Partial assessment on efficacy of a chromium chelate of DL-methionine (Availa®Cr) as a feed additive for dairy cows

Reference number RP16

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Regulated Product Dossier Assessment

Safety Assessment finalised: 18/08/2023

Summary

An application was submitted to the Food Standards Agency (FSA) in January 2021 from Zinpro Animal Nutrition ("the applicant") for the authorisation of a chromium chelate of DL-methionine (Availa®Cr) as a feed additive under the category of 'zootechnical' additives, functional group 'other zootechnical additives'.

The additive is a chromium chelate of DL-methionine, proposed to be used at a minimum dose of 0.3 mg/kg chromium (Cr) and a maximum dose of 0.5 mg/kg of complete feed (moisture content of 12%), and aims to increase milk yield in dairy cows.

To support the FSA and Food Standards Scotland (FSS) in evaluating the dossier, the Animal Feed and Feed Additives Joint Expert Group (AFFAJEG) and the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the efficacy section of the dossier and the supplementary information from the applicant. ACAF concluded that the additive has the potential to be efficacious for increasing milk yield in dairy cows at the recommended dose of 0.3-0.5 mg/kg of complete feed with a moisture content of 12%.

The views of AFFAJEG and ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

1. Introduction

The FSA and FSS have undertaken a partial risk assessment for the efficacy of a feed additive (Availa®Cr, Zinpro Animal Nutrition Inc., Unit 7, 6/7 Marine Road, Dun Laoghaire, County Dublin, Ireland), a chromium chelate of LD-methionine, under regulation (EC) No 1831/2003[1] under the category of 'zootechnical' additives, functional group 'other zootechnical additives'. To support the safety assessment by FSA and FSS, the AFFAJEG and the ACAF provided advice to the FSA and FSS outlined in this document.

With thanks to the members of the AFFAJEG and ACAF during the course of the assessment, who were: Professor John Wallace, Professor Nicholas Jonsson, Martin Briggs, Dr. Katrina Campbell, Susan MacDonald, Professor Matthew Fisher, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Dr. Helen Warren and Dr. Nick Wheelhouse.

The dossier was evaluated by the Joint Expert Group on Animal Feed and Feed Additives (AFFAJEG) at its April 2021 and October 2021 meetings. Further information was provided by the applicant in June 2021 responding to queries by the FSA. The conclusions by the AFFAJEG were reviewed and approved by its successor body, the ACAF, at their October 2022 meeting. This document outlines the discussion and conclusions of the Group's assessment on the efficacy of the additive.

In line with Article 8 of 1831/2003, the FSA/FSS has considered whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

Additive

The additive is a chromium chelate of DL-methionine, a stable, water-soluble mono-hydrochloride salt containing one molar equivalent of chromium (III) and three molar equivalents of DL-methionine, and contains a minimum of 0.1% chromium. The final product would incorporate the active substance dried onto a carrier. A minimum dose of 0.3 mg/kg Cr and a maximum dose of 0.5 mg/kg of complete feed is proposed by the applicant.

Table 1: Proposed mode of use of Availa®Cr as described in the application

Proposed mode of use in animal nutrition			
Additive		Chromium chelate of DL-methionine	
Registration number/EC No/No		4dxx	
Category(-ies) of additive		4. Zootechnical feed additive	
Functional group(s) of additive		d. Other	
Description			
Composition, description	**Chemical formula**	**Purity criteria (if appropriate)**	**Method of analysis (if appropriate)**
Chromium chelate of DL-methionine	$[CH_3S(CH_2)_2CH(NH_2)COO]$ Cr	Complies with EU feed hygiene regulation	ICP-MS or ICP-OES
Trade name (if appropriate)		Availa-Cr	
Name of the holder of authorisation (if appropriate)		--	
Conditions of use			
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**
		Chromium in mg/kg of complete feed with a moisture content of 12%	
Lactating Cows	--	0.3	0.5

2. Assessment

2.1. Section IV: Efficacy

The AFFAJEG recognised the extent of the challenge of determining the efficacy of the additive, given the difficulty of measurement of its concentration, and of separating the action of the chromium chelate from that of chromium already present in the feed.

Three efficacy studies were presented in the original application, as well as a short literature review. A compendium of the studies presented in the literature review can be consulted in Appendix 1. The applicant also provided a series of documents containing additional information as a response to previous requests made by EFSA in their assessment of the application.

2.2.1. Study 1

The first study aimed to determine the ability of chromium-DL-methionine to increase milk production in dairy cows, comparing a treatment group (8 mg Cr/animal/day) to a control group (no treatment). The applicant claimed the study demonstrated an increase in milk yield, but a reduction in protein and fat content, due to the effect of the additive. The Group noted that the study did not include data on dry matter intake, which was deemed as being of use, but not determinant, when evaluating the efficacy of the product. The study did not include parity or age as factors in the statistical analysis, potentially confounding the estimates of the efficacy of the test product.

Upon request by FSA, the applicant provided further data on dry matter intake, as well as a complete re-analysis of the data, including parity as a factor, which proved to not

significantly affect conclusions on efficacy. After consideration of the new data provided, the AFFAJEG concluded that Study 1 demonstrated the efficacy of Availa®Cr for increasing milk yield in dairy cows.

Table 2: Study 1, Milk yield (kg). Parity included as a fixed factor. SE: Standard error, Df: Degrees of freedom.

Least Square Means		Control		Treatment		Df
		Mean	SE	Mean	SE	
Milk yield (kg)	Lactation					49
	2	40.4	1.05	43.8	1.08	
	3	43.7	2.35	47.1	2.43	
	4	44.3	3.09	47.7	2.98	
	5	44.8	2.65	49.2	2.65	
P-value						
Treatment	0.0213					
Parity	0.1043					

2.2.2. Study 2

The second study aimed to determine the ability of chromium-DL-methionine to increase milk production in dairy cows, comparing a treatment group (8 mg Cr/animal/day) to a control group (no treatment). The applicant claimed the study demonstrated an increase in milk yield but no effect on body weight, milk components or feed efficiency. The Group noted that the study did not include parity or age as factors in the statistical analysis, potentially confounding the estimates of the efficacy of the test product.

Upon request by FSA, the applicant provided further data on dry matter intake, as well as a complete re-analysis of the data including parity as a factor, which on this occasion proved to be a significant factor but did not affect the overall conclusions regarding efficacy. After consideration of the new information provided, the AFFAJEG concluded that Study 2 demonstrated the efficacy of Availa®Cr for increasing milk yield in dairy cows.

Table 3: Study 2, Milk yield (kg). Parity included as a fixed factor. SE: Standard error, Df: Degrees of freedom.

Least Square Means		Control		Treatment		Df
		Mean	SE	Mean	SE	
Milk yield (kg)	Lactation					50
	2	40.4	1.40	50.2	1.51	
	3	43.7	1.82	53.8	1.87	
	4	44.3	3.42	49.9	3.19	
	5	44.8	2.72	55.9	2.72	
	6	38.5	4.56	42.5	4.55	
P-value						
Treatment	0.023					
Parity	0.047					

2.2.3. Study 3

The third study aimed to determine the ability of chromium-DL-methionine to increase milk production in dairy cows, comparing a treatment group (8 mg Cr/animal/day) to a control group (no treatment). The applicant claimed the study demonstrated an increase in milk yield, with no changes in milk components or feed efficiency. The AFFAJEG evaluated the study and identified numerous flaws in the study design and the implementation of the protocol. The applicant claimed that the cow was the experimental unit of the trial, but the Group rejected the validity of this claim given that animals were fed and housed in groups, not individually, and the dry matter intake values for the group were not provided. Furthermore, the animals in the trial were not allocated in a balanced manner, as those in the treatment group were heavier than those in the control group and animals in the control group showed more cases of digestive disturbance than those in the treatment group. Further concerns were raised about the statistical analysis carried out in the study, which included both parametric and non-parametric tests, and the reporting of the data lacked clarity and showed numerous mistakes. Based on the unsatisfactory study design and the erratic interpretation of the results, Study 3 was rejected by AFFAJEG to evaluate the efficacy of the additive.

2.2.4. Literature review

The application presented two peer-reviewed publications showing the effect of Availa®Cr in dairy cows. The Group evaluated the papers provided and concluded that a more extensive literature review would have to be carried out by the applicant following guidance recommendations to support the assessment of efficacy of the additive. Members noted the requirement to identify in the literature factors, such as chromium concentrations used, supplementation rates, background rates, and any others that would be of interest for the evaluation of the additive's efficacy. As a response to the request for further information by FSA, the applicant provided an extensive literature review, carried out following a systematic search methodology, and including several studies on the factors outlined above, as well as 10 different papers on chromium-methionine supplementation in dairy cows.

2.2.5. Section IV: Conclusions on efficacy

The AFFAJEG concluded that, based on the strong results shown in studies 1 and 2, as well as the evidence provided through the literature review, the additive is likely to be efficacious for increasing milk yield in dairy cows at the proposed dose of 0.3-0.5 mg/kg of complete feed with a moisture content of 12%.

The ACAF ratified the conclusions presented by AFFAJEG.

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for Availa Cr 10[3]:

“The feed additive is to be marketed as a grey-tan powder preparation (Availa-Cr) with a content of chromium chelate of DL-methionine of 3.4 % (w/w), including calcium carbonate and vegetable oil as carriers. The content of chromium in Availa-Cr is ranging from 1004 to 1474 mg/kg and the content of methionine in the preparation is in the range from 13800 to 17500 mg/kg. According to the Applicant the active substance of the feed additive is chromium DL-methionine.

For the quantification of the chromium DL-methionine content in the feed additive, premixtures and feedingstuffs the Applicant did not submit any method. Instead, the Applicant proposed the separate determination of the chromium and methionine contents in the above-mentioned matrices and submitted the corresponding methods.

For the quantification of the chromium content in the feed additive (Availa-Cr) the Applicant submitted a single-laboratory validated and further verified method based on inductively coupled plasma-mass spectrometry (ICP-MS). The following performance characteristics were reported in the frame of the validation and verification studies: a relative standard deviation for repeatability (RSDr) ranging from 1.0 to 4.6 %; a relative standard deviation for intermediate precision (RSDip) ranging from 1.3 to 5.9 %; and a recovery rate (Rrec) ranging from 90 to 116 %.

Based on the acceptable performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method based on ICP-MS for the quantification of the chromium content in the feed additive (Availa-Cr).

For the quantification of chromium in feedingstuffs the Applicant proposed the AOAC 2006.03 method, and an in-house method based on ICP-MS. However no experimental proof of the applicability of both methods to quantify chromium content in feed, at the proposed added levels of 0.3 to 0.5 mg/kg feedingstuffs, was submitted.

Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify the proposed added chromium content in feedingstuffs.

For the characterisation of the feed additive the Applicant proposed to quantify the methionine content by the ring-trial validated AOAC 999.13 method based on ion-exchange chromatography coupled to post-column derivatisation and colorimetric or fluorescence detection. The EURL instead identified the ring-trial validated EU and EN ISO 13903 methods based on ion-exchange high performance liquid chromatography coupled to post-column derivatisation and photometric detection (IEC-VIS), already evaluated and recommended by the EURL in the frame of a previous methionine chelate dossier.

Based on the performance characteristics available, the EURL recommends for official control the above-mentioned EU and EN ISO 13903 methods based on IEC-VIS to quantify methionine in the feed additive.

Furthermore, for proving the chelated structure of the feed additive the Applicant has proposed an additional experiment, namely the measurement of the product (Availa-Cr) by mid-infrared (IR) spectrometry.

Based on the available data, the EURL recommends for official control the measurement by mid-IR spectrometry together with the determination of the content of chromium and methionine in the product, for proving the chelated structure of the feed additive."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical methods proposed as appropriate for official controls for this feed additive.

4. Conclusions

Based on the reanalysed data of Study 1 and Study 2 presented by the applicant and the new extensive literature review containing several papers demonstrating the efficacy of the additive, AFFAJEG concluded that Availa®Cr has the potential to be efficacious for increasing milk yield in dairy cows at the proposed dose of 0.3-0.5 mg/kg of complete feed with a moisture content of 12%.

ACAF ratified the conclusions presented by AFFAJEG.

5. References

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6. Abbreviations

ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Animal Feed and Feed Additives Joint Expert Group
AOAC	Association of Official Analytical Chemists
Df	Degrees of freedom
EC	European Commission
EFSA	European Food Safety Authority
ISO	International Organization for Standardization
EU	European Union
FSA	Food Standards Agency
FSS	Food Standards Scotland
IEC-VIS	Ion exchange chromatography coupled with post-column derivatisation and photometric detection
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
No	Number

SE	Standard error

7. Appendix 1: Literature review summary tables

Table 4: Effect of supplementing Cr-Met during periparturient period on DMI, BW, BCS, milk production, blood metabolites in dairy cows (Hayirli et al., 2001)

Period	Response variable	Cr supplemental level (Cr/kg $BW^{0.75}$)				*P* value		
		0	0.03	0.06	0.12	0 vs. Cr	Linear	Quadratic
Prepartum	DMI, kg/d	10.9	11.1	11.8	12.5	0.08	0.007	0.99
	Glucose, mg/dl	58.0	63.3	61.0	59.7	0.22	0.55	0.25
	Insulin, µU/ml	13.7	16.5	16.6	16.0	0.06	0.26	0.13
	Insulin: glucose	37.5	33.5	33.5	32.9	0.30	0.40	0.58
	Glucagon, pg/ml	84.8	88.1	92.6	91.2	0.45	0.49	0.57
	NEFA, µEq/ml	289.7	247.8	228.4	149.0	0.08	0.03	0.92
Postpartum	DMI, kg/d	13.8	14.9	17.2	16.3	0.002	0.003	0.01
	BW, kg	633	621	636	642	0.94	0.27	0.60
	BCS	2.95	2.82	2.97	3.09	0.93	0.06	0.22
	Milk, kg/d	33.5	34.0	38.5	31.8	0.54	0.46	0.02
	FCM, kg/d	37.0	36.9	42.2	35.1	0.66	0.52	0.04
	Fat, kg/d	1.51	1.52	1.72	1.42	0.72	0.44	0.05
	CP, kg/d	1.08	1.05	1.19	1.06	0.83	0.92	0.21
	Lactose, kg/d	1.66	1.59	2.07	1.55	0.66	0.76	0.05
	SNF, kg/d	3.06	2.92	3.33	2.89	0.93	0.65	0.26
	Glucose, mg/dl	49.5	49.3	45.5	53.2	0.99	0.34	0.14
	Glucagon, pg/ml	120.6	118.9	112.3	119.8	0.75	0.94	0.56
	Insulin, µU/ml	9.8	5.1	5.3	6.6	0.007	0.18	0.02
	Insulin: glucose	26.1	14.1	15.2	17.7	0.006	0.14	0.02
	NEFA, µEq/ml	605.5	618.9	669.6	506.0	0.91	0.37	0.25

DMI = Dry matter intake; BW = Body weight; BCS = Body condition scores; FCM = Fat corrected milk; CP = Crude protein; SNF = Solids-not-fat; NEFA = non-esterified fatty acids

Table 5: Effect of supplementing 6.25 mg/d of Cr from Cr-Met from 6-week prepartum to 21-week postpartum on blood metabolite concentration, lactation performance and reproductive performance (Bryan et al., 2004)

Measurement	Feeding 0 mg/d of Cr from Cr-Met	Feeding 6.25 mg/d of Cr from Cr-Met	SE
Plasma/serum metabolite concentration (mmol/L)			
Plasma glucose	2.89	3.01	0.04
Serum β-hydroxybutyrate	0.72	0.67	0.02
Serum Non-esterified fatty acids[a]	0.68[b]	0.50[c]	0.05
Lactation performance			
Milk,[1] kg/d	26.7	26.0	0.4
Energy-corrected milk,[1] kg/d	32.4	31.4	0.5
Milk fat, kg/d	1.09	1.04	0.18
Milk protein, kg/d	0.94	0.92	0.18
Milk solids,[2] kg/d	2.03	1.96	0.03
Milk fat, %	5.37	5.31	0.25
Milk protein, %	4.63	4.69	0.25
Reproductive performance			
Anestrus cow,[3] %	32.0[d]	45.5[e]	-
28-d pregnancy rate, %	39.2[f]	50.0[g]	-
44-d pregnancy rate, %	54.4	61.2	-
60-d pregnance rate, %	71.2	73.1	-

[a] Time x treatment interaction (P≤0.05). [b,c] LS means lacking a common superscript letter differ (P=0.05). [d,e] Means lacking a common superscript letter differ (P≤0.05). [f,g] Means lacking a common superscript letter differ (P≤0.05). [1] Energy-corrected milk = 3.5% fat and 3.2% protein. [2] Solid= fat + protein. [3] Cows visually observed by dairy personnel to be noncycling at 7 day before planned start of mating.

Table 6: Pre- and postpartum DMI, BCS, BW, net energy balance (NEB), and milk yield and milk composition from cows fed increasing amounts of Cr-Met from 21 d prepartum through 28 postpartum (Smith et al., 2005)

Item	Cr, mg/kg $BW^{0.75}$			SE	*P*-value	
	0	0.03	0.06		Linear	Quadratic
Prepartum						
DMI, kg/d	13.6	13.9	13.6	0.2	0.97	0.21
BCS[1]	3.27	3.32	3.38	0.03	0.01	0.89
BW, kg	722	724	724	3	0.57	0.74
NEB[2], Mcal/d	6.5	7.1	6.5	0.4	0.91	0.28
Postpartum						
DMI, kg/d	18.2	18.9	19.7	0.4	0.01	0.91
BCS[1]	2.84	2.83	2.89	0.05	0.41	0.50
BW, kg	614	620	639	6	0.01	0.37
NEB[2], Mcal/d	-8.3	-8.1	-8.3	0.7	0.99	0.80
Milk, kg/d	40.3	40.5	42.8	0.8	0.03	0.29
3.5% FCM	45.4	46.0	47.8	0.9	0.05	0.58
Fat, %	4.36	4.41	4.33	0.11	0.85	0.62
True protein, %	3.34	3.37	3.15	0.11	0.22	0.34
Lactose, %	4.65	4.68	4.62	0.05	0.66	0.39
Total solids, %	13.3	13.4	13.0	0.2	0.19	0.28
SCC	355	360	495	135	0.46	0.69
MUN	14.1	13.9	14.5	0.5	0.53	0.57

[1] Cows were scored on a 5-point scale. [2] Calculated based on NRC (2001). DMI= Dry matter intake; BCS = Body condition score; NEB = Net energy balance; SCC = Somatic cell count; MUN = Milk urea nitrogen.

Table 7: Effects of Cr-Met supplementation during the periparturient period on plasma metabolites and hormones in dairy cows[1] (Smith et al., 2008)

Item	Cr[2], mg/kg $BW^{0.75}$			SE	*P*-value	
	0	0.03	0.06		Linear	Quadratic
Prepartum						
Glucose, mg/dl	59.5	61.3	59.4	0.4	0.77	0.0006
NEFA, µEq/ml	160	145	169	13	0.62	0.20
BHBA, mg/dl	5.8	5.9	5.8	0.2	0.77	0.57
Insulin, ng/ml	0.63	0.58	0.55	0.05	0.25	0.83
Glucagon, pg/ml	64.0	71.4	59.3	3.2	0.28	0.01
Insulin:glucagon (pg:pg)	11.0	11.4	10.9	1.2	0.95	0.77
Glucose:insulin (mg:ng)	1.57	1.57	1.53	0.09	0.72	0.88
Insulin:NEFA (ng: µEq)	5.82	5.88	4.72	0.54	0.15	0.35
Postpartum						
Glucose, mg/dl	45.7	45.1	43.9	1.0	0.19	0.83
NEFA, µEq/ml	367	390	386	21	0.54	0.59
BHBA, mg/dl	9.3	11.0	11.0	1.1	0.29	0.56
Insulin, ng/ml	0.21	0.17	0.19	0.02	0.68	0.18
Glucagon, pg/ml	66.1	72.8	68.9	2.5	0.44	0.09
Insulin:glucagon (pg:pg)	3.99	3.02	3.19	0.40	0.21	0.28
Glucose:insulin (mg:ng)	3.27	3.57	3.09	0.18	0.47	0.06
Insulin:NEFA (ng: µEq)	0.86	0.65	0.71	0.10	0.29	0.26

[1] Blood samples were obtained every second day throughout the peripartum period. [2] Cows received 0 (n=22), 0.03 (n=25), and 0.06 (n=25) mg of Cr-Met/kg of $BW^{0.75}$. BHBA = β-hydroxybutyrate; NEFA = non-esterified fatty acids.

Table 8: Effects of Cr supplementation and substituting barley grain with corn during periparturient period on DMI, BW, net energy balance, milk yield and milk composition in dairy cows (Sadri et al., 2009)

Item	Cr (-)[1]		Cr (+)[1]		SE	*P*-value[2]		
	BBD	CBD	BBD	CBD		Cr	G	Cr x G
Prepartum								
DMI, kg/d	11.6	12.1	12.7	11.9	0.35	0.17	0.67	0.05
DMI, % of BW	1.62	1.67	1.80	1.70	0.06	0.09	0.70	0.22
Change in DMI,[3] % of BW	-29.4	-20.2	-12.5	-31.3	8.56	0.75	0.59	0.12
Postpartum								
DMI, kg/d	16.9	18.3	18.4	17.8	0.59	0.37	0.53	0.10
DMI, % of BW	2.59	2.92	2.82	2.78	0.15	0.74	0.33	0.22
BW, kg	664.4	554.6	665.6	652.7	7.21	0.47	0.36	0.36
BW change,[4] %	-9.60	-10.9	-7.60	-6.10	1.92	0.10	0.96	0.48
NEB,[5] Mcal/d	-8.20	-6.60	-8.40	-7.70	1.62	0.69	0.47	0.79
Milk, kg/d	34.3	34.9	37.7	35.2	1.04	0.08	0.37	0.16
4% FCM, kg/d	36.1	38.2	40.5	38.2	1.56	0.18	0.98	0.17
Fat, %	4.50	4.73	4.66	4.73	0.35	0.83	0.66	0.82
Fat, kg/d	1.50	1.62	1.70	1.61	0.09	0.29	0.84	0.25
CP, %	3.05	3.19	3.00	2.98	0.06	0.03	0.35	0.15
CP, kg/d	1.01	1.11	1.10	1.03	0.05	0.90	0.78	0.09
Lactose, %	4.94	4.89	4.95	4.74	0.11	0.54	0.25	0.49
Lactose, kg/d	1.66	1.71	1.84	1.67	0.08	0.43	0.44	0.21
Total solid, %	12.8	13.1	12.8	12.7	0.36	0.67	0.84	0.60
Total solid, kg/d	4.28	4.53	4.73	4.40	0.17	0.34	0.80	0.10

[1] Treatments: Cr (-) = without supplemental Cr; Cr (+) = with supplemental Cr; BDD = barley-based diet; CBD = corn-based diet. [2] Statistical comparisons: Cr effects = Cr (+) vs. Cr (-); G effects = BDD vs. CBD; Cr x G effects = CR by G interaction. [3] Chane in DMI expressed as percentage of BW from d19 through d1 before parturition. [4] BW change was from d1 to d30 postpartum. [5] NEB calculated based on NRC (2001).

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