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Research Article

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Comparative Effects of Regular and Activated Guanidinoacetic Acid on Creatine Metabolism in Broilers Under Heat Stress and Mycotoxin Exposure

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ABSTRACT

Heat stress and mycotoxin exposure can impair either hepatic or kidney functions, oxidative balance and cellular energy metabolism in broiler chickens.

Methods: Two hundred and eighty-eight as hatched broilers were assigned to four treatments, with six replicates of twelve birds per pen. Birds were maintained under natural hot environment from day 1 to day 28. Challenged groups received both aflatoxin B1 at 0.5 mg/kg and ochratoxin A at 0.1 mg/kg of feed from day 8 to day 28. Regular guanidinoacetic acid (RGAA) or activated guanidinoacetic acid (AGAA) was supplemented at 600 mg/kg.

Results: The combined challenge induced hepatic injury and oxidative stress. Both RGAA and AGAA treatments significantly increased creatine contet in serum and pectoralis major muscle. The creatine increasing associated with AGAA was 27.64% and 33.37% greater in serum and pectoralis major muscle, respectively, when compared to RGAA.

Conclusion: Under combined hot environment and mycotoxin challenge, AGAA produced a stronger creatine-related metabolic response than RGAA at the same inclusion rate. These results highlight that AGAA improve creatine formation which may help support growth performance in broilers under challenging conditions.

List of Abbreviations

ADFI	:	Average Daily Feed Intake
ADG	:	Average Daily Gain
AFB1	:	Aflatoxin B1
ALB	:	Albumin
ALT	:	Alanine Aminotransferase
AST	:	Aspartate Aminotransferase
ATP	:	Adenosine Triphosphate
BW	:	Body Weight
FCR	:	Feed conversion Ratio
GAA	:	Guanidinoacetic Acid
GAMT	:	Guanidinoacetate Methyltransferase
GSH-Px	:	Glutathione Peroxidase
MDA	:	Malondialdehyde
OTA	:	Ochratoxin A
PCr	:	Phosphocreatine
SEM	:	Standard Error of the Mean
SOD	:	Superoxide Dismutase
TP	:	Total Protein
UA	:	Uric Acid

Keywords: Broiler Chickens, Activated Guanidinoacetic Acid, Creatine, Heat Stress, Mycotoxins, Energy Metabolism

Introduction

Heat stress and mycotoxin exposure are two of the most common field challenges in modern broiler production. Heat

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stress increases the energy expenditure for thermoregulation and negatively affects feed intake and growth rate, and it is well established as a major constraint on broiler performance and redox balance [1,2]. Furthermore, AFB1 and OTA exert toxicological pressure on hepatic and renal function, further compromising oxidative status [3,4].

The liver is fundamental to nutrient metabolism and to the conversion of GAA into creatine via guanidinoacetate methyltransferase (GAMT). Creatine can subsequently participate in the creatine–phosphocreatine system, which buffers cellular energy demand through rapid ATP regeneration. Because the liver and kidneys are primary targets of AFB1 and OTA toxicity, respectively, a combined model with high environmental temperarure and mycotoxin exposure provides a relevant setting to compare creatine-related metabolic responses between RGAA and actiGAA. The biochemical relationship among guanidinoacetic acid, creatine and phosphocreatine is well established, and dietary GAA acid can increase tissue creatine and support cellular energy metabolism in broilers [5-8].

Review of Literature

Guanidinoacetic acid is the immediate metabolic precursor of creatine. Creatine and phosphocreatine form an intracellular energy-buffering system that supports rapid regeneration of adenosine triphosphate in tissues with high and fluctuating energy demand [5,6]. In broilers, dietary guanidinoacetic acid has been associated with increased tissue creatine, improved feed efficiency, altered muscle energy status, and an arginine-sparing effect [7-10].

The response to guanidinoacetic acid may be particularly relevant during metabolic challenge. High environmental temperatures induce oxidative damage and histopathological lesions in the liver and kidneys of broilers, including fatty degeneration, necrosis, and renal fibrosis [13,14) Under chronic high ambient temperatures, guanidinoacetic acid supplementation has been reported to increase plasma and muscle creatine and phosphocreatine-related indices and, in some studies, improve feed conversion and survival [9,11]. Heat stress itself can disrupt oxidative balance and hepatic metabolism, potentially increasing the importance of efficient cellular energy buffering [1,2].

Mycotoxin exposure provides an additional metabolic burden. Aflatoxin B1 primarily targets the liver, whereas ochratoxin A has important renal and systemic toxic effects by targeting the renal tubules and combined exposure aggravates injury in both organs [15-16]. Thusboth factors negatively affect the oxidative status and compromise productive performance [3,4]. The simultaneous exposure to high environmental temperature and mycotoxins therefore provides a biologically relevant challenge model for evaluating whether different guanidinoacetic-acid-based nutritional strategies generate different creatine-related responses. Previous broiler studies have established the efficacy of RGAA, but direct evidence comparing it with AGAA under a combined high environmental temperatures and mycotoxin exposure remains limited. The present study addresses this specific evidence gap.

Materials and Methods

A total of 288 as hatched SZ901 chicks were were assigned to four dietary treatments, with six replicates per treatment and twelve birds per pen. Regular and Activated GAA (trade names: Guandino 98% and NT-PB, respectively) were provided by Numega Nutrition Pte.,Ltd, Singapore. The study was carried out to Day 28.

Table 1: Experimental Groups & Diets

Treatment	Challenge	Dietary Intervention
Control	Heat-stress background	Basal diet
Mycotoxin	Heat stress + AFB1 0.5 mg/kg + OTA 0.1 mg/kg	Basal diet
Mycotoxin + RGAA	Heat stress + AFB1 0.5 mg/kg + OTA 0.1 mg/kg	600 mg/kg
Mycotoxin + AGAA	Heat stress + AFB1 0.5 mg/kg + OTA 0.1 mg/kg	600 mg/kg

Challenge Conditions

All birds were maintained under a continuous heat-stress background from Day 1 to Day 28. Recorded daytime and overnight temperatures ranged from 31.2 to 36.7°C and 24.0 to 29.0°C respectively; daytime relative humidity ranged from 60 to 85%. Mycotoxin exposure was applied from Day 8 to Day 28.

Evaluated Outcomes and Statistical Interpretationr=

At Day 28, organ indices, serum biochemical indicators, hepatic oxidative-stress indicators, and creatine/creatinine-related responses in serum and pectoralis major muscle were evaluated. The source presentation reports results as Mean ± SEM and uses different letters to denote significant differences at $P < 0.05$. Results are reported as mean ± standard error of the mean. Differences denoted by different letters were interpreted as statistically significant at $P < 0.05$, in accordance with the source dataset.

Results

Confirmation of the Combined Stress Model

At Day 28, the challenge produced clear organ and biochemical changes. The mycotoxin group had a significantly lower liver index and bursal index than the control ($P < 0.05$), while the kidney index showed a decreasing trend ($P=0.0566$). Serum ALT increased and ALB decreased significantly ($P < 0.05$), while AST showed an increasing tendency ($P=0.057$). The AST/ALT ratio and serum uric acid were not significantly affected (Figure 1: ACBDGEFH).

Hepatic oxidative stress was also evident: MDA increased and SOD decreased in challenged birds ($P < 0.05$) (Figure 1: JK). Together, the organ-index, serum-biochemistry and hepatic oxidative-stress findings confirm that the model produced substantial hepatic and systemic stress, while the available kidney-related indicators did not demonstrate comparably strong renal impairment.

Creatine-Related Metabolic Responses

At Day 28, both RGAA and activated AGAA significantly increased serum creatine ($P < 0.05$) under mycotoxin challenge (Fig 2A). The creatine increase observed with AGAA was 27.64% greater than that observed with RGAA (Fig 2B).

A similar pattern was observed in pectoralis major muscle (Fig 2C). Both supplements significantly increased muscle creatine deposition ($P < 0.05$), while the increase associated with AGAA

was 33.37% greater than that obtained with AGAA supplementation (Fig 2D). The consistency of the serum and muscle responses supports a stronger creatine-related metabolic effect of AGAA under the conditions of this trial.

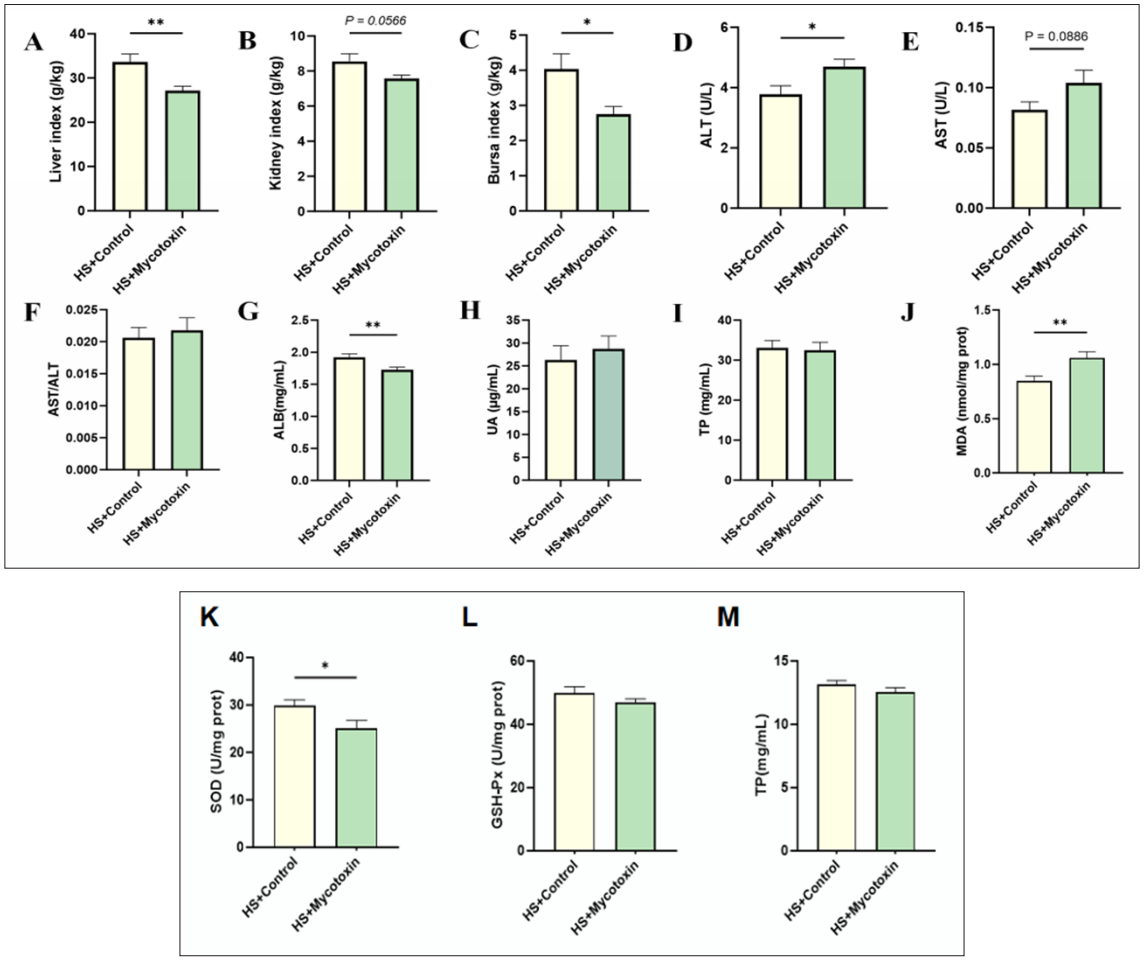

Figure 1: Organ indices and serum/hepatic biochemical parameters (A-C) Liver, kidney, and bursa of Fabricius indices, respectively. (D–I) Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT ratio, albumin (ALB), uric acid (UA), and total protein (TP). (J–M) Hepatic levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and total protein (TP). Data are presented as Mean ± SEM (n = 12 per group). * $P < 0.05$, ** $P < 0.01$ vs. control group.

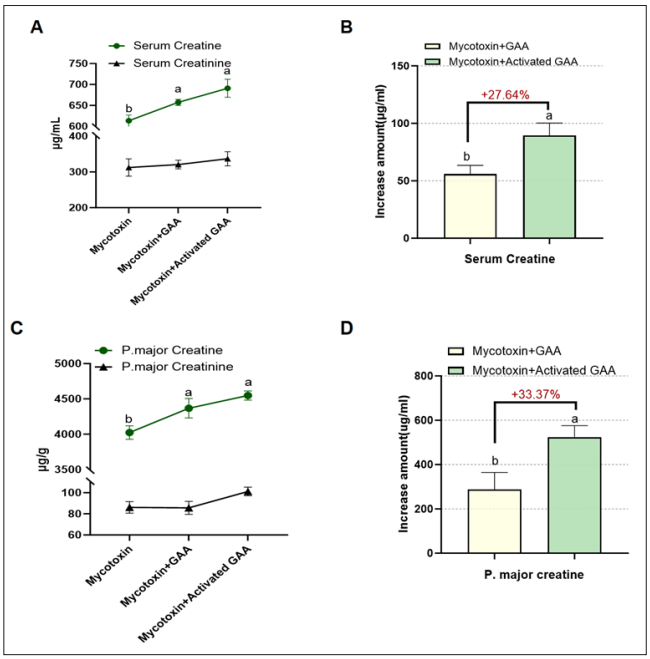

Figure 2: Creatine and creatinine concentrations in serum and pectoralis major muscle, and their relative incremental changes after mycotoxin co-treatment with RGAA or AGAA. (A) Serum creatine and creatinine levels in the mycotoxin, mycotoxin+AGAA, and mycotoxin+RGAA groups. (B) Incremental increases in serum creatine for the two treatment groups. (C) Pectoralis major

muscle creatine content under the same three conditions. (D) Incremental increases in pectoralis major muscle creatine for the two treatment groups. All values are Mean ± SEM (n = 12 per group). Different letters indicate significant differences ($P < 0.05$).

Discussion

The key finding of this study is that AGAA produced a stronger creatine-related metabolic response than RGAA under combined heat stress and mycotoxin exposure. At the same inclusion rate (600 mg/kg), AGAA resulted in greater increases in creatine than RGAA in both serum (+27.64%) and pectoralis major muscle (+33.37%). The consistent response across blood and muscle suggests a more efficient conversion of GAA into biologically available creatine.

This finding supports an important concept: the nutritional value of GAA should be evaluated not only by dietary GAA intake, but also by its conversion efficiency to creatine. Because creatine supports the phosphocreatine–ATP energy-buffering system, greater creatine availability may help maintain cellular energy metabolism when birds are exposed to metabolic stress. This interpretation is consistent with previous work linking dietary guanidinoacetic acid with tissue creatine, phosphocreatine and muscle energy status [5,7,9,11].

From a practical perspective, this mechanism may explain why AGAA can deliver more consistent production responses than RGAA under commercial conditions, where birds frequently encounter heat stress, mycotoxins, disease pressure and nutritional fluctuations. Therefore, AGAA may be better positioned as a strategy to improve creatine conversion efficiency and metabolic resilience, rather than simply as another source of RGAA. However, responses to guanidinoacetic acid vary with dietary energy, arginine supply, supplementation level and environmental conditions [8-10,12].

Findings

For poultry nutrition applications, the results suggest that the value of AGAA under severe challenge conditions may be better characterized initially through metabolic efficiency rather than through short-term growth recovery. In a model where heat stress, reduced feed intake and mycotoxin exposure occurred simultaneously, AGAA maintained a stronger creatine-related response than RGAA at the same inclusion rate.

This finding is relevant for future product validation because it identifies a measurable biological endpoint that can be tested across challenge intensity, production stage and diet type. Demonstration of a corresponding increase in phosphocreatine, ATP availability, muscle energy status or nutrient-sparing efficiency would substantially strengthen the evidence for a functional advantage.

Conclusions

The AFB1/OTA exposure negatively affect liver integrity and oxidative stress of broilers kept in hot conditions.

Both RGAA and AGAA significantly increased creatine-related responses in serum and pectoralis major muscle but at the same supplementation level, the AGAA-resulted in more 27.64% and 33.37% creatine both in serum and pectoralis major muscle, respectively.

Recommendations

Further studies should confirm these findings in larger populations and under different challenge intensities, production stages and diet types. Direct measurements of phosphocreatine, adenosine triphosphate, creatine-to-phosphocreatine dynamics, guanidinoacetate methyltransferase activity and relevant hepatic metabolic pathways are recommended. Future trials should also determine whether the greater creatine response is consistently associated with improved feed efficiency, nutrient-sparing effects, survival, carcass traits or economic outcomes.

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