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## Safety and efficacy of FRA<sup>®</sup> Octazyme C Dry (endo-1,4- $\beta$ -xylanase, mannan-endo-1,4- $\beta$ -mannosidase, $\alpha$ -amylase, endo-1,3(4)- $\beta$ -glucanase, pectinase, endo-1,4- $\beta$ -glucanase, protease, $\alpha$ -galactosidase) as a feed additive for weaned piglets and chickens for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)  
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### Abstract

The product FRA<sup>®</sup> Octazyme C Dry contains eight enzymes and is intended to be used as a zootechnical additive for chickens for fattening and weaned piglets. In a previous opinion of the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), the additive was characterised in full, including the production strains of the enzymes, the safety and the efficacy of the product. In that assessment, the FEEDAP Panel concluded that the additive was safe for the consumers of food products obtained from animals fed with the additive and that the additive would pose no risks to the environment. Regarding the safety for the users, the Panel could not conclude on the potential of the additive to be irritant to the skin and eyes or on its skin sensitising properties, but the additive should be considered a potential respiratory sensitiser. In the trials submitted to support the safety for the target species and the efficacy of the additive, the analytical results of the enzyme activities did not confirm the intended enzyme activities and therefore the FEEDAP Panel could not draw conclusions on the tolerance and the efficacy of the additive. In the current application, the applicant has provided new analysis of the feeds used in the tolerance and efficacy trials. The data have been obtained using a modified extraction procedure which has demonstrated to permit a higher recovery of the enzyme activity in supplemented and non-supplemented feeds. However, the enzyme activities obtained in the experimental diets using the new analytical method showed increases but also decreases of the enzyme activity compared to the previously submitted data. Therefore, the change in the enzyme activities could not be ascribed to the analytical method only. The Panel concluded that the new data cannot be considered as those reflecting the actual supplementation levels of the additive in the diets and therefore could not conclude on the safety and efficacy of the target species.

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**Keywords:** Zootechnical additives, digestibility enhancers, FRA<sup>®</sup> Octazyme C Dry, safety, efficacy, chickens for fattening, weaned piglets

**Requestor:** European Commission

**Question number:** EFSA-Q-2018-00798

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## 1. Introduction

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

Regulation (EC) No 1831/2003<sup>1</sup> establishes the 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, Framelco B.V., is seeking a Community authorisation of  $\alpha$ -galactosidase,  $\alpha$ -amylase, endo-1,3(4)- $\beta$ -glucanase, endo-1,4- $\beta$ -glucanase, mannan-endo-1,4- $\beta$ -mannosidase, pectinase, protease, endo-1,4- $\beta$ -xylanase as a feed additive to be used as a digestibility enhancer for piglets (weaned) and chickens for fattening (Table 1).

**Table 1:** Description of the substances

<b>Category of additive</b>	Zootechnical additive
<b>Functional group of additive</b>	Digestibility enhancers
<b>Description</b>	$\alpha$ -galactosidase, $\alpha$ -amylase, endo-1,3(4)- $\beta$ -glucanase, endo-1,4- $\beta$ -glucanase, mannan-endo-1,4- $\beta$ -mannosidase, pectinase, protease, endo-1,4- $\beta$ -xylanase
<b>Target animal category</b>	Piglets (weaned) and chickens for fattening
<b>Applicant</b>	Framelco B.V.
<b>Type of request</b>	New opinion

On 6 July 2017, 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 could not conclude on the safety and efficacy for the target species due to the limitations identified in the studies provided.

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 received on 13 September 2018.

In view of the above, the Commission asks the Authority to deliver a new opinion on  $\alpha$ -galactosidase,  $\alpha$ -amylase, endo-1,3(4)- $\beta$ -glucanase, endo-1,4- $\beta$ -glucanase, mannan-endo-1,4- $\beta$ -mannosidase, pectinase, protease, endo-1,4- $\beta$ -xylanase as a feed additive for piglets (weaned) and chickens for fattening 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 the product Fra® Octazyme C Dry as a feed additive for chickens for fattening and weaned piglets (EFSA FEEDAP Panel, 2017). The Panel could not conclude on the safety or the efficacy of the additive for the target species. The applicant has provided new data to address the limitations previously identified regarding the safety and the efficacy for the target species.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information<sup>2</sup> to a previous application on the same product.<sup>3</sup>

<sup>1</sup> 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.

<sup>2</sup> FEED dossier reference: FAD-2018-0052.

<sup>3</sup> FEED dossier reference: FAD-2014-0028.

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Fra® Octazyme C Dry (endo-1,4- $\beta$ -xylanase, mannan-endo-1,4- $\beta$ -mannosidase,  $\alpha$ -amylase, endo-1,3(4)- $\beta$ -glucanase, pectinase, endo-1,4- $\beta$ -glucanase, protease,  $\alpha$ -galactosidase) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>4</sup> and the relevant guidance documents: Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011) and Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012).

## 3. Assessment

The product FRA® Octazyme C Dry is available in powder form and contains a minimum activity of 160,000 BXU of endo-1,4- $\beta$ -xylanase (xylanase), 1,000 U<sub>M</sub> of mannan-endo-1,4- $\beta$ -mannosidase (mannanase), 10,000 U<sub>A</sub> of  $\alpha$ -amylase (amylase), 20,000 BU of endo-1,3(4)- $\beta$ -glucanase (glucanase), 1,500 U<sub>PR</sub> of protease, 3,200 U<sub>G</sub> of endo-1,4- $\beta$ -glucanase (cellulase), 2,100 U<sub>P</sub> of pectinase and 80 GALU of  $\alpha$ -galactosidase (galactosidase) per gram of product.<sup>5</sup> The additive is intended to be used as a zootechnical additive (functional group: digestibility enhancers) in feed for chickens for fattening and weaned piglets at a recommended level of 50 mg additive/kg feed (delivering 8,000 BXU xylanase units, 50 U<sub>M</sub> mannanase, 500 U<sub>A</sub> amylase, 1,000 BU glucanase, 75 U<sub>PR</sub> protease, 160 UG cellulase, 105 U<sub>P</sub> pectinase and 4 GALU galactosidase per kg feed).

In a previous opinion of the FEEDAP Panel, the additive was characterised in full, including the production strains of the enzymes; also the safety and the efficacy of the product were evaluated (EFSA FEEDAP Panel, 2017). In that assessment, the Panel concluded that the additive is safe for the consumers of food products obtained from animals fed with the additive and that the additive would pose no risks to the environment. Regarding the safety for the users, the Panel could not conclude on the potential of the additive to be irritant to the skin and eyes or on its skin sensitising properties, but owing to the nature of the active substances, the FEEDAP Panel concluded that the additive should be considered a potential respiratory sensitiser.

In the above-mentioned opinion, one tolerance and three efficacy trials each in chickens for fattening and weaned piglets were assessed. The analytical results of the diets used did not confirm the intended dosages (Appendix A). The non-supplemented diets presented in most of the cases enzyme activities close or even higher than the ones in the diets supplemented with the recommended dose. In the tolerance trials, the 100-fold level was not reached, while, in some of the efficacy trials, the supplemented diets showed enzyme activities that were higher than the intended ones (Appendix A; Table A.1). Moreover, the method of analysis used in some of the efficacy trials and for some of the enzymes had a limit of detection/quantification higher than the enzyme activity recommended (Appendix A; Tables A.2 and A.3). Considering all these limitations on the analysis of the enzyme activities present in the feeds offered to the animals, the FEEDAP Panel could not draw conclusions on the tolerance and the efficacy trials.

The applicant has provided new data to address the limitations identified regarding the enzyme activities analysed in the experimental diets.

The applicant re-analysed samples of the diets used in the tolerance and efficacy trials which had been stored since the conduct of the studies (2011–2012) under vacuum and at  $-20^{\circ}\text{C}$ . The analytical methods used in this new analysis were those previously described by the applicant but included a modification of the extraction procedure. The modification consisted in an adjustment of the temperature at which the extraction is done, the buffer used, and the stirring method applied. The feed samples were not re-analysed using the non-modified extraction procedure at the time the new analysis was conducted, which would have allowed a comparison between the two extraction methods.

The enzyme activities resulting from the new analysis are given in the tables presented in the Appendix A. The results obtained in the non-supplemented diets showed in most of the cases lower enzyme activities than those previously reported and assessed in 2017. With regard to the supplemented diets, the new analysis showed a better correspondence with the intended enzyme activities. The new

<sup>4</sup> 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.

<sup>5</sup> The details on the enzyme activities definition given in <https://ec.europa.eu/jrc/sites/jrcsh/files/finirep-fad-2014-0028-fraoctazymec.pdf>

enzyme activities were in some cases higher and in some other cases lower compared to the previously reported, with no clear tendency/pattern and with different magnitude between diets and enzymes.

The different behaviour of the new extraction step between diets and enzymes was not explained by the data submitted. Therefore, the applicant was requested to perform a comparison of the two analytical methods with the diets used in the studies to evaluate the effect of the new extraction method. This comparison was not done because no more samples of the experimental feeds were available. Instead, the applicant provided a comparison of the eight enzyme activities in feed when using the extraction procedure or not using it. The feeds used in this comparison had a similar composition to those used in the trials previously evaluated and were supplemented or not with the additive.<sup>6</sup> The results showed that the samples analysed using the new extraction method had, in all cases, higher enzyme activities than the samples analysed not using the new extraction method.

However, the results found in the re-analysis of the feeds used in the trials did not indicate such pattern (i.e. increases of the enzyme activity in all cases). Consequently, the change in the enzyme activities of the diets used in the tolerance and efficacy trials could not be ascribed only to the extraction method, but also to other non-explained factors. In the absence of a clear explanation for the differences observed, the FEEDAP Panel is not in a position to accept the new analytical results as those reflecting the actual supplementation levels of the additive in the diets. Therefore, the FEEDAP Panel reiterates its previous opinion that no conclusions on the safety and the efficacy of the additive in chickens for fattening and piglets can be drawn with the current set of data.

The FEEDAP Panel noted that the newly provided results are in some cases below the previously reported limit of quantification (LOQ), but the applicant has not properly explained how quantification was done for values below the limit of quantification.

### 3.1. Conclusions on the safety and efficacy for the target species

The FEEDAP Panel is not in the position to accept the new analytical results, as those reflecting the actual supplementation levels of the additive in the diets. Therefore, the Panel reiterates its previous opinion that no conclusions on the safety and the efficacy of the additive in weaned piglets and chickens for fattening can be drawn with the current set of data.

### 3.2. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation<sup>7</sup> and Good Manufacturing Practice.

## 4. Conclusion

The FEEDAP Panel cannot conclude on the safety and the efficacy of the additive FRA® Octazyme C Dry for weaned piglets and chickens for fattening based on the additional data submitted.

## 5. Recommendation

The Panel considers that methods allowing for quantification of the recommended enzyme activities in the feedingstuffs should be developed.

## Documentation as provided to EFSA/Chronology

Date	Event
30/07/2018	Dossier received by EFSA. FRA® Octazyme C Dry. Submitted by Framelco B.V.
11/10/2018	Reception mandate from the European Commission
24/10/2018	Application validated by EFSA – Start of the scientific assessment
20/12/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: safety for the consumer</i>
28/01/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
16/05/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

<sup>6</sup> FAD-2018-0052/Supplementary information February 2018/Annexes 2.a to 5.b.

<sup>7</sup> Regulation (EC) No 1831/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

## References

- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: Tolerance and efficacy studies in target animals. *EFSA Journal* 2011;9(5):2175, 15 pp. <https://doi.org/10.2903/j.efsa.2011.2175>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance for the preparation of dossiers for zootechnical additives. *EFSA Journal* 2012;10(1):2536, 19 pp. <https://doi.org/10.2903/j.efsa.2012.2536>
- 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 Puente S, López-Alonso M, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Dierick NA, Aguilera J and Anguita M, 2017. Scientific Opinion on the safety and efficacy of FRA®Octazyme C Dry ( $\alpha$ -galactosidase,  $\alpha$ -amylase, endo-1,3(4)- $\beta$ -glucanase, endo-1,4- $\beta$ -glucanase, mannan-endo-1,4- $\beta$ -mannosidase, pectinase, protease, endo-1,4- $\beta$ -xylanase) for chickens for fattening and weaned piglets. *EFSA Journal* 2017;15(8):4943, 20 pp. <https://doi.org/10.2903/j.efsa.2017.4943>

## Abbreviations

EFSA FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
LOQ	limit of quantification

## Appendix A – Enzyme activity in the feeds used in the tolerance and efficacy trials in target species

**Table A.1:** Mean analysed enzyme activity previously reported (previous) or newly submitted (new) for the diets used in the efficacy trials in chickens for fattening and piglets

Trial	Species	Diet	Xylanase	Mannanase	Amylase	Glucanase	Protease	Cellulase	Pectinase	Galactosidase
			BXU/kg	U <sub>M</sub> /kg	U <sub>A</sub> /kg	BU/kg	U <sub>PR</sub> /kg	U <sub>G</sub> /kg	U <sub>P</sub> /kg	GALU/kg
		<b>Expected at 50 mg/kg</b>	<b>8,000</b>	<b>50</b>	<b>500</b>	<b>1,000</b>	<b>75</b>	<b>160</b>	<b>105</b>	<b>4</b>
Tolerance	Chickens	Control – previous	5,877	110	11,027	470	< 10	< 10	61	70
		Control – new	5,724	77	514	39	0	0	0	0
		50 mg/kg – previous	10,830	139	9,603	2,640	151	< 10	133	64
		50 mg/kg – new	10,567	164	1,779	1,388	72	170	119	7
		5,000 mg/kg – previous	359,600	279	21,367	42,200	103	1,048	2,983	113
		5,000 mg/kg – new	575,098	2,333	29,274	109,323	4,857	8,826	6,930	296
Tolerance	Weaned piglets <sup>(1)</sup>	Control – previous	122,000	< 10	104,000	64,100	336	1,680	65,700	726
		Control – new	3,343	49	511	3,840	0	0	64	3
		50 mg/kg – previous	20,464	444	36,300	3,550	171	< 10	925	268
		50 mg/kg – new	9,597	226	2,591	8,050	118	287	226	49
		5,000 mg/kg – previous	759,000	638	85,650	67,950	400	1,670	51,400	682
		5,000 mg/kg – new	699,207	4,702	52,784	57,488	5,987	8,752	17,900	302

(1): Two basal diets, pre-starter and starter, were used in the study. For the control diet the enzyme activities provided for the starter diet were not considered since it was indicated that they belonged to the starter diet containing 5,000 mg additive/kg feed.



**Table A.2:** Mean analysed enzyme activities previously reported (previous) and newly submitted (new) in the diets used in the efficacy trials in chickens for fattening

Trial	Basal diet	Diet	Xylanase	Mannanase	Amylase	Glucanase	Protease	Cellulase	Pectinase	Galactosidase
			BXU/kg	U <sub>M</sub> /kg	U <sub>A</sub> /kg	BU/kg	U <sub>PR</sub> /kg	U <sub>G</sub> /kg	U <sub>P</sub> /kg	GALU/kg
		<b>Expected at 50 mg/kg</b>	<b>8,000</b>	<b>50</b>	<b>500</b>	<b>1,000</b>	<b>75</b>	<b>160</b>	<b>105</b>	<b>4</b>
Efficacy 1	Wheat	Control – previous	7,166	< 200	15,666	< 4,000	< 1,500	320	49	264
		Control – new	3,391	0	898	1,213	41	0	4	12
		50 mg/kg – previous	7,976	< 200	52,033	6,066	< 1,500	365	118	264
		50 mg/kg – new	7,674	96	18,740	2,514	89	183	78	34
	Maize	Control – previous	4,433	< 200	2,317	< 4,000	< 1,500	< 300	22	65
		Control – new	1,608	0	709	585	7	42	3	12
		50 mg/kg – previous	6,223	< 200	7,640	6,500	< 1,500	< 300	66	67
		50 mg/kg – new	6,591	157	2,849	2,668	291	200	79	49
Efficacy 2	Wheat	Control – previous	7,267	< 200	26,333	7,000	3,000	340	37	180
		Control – new	1,983	0	690	529	30	36	0	16
		50 mg/kg – previous	6,677	< 200	24,067	7,050	< 1,500	310	92	122
		50 mg/kg – new	8,225	184	6,752	916	135	294	80	42
	Maize	Control – previous	6,600	< 200	8,333	< 4,000	< 1,500	< 300	19	60
		Control – new	1,835	33	1,748	332	0	0	0	11
		50 mg/kg – previous	22,213	< 200	311,077	58,500	< 1,500	154,000	152	39
		50 mg/kg – new	12,102	141	4,214	7,701	125	368	116	24
Efficacy 3	Wheat	Control – previous	7,900	< 200	21,000	4,233	5,167	383	70	404
		Control – new	1,197	0	585	284	62	0	6	10
		50 mg/kg – previous	5,443	< 200	23,533	6,733	< 1,500	330	75	159
		50 mg/kg – new	7,141	144	4,942	1,694	209	310	92	22
	Maize	Control – previous	9,500	< 200	10,067	7,867	< 1,500	320	53	178
		Control – new	1,765	30	557	452	0	0	0	2
		50 mg/kg – previous	7,560	< 200	7,073	5,033	1,500	< 300	84	68
		50 mg/kg – new	10,209	140	2,587	1,490	91	185	101	15

**Table A.3:** Mean analysed enzyme activities previously reported (previous) or newly submitted (new) in the diets used in the efficacy trials in weaned piglets

Trial	Diet	Xylanase	Mannanase	Amylase	Glucanase	Protease	Cellulase	Pectinase	Galactosidase
		BXU/kg	U <sub>M</sub> /kg	U <sub>A</sub> /kg	BU/kg	U <sub>PR</sub> /kg	U <sub>G</sub> /kg	U <sub>P</sub> /kg	GALU/kg
	<b>Expected at 50 mg/kg</b>	<b>8,000</b>	<b>50</b>	<b>500</b>	<b>1,000</b>	<b>75</b>	<b>160</b>	<b>105</b>	<b>4</b>
Efficacy 1	Control – previous	5,550	< 200	2,150	< 4,000	< 1,500	< 300	34	31
	Control – new	628.5	0	535.5	193.5	6	56	3.5	0
	50 mg/kg – previous	4,705	< 200	6,090	5,250	< 1,500	< 300	129	58
	50 mg/kg – new	7,565	178	4,352	2,329	72	293	91	25
Efficacy 2 <sup>(1)</sup>	Control – previous	122,000	< 10	104,000	64,100	336	1,680	65,700	726
	Control – new	3,343	49	511	3,840	0	0	64	3
	50 mg/kg – previous	20,464	444	36,300	3,550	171	< 10	925	268
	50 mg/kg – new	9,597	226	2,591	8,050	118	287	226	49
Efficacy 3	Control – previous	12,550	< 200	9,735	5,840	< 150	232	135	61
	Control – new	2,543	0	583	621	11	69	14	2
	50 mg/kg – previous	12,700	< 200	2,135	5,840	< 200	215	523	31
	50 mg/kg – new	11,813	114	4,111	2,456	119	342	152	10

(1): Two basal diets, pre-starter and starter, were used in the study. For the control diet, the enzyme activities provided for the starter diet were not considered since it was indicated that they belonged to the starter diet containing 5,000 mg additive/kg feed (see tolerance trial).