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RESEARCH ARTICLE
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Supplementation of Guanidinoacetic Acid in Feed on Growth Performance, Intestine Histomorphology, Muscle Histology and Meat Quality of Native Chicken

Sri Purwanti [1*], Jasmal A. Syamsu [1,2], Nancy Lahay [1], Marhamah Nadir [1], Zaraswati Dwiyana [3], Abdul Alim Yamin [1], Raymundus Genty Laras [4], Sumiati [5] and Henny Akit [6]

[1]Department of Animal Nutrition, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia
[2]Center for Research and Development of Livestock Resources and Tropical Animals, Hasanuddin University, Makassar, Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia
[4]Behn Meyer Chemicals, Serpong, Indonesia
[5]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia
[6]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
*Corresponding author: sripurwanti@unhas.ac.id

ABSTRACT

The body naturally produces guanidinoacetic acid (GAA), an organic acid that serves as a precursor to creatine, an energy carrier in cells. An experiment was conducted to determine the response of native chickens to GAA. The GAA used is CreAMINO®, added to the feed during rearing up to 70 days of age. The native chicken was allotted to 4 treatments, each with 5 replicates of 6 chickens/pen. Four commercial diets were as control (without added GAA and Betaine): 0.06% GAA + 0.1% Betaine, 0.12% GAA + 0.1% Betaine, and 0.18% GAA + 0.1% Betaine. As a result, GAA provides additional energy for optimal growth. The criteria of responses were feed intake, feed efficiency, body weight gains, carcass weight, innards weight and length, intestinal morphology, histology on muscle, and meat quality. Supplementation of native chicken diets with 0.06 GAA resulted in the highest live weight gain, chicken wings, and chicken thighs. In 0.12 GAA, they resulted in the highest average daily gain, lowest feed conversion ratio, carcass weight, breast thighs, chicken back, and chicken lower leg. In 0.18 GAA, the lowest feed intake, feed efficiency, and meat cholesterol were found. Different GAA levels tend to give different results on native chicken performances.

Keywords: Guanidinoacetic acid; Native chicken; Feed intake; Weight gain; Meat quality; Intestinal histomorphology

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INTRODUCTION

As the biochemical precursor of creatine, guanidinoacetic acid (GAA), in its phosphorylated form, plays an important role not only as a high-energy carrier in muscle but also as an important participant in muscular energy homeostasis (Jaroslav et al., 2014; Degroot et al., 2018). GAA is formed from the amino acids glycine and arginine in the kidneys or digested from the intestines and converted into creatine in the liver, which will then be transported to muscles and other target cells. Creatine enters cells and is phosphorylated to phosphocreatine by creatine kinase, which has a role in buffering alterations in ATP (Lemme et al., 2007; Baker, 2009). Small amounts of GAA can be found in raw materials of animal origin, such as fish meal, meat and bone meal, and poultry by-products. Meanwhile, most nutritionists use raw grain ingredients, including corn, wheat, and soybean meal, for broiler feed. In contrast to animal protein, creatine is not found in plant foods and, therefore, is deficient in all plant foods. As a result, the GAA content in the diet is insufficient for the estimated increase in body weight. GAA, as a source of creatine, is more stable and cheaper than creatine itself (Ostojic, 2014). High creatine deposition will increase the phosphocreatine to adenosine triphosphate (ATP) ratio,

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thereby increasing the body's energy reserves, as a result of which the body's biochemical processes at the cell and tissue level will be more efficient (Dilger et al., 2013). In addition, the conversion ratio of energy consumed to body weight will be lower and more efficient (Majdeddin et al., 2019).

In poultry (broiler) production, optimal growth performance is sought at low cost and with minimal environmental impact. Therefore, optimizing feed efficiency is essential to avoid excessive costs and reduce environmental damage caused by supplemental nutrition. In addition, 0.6–0.8g/kg GAA can be considered a safe concentration as a feed supplement to improve the growth performance of broiler chickens, according to the European Food Safety Authority (European Food Safety Authority, 2009). Numerous studies conducted recently have proven the beneficial effects of GAA supplementation on broiler growth performance (Zhao et al., 2021).

The research results of Ahmadipour et al. (2018) showed that the addition of guanidinoacetate to broiler chicken feed can improve the development and health of the digestive tract. Broiler feed consumption, weight gain, and growth performance were better with the GAA diet (0.6g/kg) (de Souza et al., 2021). In addition, FCR is positively influenced by the addition of GAA in the diet (Lemme et al., 2007; Boney et al., 2020; Borges et al., 2021; de Souza et al., 2021). GAA supplementation has also been shown to reduce serum cholesterol and triglyceride levels, increase the surface area of microvilli in the intestine, and increase the pH of broiler chicken breast meat (Rahmawati & Hanim, 2022). These findings implied that the FCR and energy efficiency of broiler chickens could be improved by the addition of GAA. However, adding GAA to varied protein levels did not significantly improve the performance of broiler chickens but reduced cholesterol levels in chicken thigh meat (Hanim et al., 2023).

In this study, the effect of GAA (trade name CreAMINO®, Allzchem) was evaluated regarding its metabolism and its effect on the performance of native chickens (feed, carcass, intestines, and offal), which was added to all feeds starting from 0, 0.06, 0, 12 and 0.18% of the required dose. Although the mechanism of action is different from antibiotics, GAA can serve as a substitute for AGP for greater weight gain and increased FCR (European Food Safety Authority, 2009). Previously, antibiotics were often used in livestock and poultry to prevent and treat disease, increase growth, increase productivity, and increase profits (Khajali et al., 2020). However, the increasing number of bacteria and biological residues associated with animal products poses a serious threat to animal health (Roth et al., 2019; Poole & Sheffield, 2013). As a result, many countries have limited or even banned the use of antibiotics in animal feed, thereby increasing the prevalence of certain diseases in poultry (Wierup, 2001; Casewell et al., 2003).

MATERIALS & METHODS

Ethical Statement

In this study, maintenance management is referred to as the Australian Animal Welfare Standards and Guidelines for Poultry, while slaughtering native chickens is referred to as the Indonesian National Standard (INS) number 99002 of 2016 regulating halal slaughter of poultry.

Bird Management and Experimental Design

In this study, 120 native chickens were kept in closed mini cages. The research was designed with a completely randomized design in 4 treatments and five replications; each unit contained a total of six chickens. Treatments for this research were: Controlling with commercial feed (without added GAA and Betaine); 0.06% GAA + 0.1% Betaine; 0.12% GAA + 0.1% Betaine; and 0.18% GAA + 0.1% Betaine. Maintenance started at the age of 1 day (DOC) and continued until the age of 70 days, with a brooding phase for 14 days, using a lamp as a heater. Feed mixing was done once a week by weighing commercial feed, GAA, and Betaine according to the required dosage, mixed using a horizontal mixer until homogeneous. Disease was controlled by implementing biosecurity in the cage, namely controlling traffic in and out of the cage, and sanitation of the cage by cleaning and disinfection to obtain a clean, hygienic, and healthy environment. The feed used is commercial feed produced by a feed mill with the addition of GAA and Betaine according to treatment. Feeding and drinking water were *ad libitum* available. The nutritional composition of the experimental diet is shown in Table 1.

Table 1: Nutritional composition of the experimental diets

Nutrient	Unit	Control (without added GAA and Betaine)	0.06 GAA + 0.1% Betaine	0.12 GAA + 0.1% Betaine	0.18 GAA + 0.1% Betaine
Moisture	%	10.39	10.44	10.37	10.69
Crude protein	%	12.76	13.34	13.30	13.21
Extract Ether	%	6.07	5.97	6.08	5.94
Crude Fiber	%	6.00	5.53	5.90	5.63
Ash	%	6.85	6.68	6.22	6.34
Calcium	%	1.2	1.2	1.2	1.2
Phosphor	%	1.0	1.0	1.0	1.0
Amino Acid:					
Lysine	%	0.70	0.70	0.70	0.70
Methionine	%	0.27	0.27	0.27	0.27
Methionine+ Sistine	%	0.45	0.45	0.45	0.45
Threonine	%	0.17	0.17	0.17	0.17

Measurement of Growth Performances

The parameters measured in the growth performance of native chickens are body weight gain, feed intake, feed conversion ratio, live weight, feed efficiency, protein intake, and carcass weight.

Meat Quality

The cholesterol content of meat products was determined colorimetrically by the modified Liebermann-Burchard method (Abel et al., 1952; AOAC, 2000) and the total fat contents of samples were determined following Xiong et al. (2007). The fat values were given as '% w/w' and 'g fat per g of non-fat dry solid' on wet and dry bases. Samples were taken, and the pH of the meat was tested using the modified method (Bouton & Harris, 1972). Water holding capacity was determined by the method of Hamm, cooking loss, and meat tenderness with the shear press method, as a modified Warner-Bratzler method (Soeparno, 2009).

Carcass Characteristic and Histology of Muscle

On day 70, two native chickens were randomly selected from each replication and slaughtered. Feathers, feet, head, and internal organs are separated, and intestinal specimens are prepared. For commercial carcasses, weighing is carried out, namely the shoulder blades to the sternum, upper thighs, lower thighs, wings, and back (Tumiran et al., 2019). The muscle histology parameters observed were the number of myofibrils and myofibril diameter. The number of myofibrils is calculated by calculating the number of myofibril fibers contained in a certain unit area in a histological preparation, according to Ridhana (2018). Myofibril diameter measurements were carried out according to Brooke (1970). Muscle histology observations were done with a Nikon SMZ1270 microscope and the NIS-Elements Analysis D 4.40.00 program. To count the number of myofibrils, 6x magnification was used and to calculate the myofibril diameter, 8x magnification was used.

Histomorphology of the Small Intestine

Observation of the histomorphology of the small intestine by taking 1cm of the middle section of the duodenum, jejunum, and ileum of native chickens, PBS rinsed, and the specimen was preserved for 42 hours in 10% formalin phosphate buffer. The samples were dehydrated with alcohol (absolute, 70% and 95%) and embedded in wax. Two samples from each 5μm segment were cut with a microtome (MicroTec, Walldorf, Germany) and stained with hematoxylin-eosin and alcian blue (Kumar and Gill, 2010). Crypt depth and villus height were measured on stained sections with a light microscope (Carl Zeiss, Promenade, Germany) and a digital camera (U-TV1X, Olympus). A photomicrograph with a magnification of 100x was used to collect measurements. The density of goblet cells was estimated as the number per 100μm villus length by measuring villus height, crypt depth, and villus width. Calculation of the surface area of intestinal villi was done according to Iji et al. (2001)

Statistical Analysis

The data obtained were tabulated and analyzed using analysis of variance, and Duncan's Multiple Range Test was used to test variations between treatment groups (Steel & Torrie, 1993), using SPSS (Statistical Package for the Social Sciences) version 25 (IBM SPSS Statistics, USA).

RESULTS & DISCUSSION

Growth Performance

Body weight gain, feed conversion, feed efficiency and live weight did not show significant (P>0.05) differences from each treatment (Table 2). However, feed consumption, protein consumption and carcass weight were influenced by the addition of GAA and Betaine. Feed consumption with the addition of 0.06% GAA was higher than the control, 0.12% GAA and 0.18% GAA (P<0.05). The addition of 0.06% GAA to the feed significantly reduced protein consumption (P<0.05) compared to 0.12% GAA, 0.18% GAA and control. Carcass weight with the addition of 0.06 and 0.12 GAA and Betaine showed the same value as the control and was significant (P<0.05) compared with 0.18 GAA and Betaine.

In the presented study, dietary inclusion GAA and Betaine had no significant effect (P>0.05) on weight gain, FCR, feed efficiency, and live weight. In feed consumption, as the level of GAA in the feed increases, feed consumption decreases, and the highest significance (P<0.05) was at 0.06% GAA. GAA levels of 800 and 1200ppm on male broilers considerably decreased (p<0.05) average daily feed intake (ADFI) (Lemme et al., 2007). On the other hand, a decrease in ADFI could be attributed to improved energy metabolism. Energy efficiency per gram of weight gain increased following GAA supplementation. GAA supplementation boosted creatine deposition and the phosphocreatine (PCr)-to-adenosine triphosphate (ATP) ratio in muscle (Wallimann et al., 1992).

It is suspected that there is no effect of adding GAA and Betaine to the feed because the methionine content of the feed already meets adequate standards. The results of the study are in line with research conducted by Zulkifli et al. (2004) that Betaine supplementation in feed containing sufficient methionine does not affect body weight gain or the amount of feed consumed. Thus, Betaine supplementation at levels of more than 0.1% does not show a positive effect on body weight gain and feed efficiency in female broiler chickens. Research by Sakomura et al. (2013) showed that there was an increase in feed consumption due to Betaine supplementation but it had no effect on body weight. Meanwhile, Waldroup & Fritts (2005) showed that Betaine supplementation had no effect on the performance of broiler chickens.

Table 2: Effect of guanidinoacetic acid (GAA) and 0.1% betaine on the performance of native chickens

Parameter	Control	0.06 GAA	0.12 GAA	0.18 GAA	SE	P value		
						GAA	Linear	Quad
Weight gain (g/head/day)	11.93	12.59	12.71	11.91	0.21	0.457	0.971	0.120
Feed Intake (g/head/day)	50.67^{a}	51.92^{b}	51.27^{a}	50.79^{a}	0.14	0.002	0.755	0.001
Protein Intake (g/head/day)	6.46^{a}	6.18^{b}	6.82^{c}	6.71^{d}	0.05	0.000	0.001	0.000
Feed Convertion Ratio	3.42	3.312	3.23	3.448	0.06	0.628	0.997	0.229
Feed Eficiency	29.44	30.306	31.002	29.298	0.51	0.648	0.955	0.244
Live weight (g)	999.50	1034.50	1027.50	967.50	10.26	0.070	0.221	0.018
Carcass weight (g)	636.68^{ab}	680.77^{a}	690.07^{a}	617.02^{b}	10.71	0.029	0.541	0.005

$^{a-d}$ Mean values (n=5 replicates/group, 6 chickens per replicate) in the same row with different superscript letters indicate significant differences (P<0.05).

In previous research, the addition of 0.10–0.20% Betaine in high methionine feed had no effect on the feed efficiency of broiler chickens (Konca et al., 2008). The research results showed that there was a significant effect on feed consumption, protein consumption and carcass weight with the addition of GAA and Betaine. The relationship between these three parameters is that consuming sufficient protein feed will affect the growth and development of the bird's body which in turn will also affect the carcass weight of broiler chickens. If birds receive feed with good nutritional quality and sufficient amounts of protein, they tend to have better growth, which then contributes to increased carcass weight. On the other hand, a lack of nutrients or protein in the feed can limit bird growth and result in less than-optimal carcass weight.

The relationship between GAA addition and feed consumption follows the quadratic equation $y = -120.14x^2 + 21.142x + 50.773$ with a correlation coefficient $R^2 = 0.7784$ (Fig. 1). With each increase in GAA dosage levels, it can be expected that feed consumption will increase by 21.142 g/head/day, however, increasing GAA levels to a certain level (0.08-1.0%) will reduce feed consumption. Likewise, the correlation of carcass weight with the addition of GAA follows the equation $y = -8134.7x^2 + 1381.5x + 634.3$, with a correlation coefficient $R^2 = 0.9692$ (Fig. 2), with each increase in GAA levels, carcass weight decreased by 634.3g.

Slaughter Performance

The weight of commercial cuts of breast, back, wings, thigh and drumstick did not have a significant effect (P>0.05) with the addition of GAA and Betaine (Table 3). As a creatine precursor, GAA plays an important function in muscle tissue formation. In this regard, creatine monohydrate supplementation significantly improved the feed:gain ratio in broilers (Zhang et al., 2014).

The commercial cut data does not show any influence, but the relationship between the level of GAA addition to wing weight and drumstick are presented through the equation in Fig. 3 and 4. Where increasing the GAA concentration quadratically after a certain concentration will reduce the commercial cut of the wings and drumsticks.

Histomorphology of the Gastrointestinal Tract and Histology of Muscle

The addition of GAA and Betaine to the histomorphological parameters of the digestive tract segments of the duodenum, jejunum, and ileum (crypt depth, length of villi, and surface area of villi) did not have a significant effect (P>0.05) and also has no effect on muscle histology (number and diameter of myofibrils) (Table 4, Fig. 5 and 6).

The histomorphology of native chicken intestines supplemented with guanidino acetic acid at various levels and 0.1% Betaine was evaluated using duodenum, jejunum,

Table 3: Effect of guanidinoacetic acid and 0.1% betaine on the weight of commercial meat

Parameters	Control	0.06 GAA	0.12 GAA	0.18 GAA	SE	*p*-value		
						GAA	linear	Quad
Breast (g)	139.89	147.76	152.71	138.86	2.85	0.272	0.941	0.067
Back (g)	129.48	131.35	136.37	117.90	3.04	0.176	0.262	0.094
Wing (g)	86.94	94.62	92.28	82.79	1.91	0.109	0.353	0.025
Thigh (g)	86.94	94.62	92.28	82.79	2.15	0.496	0.211	0.407
Drumstick (g)	93.86	98.51	99.02	93.67	1.15	0.197	0.995	0.036

(n= 5 replicates/group, 6 chickens per replicate), GAA: Guanidinoacetic acid.

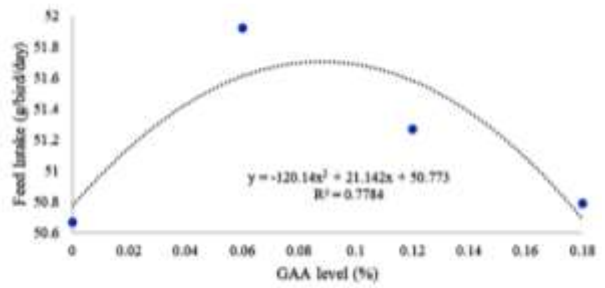

Fig. 1: Polynomial regression of the effect of GAA level on feed intake (g/head/day).

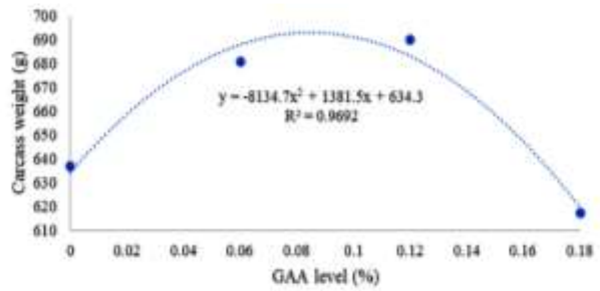

Fig. 2: Polynomial regression of the effect of GAA level on carcass weight (g).

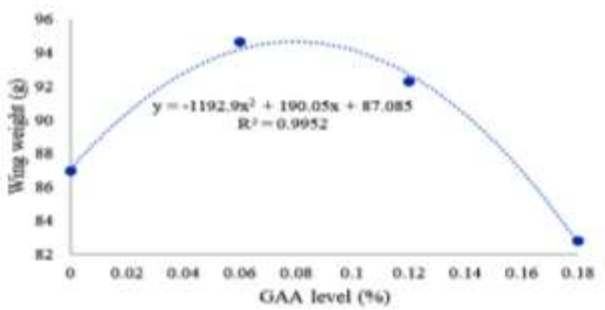

Fig. 3: Polynomial regression of the effect of GAA level on wing weight.

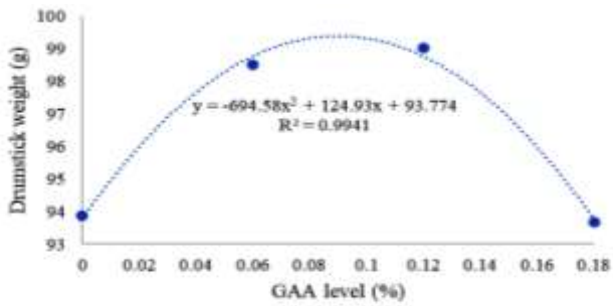

Fig. 4: Polynomial regression of the effect of GAA level on drumstick weight.

Table 4: Effect of guanidinoacetic acid and 0.1% betaine on intestinal histomorphology and muscle histology

Parameter	Control	0.06 GAA	0.12 GAA	0.18 GAA	SE	*p*-value		
						GAA	Linear	Quad
Crypt Depth (mm)								
Duodenum	568.32	520.34	454.62	426.36	34.85	0.500	0.140	0.891
Jejunum	439.84	583.54	459.39	393.73	35.62	0.287	0.409	0.150
Ileum	384.92	441.85	375.43	401.06	35.62	0.867	0.947	0.797
Villi length (mm)								
Duodenum	1394.60	1810.40	1706.83	1348.76	112.64	0.406	0.813	0.104
Jejunum	1197.06	1074.16	1003.53	929.93	53.78	0.518	0.147	0.850
Ileum	950.41	821.26	910.48	754.04	53.78	0.602	0.328	0.903
Surface area (mm^2)								
Duodenum	8047.25	13328.86	8203.83	5493.30	1223.39	0.138	0.223	0.095
Jejunum	5043.10	4066.09	4444.73	2920.78	517.79	0.559	0.225	0.800
Ileum	3063.48	2662.73	3105.47	2903.61	268.72	0.602	0.989	0.866
Number of myofibril/mm^2	6.60	5.80	5.80	4.80	0.31	0.234	0.057	0.867
Myofibril diameter (μm)	46.06	52.60	44.38	41.28	3.18	0.674	0.457	0.477

(n=5 replicates/group, 6 chickens per replicate), GAA, Guanidino Acetic acid.

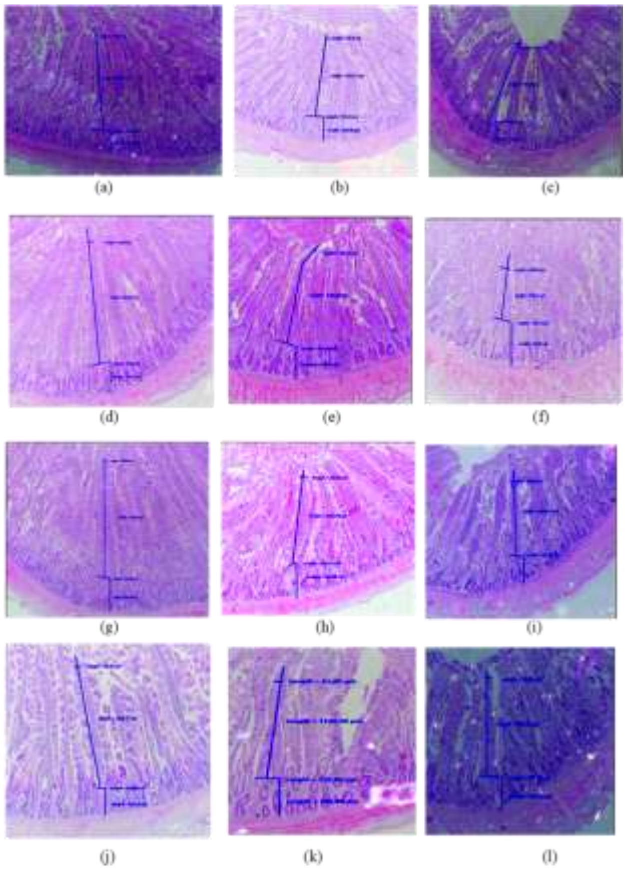

Fig. 5: Intestine (Duodenum, Jejunum and Ileum) histomorphology of native chicken. a, b, c: 0 GAA+0.1% Betaine; d, e, f: 0.06 GAA+0.1% Betaine; g, h, i: 0.12 GAA+0.1% Betaine; j, k, l: 0.18 GAA+0.1% Betaine.

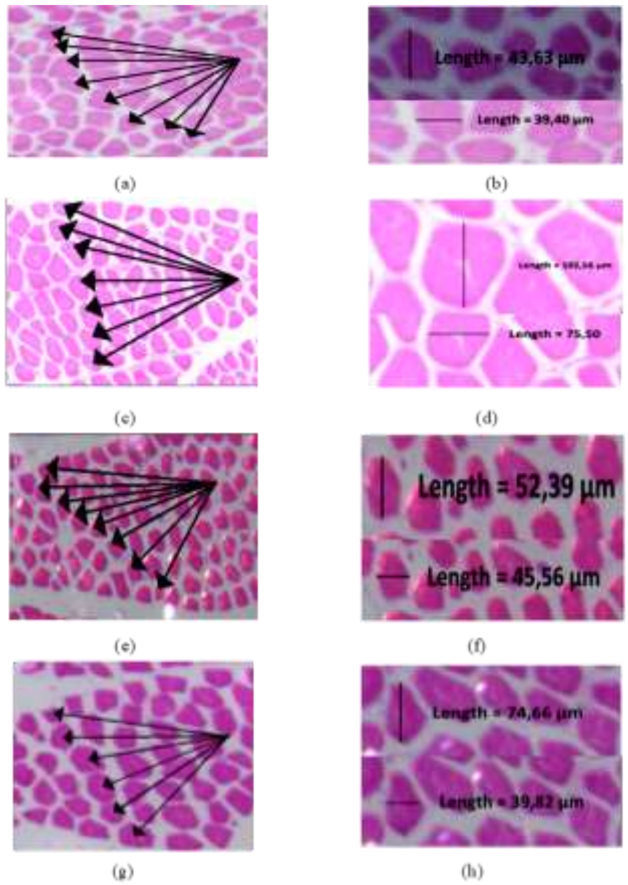

Fig. 6: Number of Myofibril and Myofibril diameter of Native Chicken. a, b: 0 GAA+0.1% Betaine; c,d: 0.06 GAA+0.1% Betaine; e,f: 0.12 GAA+0.1% Betaine; g,h: 0.18 GAA +0.1% Betaine

and ileum observation, including villi height and width, crypt depth, and surface area. Villus height, crypt depth, and the V/C ratio are crucial factors that are directly linked to the mucosa's absorptive ability and are necessary for the intestinal health of broilers (Gungor & Erener, 2020). Higher villus heights and V/C ratios are beneficial for both broiler development and intestinal nutrition absorption (Liu et al., 2022). In this study, the addition of 0.1% Betaine and 0.06 - 1.2% GAA in the feed was not able to show an increase in villi height, villous surface area and crypt depth in each segment of the small intestine. There is a tendency that with increasing levels of GAA use, the length of the villi also decreases, followed by the surface area of the villi. There is limited information available on the effect of GAA supplementation on intestinal morphology in chicken. The great majority of information has focused on arginine's role in gut shape and function. Arginine supplementation improves the morphometry of the duodenal mucosa in broiler chickens (Murakami et al., 2012; Khajali et al., 2014). Arginine supplementation (10g/kg) was shown to improve villus height, width, and absorptive surface area in the jejunum. Increased villus height increases total luminal villus absorptive area, resulting in improved digestive enzyme action and nutrient transfer at the villus surface (Laudadio et al., 2012).

Table 5: Effect of guanidinoacetic acid and 0.1% betaine on meat quality

Parameter	Control	0.06 GAA	0.12 GAA	0.18 GAA	SE	*p*-value GAA	linear	Quadratik
Crude Fat (%)	3.214	4.704	3.51	4.564	0.33	0.298	0.338	0.740
Cholesterol (mg/100g)	32.56[ab]	37.49[a]	36.77[a]	27.74[b]	1.21	0.005	0.073	0.001
Shear Force (N)	0.67	0.73	0.72	0.77	0.02	0.277	0.089	0.935
pH	6.22	6.15	6.20	6.27	0.02	0.464	0.444	0.183
Color_L	57.22	54.24	52.14	53.23	1.27	0.561	0.243	0.444
Color_A	4.17	3.24	3.87	4.63	0.32	0.508	0.494	0.211
Color_B	4.96	3.04	2.84	4.08	0.33	0.062	0.280	0.014
Tendernes (kg/cm^2)	1.37	1.30	1.35	1.41	0.02	0.352	0.400	0.146
Cooking loss (%)	37.16	23.42	24.62	24.02	3.33	0.428	0.218	0.339
Water holding capacity (%)	18.33[a]	19.08[a]	25.25[b]	26.52[b]	1.04	0.201	0.310	0.850

[a,b] Mean values (n=5 replicates/group, 6 chickens per replicate) in the same row with different superscript letters indicate significant differences (P<0.05). GAA, Guanidino Acetic acid.

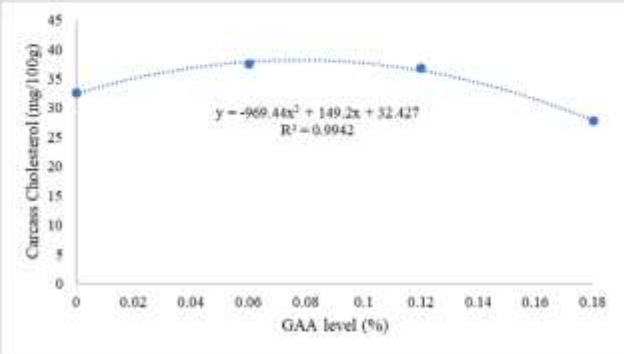

Fig. 7: Polynomial regression of the effect of GAA level on carcass cholesterol (mg/100g)

In this study it was assumed that the dose of GAA given was sufficient for function in the body. Creatine, which is produced from GAA, has been linked to increased muscle work capacity and water retention in muscle cells. This can affect the size and diameter of myofibrils because of the relationship between muscle cell volume and the availability of water and energy stored in the form of creatine phosphate.

Meat Quality

The addition of GAA and Betaine to carcass quality (Table 5) shows that observations of carcass cholesterol levels had a significant effect (P<0.05), where the control treatment did not differ from levels of 0.06, 0.12 and 0.18 additions of GAA and Betaine. However, there is a significant difference in reducing cholesterol levels at levels of 0.18 GAA and Betaine compared to levels of 0.06 and 0.12 GAA. Fat content and carcass physical quality tests (meat breaking strength, carcass pH, color, tenderness, shrinkage and water holding capacity) did not show a significant effect (P>0.05).

According to the results, the addition of GAA at various doses and 0.1% Betaine had no significant effect (P>0.05) on crude fat and physical quality of native chicken. The chemical quality of meat is closely tied to its nutritional content, which is related to the quality of what is consumed. Age, race, species, stress, nutrition, and gender all have an effect on meat's chemical quality. In this investigation, the inclusion of GAA reduced meat cholesterol levels (Lawrie 2003). Cholesterol levels in this study ranged from 27.74 to 37.49mg/100g, which is lower than the normal cholesterol level (56 mg/100mg) reported by Ponte et al. (2008).

The research results are in line with research by Hanim et al. (2023) that the addition of 1,200g/ton GAA in low protein could reduce the cholesterol levels of broiler drumstick meat, but did not significantly reduce cholesterol levels in broiler meat in the breast section (Rahmawati & Hanim, 2022). The relationship between the level of GAA addition to carcass cholesterol levels is presented through the equation in Fig. 7. The equation $y = -969.44x^2 + 149.2x + 32.427$ with $R^2 = 0.9942$ shows that when the feed is not given GAA, the expected meat cholesterol is 32.427mg/100g. For each increase in GAA dose level, meat cholesterol will increase by 149.2mg/100g, however there is a quadratic effect of -969.44 which shows that increasing the GAA concentration after a certain concentration will reduce the meat cholesterol value.

The findings of the study, which revealed that GAA supplementation did not have a significant effect on WHC, carcass pH, color, tenderness, cooking loss, but water holding capacity had a significant difference. Cooking loss estimates in this investigation varied from 26.75 to 32.37%, which is well within the expected range. High meat pH also minimizes cooking losses because it inhibits protein denaturation when the meat temperature remains high (Huff-Lonergan & Lonergan, 2005). The average tenderness value in this study varied from 1.74 to 2.55kg/cm2, and the beef tenderness value with the addition of 600g/ton GAA yielded insignificant effects with a tenderness value of 1.24kg/cm2 (Córdova-Noboa et al., 2018).

Conclusion

Supplementation of guanidinoacetic acid with different levels in native chicken diets showed different effects on the growth and performance of native chickens. The addition of 0.06% GAA resulted in the highest increase in live weight, chicken wings and chicken thighs. On the other hand, the addition of 0.12% GAA produces the highest average daily body weight gain, feed conversion ratio, carcass weight, breast thighs, chicken backs, chicken lower thighs, and 0.18% GAA results in feed intake, feed efficiency and lowest meat cholesterol. Thus, the addition of GAA can be carried out following the expected goals of farming native chickens.

Author's Contribution: Sri Purwanti, Jasmal A. Syamsu, Nancy Lahay, Zaraswati Dwiyana, Marhamah Nadir, Abdul Alim Yamin, Raymundus Genty Laras, Sumiati conducted the investigation and validated the findings; Sri Purwanti, Jasmal A. Syamsu did formal analysis and data curation; Abdul Alim Yamin, Raymundus Genty Laras prepared the original draft; Sri Purwanti, Sumiati and Henny Akit reviewed and edited the final manuscript. All authors have read and agreed to the published version of the manuscript.

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