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Safety and efficacy of a feed additive consisting of the bacteriophages PCM F/00069, PCM F/00070, PCM F/00071 and PCM F/00097 (Bafasal®) for all avian species (Proteon Pharmaceuticals S.A.)

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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 a product consisting of four bacteriophages infecting *Salmonella enterica* ser. Gallinarum B/00111, intended to be used as a zootechnical additive (functional group: other zootechnical additives) for all avian species. The additive (tradename Bafasal®) is not currently authorised in the European Union. Bafasal® is intended to be used in water for drinking and liquid complementary feed to guarantee a minimum daily dose of 2×10^6 PFU/bird, to reduce the *Salmonella* spp. contamination of poultry carcasses and load in the environment, and to improve the zootechnical performance of the treated animals. In a previous opinion, the FEEDAP Panel could not conclude on the additive's potential to be irritant or a dermal sensitiser, or on its efficacy for any avian species due to insufficient data. The applicant provided supplementary information to address these data gaps. The new data showed that Bafasal® is not a skin or eye irritant. No conclusions could be drawn on its skin sensitisation potential. The Panel was not in the position to conclude on the efficacy of Bafasal® to improve the zootechnical performance of the target species based on the available data. The additive showed the potential to decrease the counts of two strains of *Salmonella* Enteritidis in boots swabs and caecal digesta of chickens for fattening. No conclusions could be drawn on the capacity of Bafasal® to reduce the contamination of other *Salmonella enterica* strains, serovars or other species of *Salmonella*. The potential of Bafasal® to reduce the *Salmonella* spp. contamination poultry carcasses and/or the environment is limited. The FEEDAP Panel recommended a post-market monitoring plan to address the potential selection and spread of resistant variants of *Salmonella* to Bafasal®.

Keywords: zootechnical additives, other zootechnical additives, Bafasal®, bacteriophages, safety, efficacy, all avian species

Requestor: European Commission

Question number: EFSA-Q-2022-00196

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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 in particular, Article 9 thereof defines the terms of the authorisation by the Commission.

The applicant, Proteon Pharmaceuticals S.A., is seeking a Community authorisation of cocktail of bacteriophages (3sent1, 8sent65, 8sent1748 & Ssent1)[2] as a feed additive to be used as other zootechnical additive for all avian species (Table 1).

Table 1: Description of the additive

Category of additive	**Zootechnical additives**
Functional group of additive	Other zootechnical additives
Description	Preparation of bacteriophages (3sent1, 8sent65, 8sent1748 & Ssent1)
Target animal category	All avian species
Applicant	Proteon Pharmaceuticals S.A.
Type of request	New opinion

On 17 March 2021, 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 for the users and efficacy of the product:

- The Panel could not conclude on the irritancy of the additive to skin and eyes or on its dermal sensitisation potential due to lack of data.
- The Panel was not in the position to conclude on the efficacy of the additive for any avian species due to insufficient data.

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 15 February 2022.

In view of the above, the Commission asks EFSA to deliver a new opinion on preparation of bacteriophages (3sent1, 8sent65, 8sent1748 & Ssent1) as a feed additive for all avian species based on the supplementary data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

1.2. Additional information

The subject of the assessment is a product consisting of four bacteriophages (PCM F/00069, PCM F/00070, PCM F/00071 and PCM F/00097) intended to be used as a zootechnical additive (functional group: other zootechnical additives: animal welfare and food hygiene) for all avian species.

EFSA issued an opinion on the use of the additive for all avian species (EFSA FEEDAP Panel, 2021).

The additive is not authorised in the European Union.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a supplementary information[3] to a previous application on the same product.[4]

[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] Deposited at the Polish Collection of Microorganisms (PCM) with deposition numbers PCM F/00071, PCM F/00070, PCM F/00069 and PCM F/00097, respectively.
[3] FEED dossier reference: EFSA-Q-2022-00196.
[4] FEED dossier reference: FAD-2017-0039.

The dossier was received on 2 November 2022 and the general information and supporting documentation available on Open.EFSA at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00196.

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 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.[7]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of active substance (trade name of the product) is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

The additive is composed of four bacteriophages (PCM F/00069, PCM F/00070, PCM F/00071 and PCM F/00097) infecting *Salmonella enterica* ser. Gallinarum B/00111, with the tradename Bafasal®. The feed additive will be hereafter referred to as Bafasal®.

Bafasal® is intended to be used as a zootechnical additive (functional group: other zootechnical additives: animal welfare and food hygiene) for all avian species to reduce the *Salmonella* spp. contamination in broilers and in the environment,[9] and to improve the zootechnical performance of the treated animals. The additive is intended to be used in water for drinking and liquid complementary feed to guarantee a minimum daily dose of 2×10^6 plaque-forming units (PFU)/bird, equivalent to 0.04 mL additive/bird per day, during the whole life of the birds.

In a previous opinion, the FEEDAP Panel assessed the safety and the efficacy of Bafasal® when used in all avian species (EFSA FEEDAP Panel, 2021). From the information/data provided, the Panel concluded that the additive is safe for the target species, consumers and the environment. The Panel considered the product to be a potential respiratory sensitiser but could not conclude on its potential to be irritant or a dermal sensitiser. Moreover, the data provided were not sufficient to conclude on the efficacy of the additive for any avian species.

The applicant has provided new data to fill the gaps identified in the user safety assessment and to complement the information supporting the efficacy of the additive for avian species.

3.1. Safety for the user

The applicant provided *in vitro* studies to assess skin irritancy, eye irritancy and skin sensitisation potential of the additive. In all studies, the test item used was the concentrated phage preparation before the dilution step with distilled water, aimed at achieving a final product with minimum of 5×10^7 PFU/mL of product (1.25×10^7 PFU of each phage/mL additive) (EFSA FEEDAP Panel, 2021).

3.1.1. Effect on eyes and skin

The skin irritation potential of Bafasal® was determined in an *in vitro* study conducted following the OECD Testing Guideline (TG) 439.[10] The results showed that the test item is not a skin irritant and should be classified according to UN GHS as 'No Category'.

[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, pp.1–48.
[6] Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements
[7] Available at: https://open.efsa.europa.eu/questions/EFSA-Q-2022-00196
[8] 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.
[9] This claim was added in this application.
[10] Annex III.3.2.

The eye irritation or serious eye damage potential of Bafasal® was determined in an *in vitro* study conducted following the OECD TG 492.[11] The results indicated that the additive is not an eye irritant and should be classified according to UN GHS as 'No Category'.

The skin sensitisation potential of Bafasal® was investigated in an *in vitro* study conducted following the OECD TG 442D.[12] The results showed that the test item was negative in this assay, but according the OECD TG, an additional (*in vitro, in silico* or *in vivo)* test is needed to confirm this result. Therefore, another study[13] conducted following the OECD TG 442 E was performed. The results of this assay indicated that the test item is a skin sensitiser and should be classified according to UN GHS as 'Category 1'.

The FEEDAP Panel acknowledges that there are no current validated methods to test skin sensitisation of microorganisms; therefore, no conclusions can be drawn from the studies above.

3.1.2. Conclusions on safety for the user

Based on the studies submitted, the additive was shown not to be a skin or eye irritant. No conclusions can be drawn on the skin sensitisation potential of the additive.

3.2. Efficacy

The applicant claimed three effects from the intended use of the additive in all avian species: (i) improvement of the zootechnical performance, (ii) reduction of *Salmonella* spp. contamination in carcasses (by decreasing *Salmonella* carriage in birds' caeca at 35 days of age) and (iii) reduction of the environmental loading of *Salmonella* (by reducing the counts in boot swabs samples at 21 days of age).

3.2.1. Efficacy in chickens for fattening

In the previous opinion, the Panel assessed five long-term trials in chickens for fattening, which included data on the zootechnical performance of the birds, and on the *Salmonella* Enteritidis counts in boot swabs at day 21 of age and in caeca at day 35 of age (trials A–E; EFSA FEEDAP Panel, 2021). The applicant also provided a pooled analysis of these data. However, it was not considered in the assessment as one of the trials (A) was not included and no proper justification was provided.

In the current dossier, the applicant submitted two long-term trials in chickens for fattening (trials F[13] and G[14]), including data on the zootechnical performance of the birds and on the *Salmonella* Enteritidis counts in caeca at day 35 of age. The applicant also provided two pooled analyses including in each, data from the non-significant studies from the previous and current applications regarding (i) *Salmonella* Enteritidis boot swab counts at day 21 of age (trials A, D, E) and (ii) *Salmonella* Enteritidis caecal counts at day 35 of age (trials C, D, E, F).

All long-term trials shared the same experimental design in which the birds were allocated to three treatment groups, depending on the infection of the birds with *Salmonella enterica* serovar Enteritidis (strain 12, for trials A, B, C, D, F and G; and strain 65/S/10 for trial E) and/or the supplementation of Bafasal® at 2×10^6 PFU/bird per day via water for drinking. Therefore, the three experimental groups in each trial were uninfected untreated control group (UUC); infected untreated control group (IUC) and infected treated group (IT).

Experimental procedures and results of trials A–E were described in the previous opinion (EFSA FEEDAP Panel, 2021). A summary of the design of trials F and G is provided in Table 2.

[11] Annex III.3.1.
[12] Annex III.3.3.
[13] Annex_IV_4_3_7 and Reply in 2_SIn_Reply_BAFASAL_Jul22 and Annex_IV_3_Calculations_Jul22.
[14] Annex IV_4_3_8 and Reply in 2_SIn_Reply_BAFASAL_Jul22 and Annex_IV_3_Calculations_Jul22.

Table 2: Trial design of the newly submitted efficacy studies in chickens for fattening

Trial	Total N (bird/ replicate) Replicate/treatment	Breed Sex	Duration (Starter/Grower)	Composition feed (form)	Groups[(a)]
F[(b)]	528 (22) 8	Ross 308 Male	35 days (1–21/22–35)	Maize, wheat, soybean meal (mash)	UUC IUC IT
G[(c)]	528 (22) 8	Ross 308 Male	35 days (1–21/22–35)	Maize, wheat, soybean meal (mash)	UUC IUC IT

(a): UUC (uninfected untreated control group): not challenged not treated; IUC (infected untreated control group): challenged with *Salmonella* Enteritidis 12; IT (infected treated group): challenged as in the IUC group and given Bafasal® at 2×10^6 PFU/bird/day via water for drinking.
(b): Dose of infection of birds with *Salmonella enterica* serovar Enteritidis 12 in IUC and IT groups at day 5 of age is 1.0×10^8 PFU/bird.
(c): Dose of infection of birds with *Salmonella enterica* serovar Enteritidis 12 in IUC and IT groups at day 5 of age is 1.5×10^8 PFU/bird.

In these trials (F and G), the pens receiving Bafasal® were placed in a separate experimental room from the other two treatments, which were also physically separated within the same room to avoid cross-contamination. The basal diets of the respective trials, same for all treatment groups, were offered ad libitum for 35 days. Bafasal® was added at a rate of 2×10^6 PFU/bird per day to the water of the IT group (confirmed by analysis, see Table 3).[15] At day 5 of age, all birds from the IUC and IT treatments groups were infected with *Salmonella enterica* serovar Enteritidis 12 by gavage at a dose of ca. 1.0×10^8 CFU/bird (trial F) or 1.5×10^8 CFU/bird (trial G).

The general health status and mortality of birds were monitored daily, dead animals were weighed and the most likely cause of death determined. The birds were weighed at the start of the experiment (day 1 of age). Thereafter, body weight and feed intake were recorded for each pen at days 21 and 35 of age, and the water intake on a weekly basis. The average daily feed intake, average daily gain and feed to gain ratio were calculated and corrected for mortality for the periods 1–21, 22–35 and 1–35 days.

Table 3: Exposure of birds to Bafasal® in the infected treated group (IT) throughout the experiment in the newly submitted efficacy trials

Day	Trial			
	F		G	
	PFU/L water	PFU/bird/day	PFU/L water	PFU/bird/day
7	2.0×10^8	6.9×10^4	7.3×10^7	3.7×10^6
14	2.0×10^8	1.4×10^5	1.1×10^8	8.0×10^6
21	< LOQ	n/a	7.2×10^7	1.4×10^7
28	7.9×10^7	2.0×10^7	6.6×10^7	2.4×10^7
35	1.3×10^8	4.3×10^7	6.3×10^7	2.3×10^7

n/a: not analysed.

At the start of the trial, samples of feed (data not shown), meconium before allocation of birds (one per treatment, data not shown) and boot swabs (one pooled sample of two swabs) were collected to investigate the potential *Salmonella* spp. background contamination. At day 35 of age, two birds per pen were randomly selected and euthanised, and caeca sampled, pooled and analysed[16] for the presence (UUC group) or enumeration (IUC and IT groups) of *Salmonella* spp.

All data were analysed with an analysis of variance, with the dietary treatment as fixed effect. Mean values were compared with Tukey's test. Significance was declared at 0.05.

[15] 2022-08-02_EFSA-Q-2022-00196_Bacteriophages_Reply to request for supplementary information_APPL to EFSA.
[16] A miniaturised Most Probable Number (MPN) method with confirmation on xylose lysine deoxycholate (XLD) agar following ISO/TS6579-2 was used for the enumeration of *Salmonella* spp.

Zootechnical performance

In three trials of the previous opinion (B, C, D), the birds of the UUC group showed low performance (ca. 75% of expected body weight for the standards of the breed); therefore, these trials were not considered further for the assessment of the zootechnical performance. In the remaining two trials (A, E), the additive had a negative effect on performance (EFSA FEEDAP Panel, 2021).

In the newly submitted trials (F and G), the animals in the IUC group showed higher mortality, lower feed intake and final body weight and higher feed to gain ratio in comparison with the birds in the UUC group. These results suggest that the inoculation with *Salmonella enterica* serovar Enteritidis 12 impaired the birds' performance. Therefore, the Panel considers that the design of the studies is not appropriate for the evaluation of the effects of the additive on the birds' zootechnical performance. However, the results of the counts in caeca from these trials could be used to assess the effects of the additive on the reduction of the *Salmonella* spp. carriage. The same reasoning would apply for the results obtained in all pooled analyses provided.

Boots swabs counts at day 21 of age

Regarding the *Salmonella* Enteritidis load in boot swabs samples,[17] no results were included in the previous opinion, as the applicant did not claim for the reduction of the environmental load of *Salmonella*. According to the data provided in the context of the previous assessment, the IT group showed lower *Salmonella* Enteritidis counts than the IUC group in boot swabs samples taken at 21 days of age in trials B and C. The same results were obtained from the pooled analysis submitted in the current dossier, which includes data from trials A, D and E (see Table 4).

Table 4: *Salmonella* Enteritidis counts (log Most Probable Number (MPN)/boot cover) in boot swab samples at 21 days of age

Trial	Groups(1)		
	UUC	IUC	IT
B	n/d	5.88^{a}	4.55^{b}
C	n/d	4.12^{a}	2.48^{b}
Pooled A/D/E	n/d	2.77^{a}	1.86^{b}

n/d: not detected.

^{a,b}: Mean values within a row with a different superscript are significantly different $p < 0.05$.

(1): UUC (uninfected untreated control group): not challenged not treated; IUC (infected untreated group): challenged with *Salmonella* Enteritidis 12; IT (infected treated group): challenged as in the IUC group and given Bafasal® at 2×10^6 PFU/bird/day via water.

Considering all the data provided, the Panel concludes that the additive has the potential to decrease the counts of *Salmonella* Enteritidis (strains 12 and 65/S/10) in boot swabs (day 21 of age) when included in the water for drinking of chickens for fattening at the recommended use level.

Caecal counts at day 35 of age

No statistically significant differences were observed in the caecal counts of *Salmonella* Enteritidis at day 35 in trials C, D, E (data shown in previous opinion, EFSA FEEDAP Panel, 2021) or F (IUC: 1.60 vs. IT: 0.73 log MPN/g). In trials A, B and G, and the pooled analysis including data from trials C, D, E and F, the supplementation of water for drinking with Bafasal® at 2×10^6 PFU/bird per day was associated with lower *Salmonella* Enteritidis counts in caecal samples collected at day 35 in the IT group compared to the IUC group (Table 5).

[17] 1 sample/pen from 12 pens for all groups in trial A, 1 sample/pen from 6 pens for UUC and 2 samples/pen from 6 pens for IUC and IT groups in trials B, C and D and 1 sample/pen from 10 pens for all groups in trial E.

Table 5: *Salmonella* Enteritidis counts (log Most Probable Number (MPN)/g) in caecal samples at 35 days of age

Trial	Group(1)		
	UUC	IUC	IT
A	n/d	2.54[a]	1.26[b]
B	n/d	1.68[a]	0.45[b]
G	n/d	5.68[a]	1.94[b]
Pooled C/D/E/F	n/d	2.19[a]	0.97[b]

n/d: not detected.
[a,b]: Mean values within a row with a different superscript are significantly different $P < 0.05$.
(1): UUC (uninfected untreated control group): not challenged not treated; IUC (infected untreated group): challenged with *Salmonella* Enteritidis 12; IT (infected treated group): challenged as in the IUC group and given Bafasal® at 2×10^6 PFU/bird/day via water.

Considering all the data provided, the Panel concludes that the additive has the potential to decrease the counts of *Salmonella* Enteritidis (strains 12 and 65/S/10) in caecal digesta (day 35 of age) when included in the water for drinking of chickens for fattening at the recommended use level.

3.2.2. Microbiological considerations

Overall, the inclusion of Bafasal® in the water for drinking of chickens for fattening led to a statistically significant reduction of about 1 log of *Salmonella* Enteritidis caecal counts (strains 12 and 65/S/10) at the end of the experimental period (35 days of age).

Similarly, the inclusion of Bafasal® in the water for drinking of chickens for fattening led to a significant reduction (at least 1 log) of *Salmonella* Enteritidis counts (strains 12 and 65/S/10) in environmental samples. It can be reasonably assumed that a reduction of the *Salmonella* counts in faecal matter (for which the boot swabs are an acceptable sample) would also reduce the environmental contamination of *Salmonella*. However, this would only apply for the strains susceptible to the Bafasal® bacteriophages.

As regards the impact of the use of Bafasal® on the quality of the food commodities derived from the animals receiving this additive, a distinction should be made between the infection route through eggs and the one through poultry meat. The infection route through contaminated eggs is nearly exclusively due to *Salmonella* Enteritidis and constitutes an important infection route of this serovar from poultry to human. This transmission route is, however, not directly connected with the caecal route and would therefore not be directly affected by the use of Bafasal®. The only effect of a reduction of the caecal *Salmonella* content would be through a reduction of the environmental transmission to the flock. The caecal route is of main importance for poultry meat contamination due to faecal contamination during slaughter and meat processing. However, poultry carcasses may be contaminated with a high diversity of *Salmonella* serotypes and, therefore, for Bafasal® to be efficacious, it should be active towards a larger variety of *Salmonella* strains (EFSA BIOHAZ Panel, 2019).

The FEEDAP Panel notes that the bacteriophages have been tested *in vivo* against only two independent strains of *Salmonella* Enteritidis (12 and 65/S/10),[18] but not against other *Salmonella* Enteritidis strains or *Salmonella* serovars with high prevalence in chickens for fattening and other avian species for which the authorisation is sought.

To determine the host range of the Bafasal® bacteriophages, the applicant conducted spot tests with a collection of strains of several serovars (e.g. Brandenburg, Infantis, Typhimurium, Virchow, Gallinarum, Paratyphi) described as being of different origins (i.e. isolated in different geographical areas, dates, sample type).[19] The clonal relationship among some of these strains was established by pulsed-field gel electrophoresis (Zaczek et al., 2015).[20] The FEEDAP Panel has considered only the data of the strains that have been properly serotyped and for which the clonal relationship has been established and supported with analytical evidence (a total of 48).

[18] 2_SIn_Reply_BAFASAL_Jul22 and Annexex IV_1_9.
[19] 2_SIn_Reply_BAFASAL_Jul22 and Annexes IV_1_5, IV_1_6, IV_1_7, IV_1_8 and Supplementary_information_BAFASAL_02SEP22.
[20] 2_SIn_Reply_BAFASAL_Jul22 and Żaczek et al., 2015.

The results of the spot tests are reported with the codes 'cl', '+' and '−'. The '+' is explained as 'slowing down the growth of the bacterial strain' (i.e. in this case bacterial growth was observed, but it was weaker compared to that of bacterial growth, where no bacteriophages were spotted), while 'cl' is described as 'clear lysis' and '−' as no lysis. In the overall interpretation, the results 'cl' and '+' are taken together and considered as reacting to the Bafasal® treatment (Kutter, 2009). The spot tests conducted on independent strains resulted in:

- Six *Salmonella* Enteritidis strains showing clear lysis, 25 showing reduced growth and one giving intermediate results ('cl'/'+');
- One strain of Virchow and three strains of Typhimurium showing a clear lysis;
- Two Virchow, one Hadar, two Infantis, three Typhimurium, one Brandenburg strains showing reduced growth;
- One Infantis and one Typhimurium strains showing intermediate results ('−'/'cl' and 'cl'/'+'/'c l', respectively);
- One Paratyphi strain was indicated as negative (showing no lysis).

Little or no details were provided on the methodology followed (e.g. no information on incubation period, persistence of effects, no pictures made available).

The FEEDAP Panel has reservations on the biological and practical relevance of the results linked to a delayed growth (described as '+'). Therefore, these have not been considered as proof of lytic activity of the bacteriophages.

Consequently, from the data available, it can be concluded that Bafasal® could potentially reduce the load up to 20% of the tested *Salmonella* Enteritidis strains in the caecal digesta of chickens for fattening, and consequently, in the environment. As only about 1 log reduction in caecal content was seen for the two *Salmonella* Enteritidis strains tested *in vivo* and showing a clear lysis in the spot tests, it can be hypothesized that the effect on the reduction of *Salmonella* strains showing only a reduced growth in the spot test would be more limited, and probably would not be of biological relevance. In the absence of a robust evidence of lytic activity against a much larger diversity of *Salmonella* strains with high prevalence in chickens for fattening and other avian species for which authorisation is sought, it can only be concluded that the potential of Bafasal® to reduce the *Salmonella enterica* serovars contamination of poultry carcasses and/or the environment is limited.

Additionally, the FEEDAP Panel notes that the load of *Salmonella* in the caecum of poultry species is only one of the multiple factors that contribute to the *Salmonella* contamination of the carcasses.

3.3. Post-market monitoring

The FEEDAP Panel notes that the presented studies are not intended or capable of detecting the potential development of resistance to Bafasal® or the spread of the resistant varieties that might occur over a long exposure to the Bafasal® product. For this reason, a post-market monitoring plan is recommended to address the above.

4. Conclusions

Bafasal® is not a skin or eye irritant but should be considered a respiratory sensitiser. No conclusions can be drawn on the skin sensitisation potential of the additive.

The Panel is not in the position to conclude on the efficacy of Bafasal® to improve the zootechnical performance of the target species based on the available data. The additive showed the potential to decrease the counts of two strains of *Salmonella* Enteritidis in boot swabs and caecal digesta of chickens for fattening. No conclusions can be drawn on the capacity of Bafasal® to reduce the contamination of other *Salmonella enterica* strains, serovars or other species of *Salmonella*. Therefore, the potential of Bafasal® to reduce the *Salmonella* spp. contamination of poultry carcasses and/or the environment is limited. Moreover, the FEEDAP Panel notes that the load of *Salmonella* in the caecum of poultry is only one of the multiple factors that contribute to the *Salmonella* contamination of the carcasses.

5. Recommendations and/or remarks

The Panel recommends a post-market monitoring plan to address the potential selection and spread of *Salmonella* variants resistant to Bafasal®.

References

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez- Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Dewulf J, Hald T, Michel V, Niskanen T, Ricci A, Snary E, Boelaert F, Messens W and Davies R, 2019. Scientific Opinion on the *Salmonella* control in poultry flocks and its public health impact. EFSA Journal 2019;17(2):5596, 155 pp.https://doi.org/10.2903/j.efsa.2019.559

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

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.2018.5274

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances use in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Fasmon Durjava M, Lopez-Alonso M, López Puente S, Marcon F, Mayo B, Pechov AA, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Cocconcelli PS, Glandorf B, Herman L, Prieto Maradona M, Saarela M, Dierick N, Martelli G, Brantom P, Tosti L, Svensson K, Anguita M, Galobart J, Innocenti M, Pettenati E, Revez J and Brozzi R, 2021. Scientific Opinion on the safety and efficacy of a feed additive consisting on the bacteriophages PCM F/00069, PCM F/00070, PCM F/00071 and PCM F/00097 infecting *Salmonella Gallinarum* B/00111 (Bafasal®) for all avian species (Proteon Pharmaceuticals S.A.). EFSA Journal 2021;19(5):6534, 17 pp. https://doi.org/10.2903/j.efsa.2021.6534

Kutter E, 2009. Phage host range and efficiency of plating. In: Clokie MRJ and Kropinski AM (eds.). Bacteriophages: Methods and Protocols. Volume 1: Isolation, Characterization, and Interactions. 501. Humana Press, New York. pp. 141–149.

Zaczek A, Wojtasik A, Izdebski R, Gorecka E, Wojcik EA, Nowak T, Kwiecinski P and Dziadek J, 2015. PCR melting profile as a tool for outbreak studies of Salmonella enterica in chickens. BMC Veterinary Research, 11, 137. https://doi.org/10.1186/s12917-015-0451-4

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOQ	limit of quantification
PFU	plaque-forming unit