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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of guanidinoacetic acid and its preparation (GuanAMINO®) for chickens for fattening, chickens reared for laying, chickens reared for reproduction, turkeys for fattening, turkeys reared for reproduction (Evonik Operations GmbH)

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of guanidinoacetic acid (GAA) and its preparation (GuanAMINO®) as zootechnical additives for chickens for fattening, chickens reared for laying, chickens reared for reproduction, turkeys for fattening, turkeys reared for reproduction. The FEEDAP Panel concluded that GAA and its preparation GuanAMINO® are safe for chickens for fattening and reared for laying/reproduction and for turkeys for fattening and reared for reproduction when used up to the maximum proposed use level of 1200 mg guanidinoacetic acid/kg complete feed. The same conclusion applies for its use in water at 600 mg guanidinoacetic acid/L in water for drinking. However, considering the variability in water intake and the fact that no margin of safety was identified for chickens for fattening, attention should be paid to ensure that the exposure via water does not exceed the maximum safe level of the additive when administered via feed. The Panel concluded that the use of the additives under the proposed conditions of use is of no concern for the consumer and the environment. GAA and GuanAMINO® are not irritant to the skin or eyes and are not skin sensitisers. The FEEDAP Panel concludes that GAA and GuanAMINO® do not raise safety concerns for the user. The FEEDAP Panel considers that GAA and GuanAMINO® are efficacious in chickens for fattening, chickens reared for laying/reproduction and turkeys for fattening and reared for reproduction when guanidinoacetic acid is used at the minimum use level of 600 mg/kg complete feed or 300 mg/L water.

KEYWORDS

efficacy, GuanAMINO®, guanidinoacetic acid, other zootechnicals, safety, zootechnical additives

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1 | INTRODUCTION

1.1 | Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Evonik Operations GmbH[2] for the authorisation of the additive guanidinoacetic acid and a preparation of guanidinoacetic acid (GuanAMINO®), when used in feed and water for drinking for chickens for fattening, chickens reared for laying, chickens reared for breeding, turkeys for fattening, turkeys reared for laying, turkeys reared for breeding (category: zootechnical additives; functional group: other zootechnical additives).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The dossier was received on 03 May 2024 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2024-00287. The particulars and documents in support of the application were considered valid by EFSA as of 07 August 2024.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of guanidinoacetic acid and a preparation of guanidinoacetic acid (GuanAMINO®), when used under the proposed conditions of use (see **Section 3.1.3**).

1.2 | Additional information

Guanidinoacetic acid (GAA) and a preparation of GAA are currently authorised for another holder of authorisation for use in feed and water for drinking in chickens for fattening and chickens reared for laying and breeding, and for its use in feed for weaned piglets and pigs for fattening (4d372 and 4d372i).[3]

EFSA issued several opinions on the safety and efficacy of guanidinoacetic acid for chickens for fattening and for pigs (EFSA, 2009; EFSA FEEDAP Panel, 2016) and for all animal species (EFSA FEEDAP Panel, 2022). More recently, EFSA issued one opinion on the safety and efficacy of guanidinoacetic acid for turkeys for fattening and reared for breeding (EFSA FEEDAP Panel, 2025).

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of guanidinoacetic acid and its preparation (GuanAMINO®) as feed additives.

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 dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier 26 November to 17 December 2024 for which no comments were received.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 12 August to 12 November 2024 the comments received were considered for the assessment.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

[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]Evonik Operations GmbH, Rodenbacher Chausse 4, 63,457, Hanau-Wolfgang, Germany.

[3]Commission implementing regulation (EU) 2023/2628 of 27 November 2023 concerning the authorisation of guanidinoacetic acid and a preparation of guanidinoacetic acid as feed additives for chickens reared for breeding and chickens reared for laying in feed and in water for drinking, and chickens for fattening in water for drinking (holder of authorisation: Alzchem Trostberg GmbH), and correcting and amending Implementing Regulation (EU) 2016/1768. OJ L, 2023/2628, 28.11.2023, 5 pp.

[4]Dossier reference: FEED-2023-19272.

[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, p.1–48.

[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the guanidinoacetic acid in animal feed, premixtures, compound feed and water.[7]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of guanidinoacetic acid and its preparation (GuanAMINO®) is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019) and Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2024).

3 | ASSESSMENT

Guanidinoacetic acid (GAA) is intended to be used as a feed additive for chickens and turkeys for fattening and reared for laying and breeding[9] (category: zootechnical additives; functional group: other zootechnical additives: improvement of performance parameters). The applicant is asking for the authorisation of GAA and a preparation (GuanAMINO®).

3.1 | Characterisation

3.1.1 | Characterisation of the active substance and the preparation

The active substance guanidinoacetic acid (synonyms: N-(aminoiminomethyl)-glycine, N-amidino-glycine; α-guanidinoacetic acid; 2[[amino(imino)methyl]amino]acetic acid; betacyamine; glycocyamine; guanidine, (carboxymethyl)-; guanidineacetic acid; guanidylacetic acid; guanyl glycine) has the CAS number 352-97-6 and European Community (EC) number 206-529-5. Its chemical formula is $C_3H_7N_3O_2$ with a molecular weight of 117.1 Da. The structural formula of GAA is shown in Figure 1.[10]

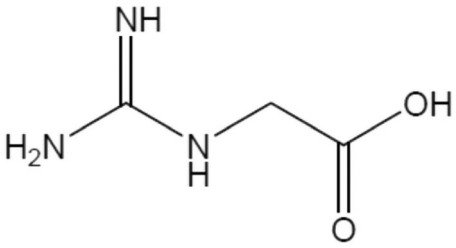

FIGURE 1 Structural formula of guanidinoacetic acid.

The specifications proposed by the applicant for GAA are ≥ 98% guanidinoacetic acid, ≤ 1.0% moisture, ≤ 0.5% ash, ≤ 1.5% glycine, ≤ 0.5% dicyandiamide, ≤ 0.03% cyanamide, ≤ 20 mg/kg melamine,[11] ≤ 40 mg/kg of the sum of melamine, ammeline, ammelide and cyanuric acid.[12]

The applicant is requesting also the authorisation of the preparation GuanAMINO®, formulated with up to 1% starch. The specifications proposed for the preparation are ≥ 96% guanidinoacetic acid, ≤ 1.0% moisture, ≤ 0.5% ash, ≤ 1.5% glycine, ≤ 0.5% dicyandiamide, ≤ 0.03% cyanamide and ≤ 20 mg/kg melamine.[13]

[7]Evaluation report received on 22/9/2025 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en.

[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]In the context of this opinion chickens reared for reproduction and turkeys reared for reproduction will be used to refer to chickens reared for breeding and turkeys reared for laying and breeding, respectively.

[10]Detailed identification and characterisation of the additive_updated.

[11]The Panel notes that the limit is in line with the limits set in the directive 2002/32.

[12]Annex_05_Specifications_Confidential.

[13]Annex_05_Specifications_Confidential.

The data provided by the applicant on the batch-to-batch variation,[14] impurities[15] and physicochemical and technological properties[16] of GAA and of GuanAMINO® are reported in Tables 1 and 2.

TABLE 1 Data on the batch-to-batch variation, impurities and physicochemical and technological properties of the additives guanidinoacetic acid (GAA) and GuanAMINO®.

	GAA	GuanAMINO®
Specifications		
Guanidinoacetic acid (%)	≥ 98.0	≥ 96.0
Moisture (%)	≤ 1.0	≤ 1.0
Ash (%)	≤ 0.5	≤ 0.5
Glycine (%)	≤ 1.5	≤ 1.5
Dicyandiamide (%)	≤ 0.5	≤ 0.5
Cyanamide (%)	≤ 0.03	≤ 0.03
Melamine (mg/kg)	≤ 20	≤ 20
Sum of melamine, ammeline, ammelide and cyanuric acid (mg/kg)	≤ 40	≤ 40
Batch-to-batch variation	[5]	[5]
Guanidinoacetic acid (%)	99.0 (98.9–99.2)	97.7 (97.5–97.9)
Moisture (%)	< 0.1	< 0.1
Ash (%)	< 0.1	< 0.1
Glycine (%)	0.2 (0.2–0.3)	0.4 (0.3–0.4)
Dicyandiamide (%)	0.04 (0.03–0.04)	0.06 (0.03–0.08)
Cyanamide (%)	< 0.01	< 0.01
Melamine (mg/kg)	0.7 (0.5–0.8)	1.5 (0.6–2.3)
Sum of melamine, ammeline, ammelide and cyanuric acid (mg/kg)	5.3 (4.3–5.9)	10.28 (1.3–16.8)
Impurities	[5]	[5]
Lead (mg/kg)	< 0.5	< 0.5
Mercury (mg/kg)	< 0.02	< 0.02
Cadmium (mg/kg)	< 0.2	< 0.2
Arsenic (mg/kg)	< 0.5	< 0.5
Fluorine (mg/kg)	< 40	< 40
Dioxins and furans (upper bound)[(1)]		
PCDD/Fs (ng WHO_{2005}-TEQ/kg)	0.16–0.17	0.16–0.17
PCDD/Fs + PCBs (ng WHO_{2005}-TEQ/kg)	0.30–0.31	0.30–0.31
nDL-PCBs (μg/kg)	1.65	1.65
Microbial contamination	[5]	[5]
Total aerobic counts (CFU/g)	1.0×10^3–1.4×10^4	1.8×10^2–2.8×10^3
Moulds (CFU/g)[(2)]	< 100	< 100
Yeasts (CFU/g)	< 100	< 100
Bacillus spp. (CFU/g)	1.7×10^2–2.1×10^4	1.3×10^2–1.2×10^3
Enterobacteriaceae (CFU/g)	< 10	< 10
Coliform bacteria (CFU/g)	< 10	< 10
Salmonella spp. (in 25 g)	Not detected	Not detected
Clostridium spp. (MPN/g)	< 1.8	< 1.8
Bacillus cereus (CFU/g)	< 100	< 100

Abbreviations: <, means below the limit of detection; nDL-PCBs, non-dioxin-like PCBs; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; RH, relative humidity; TEQ, toxic equivalent factors for dioxins, furans and dioxin-like PCBs established by WHO in 2005 (Van den Berg et al., 2006); WHO, World Health Organization;

(1)Upper bound concentrations are calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification. Values are expressed per kg of additive with 88% dry matter content.

(2)One out of the five batches for GAA had a value of < 1000 CFU/g.

[14]Batch to batch: Annex_03_CoAs_GAA_GuanAMINO_esearchable.

[15]Impurities: Annex_06_Undesirable_substances_esearchable, Annex_07_Microbio_AR_esearchable.

[16]Physical properties: Annex_08_Dusting_potential_particle_size, Annex_09_Handling_properties_AR_esearchable, Annex_11_shelf_life, Annex_12_Stability_premixtures_esearchable, Annex_13_Stability_feedingstuffs_esearchable, Annex_15_Homogeneity_esearchable_Confidential.pdf, Annex_04_AddInfo_04_2025_Clarification_stability_feedingsstuffs.

The data provided shows that the batches analysed, both for GAA and GuanAMINO® comply with the specifications set by the applicant. The Panel considers that the microbial contamination and the levels of the impurities analysed in the additives are of no concern.

TABLE 2 Data on the technological properties of guanidinoacetic acid (GAA) and GuanAMINO®.

	GAA	GuanAMINO®
Physical properties	[5]	[5]
Physical form	Solid	Solid
Bulk density (kg/m^3)	502–559	483–523
Solubility (g/L)[(1)]	4.99	4.5
Dusting potential (Stauber Heubach) (mg/m^3)	3241–5462	5439–9139
Particle size distribution (laser diffraction, size below which 90%, 50% and 10% of all particles are found) (μm)		
D90	126.2–234.4	834.5–1774.5
D50	39.8–58.3	393.7–874.1
D10	16.0–19.2	125.8–473.6
Shelf-life (% recovery)	[3]	[3]
10–25°C/20–75% RH 36 months	99.1–100.0	98.5–98.6
40°C/75% RH 36 months	99.1–100.4	98.1–98.9
Stability in premixtures (% recovery)		[3]
Vitamin-mineral premix, 10–25°C/20–75% RH, 6 months	–	97.7–100.0
Stability in feed (% recovery)		[1]
Mash, 10–25°C/20%–75% RH 3 months	–	96.9
Pelleted, 10–25°C/20%–75% RH 3 months	–	95.2
Pelleting, 85°C	–	100.0
Mash, 10–25°C/20%–75% RH 3 months	–	100.0
Stability in water (% recovery)	[3]	
25°C, 4 days	97.7–100.0	–
Homogeneity (coefficient of variation, %)		[1]
Mineral premix, 10 samples	–	7.6
Compound feed, 10 samples	–	15.1

[(1)]As provided by the applicant; no analytical data provided. Solubility was determined at 30°C for GAA solubility and at 15°C for GuanAMINO®.

Although no data on the stability of GAA in premixtures and complete feed or homogeneity have been provided, the Panel considers the data of the 96% preparation as representative also for the additive containing 98% GAA.

3.1.2 | Manufacturing process

The additive GAA is produced by chemical synthesis based on the reaction of the amino acid glycine (Chemical Abstract Services (CAS) No 56-40-6) with cyanamide (CAS No 420-04-2). [redacted][17]

The preparation GuanAMINO® is produced from crystalline GAA with an additional granulation step using 1% food-grade starch.[18]

3.1.3 | Conditions of use

Guanidinoacetic acid (GAA) and GuanAMINO® are intended to be used in feed (directly or via premixtures) for chickens for fattening, chickens reared for laying, chickens reared for reproduction and turkeys for fattening and turkeys reared for reproduction. GAA and GuanAMINO® aim to provide guanidinoacetic acid at a minimum concentration of 600 mg/kg and at a maximum concentration of 1200 mg/kg complete feed. GAA and GuanAMINO® are also intended to be used in water for

[17]Manufacturing_Process_Confidential.
[18]Manufacturing_Process_Confidential.

drinking for the same species at a minimum content of guanidinoacetic acid of 300 mg/L and a maximum of 600 mg/L.[19] The applicant states that the simultaneous use of the additives in feed and water is not foreseen.

3.2 | Safety

3.2.1 | Safety for the target species

The applicant made reference to the tolerance study already assessed by the FEEDAP Panel in the context of a previous application for the authorisation of guanidinoacetic acid for chickens for fattening (EFSA, 2009). In that assessment, the Panel concluded that the safety of guanidinoacetic acid at 1200 mg/kg complete feed was not demonstrated, but 600 mg/kg could be considered safe. The Panel notes that the composition and the conditions of use of the current additive are the same as the one previously assessed and currently authorised. Therefore, the conclusions reached in the previous assessment fully apply to the additive under assessment in the present application.

In the current submission, the applicant provided two new tolerance studies, one in chickens for fattening and another one in turkeys for fattening.

3.2.1.1 | *Safety for chickens for fattening*

A total of 960 one-day-old Ross 308 male chickens were randomly distributed in 48 floor pens and each pen allocated to one of the six experimental groups (eight replicates/treatment).[20] Birds underwent a three-phase feeding program (starter 1–11 days; grower 12–22 days; finisher 23–35 days). The diets were based on maize and soybean meal and contained 6.5–5.9-5.7 g digestible methionine/kg, 1700-1600-1500 mg choline/kg, 1.8 mg folic acid/kg and 40 μg/kg vitamin B_{12}. Experimental diets were either not supplemented (Control) or supplemented with GuanAMINO® to provide 600 (0.5× maximum recommended use level), 1200 (1×), 3600 (3×), 4800 (4×) or 6000 (5×) mg of guanidinoacetic acid/kg feed (confirmed by analysis).[21] Feeds were offered as crumbs (starter) or pellets (grower and finisher). Mortality and health status were checked daily, and dead birds subjected to necropsy. Body weight and feed intake was determined per pen basis on days 1, 11, 22 and 35. Average weight gain and feed to gain ratio was calculated. On days 35 and 38, two birds per pen were randomly selected (16 birds/treatment) for necropsy, gross pathology and tissue sampling.[22] The same birds were used to collect blood samples for haematology[23] and clinical chemistry.[24] Blood serum and tissue samples (liver, kidney, skin+fat and breast muscle) were analysed for guanidinoacetic acid, creatine, creatinine and homocysteine.

Data were statistically analysed by ANOVA using the pen as the experimental unit. Differences between groups were tested with Tukey test and the significance was set at $p < 0.05$. Additionally, linear and quadratic dose–response was analysed for each variable (Table 3).

TABLE 3 Main results of the tolerance study with guanidinoacetic acid in chickens for fattening (1–35 days).

	Control	0.5×	1×	3×	4×	5×
Guanidinoacetic acid (mg/kg)						
Intended	0	600	1200	3600	4800	6000
Analysed, starter	n.d.	565	1045	3300	4400	5500
Analysed, grower	n.d.	605	1100	3350	4300	5350
Analysed, finisher	n.d.	525	1100	3050	4250	5400
Mortality[(1)] % (n)	1.8 (3)	1.8 (3)	1.3 (2)	1.8 (3)	3.1 (5)	2.5 (4)
Zootechnical parameters						
Final body weight (g)[(2)]	2430^{a}	2464^{a}	2402^{a}	2308^{b}	2190^{b}	1929^{c}
Daily feed intake (g/bird)	90.1^{a}	91.1^{a}	87.6^{ab}	83.7^{bc}	79.9^{c}	72.6^{d}
Feed to gain ratio[(2)]	1.320^{d}	1.323^{d}	1.289^{c}	1.268^{bc}	1.253^{ab}	1.247^{a}

(Continues)

[19]Conditions of use of the additive.

[20]Annex_17_25_53_20001_Tolerance_broiler and Annex_05_AddInfo_04_2025_Annex_17_25_53_20001_corr.

[21]Annex_17_25_53_20001_Tolerance_broiler and Annex_05_AddInfo_04_2025_Annex_17_25_53_20001_corr.

[22]Organs sampled: liver, kidneys, spleen, adrenal gland, lung, proventriculus, gizzard, pancreas, small intestine, colon, caecum, thymus, thyroid gland, heart, intestinal lymph nodes, testes.

[23]Haematological measurements (1 EDTA tube), including haematocrit (HT), haemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), erythrocytes count (ERY), platelets (PLA) and white blood cell count (WBC) and differentials as well (eosinophils, basophils, etc.).

[24]Biochemical measurements (1 serum gel S tube): Sodium, potassium, chloride, calcium, phosphorus, magnesium, total protein, albumin, globulin, glucose, uric acid, cholesterol, creatinine, bilirubin, amylase, alanine aminotransferase (ALT-SGPT), aspartate aminotransferase (AST-SGOT), lactate dehydrogenase (LDH), gammaglutamyltransferase (GGT), alkaline phosphatase and creatine kinase (CPK).

TABLE 3 (Continued)

	Control	0.5×	1×	3×	4×	5×
Gross pathology[3]						
Petechia	0[b]	0[b]	0.06[b]	0.06[b]	0[b]	0.31[a]
Blood parameters						
GGT (U/L)	20.7[ab]	18.7[ab]	18.3[b]	19.9[ab]	21.9[a]	18.8[ab]
AST (U/L)[4]	481[ab]	596[a]	585[a]	459[ab]	444[ab]	412[b]
Amylase (U/L)[4]	459[b]	579[ab]	578[ab]	589[ab]	735[a]	669[b]
Creatine Kinase (U/L)[5]	44,513[a]	43,264[a]	47,289[a]	35,769[ab]	28,011[ab]	22,326[b]
Total Protein (g/L)	24.9[a]	23.9[ab]	24.1[ab]	23.6[ab]	20.4[ab]	18.8[b]
Albumin (g/L)	13.1[ab]	12.9[ab]	13.6[a]	12.8[ab]	11.8[ab]	10.9[c]
Cholesterol (mg/dL)	129.7[a]	130.6[a]	129.3[a]	113.9[b]	103.6[b]	104.3[b]
Bilirubin (mg/dL)	0.62[ab]	0.65[a]	0.62[ab]	0.48[bc]	0.41[c]	0.37[c]
MCV (fL)	231.9	219	217.6	222.3	191.4	193.7
MCH (pg/L)	51.7	47.3	45.6	46.9	40.6	40.4
Haemoglobin (g/dL)	7.29[a]	6.93[ab]	7.11[ab]	6.84[ab]	6.64[ab]	6.39[b]
Creatine (mg/dL)	1.28[d]	1.75[cd]	2.20[bc]	2.74[ab]	2.86[ab]	3.39[a]
Creatinine (mg/dL)	< 0.17	< 0.17	< 0.17	< 0.17	< 0.17	< 0.17
Guanidinoacetic acid (μmol/dL)[6]	0.7[d]	8.3[d]	13.3[d]	52.6[c]	72.8[b]	92.2[a]
Homocysteine (μmol/dL)[6]	71.8[b]	76.0[b]	66.6[b]	74.9[b]	75.4[b]	88.8[a]

Abbreviations: AST, aspartate aminotransferase; GGT, g-glutamyl transpeptidase; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

[a,b,c]Different superscripts in a row mean significant differences ($p < 0.05$) – Tukey test n.d., not detected; (limit of quantification (LOQ), guanidinoacetic acid < 50 mg/kg; limit of detection (LOD), creatine 0.17 mg/dL).

[1]Mortality includes culled birds.

[2]Data corrected for outliers by Grubb's test or interquartile box ±2.5× interquartile range.

[3]Frequency of chickens that presented lesions (total number of chickens observed = 16). Same results were observed for duodenum, jejunum and ileum.

[4]Data corrected for outliers (two birds removed from the dataset: two from 4× group for AST; one birds from 0.5× group and one from 5× group for AMY).

[5]Data corrected for outliers (four in total) and missing information (10 birds with missing results).

[6]Data corrected for outliers.

Mortality ranged from 1.3% to 3.1% and it was not treatment related. Performance was unaffected up to the maximum recommended level (1200 mg/kg), but significant reductions in body weight and feed intake occurred at 3× and higher levels (≥ 3600 mg/kg). At the highest level (5×, 6000 mg/kg), several changes in blood parameters (e.g. decreased total protein, bilirubin, albumin and haemoglobin) were detected. An increase in frequency of petechia in mucosa and serosa (either duodenum, jejunum and ileum) was observed in the 5× group relative to control. No relevant histopathological observations were identified in any of the treated groups.

The Panel concludes that the guanidinoacetic acid is safe for chickens for fattening up to the maximum recommended use level (i.e. 1200 mg guanidinoacetic acid/kg complete feed) without a margin of safety. This conclusion is extended to chickens reared for laying/reproduction.

3.2.1.2 | *Safety for turkeys for fattening*

A total of 192 one-day-old B.U.T.6 male turkeys were randomly distributed in 24 floor pens and each pen allocated to one of the four experimental treatments (six replicates/treatment; eight birds/pen).[25] The diets were based on maize, wheat and soybean meal and contained 6.5–6.1-5.7 g methionine/kg, 720 mg choline/kg, 1.2 mg folic acid/kg and 18 μg/kg vitamin B_{12}. The experimental diets were either not supplemented (Control) or supplemented with GuanAMINO® at 800 (0.66×, maximum recommended level), 1600 (1.33×) or 2400 (2×) mg guanidinoacetic acid/kg complete feed (confirmed by analysis). Birds underwent a three-phase feeding program with a starter (1–14 days), grower (15–28 days) and finisher (29–42 days) diets in which both diets were pelleted. Mortality and bird's health was monitored daily. Body weight and feed intake was determined on weekly basis. Average weight gain and feed to gain ratio was calculated. At 42 days, two birds per pen were selected (based on BW close to the group average) for haematology[26] and clinical chemistry[27] analysis. Blood analyses included the serum concentration of guanidinoacetic acid, creatine, creatinine and homocysteine. The same two birds were

[25]Annex_18_25_54_15001_Tolerance_Turkey_with_Appendices_Final and ADR submitted April 2025: Annex_07_AddInfo_04_2025_25_54_15001_Turkey_corr.

[26]The analysis included haematological parameters (erythrocytes, leukocytes, differential haemogram: lymphocytes, monocytes, eosinophils, basophiles, neutrophils, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)), serum concentrations of minerals (sodium, potassium, chlorine, calcium and inorganic phosphate).

[27]The biochemical parameters included total cholesterol, triglycerides, bilirubin, uric acid, glucose, albumin, total protein, creatine, guanidinoacetic acid and homocysteine) and enzyme activities (aspartate-amino-transferase = AST, gammaglutamyl-transferase = GGT, glutamate-dehydrogenase = GLDH).

killed and subject to necropsy, gross pathology[28] and histopathology analysis. Concentrations of guanidinoacetic acid, creatine, creatinine and homocysteine were analysed in edible tissues[29] from birds used for pathological examinations.

Data were analysed statistically by one-way ANOVA using the pen as a statistical unit. Differences between groups were tested with Tukey test and the significance was set at $p < 0.05$ (Table 4).

TABLE 4 Main results of the tolerance study with guanidinoacetic acid in turkeys for fattening (1–42 days).

	Control	0.66×	1.33×	2×
Guanidinoacetic acid (mg/kg feed)				
Intended	0	800	1600	2400
Analysed, starter	< 1	1043	2136	3056
Analysed grower	< 1	876	1785	2748
Analysed, finisher	< 1	869	1901	2703
Mortality[(1)] (%) (n)	4.2 (2)	2.1 (1)	4.2 (2)	2.1 (1)
Zootechnical parameters				
Final body weight (g)	2633^{c}	2708^{c}	2844^{b}	2940^{a}
Daily feed intake (g/day)	101.1^{c}	103.1^{cb}	105.8^{ab}	106.7^{a}
Daily weight gain (g/day)	61.1^{c}	62.9^{c}	66.1^{b}	68.4^{a}
Feed to gain ratio	1.655^{a}	1.639^{a}	1.600^{b}	1.559^{c}
Serum parameters				
Creatinine (mg/dL)	< 0.2	< 0.2	< 0.2	< 0.2
Creatine (mg/dL)	1.70^{b}	2.21^{a}	2.41^{a}	2.45^{a}
Guanidinoacetic acid (μmol/L)	1.86^{d}	8.96^{c}	15.90^{b}	26.76^{a}
Homocysteine (μmol/L)	63.6^{b}	66.2^{ab}	74.2^{a}	64.5^{ab}
Erythrocytes (counts $\times 10^{12}$/L)	1.94	1.90	1.89	1.94
Monocytes (%)	10.8^{ab}	6.83^{b}	11.00^{ab}	12.67^{a}
Mean corpuscular volume (pg)	111.0^{b}	113.4^{ab}	116.4^{a}	114.6^{a}
Total protein (g/L)	32.7	31.2	32.3	33.3

^{a,b,c}Different superscripts in a row mean significant differences ($p < 0.05$) – Tukey test. Limit of detection of creatinine: 0.2 mg/dL. Limit of quantification for GAA: 1 g/kg.
$^{(1)}$Mortality and culled birds (reasons for death or culling were anorexia, disturb equilibrium, crop paralysis).

Mortality ranged from 2.1% to 4.2% and was not treatment related. No adverse effects were observed on the performance of the animals at any supplementation level of the additive. Some differences were observed in some haematological parameters (e.g. monocytes), although they were either not treatment related or remained within the physiological ranges, and therefore, not considered adverse. No relevant differences were observed in gross pathology and histopathological examinations.

Based on the results of the tolerance study in turkeys, the FEEDAP Panel concludes that no adverse effects were observed when guanidinoacetic acid was supplemented up to 2400 mg/kg complete feed. Also, the FEEDAP Panel concludes that the additive is safe up to the maximum recommended level (i.e. 1200 mg guanidinoacetic acid/kg feed) in turkeys for fattening. This conclusion is extended to turkeys reared for reproduction.

3.2.1.3 | *Conclusions on safety for the target species*

The FEEDAP Panel concludes that guanidinoacetic acid is safe for chickens for fattening, chickens reared for laying/reproduction, turkeys for fattening and reared for reproduction up to the maximum use level of 1200 mg/kg complete feed.

The same conclusions apply for its use in water for drinking up to a maximum concentration of 600 mg guanidinoacetic acid/L. However, considering the variability in water intake and the fact that no margin of safety was identified for chickens for fattening, the Panel considers that the exposure via water should not exceed the maximum safe level of the additive when administered via feed only.

3.2.2 | Safety for the consumer

To support the safety of the additive for the consumer, the applicant referred to the studies submitted in a previous application for the same additive and already assessed by the FEEDAP Panel (EFSA, 2009). The Panel notes that the composition

[28]Digestive tract, including proventriculus, gizzard and intestine, as well as internal organs (lungs, heart, gall bladder, liver, spleen, kidneys, respiratory-, urogenital- and reproductive-tract) were examined. Additionally, weights for liver, heart, spleen and kidneys were recorded.
[29]Breast muscle, liver, kidney.

and the conditions of use of the additive under assessment are the same as those previously assessed and currently authorised. Therefore, the conclusions of the previous assessment apply to the current evaluation. In addition, the applicant submitted data on the deposition of guanidinoacetic acid and its metabolites in tissues, and an in vitro micronucleus test, which are described below.

3.2.2.1 | *Absorption, distribution, metabolism, excretion (ADME) and residues*

The absorption, distribution, metabolism, excretion (ADME) profile of guanidinoacetic acid was described by the FEEDAP Panel in its previous assessment (EFSA, 2009). No new information was made available for the current application. The Panel considers that the conclusions previously reached apply also to the present opinion.

In 2009, the FEEDAP Panel assessed the tissue deposition of guanidinoacetic acid, its metabolites creatine and creatinine and homocysteine based on data from four studies carried out on chickens for fattening. Feeding chickens at levels up to 6000 mg guanidinoacetic acid/kg feed resulted in a dose-related decrease of guanidinoacetic acid concentrations in muscle and liver (in the latter, except for the dose of 600 mg/kg feed). On the other hand, the creatine and creatinine contents in muscle and liver were found to be consistently increased by guanidinoacetic acid supplementation in those studies, when analysed. Data on homocysteine were limited to plasma but showed the positive response to guanidinoacetic acid supplementation in feed only at levels of 6000 mg/kg feed in chickens (EFSA, 2009).

In the context of the present assessment, creatine, creatinine, guanidinoacetic acid and homocysteine concentrations, following administration of GuanAMINO®, were measured in the tolerance trials in chickens for fattening and turkeys for fattening as described in Section 3.2.1. Measurements in chickens are considered relevant also for turkeys and the main results are summarised in Table 5.

TABLE 5 Deposition of guanidinoacetic acid, creatine, creatinine and homocysteine in muscle, kidney, liver and skin and fat in chickens for fattening fed GuanAMINO® for 38 days.

	Treatments Guanidinoacetic acid (mg/kg feed)					
	0	**600**	**1200**	**3600**	**4800**	**6000**
Guanidinoacetic acid (mg/kg)						
Muscle	3.09^{bc}	2.53^{cd}	1.79^{d}	2.19^{cd}	3.72^{ab}	4.61^{a}
Kidney	61^{c}	72^{bc}	79^{bc}	101^{ab}	127^{a}	127^{a}
Liver	47.5^{ab}	57.1^{a}	47.0^{ab}	22.6^{c}	21.6^{c}	30.3^{bc}
Skin and fat	1.8^{c}	5.9^{c}	10.1^{c}	37.1^{b}	45.6^{ab}	51.7^{a}
Creatine (mg/kg)						
Muscle	3914^{b}	3934^{b}	4141^{ab}	4636^{a}	4569^{a}	4496^{a}
Kidney	93^{d}	115^{cd}	136^{bc}	191^{ab}	241^{a}	254^{a}
Liver	111^{d}	172^{c}	196^{c}	393^{b}	652^{a}	716^{a}
Skin and fat	209^{b}	244^{ab}	249^{ab}	311^{a}	331^{a}	321^{a}
Creatinine (mg/kg)						
Muscle	10.1^{d}	23.2^{ab}	28.5^{a}	13.7^{cd}	20.7^{bc}	19.8^{bc}
Kidney	2.37^{b}	3.54^{ab}	3.73^{ab}	3.31^{ab}	3.45^{ab}	5.00^{a}
Liver	1.26^{d}	2.40^{c}	2.57^{c}	4.55^{b}	7.77^{ab}	10.09^{a}
Skin and fat	2.0^{b}	3.33^{ab}	3.51^{a}	2.27^{ab}	2.56^{ab}	3.27^{ab}
Homocysteine (μg/kg)						
Muscle	2.51^{c}	2.57^{bc}	2.34^{c}	2.21^{c}	3.39^{ab}	3.60^{a}
Kidney	2.28^{cd}	2.06^{cd}	1.86^{d}	2.82^{bc}	3.64^{ab}	3.99^{a}
Liver	1.40	1.53	1.77	1.59	2.00	1.78
Skin and fat	1.21^{b}	1.92^{ab}	1.74^{ab}	1.41^{ab}	1.52^{ab}	2.0^{a}

[a,b,c]Different superscripts in a row mean significant differences ($p < 0.05$) – Tukey test.

The supplementation of chickens for fattening with GAA up to the maximum use level (1200 mg guanidinoacetic acid/kg feed) resulted in a reduction of guanidinoacetic acid in muscle, an increase of creatine in liver and kidney and an increase of creatinine in muscle, liver and skin+fat. The data provided in the context of this application are in line with the observations in the previous opinion (EFSA, 2009). The limited increase in creatine and creatinine in edible tissues is an expected consequence of the use of the additive and would result in a small increase in creatine concentration, remaining within the physiological range observed in fresh meat from different animal species.

The applicant provided similar data from the tolerance study in turkeys. However, the maximum use level was not tested, and data for skin+fat was not provided. Since similar trends were seen in the turkey data, the Panel considers that the data provided for chickens for fattening is representative for turkeys.

The use of the additive up to the maximum proposed use level (1200 mg guanidinoacetic acid/kg feed) does not result in an increased exposure of consumers to residues of concern.

3.2.2.2 | *Toxicology*

In the previous opinion (EFSA, 2009), the FEEDAP Panel assessed a set of genotoxicity studies (bacterial reverse mutation test and an in vitro mammalian chromosomal aberration test) and concluded on the lack of genotoxic concern. In the same opinion, repeated dose oral toxicity studies (28-day study and 90-day in rat) were evaluated and the Panel concluded that 'the effects reported in the 28 and 90-day studies generally reflect physiological responses to high exposures to a metabolic intermediate and do not identify any novel or unexpected toxicity'.

The Panel notes that the composition and the conditions of use of the additive under assessment are the same as the one previously assessed and currently authorised, and these conclusions would apply to the current assessment.

No new oral toxicity studies were provided by the applicant for the current assessment. In line with the requirements of the current guidance on the safety for the consumer (EFSA FEEDAP Panel, 2017a, 2017b, 2017c) the applicant submitted an in vitro micronucleus test performed with GuanAMINO® (analysed guanidinoacetic acid content 97.7%) in whole blood human lymphocytes according to OECD Test Guideline (TG) 487 and claimed to be performed in line with good laboratory practices (GLP).[30]

Three concentrations of the test item (i.e. 398, 697 and 1220 μg/mL) were selected for the analysis of micronuclei applying a short treatment in the presence and absence of metabolic activation (4 + 20 h of recovery) and a continuous treatment in the absence of metabolic activation (24 + 0 h of recovery). Since no cytotoxic effects were induced by the test item, the top concentration tested (approximately to 10 mM) corresponded to the highest concentration recommended by OECD TG 487. No significant increase in the frequency of micronuclei was induced by the test item after short treatments. After continuous treatment, a significant increase was observed at the lowest dose level compared to the concurrent vehicle control (0.7% vs. 0.3% micronucleated cells, respectively). The increase was not concentration-related and the induced frequency of micronuclei was within the range of the vehicle control values (0.00%–1.14% micronucleated cells); therefore, the FEEDAP Panel considered the increase not biologically relevant. The FEEDAP Panel concludes that GuanAMINO® did not induce structural and numerical chromosomal damage under the experimental conditions applied in the present study.

Based on the results of the studies previously evaluated by the FEEDAP Panel (EFSA, 2009) showing no induction of gene mutations and chromosomal aberrations, along with the negative findings of the in vitro micronucleus test provided by the applicant for the current assessment, the FEEDAP Panel concluded that the additive does not raise safety concern for genotoxicity.

3.2.2.3 | *Conclusions on safety for the consumer*

Considering the data assessed previously and the new data provided by the applicant, the FEEDAP Panel concludes that the use of GAA and GuanAMINO® under the proposed conditions of use is safe for the consumer.

3.2.3 | Safety for the user

To support the safety of the additive for the user, the applicant made reference to the studies submitted in a previous application[31] for the same additive and already assessed by the FEEDAP Panel (EFSA, 2009). In that opinion, the FEEDAP Panel concluded based on the results of skin/eye irritation studies and a skin sensitiser study that the use of the additive did not raise safety concerns for the users. The Panel notes that the composition and the conditions of use of the additive under assessment are the same as the one previously assessed and currently authorised. Therefore, the conclusions reached in the previous assessment fully apply to the current application. Considering the composition of the preparation, the conclusions reached with GAA are valid also for GuanAMINO®.

Therefore, the FEEDAP Panel concludes that GAA and GuanAMINO® do not raise safety concerns for the user.

3.2.4 | Safety for the environment

Guanidinoacetic acid is a physiological molecule and occurs naturally in animals. The use of GAA and GuanAMINO® would not lead to a faecal excretion of molecules which are not normally present in the excreta of the target animals. The FEEDAP Panel concludes that the use of GAA and GuanAMINO® as feed additives under the proposed conditions is not expected to pose a risk to the environment.

[30] Annex_19_MNT.

[31] The applicant also made reference to the efficacy studies submitted in the context of another application (EFSA-Q-2012-00273/FAD-2011-0043) but these studies were assessed in an EFSA FEEDAP Panel opinion (2016) that is still under data protection as per the data protection clause of the Regulation 1831/2003.

3.3 | Efficacy

To support the efficacy of the additive as zootechnical additive in chickens for fattening, the applicant made reference to the efficacy studies submitted in a previous application[32] for the same additive and already assessed by the FEEDAP Panel (EFSA, 2009). In that opinion, the FEEDAP Panel concluded, based on the results of four efficacy studies in chickens for fattening, that 600 mg guanidinoacetic acid/kg complete feed improved performance characteristics. The Panel notes that the composition and the conditions of use of the additive under assessment are the same as the one previously assessed and currently authorised. Therefore, the conclusions reached in the previous assessment fully apply to the current application.

In addition, the applicant provided the results of two tolerance trials, one in chickens for fattening[33] and one in turkeys for fattening[34] (see Section 3.2.1) and two additional studies in turkeys for fattening.[35] However, these studies did not provide evidence of the efficacy of the additive in the target species at the minimum proposed use level (600 mg/kg complete feed).

Considering the above, the FEEDAP Panel concludes that GAA is efficacious in chickens for fattening at the minimum use level of 600 mg guanidinoacetic acid/kg complete feed. This conclusion is extended to chickens reared for laying/reproduction and turkeys for fattening and reared for reproduction. The conclusions reached for the use in feed apply to the use in water, considering that water intake is two to three times the dry matter feed intake (EFSA FEEDAP Panel, 2024). Therefore, the Panel considers that the GAA has the potential to be efficacious in the above target species when used in water for drinking at 300 mg of guanidinoacetic acid/L water. These conclusions apply to GuanAMINO®.

3.4 | 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[36] and Good Manufacturing Practice.

4 | CONCLUSIONS

The use of GAA and GuanAMINO® are safe for chickens for fattening and reared for laying/reproduction and for turkeys for fattening and reared for reproduction when used up to the maximum proposed use level of guanidinoacetic acid of 1200 mg /kg complete feed and 600 mg/L water for drinking. However, considering the variability in water intake and the fact that no margin of safety was identified for chickens for fattening, the Panel considers that the exposure via water should not exceed the maximum safe level of the additive when administered via feed only.

The use of the additives in animal nutrition under the proposed conditions of use is of no concern for the consumer and the environment.

Regarding user safety, the FEEDAP Panel concludes that GAA and GuanAMINO® are not irritant to the skin or eyes and are not skin sensitisers. Therefore, the FEEDAP Panel concludes that GAA and GuanAMINO® do not raise safety concerns for the user.

GAA and GuanAMINO® are efficacious in chickens for fattening, chickens reared for laying/reproduction and turkeys for fattening and reared for reproduction when used at the minimum use level of 600 mg/kg complete feed or 300 mg/L water.

5 | RECOMMENDATIONS

The Panel notes the maximum safe levels of guanidinoacetic acid in chickens for fattening, chickens reared for laying/reproduction and for turkeys for fattening and reared for reproduction are derived under the assumption that the feed contains sufficient amounts of methyl donors (other than methionine, e.g. choline, betaine and folic acid) and vitamin B_{12}. In accordance with more recent relevant opinions on GAA, the FEEDAP Panel recommends including the corresponding statement in the 'other provisions' of the authorisation for the use of GAA in feed or water for drinking in line with the current authorisation for guanidinoacetic acid.

ABBREVIATIONS

ADME	absorption, distribution, metabolism, excretion
ALT	alanine aminotransferase
AST	aspartate aminotransferase

[32]The applicant also made reference to the efficacy studies submitted in the context of another application (EFSA-Q-2012-00273/FAD-2011-0043) but these studies were assessed in an EFSA FEEDAP Panel opinion (2016) that is still under data protection as per the data protection clause of the Regulation 1831/2003.

[33]Annex_17_25_53_20001_Tolerance_broiler.

[34]Annex_18_25_54_15001_Tolerance_Turkey.

[35]Annex_23_25_54_07001_Olsztyn_turkey and Annex_22_25.54.05001_Schothorst_turkey.

[36]Regulation (EC) No 183/2005 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.

BW	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CFU	colony forming unit
CPK	alkaline phosphatase and creatine kinase
ECHA	European Chemicals Agency
ERY	erythrocytes count
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GAA	guanidinoacetic acid
GGT	gammaglutamyltransferase
HB	haemoglobin
HT	haematocrit
LDH	lactate dehydrogenase
LOD	limit of detection
LOQ	limit of quantification
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
nDL-PCBs	non-dioxin-like PCBs
OECD	Organisation for Economic Co-operation and Development
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-*p*-dioxins
PCDFs	polychlorinated dibenzofurans
PLA	platelets
RH	relative humidity
TEQ	toxic equivalent factors for dioxins, furans and dioxin-like PCBs
WBC	white blood cell count
WHO	World Health Organization

REQUESTOR

European Commission

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