Effect of Guanidinoacetic Acid in Feed with Different Protein Levels on the Performance and Internal Organ Characteristics of Broiler Chickens

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Abstract. Guanidinoacetic acid (GAA) is the only direct precursor in creating creatine in vertebrate animals. In addition, creatine plays an active role in energy metabolism by supporting the restoration of high-energy cellular ATP concentrations, such as phosphocreatine. This study evaluated the effect of GAA on the growth performance and internal organs of broilers fed a low-protein diet. A total of 1,176 day-old unsexed broiler chicks (Lohman Indian River strain) were randomly assigned to a 2x3 factorial design with seven replication pens of 28 birds each. The dietary treatment consisted of two levels of protein, i.e., high and low protein, in each growth phase and three levels of GAA, i.e., 0, 600, and 1,200 g/ton. The variables observed in the performance parameters include feed intake, feed conversion ratio, body weight, performance index, and mortality. The internal organ parameters include heart, liver, pancreas, gizzard, gallbladder, caecum, jejunum, ileum, and duodenum. The result showed that GAA did not improve broiler chickens' performance in all maintenance phases. However, we found that the high-protein treatment (21%) is optimal during the pre-starter phase, while the low-protein (19%) is optimal during the finisher phase. Moreover, 0.06% of GAA showed the highest percentage of the cecum. Overall, GAA did not have a negative effect on internal organs.

Keywords: Broiler chicken · Guanidinoacetic acid · internal organs · performance · protein levels

1 Background

The rising demand for broiler chicken meat in Indonesia compelled the livestock industry to create and introduce novel chicken feed to increase broiler chicken production. The substitution of high-protein feed ingredients with amino acids is currently viewed as more economical and feasible, and research is ongoing to identify the most influential amino acids [1]. Poultry is highly dependent on arginine availability due to its inability to synthesize the amino acid de novo due to the absence of the enzymes carbamoyl

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phosphate synthase l, arginase hepar, and ornithine carbamoyltransferase in the urea cycle [2]. Guanidinoacetic acid, or GAA, is a direct precursor of creatine in the bodies of vertebrate animals, and it can effectively substitute arginine in poultry feed.

Guanidinoacetic acid is a component of compounds derived from arginine and glycine; it is produced through synthesizing glycine cyanocide and is commercially known as CreAMINO. Creatine synthesis consumes a significant amount of arginine throughout the body. Therefore, providing creatine through animal protein ingredients is a strategy for optimizing tissue creatine storage by replacing endogenous creatine synthesis and making arginine available for other functions, such as protein synthesis. However, animal protein ingredient availability in the feed industry is reported to have low creatine concentrations [3] because crystalline creatine is not commercially available. Consequently, the addition of GAA as an additive feed is required to decrease the usage of arginine, thereby creating a direct metabolic precursor in the formation of creatine to maintain overall energy homeostasis in poultry by supporting the recovery of cellular ATP concentrations in high-energy foods such as phosphocreatine retain homeostasis ATP in myofibrils [4].

The influence of GAA on broiler chickens' performance is still regarded as unstable; hence, research on the specific influence of GAA continues. For example, adding CreAMINO at the level of 0.5–10 g/kg had no significant effect on the performance and quality of carcasses [5]. However, in another investigation, GAA was shown to significantly enhance the Feed Conversion Ratio (FCR) [6]. Therefore, this study investigated the effect of GAA with high and low protein levels in each phase on the performance and organs in broiler chickens, searching for the optimal percentage protein level for GAA usage.

2 Materials and Methods

The ingredients used in this study are basal rations and experimental rations with pre-starter phases using crude protein (CP) of 23% and 21%, starter phases using 21% and 19%, and finisher phases using 19% and 17% and addition of guanidinoacetate acid of 0 g/ton, 600 g/ton, and 1,200 g/ton. The experimental ration was applied to 1,176 broiler chickens raised for 35 days. The phases include the pre-starter phase (0–11 days), the starter phase (12–21 days), and the finisher phase (22–35 days). Broilers were raised in the UGM closed house, and during maintenance, they were not given antibiotics or additional vitamins. Weighing is performed daily. The percentage of internal organs is calculated by dividing the weight of internal organs and the weight of live chicken multiplied by 100%. Data were analyzed using analysis of variance followed by Duncan's test.

3 Results and Discussion

Table 1 shows the growth performance in the pre-starter phase. Based on the analysis variance of GAA addition with different protein levels, the pre-starter phase showed significant results for feed consumption, indicating GAA did not show significance to feed consumption. In this phase, 23% crude protein showed higher feed intakes with an

Table 1. Performance of broiler chickens given GAA with different protein levels in the pre-starter phase

Performance	Level of GAA (g/ton)	Protein Level (%)		Average
		23	21	
Feed intake cumulative (g)	0	196.59 ± 21.66^{a}	169.03 ± 12.52^{b}	182.81 ± 22.22
	600	188.49 ± 11.02^{ab}	174.24 ± 8.22^{ab}	181.37 ± 11.91
	1200	179.27 ± 15.79^{ab}	184.31 ± 25.31^{ab}	181.79 ± 20.44
Average		188.12 ± 17.45^{p}	175.86 ± 17.37^{q}	
Body weight (g/bird)	0	241.36 ± 12.04	253.76 ± 10.90	247.56 ± 12.65
	600	252.80 ± 8.53	257.36 ± 10.70	255.08 ± 9.43
	1200	244.88 ± 9.89	276.32 ± 18.06	260.60 ± 21.51
Average		246.34 ± 10.71^{q}	262.48 ± 16.27^{p}	
FCR	0	0.81 ± 0.06^{a}	0.64 ± 0.02^{c}	0.72 ± 0.09
	600	0.74 ± 0.03^{ab}	0.69 ± 0.01^{bc}	0.71 ± 0.04
	1200	0.77 ± 0.04^{a}	0.70 ± 0.04^{bc}	0.73 ± 0.05
Average		0.77 ± 0.05^{p}	0.67 ± 0.04^{q}	
Performance Index (IP)	0	178.66 ± 17.98	212.50 ± 19.28	195.58 ± 25.04
	600	187.98 ± 9.29	214.16 ± 17.15	201.07 ± 18.96
	1200	182.11 ± 129.22	227.10 ± 29.22	204.60 ± 33.45
Average		182.92 ± 15.71^{q}	217.92 ± 21.90^{p}	
Mortality (%)	0	0.42 ± 0.37	0.13 ± 0.13	0.28 ± 0.28
	600	0.05 ± 0.05	0.14 ± 0.14	0.09 ± 0.09
	1200	0.23 ± 0.23	0.19 ± 0.19	0.21 ± 0.21
Average		0.23 ± 0.23	0.15 ± 0.15	

Description: ^{a,b,c,d}different superscripts in the same column show significant results ($P < 0.05$), ^{p,q}different superscripts in the same row show significant results ($P < 0.05$).

average of 188.12 ± 17.45 compared to 21% crude protein. This result is in accordance with reports that feed containing low protein tends to be consumed in small amounts [7]. The interaction of increasing GAA with different protein levels did not show significant results in body weight, while differences in protein levels showed significant results; 23% of crude protein showed lower body weight than 21% of crude protein. The interaction of GAA addition with different protein levels showed significant results against Feed Conversion Ratio (FCR), indicating that GAA addition did not show significant results. Differences in protein levels showed significant results for crude protein; 23% had an average FCR of 0.77 ± 0.05 and crude protein of 21% with an average of 0.67 ± 0.04. In this study, lowering crude protein levels by up to 2% can reduce FCR values; lowering feed intake without body weight reduction indicates that the pre-starter phase of feed with 21% crude protein provides better feed efficiency. Normally, GAA plays a role

Table 2. Performance of broiler chickens given GAA with different protein levels in the starter phase

Performance	Level of GAA (g/ton)	Protein Level (%)		Average
		21	19	
Feed intake cumulative (g)	0	742.69 ± 45.82^{a}	661.24 ± 55.53^{b}	701.97 ± 64.39
	600	761.40 ± 58.59^{a}	694.16 ± 63.55^{ab}	727.78 ± 67.65
	1200	705.25 ± 66.05^{ab}	702.22 ± 33.61^{ab}	703.74 ± 49.43
Average		736.45 ± 58.40^{p}	686.87 ± 51.91^{q}	
Body weight (g/bird)	0	626.73 ± 10.28^{ab}	546.26 ± 57.11^{d}	586.50 ± 57.40
	600	638.54 ± 19.00^{a}	573.85 ± 29.75^{cd}	606.19 ± 41.43
	1200	575.11 ± 17.69^{cd}	590.83 ± 28.28^{bc}	582.97 ± 23.73
Average		613.46 ± 3.17^{p}	570.31 ± 42.13^{q}	
FCR	0	1.18 ± 0.07	1.21 ± 0.04	1.19 ± 0.06
	600	1.19 ± 0.06	1.21 ± 0.08	1.20 ± 0.07
	1200	1.23 ± 0.12	1.19 ± 0.08	1.21 ± 0.10
Average		1.20 ± 0.07	1.20 ± 0.08	
Performance Index (IP)	0	189.60 ± 9.48	158.88 ± 23.92	174.24 ± 23.65
	600	191.64 ± 5.85	169.79 ± 19.17	180.71 ± 17.63
	1200	161.27 ± 9.14	173.60 ± 15.98	167.43 ± 13.88
Average		180.84 ± 16.43^{p}	167.42 ± 19.57^{q}	
Mortality (%)	0	0.20 ± 0.20	0.00 ± 0.00	0.10 ± 0.10
	600	0.10 ± 0.10	0.15 ± 0.15	0.13 ± 0.13
	1200	0.26 ± 0.26	0.05 ± 0.05	0.15 ± 0.15
Average		0.19 ± 0.19	0.06 ± 0.06	

Description: ^{a,b,c,d}different superscripts in the same column show significant results ($P < 0.05$), ^{p,q}different superscripts in the same row show significant results ($P < 0.05$).

in creatine precursors and is phosphorylated into phosphocreatine which can quickly be mobilized from high-grain phosphate in poultry. This study also shows low-value mortality ranging from 0.05 to 0.42%. Mortality percentage is calculated by mortality rate and culling in maintenance during one production. In this study, neither antibiotics nor drugs were used during maintenance, and with low mortality, the percentage indicated that maintenance management was performed well. This result aligns with mortality factors, including cage sanitation and equipment, environmental cleanliness and disease, and good maintenance management standards [8].

Table 2 demonstrates the performance of broiler chickens administered with GAA at different protein levels in the starter phase (12-22d). Based on results analysis, GAA interaction with different protein levels showed significant results ($P < 0.05$) to feed intake indicating GAA addition did not show significant results. In contrast, protein levels showed significant results ($P < 0.05$). The feed with crude protein 19% decreases the feed value intake in the starter phase of broiler chickens. The interaction of GAA addition at different levels showed significant results ($P < 0.05$) to body weight (BW), adding 0.06% GAA with 21% crude protein showed the highest body weight gain with an average of 638.54 ± 19.00, while GAA 0% with gross protein 19% obtained the lowest body weight with an average of 546.26 ± 57.11. The study also indicated that the addition of GAA did not show significant results ($P > 0.05$), while differences in protein levels showed significant results ($P < 0.05$). In this study, 21% of the crude protein showed better results in the starter phase than the 19% crude protein. The study also shows that adding GAA with different protein levels showed non-significant results against FCR in the starter phase, indicating that adding GAA did not have a negative effect on FCR. It is in line with research conducted in CJ Europe which reported that GAA is not significant to the performance of broiler chickens [9]. The mortality rate also shows insignificant results, which range from 0.05–0.26%, decreased when compared to the starter phase because, in the starter phase, it has gone through a critical period in the broiler chicken maintenance phase creating the stable chicken condition.

The performance of broiler chickens administered with GAA with different protein levels in the finisher phase of broiler chickens (23–35 days) is shown in Table 3. Variance analysis shows non-significant results ($P > 0.05$) to feed intake. In the finisher phase, adding GAA did not show significant results ($P > 0.05$) to broiler chicken feed intake, while differences in protein levels showed significant results ($P < 0.05$) to the broiler chicken feed intake. This result is in line with a previous study reporting that adding GAA up to 0.12% does not affect feed intake [10]. The interaction of GAA addition with different protein levels did not show significant results against body weight in the finisher phase, indicating that adding GAA with low protein showed insignificant results on broiler chicken body weight at five weeks [10]. The study also shows that the interaction of GAA addition with different protein levels did not show significant results ($P > 0.05$), while differences in protein levels showed significant results ($P < 0.05$) against FCR in the finisher phase. The study is also in line with previous research, reporting that GAA does not negatively affect FCR when GAA is added to feed [9]. The 19% crude protein feed showed better results than 17% crude protein. As a result of variance analysis, GAA addition with different protein levels also showed insignificant results ($P < 0.05$) to mortality, with an average of 0.05–0.26% mortality rate in the finisher phase. In this study, chickens were not given antibiotics or drugs, showing that adding GAA with different protein levels in the finisher phase with standard maintenance management does not negatively influence the chickens' health and mortality rate.

The percent relative weight of organs in broiler chickens given GAA with different protein levels is presented in Table 4. The analysis did not show significant ($P > 0.05$) to the weight of the heart, liver, gizzard, and pancreas but showed significant results ($P < 0.05$) against the weight of the cecum and bile. Previous studies state that the giblet results from participation in poultry consisting of liver, heart, and gizzard [10]. In comparison, those that affect the giblet are the nation, age, body weight, drugs, and rations, especially its rough fiber content. The highest average weight of the cecum is indicated by adding GAA with a level of 600 g/ton with an average of 0.53 ± 0.24. A previous study declares that because of adding GAA in food fodder, broiler chicken

Table 3. Performance of broiler chickens given GAA with different protein levels in the finisher phase

Performance	Level of GAA (g/ton)	Protein Level (%)		Average
		19	17	
Feed intake cumulative (g)	0	2194.51 ± 91.66	2019.69 ± 71.02	2107.10 ± 120.27
	600	2179.12 ± 150.32	2002.71 ± 102.46	2090.92 ± 152.81
	1200	2124.86 ± 72.22	2068.49 ± 144.92	2096.68 ± 111.96
Average		2166.16 ± 106.31^{p}	2030.30 ± 106.18^{q}	
Body weight (g/bird)	0	1333.61 ± 15.80	1112.42 ± 60.66	1223.01 ± 123.84
	600	1311.18 ± 49.28	1100.71 ± 101.38	1205.94 ± 133.98
	1200	1296.87 ± 28.80	1117.59 ± 54.58	1207.23 ± 103.06
Average		1313.89 ± 35.31^{p}	1110.24 ± 69.95^{q}	
FCR	0	1.64 ± 0.06	1.81 ± 0.06	1.73 ± 0.10
	600	1.66 ± 0.13	1.82 ± 0.12	1.74 ± 0.14
	1200	1.64 ± 0.05	1.85 ± 0.09	1.74 ± 0.13
Average		1.64 ± 0.08^{q}	1.83 ± 0.09^{p}	
Performance Index	0	153.80 ± 2.18	113.19 ± 7.47	133.50 ± 22.06
	600	152.44 ± 18.08	98.89 ± 5.63	125.67 ± 30.92
	1200	152.29 ± 5.36	115.23 ± 10.79	133.76 ± 21.12
Average		152.85 ± 10.17^{p}	109.10 ± 10.77^{q}	
Mortality (%)	0	0.20 ± 0.20	0.10 ± 0.10	0.10 ± 0.10
	600	0.10 ± 0.10	0.15 ± 0.15	0.13 ± 0.13
	1200	0.26 ± 0.26	0.05 ± 0.05	0.15 ± 0.15
Average		0.19 ± 0.19	0.06 ± 0.06	

Description: ^{p,q}different superscripts in the same column and row show significant results ($P < 0.05$).

breed has heart relative weight ranging from 0.60–0.68% and relative weight of liver ranges from 1.96–2.16% [11]. In this study, the relative weight of the heart ranged from 0.60–0.68%, in line with Ahmadipour *et al.* (2018) [11]. The heart has the primary function of circulating blood throughout the body. The weight percentage of heart in normal broiler chickens ranges from 0.5%-1.42% [12], stating that this study is still within the normal range. This research also indicates that the percentage of heart weight in high CP feed showed lower yields with an average of 0.58%, compared to lower CP with an average of 0.69%. This study aligns with Nastain *et al.* (2021) [13], who reported that the percentage of heart weight with CP of 22% has an average of 0.65% lower than CP of 19.5%, with an average of 0.70%. The previous study also declared that the liver also plays an essential role in the digestion, metabolism, and regulation of

Table 4. Percentage of internal organs in the body of broiler chickens

Content in body weight (%)	Level of GAA (g/ton)	Protein Level (%)		Average
		23	21	
Heart (%)	0	0.57 ± 0.05	0.62 ± 0.05	0.60 ± 0.05
	600	0.58 ± 0.07	0.77 ± 0.18	0.68 ± 0.16
	1200	0.59 ± 0.12	0.68 ± 0.09	0.63 ± 0.11
Average		0.58 ± 0.08^{q}	0.69 ± 0.13^{p}	
Liver (%)	0	2.17 ± 0.41	2.17 ± 0.22	2.17 ± 0.31
	600	2.13 ± 0.52	2.04 ± 0.53	2.09 ± 0.50
	1200	2.56 ± 0.26	2.48 ± 0.36	2.52 ± 0.30
Average		2.29 ± 0.43	2.23 ± 0.41	
Gizzard (%)	0	1.69 ± 0.35	1.88 ± 0.14	1.79 ± 0.27
	600	1.89 ± 0.29	2.02 ± 0.26	1.96 ± 0.27
	1200	1.50 ± 0.17	2.11 ± 0.42	1.81 ± 0.44
Average		1.70 ± 0.31^{q}	2.00 ± 0.29^{p}	
Pancreas (%)	0	0.54 ± 0.08	0.52 ± 0.06	0.53 ± 0.07
	600	0.55 ± 0.11	0.55 ± 0.11	0.55 ± 0.11
	1200	0.49 ± 0.12	0.41 ± 0.09	0.45 ± 0.11
Average		0.53 ± 0.10	0.49 ± 0.10	
Caecum (%)	0	0.38 ± 0.06^{bc}	0.42 ± 0.08^{bc}	0.40 ± 0.07^{b}
	600	0.35 ± 0.11^{c}	0.71 ± 0.21^{a}	0.53 ± 0.24^{a}
	1200	0.49 ± 0.11^{bc}	0.53 ± 0.09^{b}	0.51 ± 0.10^{ab}
Average		0.41 ± 0.11^{q}	0.56 ± 0.18^{p}	
Gallbladder (%)	0	0.12 ± 0.04^{a}	0.09 ± 0.01^{ab}	0.11 ± 0.03
	600	0.09 ± 0.04^{ab}	0.10 ± 0.03^{a}	0.10 ± 0.03
	1200	0.05 ± 0.02^{b}	0.12 ± 0.01^{a}	0.09 ± 0.04
Average		0.09 ± 0.04	0.11 ± 0.02	
Abdominal fat (%)	0	0.74 ± 0.20^{ab}	0.80 ± 0.10^{ab}	0.77 ± 0.15
	600	0.35 ± 0.14^{c}	0.97 ± 0.31^{a}	0.66 ± 0.39
	1200	0.54 ± 0.21^{bc}	0.78 ± 0.31^{ab}	0.66 ± 0.28
Average		0.55 ± 0.24^{q}	0.85 ± 0.25^{p}	
Jejunum (%)	0	0.66 ± 0.06	0.67 ± 0.13	0.67 ± 0.09
	600	0.69 ± 0.15	0.67 ± 0.12	0.68 ± 0.13

(*continued*)

Table 4. (*continued*)

Content in body weight (%)	Level of GAA (g/ton)	Protein Level (%)		Average
		23	21	
	1200	0.61 ± 0.08	0.81 ± 0.26	0.71 ± 0.21
Average		0.65 ± 0.10	0.72 ± 0.18	
Length Jejunum (cm)	0	32.00 ± 2.00	29.00 ± 1.00	30.50 ± 2.17
	600	32.80 ± 2.28	32.40 ± 2.60	32.60 ± 2.31
	1200	31.40 ± 1.94	33.40 ± 4.09	32.40 ± 3.20
Average		32.06 ± 2.01	31.60 ± 3.29	
Ileum (%)	0	1.45 ± 0.09	1.99 ± 0.12	1.72 ± 0.30
	600	1.59 ± 0.27	1.66 ± 0.37	1.63 ± 0.31
	1200	1.70 ± 0.28	1.83 ± 0.12	1.77 ± 0.22
Average		1.58 ± 0.24^{q}	1.83 ± 0.26^{p}	
length Ileum (cm)	0	97.00 ± 4.00	94.00 ± 14.84	95.50 ± 10.37
	600	99.20 ± 9.47	103.80 ± 6.83	101.50 ± 8.15
	1200	103.20 ± 7.91	105.40 ± 8.11	104.30 ± 7.64
Average		99.80 ± 7.42	101.06 ± 11.06	
Duodenum (%)	0	1.25 ± 0.23	1.36 ± 0.03	1.30 ± 0.17
	600	1.32 ± 0.38	1.31 ± 0.26	1.31 ± 0.31
	1200	1.35 ± 0.13	1.37 ± 0.06	1.36 ± 0.10
Average		1.31 ± 0.25	1.34 ± 0.14	

Description: ^{a,b,c,d} different superscripts in the same column show significant results ($P < 0.05$), ^{p,q} different superscripts in the same row show significant results ($P < 0.05$).

broiler chicken production, and several factors that can affect the liver, including feed, feed restriction, anti-nutritional substances, and feed additives that are reported to affect the metabolic structure and function of the liver [14]. The physiological functions of the liver are the secretion of bile to emulsify fat, neutralize toxins, energy storage for glycogen, and decompose remaining protein into uric acid, which will then be excreted by the kidneys [15]. The analysis showed a non-significant difference in crude protein in feed ($P > 0.05$) to the liver's relative weight, suggesting that the difference in crude protein did not interfere with liver performance. The liver's relative percentage weight in this study ranged from 2.04–2.56%, while another study conducted by Mousavi *et al.* (2013) [16] reported that the relative weight of the liver in his study with adding GAA in different energy levels ranged from 2.05–2.53%.

Bile weight is influenced by nutritional status, feed type, blood flow, and enterohepatic bile circulation [17]. However, the primary function of bile is the absorption of feed fat and the release of indigestible products. Another study by Farida *et al.* (2019)

[18] reported an average bile weight of 0.3–1.15%. In this study, the average relative weight of bile ranges from 0.9–0.12%. The analysis also showed non-significant results ($P > 0.05$) against the relative weight and length of intestines for jejunum, ileum, and duodenum. In comparison, abdominal fat showed significant results ($P < 0.05$), and the outcome of high crude protein studies with the addition of 600 g/ton GAA showed the smallest abdominal fat result compared to the addition of 0 g/ton and 1,200 g/ton. While low crude protein leads to the opposite result, adding 600 g/ton GAA shows the highest abdominal fat compared to adding 0 g/ton and 1,200 g/ton. In addition, protein content has a direct or indirect role in regulating lipid metabolism [19]. The relative weight of the duodenum on low crude proteins also showed greater yields than on high crude proteins. It is in accordance with Barekatain *et al*. (2019) [20] reporting that the relative weight of the duodenum at low protein is greater than that of high-protein feed.

4 Conclusion

In all phases, i.e., pre-starter, starter, and finisher, adding guanidinoacetic acid (GAA) does not improve the performance of broiler chickens. In the pre-starter phase, the crude protein with a level of 21% gives optimal results, whereas it does not affect the starter phase. Meanwhile, 19% crude protein provides optimal results in the finisher phase. Furthermore, the addition of 0.06% GAA indicates the highest percentage of the cecum and lowering protein levels by two percent could increase the percentage of the heart, gizzard, cecum, abdominal fat, and ileum. Overall, the addition of GAA does not have a negative impact on the internal organs of broiler chickens.

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References

1. Dilger R N *et al*., 2013 Dietary guanidino acetic acid is an efficacious replacement for arginine for young chicks, *Journal Poultry Science* 92, pp. 171–177
2. Abudabos A M, *et al*., 2014 The relationship between guanidino acetic acid and metabolizable energy level of diets on performance of broiler chickens, *Italian Journal Animal Science* 13, pp. 548–556
3. B. Dobenecker, U. Braun, 2015 Creatine and creatinine contents in different diet types for dogs-effects of source and processing. *Journal Animal Physiology Animal Nutrition* 99, pp. 1017–1024
4. A. A. Degroot, U. Braun, R. N. Dilger, 2018 Efficacy of guanidinoacetic acid on growth and muscle energy metabolism in broiler chicks receiving arginine-deficient diets *Journal Poultry Science* 97, pp. 890–900
5. I. Halle, M. Henning, P. Kohler, 2006 Studies of the effects of creatine on performance of laying hens, on growth and carcass quality of broilers. *Landbauforschung Volkendrode* 56, pp. 11–18

6. C.A. Stahl, M. W. Greenwood, E. P. Berg, 2003 Growth parameters and carcass quality of broilers fed a corn-soybean diet supplemented with creatine monohydrate *Journal Poultry Science* 2, pp. 404–408
7. S. A. Sitompul, O. Sjofjan, H. Djunaidi, 2016 Effect of several types of commercial feed on quantitative and qualitative production performance of broilers*Buletin Peternakan* 40, pp. 187–196
8. Kusnadi E, *et al.,* 2006 Giving antanan (*Centella asiatica*) and vitamin c as an effort to overcome the effect of heat stress on broilers. *Journal of the Indonesian Tropical Animal Agriculture* 33 pp. 133–140
9. B. Saremi, 2021 GAA does not provide energy saving effects in broilers *Poultry World* pp. 1–4
10. El-Faham A *et al*., 2019 Effect of graded levels of guanidine acetic acid in low protein broiler diets on performance and carcass parameters. *Journal Nutrition Feed* 22, pp. 223–233
11. T. Kurtini, K. Nova, D. Septinova, Buku ajar produksi ternak unggas, anugrah utama raharja, Universitas Lampung, 2014
12. B. Ahmadipour, F. Khajali, M. R. Sharifi, 2018 Effect of guanidinoacetic acid supplementation on growth performance and gut morphology in broiler chickens*Journal Poultry Science* 6, pp. 19–24
13. R. Nickle, A. Schummer, E. Seifrle, Anatomy of domestic bird, 1990
14. Nastain F S *et al*., Visceral organ weight of broiler chicken fed different level protein and protease enzyme supplementation diet. IOP Conference Series: Earth Environment Science (2021) 788 pp. 1–8
15. F. Zaefarian, M. R. Abdollah, A. Cowieson, V. Ravindran, Avian liver: the forgotten organ animal. 9 (2019) pp. 1–23
16. J. Blakely, Blade, Ilmu peternakan, Gadjah Mada University, 1991
17. S. N. Mousavi, A. Afsar, H. Lotfollahian, 2013 Effects of guanidinoacetic acid supplementation to broiler diets with varying energy contents. *Journal of Applied Poultry Research.* 22 pp. 47–54
18. E. Suprijatna, A. Umiyati, K. Ruhyat, Ilmu ternak unggas, cetakan 1, Penerbar Swadaya, 2005
19. Fouad A M*et al*., Dietary L-arginine supplementation reduces abdominal fat content by modulating lipid metabolism in broiler chickens. 7 (2013) 1239–1245
20. Barekatain R *et al*., 2019 Reduced protein diet and amino acid concentration alter intestinal barrier function and performance of broiler chickens with or without synthetic glucocorticoid *Poultry Science* 98, pp. 3662–3675