5 mg Maα/kg feed (confirmed by analysis) for 9

[9] Field isolates collected: AST-1: in November 2017, Belgium, estimated dose per bird: 232,000 *E. meleagrimitis*, 4,800 *E. adenoeides;* AST-2: in January 2018, Belgium, estimated dosage per bird: 55,200 *E. meleagrimitis*, 3,840 *E. adenoeides*; and AST-3: in August 2018, Norway, estimated dosage per bird: 174,000 *E. meleagrimitis*, 22,500 *E. adenoeides*.

days. Two days after the start of dietary treatment, all birds were inoculated via syringe with field isolates of pathogenic *Eimeria* species (collected in August 2018 in Norway). Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured at the beginning and at the end of the study, feed to gain ratio was calculated. Intestinal lesion scoring was performed on all birds at the end of the treatment (over five days). On the same days, excreta were analysed for oocyst content. The results were statistically analysed using analysis of variance (ANOVA). The level of significance was set at $p < 0.05$ using two-sided tests.

The IT group had significantly lower lesion scores than IUC for *E. adenoeides* on days 20 (0.4 vs. 1.4) and 21 (1.4 vs. 2.3), and for *E. meleagrimitis* on day 20 (1.2 vs. 1.9). On days 22 and 23, the IT group had significantly lower mortality related to coccidiosis as compared to IUC (no losses vs. 16 out of 140). Compared to IUC, the IT group had significantly higher average daily weight gain and significantly lower feed to gain ratio. Feed intake was not significantly different.

This study is not considered as an AST due to the lack of a UUC group, however it is considered as supporting evidence of the anticoccidial efficacy of the additive.

4. Conclusions

A positive effect of Cygro® 10G (Maα) in preventing coccidiosis in turkeys was shown in three ASTs. However, owing to the lack of floor pen studies showing a positive effect, the FEEDAP Panel is not in the position to conclude on the efficacy of Cygro® 10G (Maα) for turkeys for fattening.

5. Documentation as provided to EFSA/Chronology

Date	Event
03/01/2019	Dossier received by EFSA. Cygro® 10G in turkeys for fattening submitted by Zoetis Belgium SA
17/01/2019	Reception mandate from the European Commission
29/01/2019	Application validated by EFSA – Start of the scientific assessment
11/04/2019	Spontaneous supplementary information received by EFSA
05/06/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: efficacy studies*
06/09/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
26/11/2019	Request of clarification to the applicant by email – *Issues: efficacy studies*
3/12/2019	Reception of the reply to clarification request
9/1/2020	Request of clarification to the applicant by email – *Issues: efficacy studies*
7/2/2020	Reception of the reply to clarification request
19/3/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA analysis of variance
AST anticoccidial sensitivity test
FEEDAP EFSA Panel on Additives and Products or Substances used in Animal Feed
OPG oocyst count per gram of excreta