Performance, carcass traits, and relative organ weight of broiler supplemented by guanidinoacetic acid: A meta-analysis

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Abstract. Guanidinoacetic acid (GAA) is formed by the arginine and glycine that are catalysed by arginine:glycine amidinotransferase in the kidney. In the liver, GAA is methylated by s-adenosyl methionine and converted to creatine, then deposited into muscle as energy supply. This meta-analysis was done by integrating 20 articles from various journals. Supplementation doses ranged from 0 to 8000 ppm/kg feed. The mixed model methodology was employed with GAA level and broiler strain as fixed effects and studies as random effects. The results showed that increasing GAA level improved average daily gain day 0-21 and reduced feed conversion ratio day 0-35 (P<0.05). A higher GAA also accompanied by decreasing relative liver weight (P<0.05). GAA supplementation did not affect average daily feed intake and percentage of carcass traits (carcass, legs, breast, wings, drum, thigh) and other parameters such as abdominal fat, gizzard, heart, bursa, thymus and spleen (P>0.05). It was concluded that supplementation of GAA improved the performance of broilers.

1. Introduction
Guanidinoacetic Acid (GAA) is a precursor of creatine [1] that plays a role in energy metabolism. GAA is formed in the kidney by arginine and glycine catalyzed by the enzyme arginine:glycine amidinotransferase (AGAT), then GAA transferred to the liver via blood circulation [2]. In the liver, GAA is methylated by s-adenosyl methionine and converted to creatine, then stored in the muscles. Creatine can affect muscle development [3] and supports the energy supply in adenosine triphosphate (ATP) and adenosine diphosphate (ADP) form [4]. Creatine will be converted into creatinine every day and it’s irreversible, therefore the needs of creatine must be supplied to meet the requirement.

GAA supplementation in poultry feed is used more often than creatine. GAA has more stable molecules compared to direct creatine supplementation [5]. In addition, GAA was also resistant to pelleting temperatures [6]. GAA recovery after receiving heat treatment from 100°C to 190°C was still good (97-100%). Additional GAA in broiler feed was able to increase performance and carcass percentage. Supplementation of 600 ppm in the feed with energy reduction up to four percent, had a better feed conversion ratio (FCR) than control and also higher carcass percentage, breast and thigh yield [4]. According to another research [7], there was an increasing daily weight gain at 0-48 and 0-
55 days of age. This improvement might be caused by its ability to increase villus height, villus width and crypt depth [8] thereby increased ability to absorb nutrients for maintenance and production.

GAA can be found in animal derived raw materials such as poultry by product, meat bone meal and fish meal, even in small quantities. Meanwhile, when performing broiler feed formulation, the majority nutritionist used grain raw materials such as corn, wheat and soybean meal. Thus, it has an impact on low GAA content in the feed and it’s not sufficient to support the desired body weight gain. Moreover, if broilers are exposed to heat stress, creatine biosynthesis is low [9]. Based on this, GAA supplementation is urgently needed to support energy utilization in the body [10].

The use of antibiotics in feed as an antibiotic growth promoter (AGP) has been banned in many countries, including Indonesia since 2018. The use of antibiotics is allowed for therapeutic purposes with maximum seven days implementation and it should follow withdrawal time regulation for each antibiotic. Until now, searching for alternative AGP is still the main topic for feed producers to provide feed with similar performance as when antibiotics are used. Although it has different modes of action with antibiotics, GAA has potential to be an alternative of AGP to increased body weight gain and improved FCR [10]. There was no standard for GAA requirements in the feed. Therefore, it is necessary to analyze the relationship of level GAA to broiler performance.

2. Materials and methods

2.1. Development of database
A database was gathered from various published articles. Articles were searched through google search engines using keywords “Guanidinoacetic acid” and “Broiler”. The parameter included in the database were average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), carcass traits (carcass, leg, breast, drum, thigh), abdominal fat and relative organs weight (liver, gizzard, heart, bursa, thymus, spleen). A total of 21 articles were found after searching the keywords. The next step was article evaluation, each article should mention one of desired variable responses and also broiler strain and GAA level. In the tabulating process, similar variables were converted in the same measurement units. Variable responses ADG and ADFI were measured in g/day unit, and FCR was measured in g/g unit. Variable leg, breast, wings, drum, and thigh were measured in % carcass unit. All parameters of relative organs weight, carcass and abdominal fat were measured in % body weight unit. After the evaluation step, one article was removed due to not mentioning broiler strain. Finally, 36 studies from 20 articles were used and tabulated into database.

2.2. Analysis of data
The data was analysed using the mixed-model methodology [13][14]. Different studies were grouped as random effects, then broiler strain and level of guanidinoacetic acid were grouped as fixed effects. Replication in each study was calculated as a weighting factor during data analysis. The model used in this study was based on the p-value. It was significant when the p-value was <0.05 and it was considered tendency to be significant when p-value was between 0.05 and 0.1. All statistical analysis was done using SAS OnDemand for Academics.

3. Results and discussion

3.1. Database of studies
Compilation of studies included in this meta-analysis shown in Table 1. Levels of GAA ranged from 0 to 8000 ppm. This range was higher than recommendation [15] that was in range from 600 to 1200 ppm. Various strains of broiler were used in the studies such as Hubbard, Ross 308, Ross 708, Cobb 500, Hubbard x Cobb 500 and Arbor Acres. Majority of the studies used male broiler rather than...
female or mixed sex. Replication of the studies ranged from 4 to 16 replications each treatment. The studies of GAA were relatively new, it was started in 2007 and then continued in 2013 until 2020.

Table 1. Studies included in meta-analysis.

| No. | Reference                | Year | Study | Rep. | Broiler strain   | Sex   | GAA level (ppm) |
|-----|--------------------------|------|-------|------|------------------|-------|-----------------|
| 1   | El-Faham et al [16]      | 2019 | 1-2   | 6    | Hubbard          | Male  | 0-1200          |
| 2   | Majededin et al [17]     | 2020 | 3     | 12   | Ross 308         | Male  | 0-1200          |
| 3   | Lemme et al [18]         | 2007 | 4     | 8    | Ross 308         | Male  | 0-1200          |
| 4   | Lemme et al [18]         | 2007 | 5     | 8    | Ross 308         | Female| 0-1200          |
| 5   | Tossenberger et al [6]   | 2016 | 6     | 8    | Ross 308         | Male  | 0-6000          |
| 6   | Cordova et al [19]       | 2018 | 7-8   | 16   | Ross 708         | Male  | 0-600           |
| 7   | Heger et al [4]          | 2014 | 9-13  | 6    | Ross 308         | Male  | 0-600           |
| 8   | Ahmadipour et al [20]    | 2018 | 14    | 4    | Ross 308         | Male  | 0-2000          |
| 9   | Ahmadipour et al [21]    | 2018 | 15    | 4    | Ross 308         | Male  | 0-1500          |
| 10  | Mohabibifar et al [22]   | 2019 | 16    | 8    | Cobb 500         | Male  | 0-1800          |
| 11  | Ahmadipour et al [23]    | 2018 | 17    | 4    | Ross 308         | Male  | 0-2000          |
| 12  | Majededin et al [12]     | 2018 | 18    | 6    | Ross 308         | Male  | 0-1200          |
| 13  | Boney et al [24]         | 2020 | 19-20 | 12   | Hubbard x Cobb 500 | Mix  | 0-600           |
| 14  | Mousavi et al [25]       | 2013 | 21-23 | 6    | Cobb 500         | Mix   | 0-600           |
| 15  | Majededin et al [26]     | 2019 | 24    | 6    | Ross 308         | Male  | 0-1200          |
| 16  | Kodambashi et al [27]    | 2017 | 25-26 | 6    | Ross 308         | Male  | 0-1200          |
| 17  | Cordova et al [28]       | 2018 | 27-28 | 10   | Ross 708         | Male  | 0-600           |
| 18  | Abudabos et al [29]      | 2014 | 29-32 | 5    | Ross 308         | Male  | 0-600           |
| 19  | Amiri et al [30]         | 2019 | 33-34 | 6    | Ross 308         | Male  | 0-1200          |
| 20  | Zhang et al [31]         | 2017 | 35    | 6    | Arbor Acres      | Male  | 0-8000          |
| 21  | Fosoul et al [32]        | 2019 | 36    | 5    | Ross 308         | Male  | 0-1800          |

Rep.: Replication

3.2. Effect of GAA level to performance parameters
The effect of GAA level on performance parameters shown in table 2. The level of GAA was significantly increased ADG day 0-21 and reduced FCR day 0-35 (p<0.05). These results were similar with previous findings [1] [4] [17] [18]. In present study, GAA level was not significant on ADFI (p>0.05) at all day parameters. This was in contrast with previous finding [18], that additional GAA at level 800 and 1200 ppm on male broilers significantly reduced ADFI (p<0.05). Supplementation of GAA more than 1200 ppm can alter the taste of feed. In other hand, reduction of ADFI might be caused by improvement of energy metabolism. Energy efficiency per gram of weight gain increased after supplementation of GAA [4]. Supplementing GAA increased creatine deposition and ratio of phosphocreatine (PCr) to adenosine triphosphate (ATP) in the muscle. This indicated that buffering capacity of PCr to ATP hydrolysis was increased and muscle capacity to growth was gained [11]. Furthermore, biochemical processes in tissue and cell such as cell metabolism, cell motility, and muscle contraction will be more efficient when PCr to ATP ratio increased [12].

3.3. Effect of GAA level to carcass traits and relative organs weight
GAA level was significantly reduced relative liver weight (p<0.05), but not significant on gizzard, heart, bursa, thymus, spleen and all carcass traits (p>0.05). Liver is involved in detoxification, removal of waste products and metabolism of fat, carbohydrates, protein, vitamin and mineral in the body [33]. Proportion of liver depending on species, body weight and age of animal. Reduction of relative liver weight indicated lipogenesis was reduced and the percentage of abdominal fat might be limited [23]. In the present study, abdominal fat was not affected by GAA level (P>0.05), but there was interaction between broiler strain and GAA level (P<0.05). The regression model had negative slope means in particular strain, abdominal fat was reduced with additional GAA. As mentioned in table 3, there was interaction between broiler strain and GAA level to variable thigh (P<0.05). It means supplementation
of GAA in particular strain reduced thigh yield and increased another commercial cut such as breast meat. Breast yield can be influenced by manipulation of energy density in the feed [4].

Table 2. Effect of GAA level to performance parameter.

| Response variable | Unit | Parameter estimates | Model Statistics |
|-------------------|------|---------------------|-----------------|
|                   |      | intercept | SE intercept | slope | SE slope | p-value | AIC | Strain x level |
| ADG day 0-10      | g/day | 20.874    | 0.846       | 0.0002 | 0.000183 | 0.744   | 150.70 | 0.692 |
| ADG day 0-21      | g/day | 31.118    | 0.975       | 0.0009 | 0.000521 | 0.045   | 112.10 | 0.040 |
| ADG day 0-35      | g/day | 72.434    | 1.227       | 0.0012 | 0.001013 | 0.125   | 218.00 | 0.019 |
| ADG day 0-42      | g/day | 53.978    | 1.653       | 0.0020 | 0.000575 | 0.408   | 236.40 | 0.004 |
| ADFI day 0-10     | g/day | 26.895    | 0.382       | -0.0004 | 0.000235 | 0.849   | 136.10 | 0.442 |
| ADFI day 0-21     | g/day | 45.410    | 2.161       | 0.0004 | 0.000444 | 0.223   | 112.40 | 0.133 |
| ADFI day 0-35     | g/day | 105.180   | 1.521       | -0.0001 | 0.001254 | 0.159   | 234.70 | 0.415 |
| ADFI day 0-42     | g/day | 95.881    | 2.059       | 0.0005 | 0.000681 | 0.592   | 255.40 | 0.831 |
| FCR day 0-10      | g/g   | 1.269     | 0.045       | -0.0000 | 0.000011 | 0.593   | -48.80 | 0.276 |
| FCR day 0-21      | g/g   | 1.452     | 0.043       | -0.0000 | 0.000022 | 0.121   | -1.30  | 0.266 |
| FCR day 0-35      | g/g   | 1.449     | 0.043       | -0.0000 | 0.000026 | 0.006   | -57.00 | 0.066 |
| FCR day 0-42      | g/g   | 1.824     | 0.025       | -0.0001 | 0.000015 | 0.244   | -43.70 | 0.001 |

ADG: average daily gain, ADFI: average daily feed intake, FCR: feed conversion ratio, SE: standard of error, AIC: Akaike information criterion (smaller is better)

Table 3. Effect of GAA level to carcass traits and relative organs weight.

| Carcass traits    | Unit | Parameter estimates | Model Statistics |
|-------------------|------|---------------------|-----------------|
|                   |      | intercept | SE intercept | slope | SE slope | p-value | AIC | Strain x level |
| Carcass % BW      |      | 78.574    | 1.5328      | 0.00009 | 0.00046 | 0.130   | 327.10 | 0.856 |
| Leg % carcass     |      | 28.2777   | 1.7251      | -0.00002 | 0.00036 | 0.954   | 237.00 | 0.958 |
| Breast % carcass  |      | 35.5910   | 2.5687      | 0.00016 | 0.00050 | 0.058   | 464.80 | 0.636 |
| Wings % carcass   |      | 8.7289    | 0.5560      | 0.00010 | 0.00019 | 0.909   | 113.30 | 0.437 |
| Drum % carcass    |      | 13.5049   | 0.4007      | -0.00014 | 0.00053 | 0.403   | 143.70 | 0.322 |
| Thigh % carcass   |      | 18.1898   | 0.8071      | -0.00064 | 0.00057 | 0.155   | 180.90 | 0.044 |
| Abd_Fat % BW     |      | 1.3136    | 0.0970      | -0.00016 | 0.00003 | 0.904   | 43.10  | 0.007 |
| Relative organs weight | | | | | | | | |
| Liver % BW       |      | 2.7328    | 0.2736      | -0.00015 | 0.00003 | 0.016   | 39.90  | 0.128 |
| Gizzard % BW     |      | 1.4340    | 0.3007      | -2.3E-06 | 0.00039 | 0.862   | 43.40  | 0.867 |
| Heart % BW       |      | 0.6940    | 0.0661      | -0.00003 | 0.00005 | 0.586   | 32.20  | 0.945 |
| Bursa % BW       |      | 0.1338    | 0.0179      | 8.33E-06 | 0.00001 | 0.948   | -12.80 | 0.296 |
| Thymus % BW      |      | 0.2800    | 0.0542      | -6.32E-06 | 0.00005 | 1.000   | 12.50  | na   |
| Spleen % BW      |      | 3.3E-06   | 0.0000      | 3.33E-06 | 0.00001 | 0.515   | -15.70 | 0.255 |

BW: body weight, Abd_Fat: abdominal fat, SE: standard of error, AIC: Akaike information criterion (smaller is better), na: not available

4. Conclusions
The results showed that increasing GAA level improved average daily gain day 0-21 and reduced feed conversion ratio day 0-35 (P<0.05). A higher GAA also accompanied by decreasing relative liver weight (P<0.05). GAA supplementation did not affect average daily feed intake and percentage of carcass traits (carcass, legs, breast, wings, drum, thigh) and other parameters such as abdominal fat, gizzard, heart, bursa, thymus and spleen (P>0.05). It was concluded that supplementation of GAA improved the performance of broilers.
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