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Safety and efficacy of AviMatrix[®] (benzoic acid, calcium formate and fumaric acid) for chickens for fattening, chickens reared for laying, minor avian species for fattening and minor avian species reared to point of lay

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Abstract

AviMatrix[®] (benzoic acid, calcium formate and fumaric acid) is intended to be used as a zootechnical additive (functional group: other zootechnical additives) in feed of chickens for fattening, chickens reared for laying, minor avian species for fattening and minor avian species reared to point of lay to increase performance. The additive has not been authorised in the EU. In 2014, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted an opinion in which, owing to insufficient tolerance data and the limited and inconsistent evidence of efficacy, a definitive conclusion on the safety and efficacy of the additive as a zootechnical additive could not be drawn. Additional data have been provided by the applicant related to the safety and the efficacy of the additive for the target species, which is the subject of this opinion. The FEEDAP Panel considers that the additive is tolerated by chickens for fattening up to the maximum recommended dose (1,000 mg/kg complete feed); this conclusion can be extended to chickens reared for laying. However, since no conclusion could be drawn on the margin of safety, the safety of the additive to minor avian species for fattening and to point of lay cannot be extrapolated. Data from three trials provided evidence that AviMatrix[®] at 500 mg/kg complete feed had the potential to improve the performance of chickens for fattening. The dose proposed for use with chickens reared for laying and minor poultry species for fattening and to point of lay is the same as that demonstrated being efficacious in a physiologically similar major species (chickens for fattening) and it can be reasonably assumed that the mode of action is the same. Consequently, the conclusion on efficacy for chickens for fattening can be extended to chickens reared for laying and extrapolated to minor poultry species for fattening and to point of lay.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Regulation (EC) No 1831/2003¹ establishes the rules governing the Community authorisation of additives for use in animal nutrition and in particular, Article 9 defines the terms of such authorisation by the Commission.

The applicant Novus Europe SA is seeking a Community authorisation of a preparation of benzoic acid, calcium formate and fumaric acid to be used as zootechnical additive for chickens for fattening, chickens reared for laying, minor avian species for fattening and minor avian species to point of lay (Table 1).

Table 1: Description of the substances

Category of additive	Zootechnical additives
Functional group of additive	Other zootechnical additives
Description	Preparation of a lipid matrix containing benzoic acid, calcium formate and fumaric acid
Target animal category	Chickens for fattening, chickens reared for laying, minor avian species for fattening and minor avian species to point of lay
Applicant	Novus Europe S.N.A./S.V.
Type of request	New Opinion

On 2 July 2014, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA) ('Authority'), in its opinion on the safety and efficacy of the product, concluded that limited and inconsistent evidence of efficacy and insufficient tolerance data had been provided, so the FEEDAP Panel was not in a position to draw a definitive conclusion on the safety and efficacy of the additive.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and allow a revision of the Authority's opinion. The new data have been received on 28 November 2016.

In view of the above, the Commission asks the Authority to deliver a new opinion of AviMatrix® (benzoic acid, calcium formate and fumaric acid) as a zootechnical additive for chickens for fattening, chickens reared for laying, minor avian species for fattening and minor avian species to point of lay based on the additional data submitted by the applicant.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information² to a previous application on the same product.³

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of AviMatrix® is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011).

3. Assessment

AviMatrix® is a granulated preparation of benzoic acid, calcium formate and fumaric acid encapsulated in a lipid matrix. It is proposed to be used as a zootechnical additive (functional group: other zootechnical additives) in chickens for fattening, chickens reared for laying, minor avian species for fattening and minor avian species reared to point of lay, with the aim of affecting favourably their performance.

¹ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

² FEED dossier reference: FAD-2017-0007.

³ FEED dossier reference: FAD-2012-0037.

In the supplementary information dossier, the applicant proposes to express the specifications of the additive in terms of minimum or maximum contents and not as a range as expressed in the previous opinion (EFSA FEEDAP Panel, 2015).⁴ Therefore, the additive is specified to contain $\geq 42.5\%$ benzoic acid, $\geq 2.5\%$ calcium formate, $\geq 0.8\%$ fumaric acid and $\geq 45\%$ palm stearin. It is also proposed that silica or calcium carbonate can be used interchangeably as excipients, at a total maximum level of 3%.⁵ The different way of expression of the specification will not have implications on the outcome of the previous assessment, as the product is the same, i.e. the results of the batch to batch variation previously submitted match with the new format of specification proposal. Similarly, it is considered that the inclusion of calcium carbonate as an alternative excipient that can be used replacing silica does not have an impact on either the safety or the efficacy of the product.

The additive is intended to be used in feedingstuffs for chickens for fattening, chickens reared for laying, minor avian species for fattening and minor avian species to point of lay at a recommended use level ranging between 500 (minimum content) and 1,000 (maximum content) mg/kg complete feed. The FEEDAP Panel notes that the applicant has modified the minimum content (formerly 250 mg additive/kg complete feed) and recommends a maximum use level. Both proposals will be considered in the context of the current assessment.

In its previous opinion, the FEEDAP Panel concluded that limited and inconsistent evidence of efficacy and insufficient tolerance data had been provided, so the FEEDAP Panel was not in a position to draw a definitive conclusion on the safety and efficacy of the additive (EFSA FEEDAP Panel, 2015).

The applicant has submitted additional information related to the safety of the additive for the target species and on the efficacy of the additive and this new information is the subject of this opinion.

3.1. Safety for the target species

In its previous assessment (EFSA FEEDAP Panel, 2015), the FEEDAP Panel was not in a position to conclude on the safety of AviMatrix® for the target species considering the weakness of the tolerance study (e.g. lack of clinical biochemistry and pathology data for the proposed recommended dose), the increased feed-to-gain ratio with all doses of AviMatrix® and the statistically significant differences in some of the serum enzyme activities.

The applicant provided a new tolerance study to evaluate the safety of AviMatrix® in chickens for fattening.⁶ A total of 1,800 1-day-old male Ross 308 chickens distributed into 45 floor pens of 40 birds each were used. Chickens were fed a starter diet from day 1 until day 21 and a grower diet from day 22 until day 35, both based on wheat, maize and soybean meal, and presented in mash form. AviMatrix® was incorporated into the basal diets at 0 (control), 250, 500, 1,000 or 3,000 mg/kg complete feed, corresponding to 0-, $\times 0.25$ -, $\times 0.5$ -, $\times 1$ - and $\times 3$ -fold the maximum recommended dose, respectively. Benzoic acid was analysed in all the diets to confirm concentrations of additive in feed. Therefore, there were five experimental treatments (levels of additive inclusion) with nine replicates (pens) per treatment. Feed and water were available *ad libitum* over the experimental period of 35 days. Body weight (BW) and feed intake (FI) were recorded on days 0, 21 and 35, and average daily gain (ADG), average daily feed intake (ADFI) and feed-to-gain ratio were calculated. Mortality was checked daily and the most probable cause of death and culling was recorded.

On day 35, 45 chickens (one per pen) were randomly selected for haematological⁷ and serum biochemistry⁸ parameters. These 45 chickens were killed and examined for gross lesions in the oral cavity, crop and intestinal tract. The experiment was laid out as a randomised block design and data were subjected to analysis of variance considering the pen as the experimental unit. Statistical significances were declared at $p < 0.05$.

Overall mortality rate was low ($< 3.5\%$) and was not treatment related. Performance parameters were not adversely affected and no significant differences between treatments were observed over the whole experimental period. Across all the experimental treatments, average final BW was 2.39 kg

⁴ Text from the previous EFSA Opinion (EFSA FEEDAP Panel, 2015): The additive is specified to contain 42.5–50% benzoic acid, 2.5–3.5% calcium formate, 0.8–1.2% fumaric acid, 3% precipitated silica and 45% palm stearin.

⁵ Technical dossier/Supplementary information November 2016/Annex A.

⁶ Technical dossier/Supplementary information November 2016/Avimatrix_Tolerance_broilers.pdf.

⁷ Red blood cell counts (RBC), reticulocyte count, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), haemoglobin (Hb), haematocrit/packed cell volume (PCV), white blood cell counts (WBC) and white blood cell differentials (basophils, heterophils, lymphocytes, monocytes, eosinophils).

⁸ Alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), glutamic oxalacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), lactate dehydrogenase (LDH), creatinine kinase (CK), glucose, uric acid, total protein, albumin.

(range 2.37–2.42 kg), ADG 66.9 g/day (range 66.3–67.6 g/day), ADFI 102.6 g (range 101.8–103.5 g) and feed-to-gain ratio 1.54 (range 1.53–1.56). No significant effects of AviMatrix® supplementation were observed in blood biochemistry and most haematological parameters. However, for some haematological parameters, there were significant ($p < 0.05$) differences between the control and the $\times 3$ dose treatments. Compared with the unsupplemented control, in the $\times 3$ level, the number of leucocytes (15.3 vs $29.7 \times 10^6/\text{mL}$) and heterophils (5.5 vs $17.4 \times 10^6/\text{mL}$) were decreased, the percentage of lymphocytes was increased (44% vs 27%) and the percentage of heterophils was decreased (36% vs 57%). There was no evidence at necropsy that AviMatrix® exerted adverse effects on the gastrointestinal tract.

The FEEDAP Panel notes that the ratio of neutrophils/lymphocytes was greater in the control group, what may indicate a situation of stress (Davis et al., 2008) in these birds. If this was the case, the haematological profile would be less favourable in the control than in the overdosed treatment. However, in the lack of reliable reference values, the Panel considers that the decrease in the number of total leucocytes and heterophils in the $\times 3$ dose might be an adverse effect.

The FEEDAP Panel considers that the additive is tolerated by chickens for fattening up to the maximum recommended dose (1,000 mg/kg complete feed). This conclusion can be extended to chickens reared for laying. However, no conclusion can be drawn on the margin of safety given the observed changes in the number of white blood cells at threefold overdose of AviMatrix®. Consequently, the conclusion cannot be extrapolated to minor avian species for fattening and to point of lay.

3.2. Efficacy

3.2.1. Efficacy for chickens for fattening

In its previous assessment (EFSA FEEDAP Panel, 2015), the Panel concluded that 'only two studies showed a positive effect of AviMatrix® supplementation on the performance of chickens for fattening. In addition, because of the significant treatment \times study interactions, the results of the meta-analysis cannot be used to assess the efficacy of AviMatrix®. Therefore, the FEEDAP Panel is not in the position to conclude on the efficacy of AviMatrix® in chickens for fattening'.

The applicant provided an additional efficacy study with chickens for fattening. A total of 1,100 one-day-old male Ross 308 chickens were into 50 pens of 22 birds each. The animals were fed a basal wheat–barley–soybean meal diet supplemented with AviMatrix® at either 0 or 500 mg/kg feed. Each treatment was applied to 25 pens (experimental units). Broilers were fed a starter diet (2,950 kcal ME/kg) from day 1 until day 21 and a grower/finisher diet (3,050 kcal ME/kg) from day 22 until day 35. All feeds were in mash form and offered *ad libitum*.

Body weight was recorded at 1, 21 and 35 days. Average daily weight gain and feed-to-gain ratio were calculated for the periods 1–21, 22–35 and 1–35 days. Mortality, culling and the most probable cause of mortality were recorded. Data were subjected to analysis of variance considering the pen as the experimental unit. Statistical significance was declared at $p < 0.05$.

Mortality was low ($< 3\%$) and no treatment related. Supplementation with AviMatrix® resulted in a significant improvement ($p < 0.05$) in final BW, ADG and feed-to-gain ratio over the whole study period (Table 2). No differences were observed in the ADFI.

Results of the two positive studies (studies 1 and 2) assessed in the previous opinion together with the newly submitted study (study 3) are shown in Table 2.

Table 2: Effects of AviMatrix® on the performance of chickens for fattening

Trial (days)	AviMatrix® (mg/kg feed)	Total number of birds (replicates/treatment \times birds/replicate)	Sex	Final weight (kg)	Feed intake (kg/bird)	Feed-to-gain ratio	Mortality (%)
1 ^d (42)	0	1,600 (10 \times 40)	Male	2.51 ^a	4.47 ^a	1.82 ^a	0.3
	266			2.69 ^b	4.65 ^b	1.76 ^b	0.3
	532			2.79 ^c	4.84 ^c	1.76 ^b	0.5
	1,064			2.76 ^{bc}	4.78 ^{bc}	1.76 ^b	1.5

Trial (days)	AviMatrix® (mg/kg feed)	Total number of birds (replicates/treatment × birds/replicate)	Sex	Final weight (kg)	Feed intake (kg/bird)	Feed-to-gain ratio	Mortality (%)
2 ^e (42)	0	800 (10 × 20)	Male	2.61	4.18	1.62 ^a	2.0
	250			2.66	4.24	1.62 ^a	5.0
	500			2.69	4.22	1.59 ^b	4.5
	1,000			2.66	4.20	1.60 ^{ab}	4.0
3 ^f (35)	0	1,100 (25 × 22)	Male	2.19 ^a	3.27	1.51 ^a	1.6
	500			2.26 ^b	3.30	1.48 ^b	2.7

a,b,c: Within each trial and column, values with no common superscript differ significantly ($p \leq 0.05$).

d: Technical dossier/Section IV/Annex_IV_3.1. (Dossier FAD-2012-0037).

e: Technical dossier/Section IV/Annex_IV_3.3. (Dossier FAD-2012-0037).

f: Technical dossier/Supplementary information November 2016/Avimatrix_Efficacy_Broilers.

Considering the results of the current study, along with the two efficacy studies already assessed in the previous opinion (EFSA FEEDAP Panel, 2015), in which the performance of chickens for fattening was positively affected as a result of the supplementation with the additive at 500 mg/kg complete feed, the FEEDAP Panel concludes that AviMatrix® at 500 mg/kg complete feed has the potential to improve the growth of chickens for fattening.

3.2.2. Conclusions on efficacy

Data from three trials provide evidence that the inclusion of AviMatrix® at 500 mg/kg complete feed has the potential to improve the performance of chickens for fattening.

The dose proposed for use with chickens reared for laying and minor poultry species for fattening and to point of lay is the same as that demonstrated being efficacious in a physiologically similar major species (chickens for fattening) and it can be reasonably assumed that the mode of action is the same. Consequently, the conclusion on efficacy for chickens for fattening can be extended to chickens reared for laying and extrapolated to minor poultry species for fattening and to point of lay without a need for specific studies and when used at the same dose.

4. Conclusions

The FEEDAP Panel considers that the additive is tolerated by chickens for fattening up to the maximum recommended dose (1,000 mg/kg complete feed). This conclusion can be extended to chickens reared for laying. However, no conclusion can be drawn on the margin of safety given the observed changes in the number of white blood cells at threefold overdose of AviMatrix®. Consequently, the conclusion cannot be extrapolated to minor avian species for fattening and to point of lay.

Data from three trials provide evidence that the inclusion of AviMatrix® at 500 mg/kg complete feed has the potential to improve the performance of chickens for fattening. The dose proposed for use with chickens reared for laying and minor poultry species for fattening and to point of lay is the same as that demonstrated being efficacious in a physiologically similar major species (chickens for fattening) and it can be reasonably assumed that the mode of action is the same. Consequently, the conclusion on efficacy for chickens for fattening can be extended to chickens reared for laying and extrapolated to minor poultry species for fattening and to point of lay.

Documentation provided to EFSA

- 1) AviMatrix® (preparation of benzoic acid, calcium formate and fumaric acid) for chickens for fattening, chickens reared for laying, minor avian species for fattening and to point of lay. November 2016. Submitted by Novus Europe S.A.
- 2) AviMatrix® (preparation of benzoic acid, calcium formate and fumaric acid) for chickens for fattening, chickens reared for laying, minor avian species for fattening and to point of lay. January 2013. Submitted by Novus Europe S.A.

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FI	feed intake