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Dietary guanidinoacetate reduces spaghetti meat myopathy risk in the breast muscle of broiler chickens

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ABSTRACT

The global demand for white chicken meat along with the increase in the occurrence of growth-related breast muscle myopathies (**BMMs**) [namely white striping (**WS**), wooden breast (**WB**), and spaghetti meat (**SM**)] highlights the need for solutions that will improve meat quality while maintaining the high productivity of modern broilers. Guanidinoacetate (**GAA**), a precursor of creatine, is used as a feed additive and has previously shown the potential to affect the quality of breast meat. This study investigated growth performance, meat quality and the risk ratio for the development of BMMs in broilers assigned to two dietary treatments: control (**CON**) group, fed a commercial basal diet, and supplemented GAA (**sGAA**) group, receiving the control diet supplemented on top with 0.06% GAA. Growth performance indicators such as BW, daily weight gain, daily feed intake, feed conversion ratio and cumulative feed conversion ratio were recorded on a pen basis. As a trait affecting animal welfare, the occurrence of foot pad dermatitis was also evaluated. At day 43, birds were processed, and breasts were scored for the incidence and severity of BMMs (n = 166 and 165 in CON and sGAA groups, respectively). Quality traits (ultimate pH, colour) and technological properties (i.e., drip and cooking losses, marinade uptake, shear force, and oxidation levels of the lipid and the protein fractions) of breast meat were assessed in both treatments on samples not showing any macroscopic sign of BMMs (n = 20 breast fillets per group). Data of myopathy risk ratio were analysed as the risk for each group to develop WS, WB, and SM myopathies. Our results show that while sGAA and control groups did not differ significantly in growth performance, a remarkably beneficial effect of GAA was observed on the incidence of BMMs with significantly reduced risk of sGAA group to develop SM myopathy. The risk of sGAA group to develop SM was 30% lower compared to CON (P = 0.028). Finally, a significantly lower drip loss was observed in sGAA in comparison with CON (1.78 vs 2.48%, P = 0.020). Together, our results show that the inclusion of 0.06% GAA in feed can improve the water-holding capacity of meat and reduce the risk to develop SM myopathy without compromising the performance of broilers.

Implications

The significance of this study lies in its ability to address one of the major challenges faced by the poultry industry, marked by an increasing prevalence of growth-related breast muscle myopathies, resulting in meat quality issues and substantial economic losses worldwide. Dietary inclusion of guanidinoacetate demonstrated a reduction in the risk of spaghetti meat myopathy and an enhanced water-holding capacity, a key trait for meat during storage and processing. Notably, these improvements were achieved without reducing broiler performance, thus offering a sustainable and practical solution to meet demands for high-quality meat and to tackle relevant breast muscle myopathies challenges.

Introduction

White striping (**WS**), wooden breast (**WB**), and spaghetti meat (**SM**) abnormalities belong to a group of growth-related myopathies that affect the integrity of the breast muscle (*Pectoralis major*) of modern broiler chickens. WS includes the appearance of white striations parallel to the muscle fibres (Kuttappan et al., 2013), WB manifests with the presence of out-bulging and pale areas of hardened consistency (Sihvo et al., 2014), and SM, is characterised by the tendency for muscle fibre bundles separation (Sirri et al.,

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2016). Various studies over the past 10–15 years have suggested a possible connection between these newly emerged myopathies and the high growth rates of modern broilers, which have undergone intense selective breeding since the 1950 s (Velleman and Clark, 2015; Petracci et al., 2019; Soglia et al., 2021). The high incidence rate of these breast muscle myopathies (**BMMs**) triggered an increasing concern in the poultry industry and research community. Indeed, issues of consumers' acceptance as well as those related to meat quality and processability (e.g., poor texture, lower marinade uptake, higher drip and cooking losses) have forced producers to increase the rejection rate or downgrading of fresh breast meat (Kuttappan et al., 2016; Petracci et al., 2019). The negative economic impact for the poultry industry is estimated at an annual cost of up to 1 billion dollars in North America alone, mainly associated with sorting out, trimming or diverting meat to manufacture ground and emulsified processed products, thus reducing the profits on affected breast fillets (Che et al., 2022).

The onset of BMMs is characterised by a complex aetiology, involving several metabolic and biologic processes (Soglia et al., 2021). Overall, the association observed between high breast yield and growth rate and the distinctive phenotypes of BMMs supports the assumption of a genetic basis of these defects (Bordignon et al., 2022). However, as genetic correlations and heritability estimates suggest a modest genetic component to the manifestation of BMMs (Bailey et al., 2020), other factors should be considered as means to reduce the prevalence of BMMs. These factors include incubation, brooding, ventilation, temperature, gender, nutrition, and slaughter age (Bordignon et al., 2022). Various nutritional strategies are suggested as a useful solution to reduce myopathic conditions (Bailey et al., 2020; Trocino et al., 2023). These include reduction of feed intake, limiting the feed availability or feeding diets with low energy or amino acids content to restrain the growth rate of broilers and allow optimal development of muscle support structures (i.e., the vascular system and connective tissues). However, as these strategies pose a negative impact on production efficiency through poor feed conversion and growth, the use of targeted nutrients (e.g., specific amino acids) is advised as a more effective solution (Meloche et al., 2018a,b; Zampiga et al., 2019; Lackner et al., 2022).

A candidate natural amino acid is guanidinoacetate (**GAA**), a direct endogenic precursor of creatine which supports muscle function by allowing rapid provision of ATP through the creatine/phosphocreatine system (Bessman and Carpenter, 1985). The potential of GAA to reduce myopathies in poultry was recently reviewed by Oviedo-Rondón and Córdova-Noboa (2020). GAA inclusion in feed was shown to reduce the incidence and severity of WB and WS (Córdova-Noboa et al., 2018a, b; Maynard et al., 2023). This is probably due to the role of GAA in reducing insulin resistance, lowering calcium levels and improving antioxidant capacity (Ostojic et al., 2020), increasing vasodilation through sparing of arginine (Zampiga et al., 2019), and promoting the energetic balance in muscles due to increased levels of creatine to allow optimal muscle function during growth (Baldi et al., 2021). To date, there is limited or no evidence on dietary GAA and its ability to ameliorate the prevalence of SM myopathy in broilers. This is probably linked to the recentness of the SM condition, and its incidence that is problematic to predict compared to WB and WS myopathies, making it more difficult to study (Baldi et al., 2021).

This study aimed to examine the effect of GAA supplementation on the risk ratio for the development of WS, WB, and SM myopathies, and on meat quality. Accordingly, we followed broilers assigned to two dietary treatments: control (**CON**) group, fed a commercial basal diet, and supplemented GAA (**sGAA**) group, receiving the same basal diet supplemented on top with 0.06% GAA. Growth performance was recorded throughout the experiment including traits of BW, daily weight gain, daily feed intake and feed conversion ratio. As a trait affecting performance (Hashimoto et al., 2013), the occurrence of foot pad dermatitis was also evaluated in this experiment. On day 43, birds were evaluated for the risk of each group to develop WS, WB, and SM myopathies, in addition to the main quality traits and technological properties of the breast meat (Dayan et al., 2023 abst., show preliminary results).

Material and methods

Animals and husbandry

A total of 420 male broiler chickens (Ross 308) were reared from the day of hatch until day 43 and assigned to two dietary treatments (seven pens/treatment and 30 birds/pen). The birds were housed in randomly distributed concrete-floor pens (2.5 m^2 each) using wood shavings (2 kg/m^2) as litter material. Stocking density followed the European legislation (maximum of 33 kg/m^2). Each pen was provided with a circular pan feeder able to guarantee at least 2 cm of front space/bird and an independent drinking system with one nipple/five birds. The environmental temperature, humidity and lighting were defined according to the management guide provided by the breeding company. Twice daily observations recorded general flock condition, temperature, lighting, water, feed, litter condition and mortality. A 3-phase feeding programme was used (starter: 0–14 d; grower: 15–28 d; and finisher: 29–43 d), no antibiotics or coccidiostats were included, and feed was administered in a mash form for ad libitum consumption (water was supplied on ad libitum basis as well). Birds of CON were fed a commercial basal diet formulated to meet the current recommendations for fast-growing broilers (Table 1). Birds of sGAA treatment received the same commercial basal diet supplemented on top with 0.06% GAA (Alzchem Trostberg GmbH, Germany). Growth performance was recorded and calculated on a pen basis at placement (day 0), at each diet change (14 and 28 d), at slaughter (43 d) and for the overall period. The following traits were determined: BW, daily weight gain, daily feed intake and feed conversion ratio. At day 43, all birds were processed in a commercial plant, where the overall carcass weight of each group was recorded after air-chilling and carcass yield was calculated as the percentage of body live weight. Similarly, skinless, and deboned breast was mechanically obtained from the carcass and yield was calculated on a group basis as percentage of carcass weight.

Occurrence of foot pad dermatitis and breast meat abnormalities

At day 43, the incidence and severity of foot pad dermatitis (**FPD**) were macroscopically evaluated (n = 166 and 165 observations in CON and sGAA groups, respectively, one foot/bird) according to a 3-point scale evaluation system (Ekstrand et al., 1998): score 0 = no lesions, score 1 = mild lesions (< 0.8 cm), and score 2 = severe lesions (> 0.8 cm).

Also, the incidence and severity of WS, WB, and SM abnormalities were evaluated approximately 24 h after processing at a commercial plant (n = 166 and 165 observations in CON and sGAA groups, respectively). For each defect, a 3-point scale evaluation system was used to classify the magnitude of the myopathy with a score of 0 representing fillets with no myopathy, a score of 1 for moderate myopathy, and a score of 2 for severe myopathy. All the evaluations were performed by the same well-trained operators in analogous environmental conditions. For WS, the classification criteria were based on the thickness and distribution of white striations on the muscle surface (Kuttappan et al., 2013), whereas the hardness at palpation was used to evaluate the WB defect (Sihvo et al., 2014). Finally, the proneness to show muscle

Table 1
Composition of basal diet (control group) for broiler chickens in starter, grower, and finisher feeding phases[1].

Item	Feeding phase		
	Starter 0–14 d	Grower 15–28 d	Finisher 29–43 d
Ingredient (%)			
Wheat	25.1	30.1	34.3
Corn	25.7	22.5	20.0
White corn	0.00	5.01	7.03
Soybean meal	22.8	15.8	8.58
Soybean full-fat	10.0	13.1	17.0
Pea	3.01	3.01	3.01
Sunflower meal	3.01	4.01	3.01
Corn gluten	3.01	0.00	0.00
Soybean oil	3.36	3.49	4.27
Calcium carbonate	0.37	0.42	0.78
Dicalcium phosphate	1.05	0.39	0.00
Salt	0.36	0.24	0.18
Sodium bicarbonate	0.06	0.17	0.25
Choline	0.10	0.10	0.06
Lysine sulfate (50%)	0.68	0.61	0.52
L-Threonine (98%)	0.20	0.16	0.13
DL-Methionine	0.33	0.31	0.27
Amino acid mix (Arg + Val + Ile)	0.20	0.22	0.22
Vitamin-mineral premix[2]	0.50	0.45	0.30
Phytase	0.10	0.10	0.10
Xylanase	0.05	0.05	0.05
Mycotoxin binder	0.10	0.10	0.10
Chemical composition expressed on fed basis (%)			
DM	88.6	88.5	88.7
CP	23.4	20.3	18.3
Total lipid	6.78	7.53	9.07
Crude fibre	3.17	3.26	3.07
Ash	5.01	4.27	4.00
Calcium (total)	0.74	0.58	0.57
Phosphorous (total)	0.58	0.45	0.36
Lysine (total)	1.48	1.31	1.15
Met + Cys (total)	1.09	0.98	0.88
Threonine (total)	1.03	0.88	0.77
AME (kcal/kg)	3 087	3 166	3 311

Abbreviations: AME, apparent metabolisable energy.
[1] The guanidinoacetate-supplemented group received the same basal diet (as control) and was supplemented on top with 0.06% of guanidinoacetate (Alzchem Trostberg GmbH, Germany).
[2] Provided the following per kg of diet: vitamin A (retinyl acetate), 13 000 IU; vitamin D3 (cholecalciferol), 4 000 IU; vitamin E (DL-α_tocopheryl acetate), 80 IU; vitamin K (menadione sodium bisulfite), 3 mg; riboflavin, 6.0 mg; pantothenic acid, 6.0 mg; niacin, 20 mg; pyridoxine, 2 mg; folic acid, 0.5 mg; biotin, 0.10 mg; thiamine, 2.5 mg; vitamin B12 20 µg; Mn, 100 mg; Zn, 85 mg; Fe, 30 mg; Cu, 10 mg; I, 1.5 mg; Se, 0.2 mg; ethoxyquin, 100 mg.

deconstruction in response to an external stimulus (finger pinching), as described by Baldi et al. (2021), was used to score the breasts according to the SM defect.

Meat quality traits

As for the main quality traits of fresh meat, data were assessed in both groups on samples not showing any macroscopic sign of BMMs (n = 20 breast fillets per group).

The ultimate pH was measured on the antero-dorsal position of each *Pectoralis major* muscle by using a portable pH-meter (HI-98163, Hanna Instruments Ltd., UK), previously calibrated with standard buffer solutions at pH 4.01 and 7.01. Colour was measured in triplicate on the same position using a portable colorimeter Chroma Meter CR-400 Konica Minolta Sensing device (Minolta Sensing Inc., Osaka, Japan) under standardised conditions (i.e., illuminant Phu D65 and CIE 2° standard observer). Colour was then expressed as lightness (L*), redness (a*), and yellowness (b*) (Commission Internationale de l'Éclairage, 1976).

Water holding capacity was assessed by measuring drip and cooking losses. A parallelepiped meat cut (8 × 4 × 3 cm, weighing about 80 g) was excised from the antero-ventral position of the cranial area of each breast muscle. After being individually weighed, samples were placed in plastic boxes over sieved plastic racks and stored for 48 h at 4 ± 1 °C. Then, samples were blotted (to remove the eventual fluid on the surface), weighed, and drip loss was calculated as the percentage of weight lost during refrigerated storage (Petracci and Baéza, 2011). Each sample was individually packaged under vacuum and cooked in a water bath (75 °C) until reaching 72 °C in the inner core and cooking loss was calculated as the percentage of weight lost during cooking.

Shear force was determined on a parallelepiped sub-sample (4 × 1 × 1 cm), excised following the direction of the fibres, by using a TA-HDi Heavy Duty texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, the UK), equipped with 5-kg load cell and a Warner-Bratzler shear cell set at a cross head speed of 2 mm/sec. The shear values were then expressed as kg.

As for the occurrence of oxidative phenomena affecting the protein and the lipid fraction, carbonyls content and thiobarbituric acid reactive substances were measured following the procedures described by Bao and Ertbjerg (2015) and Soglia et al. (2016), respectively.

Statistical analyses

Growth performance data were analysed using one-way ANOVA with blocks (Group = experimental factor; Replicate = ex perimental unit). Data concerning meat quality were analysed using one-way ANOVA and considered significant with a *P*-value lower or equal to 0.05 ($P \leq 0.05$).

Data of BMMs and FPD were analysed with Pearson's chi-squared test using the sampled animal as the experimental unit. Then, data were arranged in two by two contingency tables aligning the levels of the group (i.e., CON and sGAA) and having binarily aggregated BMMs or FPD scores in columns (i.e., "presence" as a sum of score 1 and score 2 counts, while "absence" as score 0 counts). The incidence risk ratio was computed on these tables with the package epiR of the software R. The risk of developing a specific BMMs or FPD was calculated as incidence risk ratio minus 1 and expressed in percentage. A confidence interval of 95% and Pearson's chi-squared test were used to test the significance of the incidence risk ratio. In addition, BMMs data were aggregated for the acceptability assay as follows: fillets severely affected by WS, WB, or SM (i.e., getting a score of 2 at least for one BMMs) were referred to as downgraded, while those without or with moderate-degree BMMs (i.e., getting only score 0 or 1) were graded as normal. The incidence risk ratio was computed on the acceptability data as described before. All data were analysed using 'R' software (R Core Team, 2020).

Results

Growth performance

During the feeding trial, apart from a significant difference in hatching weight between groups (42.26 vs 41.75 g for CON and sGAA, respectively; *P* = 0.040], there were no significant differences in performance parameters. These traits include BW, daily weight gain, daily feed intake, feed conversion ratio, and mortality (Table 2). Also for slaughter yields, no remarkable differences were found between CON and sGAA groups with eviscerated carcass of 71.9 and 71.8%, respectively. Also for cut-up yields out of eviscerated carcass, no substantial differences were found between CON and sGAA groups with breast muscle percentage of 32.8 and

Table 2
Growth performance in chicken broilers from control and guanidinoacetate-supplemented groups.

Trait	Group		SEM	*P*-value
	CON	sGAA		
Hatchling weight (g/bird)	42.26	41.75	0.36	0.040
Starter period (0–14 d)				
BW (g/bird)	445.2	439.0	27.45	0.690
Daily weight gain (g/bird/d)[1]	28.51	28.14	1.92	0.729
Daily feed intake (g/bird/d)[1]	39.36	38.86	1.47	0.548
Feed intake (g/bird)[1]	551	544	20.6	0.548
Feed conversion ratio[1]	1.382	1.383	0.07	0.977
Mortality (%)	0.48	0.48	0.07	1.000
Grower period (15–28 d)				
BW (g/bird)	1 567	1 535	74.27	0.450
Daily weight gain (g/bird/d)[1]	79.91	78.15	4.13	0.457
Daily feed intake (g/bird/d)[1]	116.1	115.4	3.94	0.754
Feed intake (g/bird)[1]	1 626	1 616	55.1	0.754
Feed conversion ratio[1]	1.454	1.479	0.06	0.500
Mortality (%)	0.51	0.49	0.00	0.356
Finisher period (29–43 d)				
BW (g/bird)	3 259	3 205	114.1	0.406
Daily weight gain (g/bird/d)[1]	112.8	111.6	4.08	0.595
Daily feed intake (g/bird/d)[1]	204.9	202.1	6.93	0.466
Feed intake (g/bird)[1]	3 074	3 031	104.0	0.466
Feed conversion ratio[1]	1.820	1.812	0.07	0.838
Mortality (%)	0.00	0.49	0.05	0.356
Entire trial (0–43 d)				
BW (g/bird)	3 259	3 205	114.1	0.406
Daily weight gain(g/bird/d)[1]	74.74	73.48	2.65	0.409
Daily feed intake (g/bird/d)[1]	120.6	119.1	3.19	0.395
Feed intake (g/bird)[1]	5 251	5 191	160.4	0.511
Feed conversion ratio[1]	1.634	1.639	0.04	0.855
Mortality (%)	0.95	1.43	0.09	0.604

Abbreviations: CON = control group; sGAA = guanidinoacetate supplemented group.
[1] Data are presented as the mean of seven replicate pens/group and corrected for mortality.

33.3, respectively, leg percentages of 41.4 and 41.8, respectively, and wing percentages of 18.8 and 18.9, respectively. Overall, it can be concluded that the inclusion of 0.06% GAA in feed did not affect the growth performances of broilers.

Risk to develop breast muscle myopathies

A total of 166 and 165 breast fillets were evaluated for the incidence of WS, WB and SM abnormalities in CON and sGAA groups, respectively. Their incidence and severity rates are presented in Fig. 1. Results showed a positive trend of GAA to increase the percentage of breasts with no WS and SM defects and to reduce the occurrence of severe WS cases (P = 0.108 and 0.056, respectively, for WS and SM). No significant effect of the dietary treatment was observed on WB incidence and overall acceptability of breast fillets (Fig. 2). Risk analysis (Table 3) revealed a significant difference in the P of sGAA broilers for developing SM myopathy with 30% lower risk compared to the control (P = 0.028). As for the other growth-related myopathies, no significant changes were observed. The parameter of P for downgraded fillets was not significantly different between treatments.

Risk to develop foot pad dermatitis

Similarly to BMMs analysis, FPD incidence evaluation included 166 and 165 observations in the CON and sGAA groups, respectively. The incidence and severity of FPD are presented in Fig. 3. Risk analysis (Table 3) revealed that the dietary treatment did not significantly affect the risk to develop FPD.

Meat quality

The findings concerning the effect of the dietary supplementation of GAA on the main quality traits and technological properties of raw breast meat are shown in Table 4. Overall, supplementing the diet with GAA only marginally affected the main meat quality traits (i.e., ultimate pH and colour). In detail, if compared to CON, a significantly lower redness (a*) was found in sGAA (2.19 vs 1.76; $P < 0.05$). Although not statistically significant, it is interesting to point out the tendency observed for ultimate pH and lightness (L*) (P = 0.071 and P = 0.078, respectively). Indeed, if compared to CON, these traits tended to be respectively lower and higher in the breast meat belonging to sGAA group. As for the main technological properties of both raw and marinated breast meat, no significant differences ascribable to the dietary treatment were found with the only exception of the amount of fluid lost during refrigerated storage (i.e., drip loss). As for this parameter, the dietary supplementation with GAA resulted in a remarkably higher ability of meat to retain its constituting water during storage, as depicted by the significantly lower drip loss assessed in sGAA group in comparison with CON (1.78 vs 2.48%; P = 0.020). No differences were found in the cooking losses as well as oxidation level of both the lipid and the protein.

Discussion

In the current study, we followed the growth performance, meat quality and the risk ratio for the development WS, WB, and SM myopathies of broilers fed a commercial basal diet, or the same basal diet supplemented with 0.06% GAA. Our results show that while the dietary treatment did not exert any significant effect on the productive performance, a remarkably beneficial effect of GAA was observed on the incidence of SM with significantly

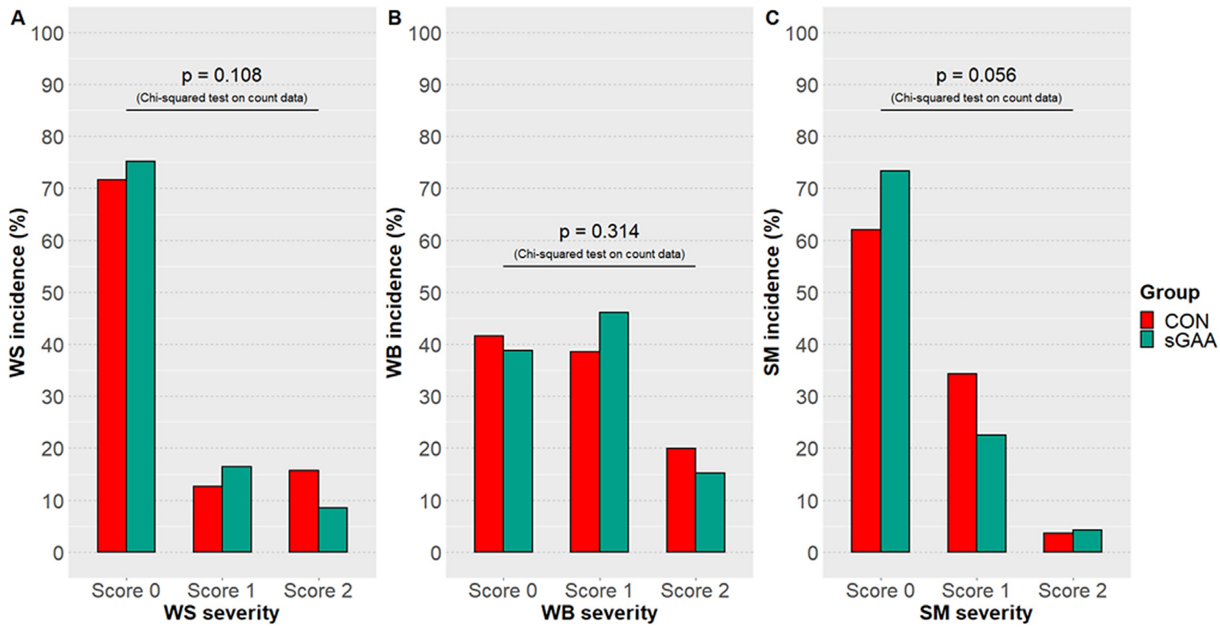

Fig. 1. Breast muscle myopathies (BMMs; white striping – WS; wooden breast – WB; spaghetti meat – SM) incidence and severity in 43-day-old chicken broilers from control (CON) and guanidinoacetate supplemented (sGAA) groups. Count data of BMMs, with severity rate in a score of 0 (non), 1 (moderate), and 2 (severe), were analysed with Pearson's chi-squared test (confidence interval of 95%). N = 166 and 165 observations in CON and sGAA groups, respectively.

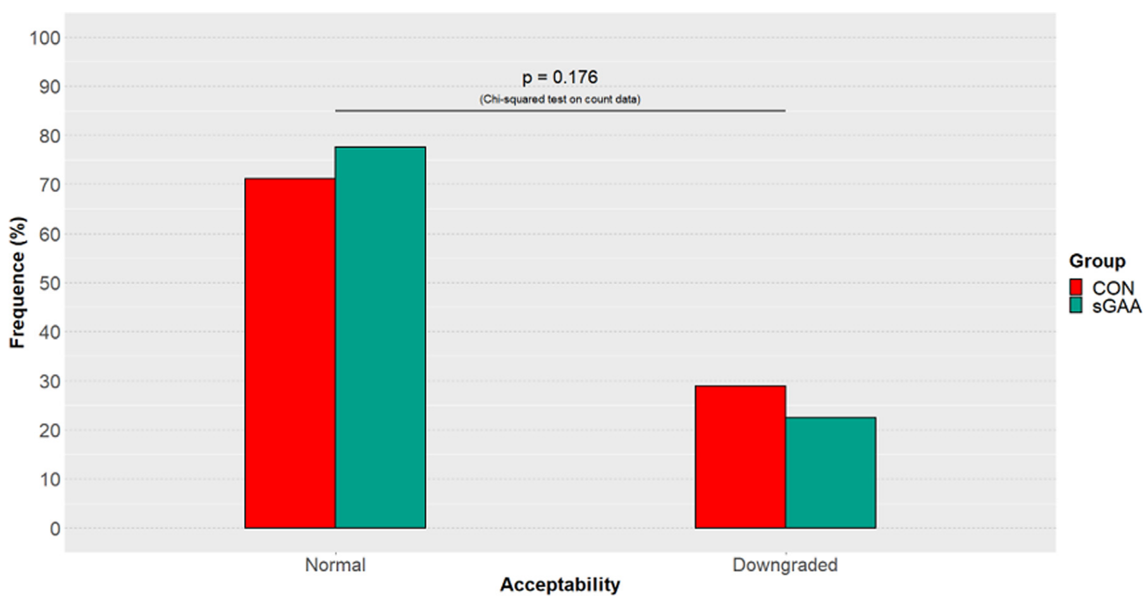

Fig. 2. Breast muscle myopathies (BMMs) acceptability rate in 43-day-old chicken broilers from control (CON) and guanidinoacetate GAA supplemented (sGAA) groups. Data were aggregated for the acceptability assay as follows: fillets severely affected with a score of 2 at least for one BMM were referred to as downgraded, while those without or with a moderate degree of BMMs (with a score of 0 or 1) were graded as normal. A confidence interval of 95% and Pearson's chi-squared test were used to test the significance of the incidence risk ratio for a downgraded breast fillet. N = 166 and 165 observations in control CON and sGAA groups, respectively.

reduced risk of the sGAA group to develop such myopathy. As for the measurements of meat quality, the drip loss percentage was significantly lower in sGAA group. Together, our results demonstrate the beneficial effect of dietary GAA on the quality of meat and the risk to develop BMMs without compromising the performance of broilers.

To analyse the risk to develop BMMs, for each myopathy, data were aggregated as "presence" including a sum of score one and two counts (i.e., moderate and severe lesions, respectively), and as "absence" including a score of zero counts. This statistical approach could be useful to further explore the data concerning muscle abnormalities, providing information about the *P* of developing BMMs in broilers fed the GAA-supplemented diet compared to those receiving the CON one. To our knowledge, this is the first study to show the beneficial effect of GAA to reduce the incidence rate of SM myopathy, with a 30% lower risk for developing such muscle abnormality. As for WS and WB myopathies, no significant reduction in their occurrence was observed. These findings differ from those reported by Córdova-Noboa et al. (2018a, b) who reported a positive effect on WB prevalence, and Maynard et al. (2023) who evidenced a positive effect on the prevalence of both WB and WS myopathies. One explanation could be the fact that Córdova-Noboa et al. (2018a, b) and Maynard et al. (2023) examined a different strain of broilers (Ross 708 compared to Ross 308

Table 3
Incidence risk analysis in 43-day-old broiler chickens from control and guanidinoacetate supplemented groups. Incidence risk ratio was calculated for each breast muscle myopathy, overall risk for downgraded breast fillet and foot pad dermatitis.

Trait	Contrast[1]	Risk ratio[2]	% Risk[3]	χ^2 *P*-value
WS Myopathy	sGAA vs CON	0.88 (0.61, 1.26)	−12%	0.476
WB Myopathy	sGAA vs CON	1.05 (0.88, 1.25)	5%	0.606
SM Myopathy	sGAA vs CON	0.70 (0.51, 0.97)	−30%	0.028
Downgraded fillet	sGAA vs CON	0.78 (0.54, 1.12)	−22%	0.176
FPD	sGAA vs CON	0.81 (0.54, 1.22)	−19%	0.314

N = 166 and 165 observations in control and guanidinoacetate-supplemented groups, respectively.
Abbreviations: WS = white striping; WB = wooden breast; SM = spaghetti meat; FPD = foot pad dermatitis; CON = control group; sGAA = guanidinoacetate supplemented group.
[1] The inverse contrast produces a risk ratio equals to the reciprocal of the risk ratio shown.
[2] 95% confidence interval is given in brackets.
[3] The risk was calculated as risk ratio minus 1 and is expressed in percentage.

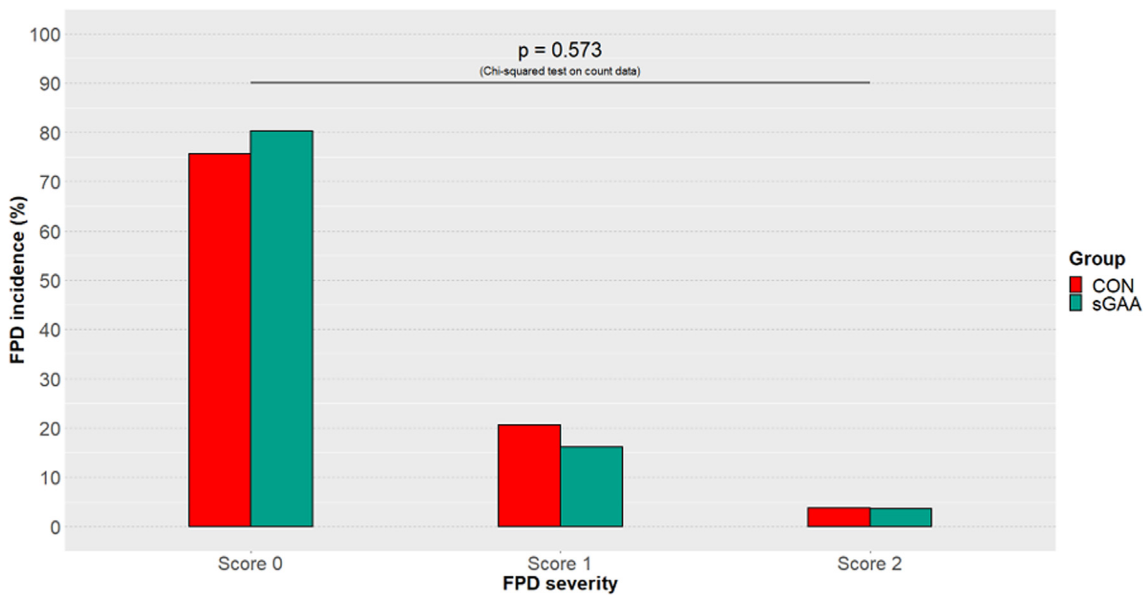

Fig. 3. Footpad dermatitis (FPD) incidence and severity in 43-day-old chicken broilers from control (CON) and guanidinoacetate GAA supplemented (sGAA) groups. Count data of FPD with severity rate in a score of 0 (non), 1 (moderate), and 2 (severe), were analysed with Pearson's chi-squared test (confidence interval of 95%). N = 166 and 165 observations in control CON and sGAA groups, respectively.

Table 4
Meat quality in 43-day-old broilers from control and guanidinoacetate-supplemented groups.

Trait	Group		SEM	*P*-value
	CON	sGAA		
Fillet weight (g)	251.3	243.4	4.3	0.556
Ultimate pH	5.96	5.88	0.03	0.078
Lightness – L*	55.51	57.50	0.41	0.071
Redness – a*	2.19[a]	1.76[b]	0.09	0.033
Yellowness – b*	5.56	6.54	0.21	0.076
Technological properties – raw meat				
Drip loss (%)	2.48[a]	1.78[b]	0.11	0.020
Cooking loss (%)	19.3	20.4	0.4	0.260
Shear force (kg)	1.66	1.66	0.05	0.983
Technological properties – marinated meat				
Uptake (%)	15.2	14.3	0.5	0.640
Cooking loss (%)	16.7	17.2	0.3	0.540
Shear force (kg)	1.22	1.24	0.05	0.901
Oxidation				
Carbonyls (nmol/mg of proteins)	1.93	2.05	0.07	0.786
Thioarbituric acid reactive substances (mg MDA/kg of meat)	0.25	0.28	0.03	0.715

Abbreviations: CON = control group; sGAA = guanidinoacetate supplemented group.
[a,b] Values within a row with different superscripts denote for means that are significantly different between groups, as derived from a one-way ANOVA analysis ($P \leq 0.05$).
N = 20 breast fillets per experimental group.

in our study). Bedford et al. (2017) suggests that the altered response to dietary GAA is the result of the metabolic differences between the Ross 308 and 708 strains, particularly different patterns in hepatic expression of genes associated with lipid metabolism and the higher rate of muscle fat deposition in the Ross 708 strain. Another explanation could be the age and weight differences between studies, which may have resulted in lower initial susceptibility of birds from our experiment to exhibit WB and WS myopathies. While we examined lighter 43-day-old birds, Córdova-Noboa et al. (2018a, b) and Maynard et al. (2023) examined heavier 56-day-old birds. BW and age at slaughter are two well-known factors correlated to higher incidence and severity of WB and WS myopathies (Petracci et al., 2019). Overall, the distribution between the three myopathies found in our study resembles previously reported data from large-scale surveys (Petracci et al., 2019; Che et al., 2022), with the highest number of fillets detected with WS, lowest number of fillets with WB, and SM with intermediate number. In this context, given that females exhibit a different occurrence distribution compared to males in each of the three myopathies, with an overall higher likelihood of developing SM myopathy (Bordignon et al., 2022), exploring whether dietary supplementation of GAA to females can produce similar results to ours, with a significant reduction in SM prevalence, would be particularly interesting.

The question regarding how GAA contributed to a lower incidence of SM myopathy still remains. A possible explanation could be related to the lower levels of the vimentin protein, directly involved in maintaining sarcomere cytoarchitecture, which was found in SM muscles (Soglia et al., 2020). In detail, a potential effect of creatine to increase the levels of the vimentin protein in mouse myotube cultures demonstrated by Young et al. (2010) allows to hypothesise a possible role of GAA in promoting the synthesis of vimentin that could be likely associated with the reduced manifestation of the SM phenotype. Our findings demonstrated an improvement in water-holding capacity of sGAA fillets as the drip loss percentage was significantly lower compared to the control. The improved water-holding capacity could be related to GAA-driven creatine attracting water into the muscle cells leading to super-hydrated muscle (Michiels et al., 2012). It was found that super-hydrated muscle may reduce drip loss through triggered protein synthesis, minimised protein breakdown (Häussinger, 1996), and increased glycogen synthesis (Young et al., 2007).

As for the performance of broilers, in accordance with Córdova-Noboa et al. (2018a, b) and Maynard et al. (2023), our findings revealed similarity in all examined performance traits, including BW, daily weight gain, daily feed intake, feed conversion ratio, and mortality. In this study, we also examined the occurrence of FPD as a trait affecting performance (Hashimoto et al., 2013) and results indicate that the dietary treatment did not remarkably affect the incidence and severity of FPD. Altogether, our results suggest that dietary GAA can be used as a strategy for improving meat quality without negatively affecting growth performance.

In conclusion, our results demonstrated the potential of dietary GAA in reducing the risk to develop SM myopathy in male Ross 308 broilers. In addition, dietary GAA was shown to improve the water-holding capacity of breast fillets, a relevant parameter of meat quality. These improvements were achieved without impairing the performance of broilers, thus offering a sustainable and practical solution to the poultry industry, struggling nowadays with the increased prevalence of SM myopathy.

Ethics approval

The experimental protocol was designed according to the guidelines of the current European and Italian laws on the care and use of animals kept for experimental purposes and approved by the Ethical Committee of the *Alma Mater Studiorum* - University of Bologna and by the Italian Minister of Health (n. 614/2021-PR).

Data and model availability statement

None of the data were deposited in an official repository. Data analysed in the current study are included in the article; further inquiries can be directed to the corresponding author.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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