Assessment of the safety and efficacy of an additive of chromium chelate of DL-methionine (Availa®Cr) as a feed additive for dairy cows

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Regulated Products Risk Assessment Unit
Science, Evidence and Research Division, FSA

Risk Assessment Team
Science Division, FSS

Regulated Product Dossier Assessment
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Abbreviations

Acronym	Definition
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
DL-Met	DL-methionine
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Reference Laboratory
FCR	Feed conversion ratio
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland
GB	Great Britain
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
ISO	International Organization for Standardization
NOAEL	No observed adverse effect level
R_{Rec}	Recovery rate
RSD_{ip}	Relative standard deviations for intermediate precision
RSD_r	Relative standard deviations for repeatability
SE	Standard error

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 0.2 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.

This feed additive had its application of authorisation assessed by the European Food Safety Authority (EFSA), which was published in 2020. FSA/FSS have reviewed the information available, including the EFSA opinion[1], to conclude on the Identity and Characterisation, and Safety sections of the dossier. No conclusion could be reached on the Efficacy of the additive.

The Animal Feed and Feed Additives Joint Expert Group and its successor body, the Advisory Committee on Animal Feedingstuffs (ACAF), were asked to review the Efficacy section of the dossier and the supplementary information submitted by the Applicant, and to advise the Food Standards Agency and Food Standards Scotland (FSA/FSS) in evaluating the dossier.

The FSA/FSS concluded, based on their review of the information available, including the EFSA opinion, and confirmed that the additive, when used at a maximum dose of 0.5 mg Cr/kg of complete feed, is safe for the target species with a 10-fold margin of safety. The additive can be considered safe for the environment. Based on the weight of evidence of *in vivo* genotoxicity studies together with previous evaluations of the genotoxicity and carcinogenicity of Cr (III) and its salts, and the limited exposure expected through the diet, the additive can be considered safe to consumers when used in dairy cows at a maximum level of 0.5 mg/kg of complete feed. The additive is not an eye or skin irritant, and is a skin sensitiser. Given the uncertainties and the high dusting potential of the additive, measures should be taken to minimise exposure to users and workers through inhalation.

The FSA/FSS concluded, based on the ACAF's advice, that the additive has the potential to be efficacious for increasing milk yield in dairy cows at the recommended dose of 0.2-0.5 mg/kg of complete feed with a moisture content of 12%.

The views of AFFAJEG/ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS. This document replaces the previous Safety Assessment published in 2023.

1. Introduction

The FSA/FSS have undertaken an assessment of a feed additive (Availa®Cr, Zinpro Animal Nutrition Inc., Unit 7, 6/7 Marine Road, Dun Laoghaire, County Dublin, Ireland), of chromium chelate of LD-methionine, under regulation (EC) No 1831/2003[2] under the category of 'zootechnical' additives, functional group 'other zootechnical additives'.

Whilst it was a Member State of the EU, the UK accepted the assessments of EFSA in support of authorisations for regulated food and feed products. Since the end of the transition period, FSA/FSS has adopted equivalent technical guidance and quality assurance processes to make independent GB safety assessments.

A number of applications have been received by GB where EFSA, prior to the end of the transition period, evaluated an application for the product. FSA/FSS has decided to make use of the EFSA risk assessment, where this is appropriate, in forming its own independent opinion. Therefore, FSA/FSS safety assessors have reviewed the EFSA opinion[1] for this application in the context of intended GB use.

In reviewing the output of the EFSA risk assessment the reviewers have verified that the standard approach as outlined in the relevant guidance has been followed and the arguments made are consistent with the data summarised. Consideration has been given to the processes undertaken to ensure the outcome is robust and whether there are any aspects that would require further review such as specific issues for the countries of the UK. The result of the assessment is that the EFSA scientific opinion on the safety of the additive is adequate also for UK considerations.

To support the assessment of the Efficacy of the additive, the AFFAJEG/ACAF were asked to provide advice to the FSA/FSS as outlined in this document.

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.

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 efficacy section of 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.

This document replaces the previous Safety Assessment published in 2023, and sets out the findings of the conclusions of the review of the EFSA opinion for the assessment on the safety of the feed additive, as well as the findings of the Committee's assessment of the efficacy section, on which the FSA/FSS have made their opinion for the request of a new authorisation.

2.Assessment of Sections II and III

2.1. Methodology applied in the EFSA opinion

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed the safety and the efficacy of Availa®Cr in accordance with EFSA FEEDAP Panel

guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives[3], Guidance on zootechnical additives[4], Technical guidance: Tolerance and efficacy studies in target animals[5], Guidance on the assessment of the safety of feed additives for the target species[6], Guidance on the assessment of the safety of feed additives for the consumer[7], Guidance on studies concerning the safety of use of the additive for users/workers[8], Technical Guidance for assessing the safety of feed additives for the environment[9] and principles in Regulation (EC) No 429/2008[10].

2.2. Section II: Identity, characterisation and conditions of use

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 0.2 mg Cr/kg and a maximum dose of 0.5 mg Cr/kg of complete feed is proposed by the applicant, equivalent to 300 and 500 mg Availa®Cr/kg of complete feed.

The applicant provided data from several batches on the composition and impurities of the additive. No causes for concern were identified. The product was shown to be quite dusty, with a dusting potential of 3.5-5.6 g/m^3 tested through the Stauber-Heubach method. Both chromium and methionine in the additive showed stability at 25°C, 60% relative humidity and 40°C, 70% relative humidity for 36 months. Stability in premixtures and feed was not evaluated. Homogeneity was demonstrated in premixtures and feed.

2.3. Section III: Safety

The opinion under review refers to a previous evaluation of 2009 by the EFSA FEEDAP Panel[11], in which the safety of chromium (III) was evaluated. In the 2009 opinion, a subchronic toxicity study was evaluated, for which a NOAEL of 34 and 39 mg Cr DL-Met/kg bw/day was observed for males and females, respectively.

The FEEDAP panel cited an evaluation of Chromium (both Cr (III) and Cr (VI)) by the CONTAM Panel,[12] in which it was concluded that several positive results had been reported in developmental toxicity with Cr (III), with a lowest observed adverse effect

level (LOAEL) of 30 mg/kg bw/day. In this same evaluation, the CONTAM Panel concluded that there is conflicting evidence in the literature on the genotoxic potential of Cr (III) with variable results *in vitro*, mainly non-guideline studies, but negative results in *in vivo* guideline studies. This difference in results between *in vitro* and *in vivo* studies was concluded to be due to the poor uptake capacity Cr (III) by animal cells limiting access to intracellular DNA, combined with the fact that Cr (III) is known to be poorly absorbed from the gastrointestinal tract. In addition, the CONTAM panel concluded that Cr (III) showed no evidence of carcinogenicity. Overall, the EFSA FEEDAP panel concluded that chromium (III) is not carcinogenic and that Cr DL-Met is unlikely to pose a carcinogenic risk at levels occurring in the diet.

In the 2020 opinion, EFSA evaluated a newly submitted *in vitro* micronucleus study and concluded that Cr DL-Met was genotoxic in this assay in the absence of metabolic activation. An *in vivo* micronucleus assay, previously evaluated in the 2009 opinion, had tested up to the top dose recommended in OECD Guideline 474 of 2000 mg/kg bw/day, due to the absence of signs of toxicity, with negative results. However, no evidence of bone marrow exposure was observed. EFSA noted that in this case, the genotoxic effects at other potentially relevant targets, such as the site of first contact, should be evaluated. Since these data were not available, the potential for genotoxic effects of chromium DL-Met at the site of first contact could not be assessed.

To evaluate the safety for the target species, the FEEDAP considered a new tolerance study in dairy cows presented by the applicant. Despite no observation of adverse effects at the intended use level of 8 mg Cr/cow/day (estimated by EFSA to be equivalent to 0.4 mg Cr/kg of complete feed), a 5-fold overdose level of 40 mg Cr/cow/day or a 10-fold overdose level of 80 mg Cr/cow/day, the tolerance study was only considered as supportive evidence due to significant weaknesses in the design and reporting of the study. The Panel also considered the subchronic toxicity study evaluated in 2009 as part of the evidence. In this study, a NOAEL of 34 mg Cr DL-Met/kg bw/day was observed. Applying an uncertainty factor of 100, and default values for feed consumption and body weight in the EFSA's Guidance on the assessment of the safety of feed additives for the target species[6], the estimated safe level for dairy cows would be 9.7 mg supplemental Cr/kg complete feed. Taking into account the background concentration of chromium in feed, and presuming the same qualitative

and quantitative bioavailability as shown for Cr DL-Met, the total dietary exposures of dairy cows supplemented with the maximum intended use level of this additive of 0.5 mg Cr/kg would still be at least 10 times lower than this estimated safe level. Overall, EFSA concluded that the maximum recommended use level of 8 mg Cr/cow/day from Cr CL-Met, which they considered to correspond to 0.4 mg Cr/kg of complete feed from Cr DL-Met, is safe for the target species.

The additive was concluded to be safe for consumers and the environment, as its use in dairy cows at the proposed dose is not expected to increase consumer exposure to trivalent chromium or significantly increase the concentration of Cr in soil, sediments and water.

In the previous evaluation in 2009 by EFSA[11], prolonged inhalation exposure was shown to be associated with an increased risk of genotoxic effects in the respiratory system. Based on the dusting potential, the exposure to Cr in the dust (0.04 mg Cr/m^3) was shown to exceed the recommended threshold limit value[13] for inhalable inorganic chromium (III) compounds (0.003 mg/m^3) by an order of magnitude. Based on this, the FEEDAP concluded that the additive poses a risk to the user/worker if inhaled. It was also concluded that the additive is not irritant for skin or eyes, but that, based on the presence of nickel in the dust, it is a skin sensitiser.

2.4. Caveats and uncertainties

For safety for the target species, due to weaknesses of the study design in the dairy cow tolerance study, the FEEDAP Panel also took the NOAEL into account from the subchronic toxicity study in rats, from which they estimated a safe level for dairy cows of 9.7 mg supplemental Cr/kg complete feed. Taking into account the background concentration of chromium in feed, and presuming the same qualitative and quantitative bioavailability as shown for Cr DL-Met, the total dietary exposures of dairy cows supplemented with the maximum intended use level of this additive of 0.5 mg Cr/kg would still be approximately 10 times lower than this estimated safe level. However, as the applicant had indicated that the maximum intended use level was 8 mg/cow, which EFSA estimated was equivalent to 0.4 mg Cr/kg of complete feed, EFSA concluded that the maximum intended use level of 8 mg/cow, equivalent to 0.4 mg Cr/kg of complete feed, was safe, and did not specifically conclude on the safety of 0.5

mg Cr/kg of complete feed. Therefore, no conclusion was made on the maximum dose of 0.5 mg Cr/kg of complete feed proposed by the applicant.

Within the assessment of consumer safety, the FEEDAP panel referred to the conclusions by the CONTAM panel on developmental toxicity, which said that several positive results had been reported, with a lowest observed adverse effect level (LOAEL) of 30 mg/kg bw/day. It is also concluded that these results should be taken into account, given no adequate data was generated to characterise further the developmental toxicity of the additive.

2.5. FSA/FSS conclusion for GB risk analysis

The conclusions of the EFSA opinion have been reviewed in detail by FSA/FSS and are considered appropriate and consistent within the identified caveats and uncertainties identified in the opinion and would be applicable to GB.

The FSA/FSS considered the conclusions from the EFSA FEEDAP Panel on the safety for the target species. The applicant has proposed a min-max dose of 0.2-0.5 mg/kg of complete feed, where EFSA was able to conclude that the additive is safe for the target species at 0.4 mg/kg of complete feed. This was because the applicant had indicated that the intended maximum use level was 8 mg/cow and EFSA considered that this was equivalent to 0.4 mg/kg of complete feed rather than 0.5 mg/kg of complete feed. The FSA/FSS evaluated the possibility to extrapolate the EFSA conclusions based on the 0.4 mg/kg dose to the maximum proposed dose by the applicant of 0.5 mg/kg.

The new tolerance study evaluated by EFSA was undertaken using a dose equivalent to 0.4 mg/kg of complete feed, as well as 5-fold and 10-fold overdose groups. Although it was only considered as supporting evidence, no adverse effects for body weight, milk yield, haematology and blood chemistry were observed at any dose level.

In their evaluation of the subchronic toxicity study presented in 2009, EFSA estimated the safe dose for supplemental Cr for dairy cows would be 9.7 mg/kg of complete feed, 19 times higher than the maximum proposed dose of 0.5 mg/kg of complete feed. Taking into account the background concentration of chromium in feed, and presuming the same qualitative and quantitative bioavailability as shown for Cr DL-

Met, the total dietary exposures of dairy cows supplemented with the maximum intended use level of this additive would still be at least 10 times lower than this estimated safe level.

Based on this observed margin of safety, and following the principles set out in the Guidance on the assessment of the safety of feed additives for the target species[6], FSA/FSS consider that there is sufficient evidence to conclude that the additive is safe for the target species when used at the proposed dose of 0.2 - 0.5 mg Cr/kg of complete feed with a moisture content of 12%, with a margin of safety of 10.

The FSA/FSS considered the conclusions from the EFSA FEEDAP Panel on the safety for the consumer. Several positive results had been reported in the literature for developmental toxicity, which the applicant acknowledged, providing the relevant documents. Conflicting evidence was also reported in the literature regarding the genotoxic potential of Cr (III) *in vitro*. This genotoxic potential was not reflected *in vivo*, where only one non-standard study out of an extensive literature review reported positive effects. It was concluded by the CONTAM Panel that the lack of genotoxicity *in vivo* is likely due to the capacity of animal cells to limit the access of Cr (III) to intracellular DNA. The CONTAM panel also concluded that Cr (III) is not carcinogenic.

Based on the toxicological data available, the CONTAM panel established a tolerable daily intake (TDI) of 300 µg/kg bw/day and compared it to the mean dietary exposure from food, drinking water and supplements, observing exposure was under 10% of the TDI. The mean dietary exposure was estimated from data from multiple literature references, following an inclusion/exclusion criteria based on limit of quantification (LOQ) cut-off points. These cut-offs were based on the distribution of LOQs of different food types from the FoodEx classification[14]. The applicant also presented references for three residue studies carried out in cows, one in milk and two in muscle, liver and kidney, showing no significant increase of Cr concentration in these tissues after supplementation with up to 0.83 mg/kg of complete feed. Based on this evidence, it can be concluded that the additive chromium DL-Met, when used at the maximum proposed level of 0.5 mg/kg is not expected to significantly increase consumer exposure to Cr (III). Based on the available weight of evidence of an *in vivo* genotoxicity study together with a previous evaluation of the genotoxicity and

carcinogenicity of Cr (III), the limited absorption potential of Cr (III), and the very limited exposure to consumers through the diet, the FSA/FSS consider the additive can be considered safe for consumers.

Based on the dusting potential of the additive, the exposure to Cr in the dust (0.04 mg Cr/m^3) was shown to exceed by an order of magnitude the recommended threshold limit value set by the American Conference of Governmental Industrial Hygienists (ACGIH)[13] for inhalable inorganic chromium (III) compounds (0.003 mg/m^3). Based on this and the existing uncertainties, measures should be put in place to minimise exposure to the additive through inhalation.

3. Assessment of Section IV

3.1. 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 1: 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 2: 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 0.2-0.5 mg/kg of complete feed with a moisture content of 12%.

The ACAF ratified the conclusions presented by AFFAJEG.

4.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[15]:

> "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.

5.Conclusions

FSA/FSS has reviewed the applicant's application, supporting documentation, and the EFSA risk assessment opinion (2020) and consider sufficient evidence has been demonstrated to conclude without further questions or risk assessment for sections II and III of the dossier.

The FSA/FSS conclude that the feed additive Availa®Cr, as described in this application, is safe for the target species, consumers and the environment. The additive can be considered safe for the target species used at a maximum dose of 0.5 mg Cr/kg of complete feed with a moisture content of 12%, with a 10-fold margin of safety. Based on the weight of evidence of *in vivo* genotoxicity studies together with previous evaluations of the genotoxicity and carcinogenicity of Cr (III) and its salts, and the limited exposure expected through the diet, the additive can be considered safe to consumers when used in dairy cows at a maximum level of 0.5 mg/kg of

complete feed. The additive is not an eye or skin irritant, and is a skin sensitiser. Given the uncertainties and the high dusting potential of the additive, measures should be taken to minimise exposure to users and workers through inhalation.

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 0.2-0.5 mg/kg of complete feed with a moisture content of 12%. ACAF ratified the conclusions presented by AFFAJEG.

The FSA/FSS agree with the conclusions reached by the ACAF. FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

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7. Appendix 1: Literature review summary tables

Table 3: 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 4: 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 5: 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 6: 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 7: 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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