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Effects of Guanidinoacetic Acid (GAA) Supplementation on Zootechnical Performance and Biochemical Parameters of Broiler Chickens in Dakar Region, Senegal

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Abstract: Creatine, a natural component synthesized in liver and kidneys from guanidinoacetic acid (GAA), is mainly used in muscle tissue, due to its major role in energy metabolism. Approximately 50% of the daily requirement should be provided by the diet, yet this creatine is not stable as a feed additive under current tropical feed manufacturing conditions. This study was therefore undertaken from April to June 2021 in Dakar region, to assess the effects of GAA supplementation on growth performance and biochemical parameters of broilers in Senegal. It involved 550 unsexed day-old broiler chicks of $Cobb_{500}$ strain with an average live weight (ALW) of 44.8 g. They were randomly divided into 2 batches of 275 birds each, subdivided into 5 replicates of 55 birds, corresponding respectively to two iso-nutritional dietary treatments, T-GAA_0 (control diet) and S-$GAA_{0.06}$ (control supplemented at 0.06% GAA). Raised according to recommended densities during the different rearing phases, the birds were *ad libitum* watered with tap drinking water and fed with these two diets presented in crumb (at start-up) and in pellet forms (during growth-finishing). Data collected or calculated, i.e. ALW, average daily gain (ADG), daily feed intake (DFI) and water consumption (DWC), feed conversion ratio (FCR), mortality rate (MR), carcass weight (CW), dressing carcass (DC), organ weights, blood total protein, albumin, creatinine, uric acid, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) levels per dietary treatment, were subjected to Student's t analysis using SPSS software at the 5% threshold to compare means. Results showed that supplementing diet with GAA (at 0.06%) had no adverse effect on broilers' health and their growth performance. It significantly ($p<0.05$) increased ALW (1923 vs.1796 g), ADG (45 vs. 42 g/day), DFI (93 vs. 91 g/bird/day), DWC (286 vs. 275 mL/bird/day), CW (1623 vs. 1526 g) and heart and gizzard weights, while reduced FCR (2.13 vs. 2.46), DC (84.4 vs. 85%) and liver weight (56 vs. 61 g) in birds compared to controls. It was also accompanied by a significant increase in blood albumin (17.5 vs. 16.9 g/L), creatinine (114.3 vs. 112.9 µmol/L), uric acid (0.86 vs. 0.78 mmol/L) and ALAT (34.3 vs. 27.5 UI/L) levels in birds concerned compared with controls, whereas total protein and ASAT levels were similar ($p>0.05$). Indeed, all biochemical parameters levels studied were in line with the corresponding reference values in broilers both for controls and supplemented birds, with the exception of uric acid content in blood which were higher.

Keywords: Broilers, Guanidinoacetic Acid, Growth performance, Feeding, Biochemical Parameters, Senegal

1. Introduction

Semi-industrial poultry farming has been one of the livestock sector's fastest-growing sectors in recent decades, since 2005 with the embargo on poultry materials and products importation as part of the measures taken by the Senegalese government to control and protect against avian influenza disease [1, 2]. The industry is showing and maintaining good momentum, with broiler poultry numbers rising from 3,994,800 head in 2004 to 11,386,100 in 2008, reaching 51,364,500 chicks in 2019, accompanied by a substantial improvement in various production factors such as farm buildings, compliance with technical standards and biosafety measures, rearing conditions and training for actors, etc. Also, with feed accounting for the lion's share of costs associated with chicken production, some actors (researchers, technicians, etc.) are increasingly seeking to use additive and alternative ingredients as substitutes to improve productivity and profitability in poultry farming [1, 3]. We are thus witnessing a growing use of various feed additives (pre/probiotics, enzymes, mineral-vitamin supplements, synthetic amino acids and derivatives, etc.) likely to reduce the effects of heat stress and/or improve the well-being and growth performance of broilers. In this regard, studies have reported that the inclusion of guanidinoacetic acid - GAA (creaminoND), a creatine precursor in the broiler diet, substantially improved their zootechnical performance [4-8]. Indeed, creatine, a natural component of vertebrate tissues, is a natural amino acid derivative synthesized by the kidneys, liver and pancreas from GAA, and mainly used in muscle tissue and the brain due to its major role in energy metabolism [9, 10]. Around 50% of animal's daily creatine requirement should come from the diet, whereas this creatine is not stable as a feed additive under current tropical feed manufacturing conditions compared to its precursor, GAA (creaminoND containing 96% GAA) which is more stable. This latter has then become an alternative in animal nutrition, where its incorporation into the poultry diet (0.06 - 0.78%) by some authors has induced a significant improvement in growth performance, particularly the feed conversion ratio in different strains of broilers [11-15].

Moreover, most of works on guanidinoacetic acid (GAA) using in poultry feed have mainly been carried out in cooler climatic conditions areas (China, Belgium, Turkey, USA, etc.), compared with the hot tropics of sub-Saharan Africa such as Senegal, where almost no investigations have been realized. It is for this in mind that, this study was undertaken to assess the effects of GAA supplementation on productivity and biochemical parameters in broiler chickens at Dakar region in Senegal.

2. Materials and Methods

2.1. Experimental Diets and Proximate Analyses

Maize, soybean oil, soybean cake-48, sunflower cake-36, dried distillers grains with solubles (DDGS-28), fish meal-65, oyster shell meal, phosphate, synthetic amino acids, mineral and vitamin supplement and CreAMINOND, were the main ingredients used. Based on the results of their nutrient contents, two iso-protein and iso-energetic diets, one control (T-GAA$_0$) and one test (control diet supplemented at 0.06% GAA, S-GAA$_{0.06}$) for broilers were formulated each for their three rearing phases respectively start-up, growth and finishing. These experimental rations formulated, were mainly produced and have been providing freely for this trial by NMA-Sanders feed industry, with the starter diets presented in crumb form, and the growth and finishing diets in pellet form. These different diets used were analyzed at the Laboratoire d'Analyses des Aliments et de Nutrition Animales (LANA) of the Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV) in Dakar. Analyses were carried out to determine dry matter (DM), crude ash or mineral matter (MM), crude protein (CP), ether extract (EE), crude fiber (CF) and some mineral elements (calcium and phosphorus). The DM content was determined according to the AFNOR [16] method, while the MM or ash content was determined according to AFNOR [17]. The crude protein content was determined according to the same standard, but based on the Kjeldhal - Nx6.25 methods [18], while EE content was determined using the reflux extraction method with ethyl ether using the Soxhlet apparatus, described by AFNOR [19]. Crude fiber content was determined according to AFNOR [20], based on the Wende method. Calcium content was measured using the flame atomic absorption spectrophotometric method [21], while phosphorus content was measured using the absorption spectrophotometric method at 430 nm of AFNOR [22]. Metabolizable energy (ME) was calculated using the regression equation (1) described by Sibbald et al. (1980) and cited by Leclercq et al. [23], i e.:

$$\text{ME (kcal/kg DM)} = [3951 + (54.4 \text{ x EE}) - (40.8 \text{ x MM}) - (88.7 \text{ x CF})] \quad (1)$$

with EE, Ash and CF expressed in %DM.

2.2. Animal and Experimental Design

Experiment was conducted at the EISMV farm located at Keur Ndiaye LO, Sangalkam in Rufisque department, about 30 km from Dakar. The experimental procedures, animal handling and the collection of samples were reviewed and approved by the Committee of Ethics and Animal Welfare in Research and Training (CERE) of EISMV-Dakar. The trial was undertaken during the period from April to June 2021 in a semi-open building with a double-sloped roof made of aluminum sheeting. Two weeks before the arrival of the chicks, the building and rearing equipment (feeders, drinkers and wire frames used to make up chicks' batches) were cleaned, disinfected and sanitized with soapy water and bleach. One week before chick's arrival, poultry house, wire frames and all rearing equipment were washed and disinfected with VIRUNET-10% solution (a disinfectant with virucidal, bactericidal and fungicidal properties), with the house and wire frames brushed with a layer of quicklime. On the eve of chicks' arrival, the brooding area was set up and delimited and

partitioned into two (2) groups by the wire frames and covered with a thick layer (about 3 cm) of wood shavings litter a rate of 4 kg/m^2. A radiant heater of 1400 kW suspended about one meter from the ground was used to heat the living area to an ideal temperature (31-33°C), measured by the mini-maxi thermometer specially installed. Lighting in the building was permanent throughout the trial, and was provided by natural light during the day, completed by night artificial lighting with electric lamps. In addition to the footbath filled with cresyl solution installed at the entrance of poultry house to ensure compliance with sanitary prophylaxis, a scale and data collection sheets were also placed in the coop building.

The trial involved 550 unsexed day-old broiler chicks of $Cobb_{500}$ strain purchased from a local hatchery. On receipt, these chicks underwent quality control (checking numbers, live body weighing, vivacity, homogeneity, umbilical condition, legs and liveliness) before being placed in the brooding house. They were then randomly divided into 2 batches of 275 subjects each, corresponding respectively to the two dietary treatments, the control (T-GAA_0) and the supplemented (S-$GAA_{0.06}$) diets containing respectively 0 and 0.06% of guanidinoacetic acid (GAA). During the first two weeks of the trial, chicks of each of two batches were reared together based on a density of 40 birds/m^2, and fed with the corresponding starter experimental diets (T-GAA_0 and S-$GAA_{0.06}$) before being subdivided into 5 sub-batches of 55 chicks each as a repetition from the 3rd week of age until the end of the trial at 6 weeks of age. Throughout the growth (3-4 weeks) and finishing (5-6 weeks) periods, the sub-batches of control and supplemented chicks were arranged alternately throughout the house to limit the wall or corner effect on them, and were fed respectively with the two types of diets (T-GAA_0 and S-$GAA_{0.06}$) for growth and finishing applying the densities 25 birds/m^2 and 10 birds/m². Water and feed were distributed *ad libitum*, although the amount of feed distributed daily was controlled to ensure that the feeders were empty for one hour ideally. Between the two rearing phases, a four-day feed transition was carried out, consisting of gradually reducing (75, 50, 25 and 0%) in the mixed feed, the old diet (from the previous phase) usually served in favor of a new experimental diet (from the next phase). In terms of health, the birds were monitored and vaccinated against Newcastle and Gumboro diseases, and received vitamins and preventive treatment against avian coccidiosis in accordance with the medical prophylaxis program in force in Senegal.

2.3. Data Collection and Zootechnical and Biochemical Parameters Determination

The main data collected during the trial were ambient temperature (recorded three times a day in the poultry house using thermometer installed for this purpose), live weights, feed and water consumption, mortalities, carcass and organ characteristics and biochemical parameters (total protein - TP, albumin, uric acid, creatinine, alanine aminotransferase - ALAT, aspartate aminotransferase - ASAT). Individual live weights of the birds were taken on the first day and weekly on an empty stomach using an SF-400 electronic balance with a maximum capacity of 5 kg and 10 g accuracy. Feed and water intake (quantity distributed - quantity refused) and mortality of birds were measured daily.

At the end of the trial, 60 broilers (30 birds per dietary treatment, i e. 6 birds/sub-batch) of a similar weight to the average for the batch, were randomly selected and slaughtered by severing the jugular vein of the neck in order to assess carcass and organ characteristics of the birds between treatments. After plucking with hot water and evisceration, during which the crop and intestine were removed, the carcasses of headless chickens containing some organs (liver, gizzard, spleen) and detached organs were individually weighed per dietary treatment.

For biochemical parameters determination, 120 blood samples were taken at 29 and 42 days old from different chickens chosen randomly from of the two batches, i.e. 60 blood samples per dietary treatment. These samples were taken in dry tubes from the birds' wing veins, then kept cool in a cooler containing carboglass. They were immediately sent for biochemical analysis in the Laboratory of Endocrinology, Radioimmunology and Molecular Biology (LERBIOM) of EISMV-Dakar. After centrifuging the blood samples for 10 minutes using a 3000 rpm centrifuge, the sera collected per dietary treatment were used to determine the previous biochemical parameters. Total plasma protein and albumin were determined by spectrophotometric methods using Biuret reaction at 545 nm [24] and bromocresol green at 520 nm wavelengths [25] respectively. Creatinine and uric acid contents were also determined by spectrophotometric methods using the Jaffe reaction at 492 nm [26] and the urease reaction at 630 nm wavelengths [27]. ALAT and ASAT contents were determined by spectrophotometric method using coupled lactate dehydrogenase and malate dehydrogenase reactions at 349 and 340 nm wavelengths respectively [28].

All data collected per dietary treatment were recorded in a Microsoft Excel spreadsheet, which was used to calculate zootechnical and biochemical parameters such as average live weight (ALW), average daily gain (ADG), average daily feed intake (DFI) and water consumption (DWC), feed conversion ratio (FCR), mortality rate (MR), carcass weight (CW) and dressings carcass (DC) and organ weight, average biochemical element content (BEC) according to the formulas 2 to 9 below.

$$\text{ALW (g/bird)} = [\text{Sum of Live weights of birds per group (g)} \div \text{Number of birds in the group}] \quad (2)$$

$$\text{ADG (g/d)} = [\text{Live weight gain of the period (g)} \div \text{Length of the period (days)}] \quad (3)$$

$$\text{DFI (g/bird/d)} = [(\text{Quantity of feed offered} - \text{Quantity of feed refused})/\text{day} \div \text{Number of birds}] \quad (4)$$

$$\text{DWC (mL/bird/d)} = [(\text{Quantity of water offered} - \text{Quantity of water refused})/\text{day} \div \text{Number of birds}] \quad (5)$$

$$\text{FCR} = [\text{Feed intake during a period (g)} \div \text{Weight Gain of the period (g)}] \quad (6)$$

$$\text{MR (\%)} = [(\text{Initial number of birds} - \text{Final number of birds}) \div \text{Initial number of birds}] * 100 \quad (7)$$

$$\text{DC (\%)} = [(\text{Carcass weight of the bird} \div \text{Live body weight of the bird}) * 100] \quad (8)$$

$$\text{BEC} = [\text{Sum of concerned element contents in the batch} \div \text{Number of birds sampled in the batch}] \quad (9)$$

2.4. Statistical Analyses

Zootechnical and biochemical data obtained and/or calculated at the end of the trial, were analyzed using SPSS version 23 Statistical Software Program (SPSS, Inc., IBM, Chicago, Illinois, USA) where they were subjected to a Student's t-test analysis for comparing means between control ($T\text{-}GAA_0$) and supplemented ($S\text{-}GAA_{0.06}$) birds at 5% error risk level. Difference of $p < 0.05$ between dietary treatments was considered statistically significant.

3. Results

3.1. Nutritient Composition of Experimental Diets

The nutritive values of experimental diets used are reported in Table 1. The latter shows that the control ($T\text{-}GAA_0$) and supplemented ($S\text{-}GAA_{0.06}$) rations are iso-nutritional, with metabolizable energy-protein [ME/CP] ratio of 14.5, 14.12 and 14.4 for starter, growth and finishing diets respectively.

Table 1. *Nutrient composition of control ($T\text{-}GAA_0$) and supplemented ($S\text{-}GAA_{0.06}$) diets used during start-up, growth and finishing phases of broiler in the Dakar region, Senegal.*

Nutritient composition	Stater diets		Grower diets		Finishing diets	
	$T\text{-}GA_0$	$S\text{-}GA_{0.06}$	$T\text{-}GA_0$	$S\text{-}GA_{0.06}$	$T\text{-}GA_0$	$S\text{-}GA_{0.06}$
Dry matter, DM (%)	85.95	85.31	89.78	89.81	87.38	87.37
Crude ash (%)	9.61	9.62	10.37	10.36	11.03	11.02
Crude Protein, CP (%)	22.75	22.75	21.86	21.87	21.76	21.74
Ether Extract, EE (%)	3.23	3.24	3.59	3.58	3.03	3.02
Crude fiber, CF (%)	4.84	4.83	7.14	7.15	6.1	6.1
Calcium, Ca (%)	1.02	1.07	0.99	0.96	0.93	0.91
Phosphorus, P (%)	0.52	0.52	0.55	0.56	0.59	0.58
Energy (kcal ME/kg)	3305.32	3306.34	3089.88	3088.86	3124.7	3124.60
Ratio ME/CP (kcal/g)	14.53	14.53	14.13	14.12	14.36	14.37

ME: metabolizable energy

3.2. *Ambient Temperature and Mortality of Broilers Fed Experimental Diets*

The average ambient temperature recorded in the barn during the trial ranged from 23.97°C to 32.02°C, with the highest values obtained in the middle of the day (31.42 - 32.60°C) and the lowest in the mornings and/or evenings (23.97 - 28.58°C). A total of 101 cases of broiler chicken mortality were recorded throughout the trial period, giving an overall mortality rate of 18.36%. These mortalities were higher, especially during the 5th and 6th weeks of age, and were slightly more marked in broilers fed the supplemented diet (59 birds ≈ 21.45%) than those fed the control diet (42 birds ≈15.27% mortality).

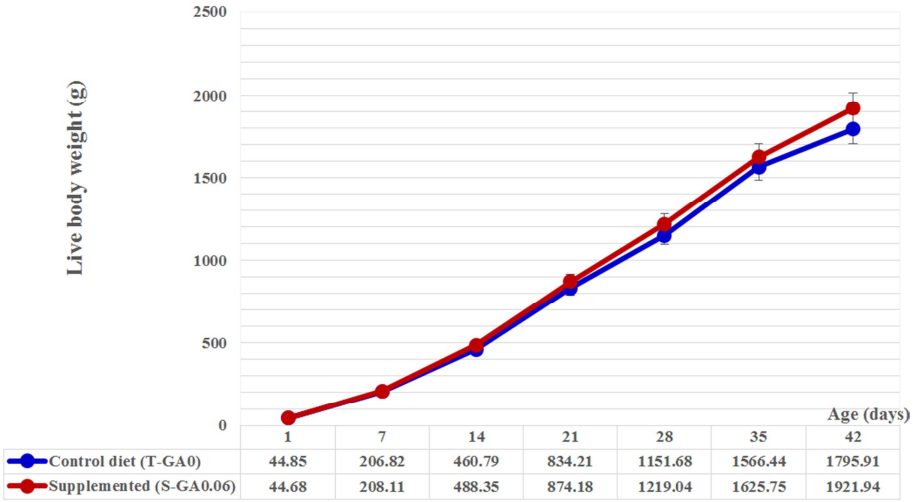

Figure 1. *Average live body weights progression as a function of age in broilers fed experimental control ($T\text{-}GAA_0$) and supplemented guanidinoacetic acid ($S\text{-}GAA_{0.06}$) diets in the Dakar region, Senegal.*

3.3. Zootechnical Performance of Broilers Fed Experimental Diets

The average live weight (ALW) variation of broiler chickens in the control and supplemented dietary treatments over the course of the trial is illustrated in figure 1. Although the control birds had a significantly higher initial ALW than the supplemented birds, it was noted from the 2nd to the 6th week of age that the broilers fed the supplemented ration (S-$GAA_{0.06}$) had significantly higher ALW than their counterparts fed only the control diet (T-GAA_0), with a persistent growth superiority of almost 7% at the end of the trial.

Table 2, reporting others parameters such as average daily gain (ADG), daily feed intake (DFI) and water consumption (DWC), feed conversion ratio (FCR), carcass weight (CW) and dressing carcass (DC), shows that supplementing broiler ration with GAA significantly increased ADG by 7.2% (44.7 vs. 41.7 g/d), DFI by 1.59% (92.5 vs. 91.1 g/bird/d), DWC by 4.15% (286.34 vs. 274.92 mL/bird/day), CW (1622.6 vs. 1526.1 g), while reducing the FCR by around 15.49% (2.13 vs. 2.46) and the DC by 0.61% (84.43 vs. 84.95%) in these subjects compared with control birds.

Table 2. *Zootechnical parameters and carcass characteristics in broiler chickens fed control (T-GAA_0) and GAA-supplemented (S-$GAA_{0.06}$) diets in Dakar region, Senegal.*

Zootechnical parameters	Age (weeks)	Dietary treatments		p values
		T-GAA_0	S-$GAA_{0.06}$	
Average daily weight gain, ADG (g/day)	1-2 weeks	29.71 ±4.99[a]	31.69 ± 4.66[b]	0.000
	3-4 weeks	49.35 ±8.96[a]	52.19 ± 10[b]	0.000
	5-6 weeks	46.02 ±13.39[a]	50.21 ± 8.10[b]	0.000
	1-6 weeks	41.69 ±9.11[a]	44.70±7.59[b]	0.000
Daily feed intake, DFI (g/bird/day)	1-2 weeks	40.86 ± 10.15	41.69 ± 10	0.342
	3-4 weeks	94.54 ± 12.79[a]	97.74 ± 11.32[b]	0.002
	5-6 weeks	137.76 ± 15.81	138.06 ± 14.87	0.822
	1-6 weeks	91.05±12.92[a]	92.5±12.06[b]	0.000
Daily water consumption, DWC (mL/bird/day)	1-2 weeks	115.65 ±23.51[a]	120.19 ±23.29[b]	0.025
	3-4 weeks	232.17 ±28.85[a]	240.26 ±30.84[b]	0.002
	5-6 weeks	476.94 ± 49.65[a]	498.57 ±51.57[b]	0.000
	1-6 weeks	274.92 ± 34[a]	286.34±35.23[b]	0.001
Feed conversion ratio, FCR	1-2 weeks	1.28 ± 0.18[b]	1.22 ± 0.22[a]	0.000
	3-4 weeks	2.06 ± 0.33[b]	1.97 ± 0.51[a]	0.026
	5-6 weeks	4.05 ± 2.98[b]	3.19 ± 0.73[a]	0.000
	1-6 weeks	2.46 ±1.16[b]	2.13 ±0.49[a]	0.000
Carcass characteristics				
Carcass weight, CW (g/bird)	6 weeks	1526.13 ±314.38[a]	1622.59 ±241.45[b]	0.000
Dressing carcass, DC (%)	6 weeks	84.95 ±1.59[b]	84.43 ±1.02[a]	0.000

(a, b): Means with different superscript letters on the same line are significantly different at the 5%.

3.4. Organ Characteristics and Biochemical Parameters in Broilers Fed Experimental Diets

Organ weights and biochemical parameters (total protein, albumin, uric acid, creatinine, ALAT and ASAT) recorded in the broiler chickens are reported in Table 3. The latter shows that the heart weight in supplemented chickens was significantly higher ($p < 0.05$) than that of control subjects (11.13 vs. 10.33 g), in contrast to liver weight (55.78 vs. 60.75 g). Concerning biochemical parameters, the broiler's diet GAA supplementation, did not affect total protein and ASAT levels in the blood, which remained similar between the two dietary treatments. However, GAA supplementation had significantly ($p < 0.05$) increased levels in blood of albumin (17.46 vs. 16.87 g/L), creatinine (114.26 vs. 112.93 µmol/L), uric acid (0.86 vs. 0.78 mmol/L) and ALAT (34.31 vs. 27.54 UI/L) in broilers compared with control subjects. Indeed, the levels of all other biochemical parameters studied were in line with the corresponding reference values in broilers both for controls or supplemented birds, with the exception of uric acid content in blood which were higher than these reference values.

Table 3. *Organ characteristics and biochemical parameters in broiler chickens fed control (T-GAA_0) and GAA-supplemented (S-$GAA_{0.06}$) diets in Dakar region, Senegal.*

Determined parameters	Reference values [29-31]	Dietary treatments		p values
		T-GAA_0	S-$GAA_{0.06}$	
Organ characteristics				
Liver weight (g)	-	60.75±12.94[b]	55.78±8.50[a]	0.000
Heart weight (g)	-	10.33±2.28[a]	11.13±2.05[b]	0.000
Total organ weight (g)	-	136.94±28.80	137.45±21.02	0.816
Biochemical parameters				
Total protein (g/L)	[30 - 60]	30.47±2.98	30.08±1.71	0.066
Albumin (g/L)	[13 - 35]	16.87±1.17[a]	17.46±1.27[b]	0.000

Determined parameters	Reference values [29-31]	Dietary treatments		p values
		T-GAA$_0$	S-GAA$_{0.06}$	
Craatinine (µmol/L)	[79 - 159]	112.93±2.44^a	114.26±1.67^b	0.025
Acide urique (mmol/L)	[0.15 - 0.59]	0.78±0.14^a	0.86±0.07^b	0.000
Alanine aminotransferase (UI/L)	[10 - 50[	27.54±14^a	34.31±19.02^b	0.000
Aspartate aminotransferase (UI/L)	[88 - 230]	176.18±41.73	179.68±36.50	0.301

(a, b): Means with different superscript letters on the same line are significantly different at the 5%.

4. Discussion

The crude protein and metabolizable energy contents of the control and supplemented diets used for start-up, growth and finishing are in line with the recommendations of Leclercq et al. [32], except for the crude protein content in the finishing rations, which remains much higher (21.8 vs. 19.5%). The ambient temperatures recorded during the trial (23.97 to 32.02°C) are well above those (20.63 - 28°C) obtained by Ayssiwede et al. [33] in the same area, as well as those (19 to 27°C) recommended by ITAVI [34] for good broiler performance. This variation in temperature can be explained by the fact that our experiment took place during the period from April to June, corresponding to the start of the hot season (June to October) in Senegal, unlike the first authors who conducted their trial during a relatively cooler period, from November to March. It is therefore clear that the birds were kept in a condition of natural thermal stress, which could certainly have a negative impact on them. Moreover, the higher overall mortality rate (18.4%) obtained in this study, compared with the accepted norm (5%), would be due to the thermal stress that prevailed during the trial, as confirmed by the variation in temperatures recorded (24-32°C). The higher mortality rate noted in supplemented birds (21.45 vs. 15.3%) compared with controls at the end of the trial, is contrary to the results of Mohebbifar et al. [35] in Iran, who recorded similar mortality rates (≈ 27.5%) in control and subjects supplemented at 0.06 and 0.18% of GAA. Although this high mortality rate in the supplemented chickens in our study was lower than that obtained by these authors [35], it can be mainly explained by the lower heat resistance capacity of these birds due to their significant superior live body weight. In fact, even a GAA-based diet supplemented with T3 thyroid hormone hardly generates any serious hepatic lesions that may be promoted by this T3 and likely to increase the risk of ascites mortality in broiler chickens, compared with the control diet without GAA [12].

The significant improvement of average live weight (ALW) and growth rate (ADG) obtained in GAA-supplemented chickens compared with controls in our study is in line with the results of Ren et al. [5] in China, Herger et al. [7] in Turkey, Digler et al. [14] in the USA and Michels et al. [8] in Belgium when feeding broiler chickens with diets containing 0.04 to 0.78% GAA. Also, Ceylan et al. [11] in Turkey, Boney et al. [4] and Yazdi et al. [13] in Iran, and Tossenberger et al. [6] in Hungary, noted a non-significant improvement in average daily weight gain (ADG). Indeed, the final ALW and ADG obtained in this trial were higher than those (1550 g and 25.6 g/d) of Ren et al. [5], they remain well below those (2123 g and 66.8 g/d; 2707 g and 68.3 g/d) recorded respectively by Herger et al. [7] and Michels et al. [8] at 35 and 39 days of age in supplemented chickens. This variation in weight performance and ADG can be explained by age, chicken breed or difference in climatic conditions between trial area locations (Senegal vs. Turkey, USA and Belgium where it is relatively much cooler). Our results are contrary to the observations of Mohebbifar et al. [35] who obtained, compared to controls, a significant reduction in ADG in broilers fed diets supplemented at 0.06, 0.12 and 0.18% GAA. The improved growth performance of supplemented subjects can be explained by the increased availability of creatine to muscle tissue following the methylation reaction catalyzed in the liver by the enzyme guanidinoacetate methyltranferase (GAMT; EC 2.1.1.2) between the supplied GAA and S-adenosyl-methionine (SAM). Creatine transported from liver to muscle is then phosphorylated in the presence of creatine kinase (CK; EC 2.7.3.2) to phosphocreatine, a high-energy phosphate store, able of immediately replenishing ATP to skeletal muscle cells and brain from ADP, which would have increased energy utilization efficiency to support rapid growth in broilers [9-10, 12-13].

The significantly higher feed intake (+1.59%) of GAA-supplemented chickens compared with control subjects in this study is in line with the findings of Digler et al. [14], who had also noted an increase in daily feed intake (DFI) of these birds by supplementing their Arginine-deficient ration with GAA at 0.06, 0.12, 0.39 and 0.78%. Certainly, Boney et al. [4], Yazdi et al. [13] and Tossenberger et al. [6] had noted that supplementing ration with GAA at 0.06% induced a slight, but non-significant increase in DFI in broiler chickens. The same applies to Ceylan et al. [11], Lemme et al. [15] and Ringel et al. [36], who supplemented respectively broiler diets with 0.06%, 0.04 to 0.12% and 0.031 to 0.13% GAA or 0.04 to 0.12% creatine monohydrate (CMH). However, this increase in DFI is contrary to the findings of Mohebbifar et al. [35], Ren et al. [5] and Herger et al. [7] who reported a significant reduction of DFI in subjects fed diets supplemented with 0.04 - 0.12% GAA. The DFI of GAA-supplemented subjects (92.7 g/day) in our study remains higher than those (84; 88.2 and 79.64 g/day) obtained respectively by Zarghi et al. [37], Yazdi et al. [13] and Mousavi et al. [38] in contrast to Michiels et al. [8] who had reported a higher feed intake (110 g/day/bird).

The better feed conversion ratio (FCR) recorded in chickens supplemented with 0.06% GAA compared to controls (2.13 vs. 2.46) in this study, is in agreement with the results reported by many authors [4, 8, 12-13, 15, 36-37, 39] who had applied this GAA at the same rate, or even higher (0.18%) in broiler feed, even though the FCR (1.55 -1.8) reported by the latter remain well below ours. Ceylan et al. [11] and Digler et al. [14] had

reported similar results after supplementing low-energy diets with 0.06% GAA and Arginine-deficient diets with 0.06, 0.12, 0.39 and 0.78% respectively. In China, Ren et al. [5], by including 0.04, 0.08 and 0.12% GAA in the ration of 60-day-old chickens, had also obtained a significant improvement in FCR (2.15 vs. 2.44) in these subjects, compared with controls. However, Tossenberger et al. [6] and Michiels et al. [8] had noted no significant difference between the FCR of birds in the control and 0.06 and 0.12% GAA-supplemented treatments. Our results are contrary to those of Mohebbifar et al. [35], who had reported a significant deterioration in FCR (1.42 vs. 1.35) in chickens fed diets supplemented with 0.06, 0.12 and 0.18% GAA compared with controls. Indeed, the FCR improvement in supplemented broilers corresponding to a better valorization of their diet into product compared to controls, can be explained by GAA's ability to increase the availability of phosphocreatine at the level of muscle tissue, which rapidly converts it when needed into ATP, which would have promoted better weight growth in the birds [8-9, 37].

The high water consumption recorded in all chickens, with a ratio [water/feed 3], and more particularly the significantly higher consumption of supplemented birds compared to controls in this study can be explained on the one hand, by the high heat environment that prevailed during the trial, and on the other hand, by the higher feed intake in these subjects for better feed digestion. In fact, water consumption in poultry under normal conditions is almost double that of the feed, with a [water/feed] ratio that could well exceed 2 to reach 3-4, particularly under heat stress conditions [34], as we've observed in this study.

The significantly higher carcass weight, and even organ weights, obtained in supplemented chickens compared with controls, is in line with the findings of Khalil et al. [12]. This advantage could be explained by the superior live weight of these supplemented birds, especially as the carcass and organ weights of the subjects generally remain closely related to their live weights. Indeed, authors have previously reported that supplementing chicken diets with GAA significantly improved the development and yield of brevis muscles. The latter, known as fast muscles with a high glycolytic metabolism, are affected by the addition of GAA to the ration by the increase in their phosphocreatine content, then in ATP, in order to provide them the energy required for growth [4, 8, 15, 36]. This confirms, as stated above, that supplementing the diet with GAA reduced muscular oxidative stress and improved muscle growth or regeneration, or even the carcass weight of subjects [40]. The significantly lower dressing carcass obtained in GAA-supplemented broiler chickens compared with controls (84.4 vs. 84.95%) is contrary to the findings of Boney et al. [4], Michiels et al. [8], Ceylan et al. [11], Zarghi et al. [37] and Abudabos et al. [39] who recorded lower dressing carcasses - 78.70; 73.2; 74.54; 67 and 70.7% respectively - but similar between dietary treatments. This difference in dressing carcass can probably be explained by the duration of the trial or the final live weight of the birds, but above all by the way in which the carcasses studied were prepared. For these authors, the carcasses were stripped of legs, skin and viscera as a whole, whereas in our study, only the digestive tract without gizzard was removed.

The significant decrease in liver weight (-2.90%) recorded in supplemented chickens compared to controls in our study is in agreement with the results of Abudabos et al. [39] and Mousavi et al. [38] who also noted respectively a reduction in weight of this organ of around 7.5 and 2.23% in the same birds at 35 and 42 days of age. However, our results are contrary to those of Khalil et al. [12], who reported that supplementation of chicken ration with GAA induced a significant increase in heart weight, but in no way affected liver weight, which remained similar in control and supplemented birds.

Total protein levels (≈ 30.30 g/L) obtained in this study were similar between control and supplemented dietary treatments as previously reported by Mohebbifar et al. [35], but remain lower than those (33 g/L) of these authors who, in contrast to our study, recorded lower and similar blood albumin contents between control birds and those fed diets containing 0.06 - 0.18% GAA. The significantly higher albumin, creatinine, uric acid and alanine aminotransferase (ALAT) levels in blood obtained in GAA-supplemented broilers compared with controls, corroborate the findings of Tossenberger et al. [69], in contrast to the observations of Khalil et al. [12], who had noted no significant difference in creatinine, ALAT and ASAT contents between control and supplemented subjects. But although albumin, creatinine, uric acid and ALAT levels in supplemented chickens were significantly higher than those in control subjects, these parameters in both types of birds, including total protein and ASAT, were well within the usual reference values for these birds, except for uric acid levels which remained well above them.

The higher creatinine levels observed in supplemented broiler chickens compared to controls are thought to be due to the addition of GAA to their ration, whose metabolism in the liver leads to the production and increased availability of creatine, converted into phosphocreatine in muscle tissue. To obtain the energy they need for growth, muscle tissue then metabolizes phosphocreatine with ADP to produce ATP and creatine. The irreversible degradation of this latter then leads to creatinine, which is released into the bloodstream and transported via the liver to the kidneys, where it is eliminated in the urine [36-37, 40-42]. Also, as urea is the main form of nitrogenous waste elimination during protein catabolism (mobilized endogenous or excess dietary proteins) in the liver, the higher blood uric acid levels noted compared with reference values in both types of birds can be justified by the relatively high crude protein content of the diets used, particularly in finishing (21.75%) compared with the requirement recommended (19.5%) in these chickens [32]. Since supplemented broilers had the best weight performance and were fatter, the significantly higher levels of ALAT (a specific marker of hepatic cytolysis synthesized by the liver, cardiac and skeletal muscles) in these subjects compared to controls, may be explained by an important accumulation of intra-hepatocyte fat, which would have led to eventual

hepatocyte suffering resulting in leakage of ALAT into the bloodstream [43]. This suffering of liver cells due to lipid infiltration between cells could explain the drop in liver weight observed in supplemented subjects.

5. Conclusion

Supplementing the diet with 0.06% GAA (0.6 g/ kg feed) had no adverse effect on the broilers health, who significantly improved their growth performance (live weight, average daily gain, feed and water consumption, feed conversion ratio, carcass weight) compared with control subjects. It was accompanied by a significant increase in blood albumin, creatinine, uric acid and alanine aminotransferase (ALAT) levels in the subjects concerned compared with controls. However, given the relatively higher mortality rate obtained (21.5 for $GAA_{0.06}$ vs. 15.3% for control) compared with the recommended standard (5%) and especially attributable to the high heat condition recorded (24-32°C) during the trial, it would be desirable for another study to be carried out, particularly in cooler period, with a supplementation rate around 0.06 to 0.12%, in order to confirm these results.

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Conflicts of Interest

The authors declare no conflict of interest.

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