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PAPER



# Supplementation of guanidinoacetic acid and betaine improve growth performance and meat quality of ducks by accelerating energy metabolism

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**ABSTRACT**

This study was conducted to investigate the effects of guanidinoacetic acid (GAA) and betaine on growth performance, meat quality and metabolism of ducks. A total of 384-one-day-old Cherry Valley meat ducks (55.75 ± 0.55 g) were randomly assigned to four treatments with 6 replicates of 16 ducks. Ducks were fed a basal diet (control) or a test diet supplemented with 0.6 g GAA/kg, 2.0 g betaine/kg, or 0.6 g GAA/kg + 2.0 g betaine/kg. The results showed that compared with the control group, GAA and betaine improved feed conversation ratio from day 1 to 14 and 1 to 35 and body weight at 35 days of age ($p < .05$). GAA and betaine increased breast muscle percentage while reduced abdominal fat percentage, and drip loss and shear force of breast muscle ($p < .05$). GAA and betaine lowered free serine, methionine, tryptophan and total amino acid contents in muscles, increased free serine, aspartic acid, phenylalanine and tryptophan contents in plasma ($p < .05$). There was a synergistic effect on non-esterified fatty acid, lactic acid and uric acid between GAA and betaine. GAA and betaine increased the creatine kinase activity in plasma and creatine and ATP content in breast muscle, decreased the lactate dehydrogenase and L-arginine: glycine amidino transferase activities ($p < .05$). In conclusion, supplementation of GAA and betaine improved the growth performance and meat quality of ducks by accelerating energy metabolism. There was a synergistic effect on NEFA, LA and UA concentrations in plasma of ducks between GAA and betaine.

**HIGHLIGHTS**

- Supplementing GAA and betaine improved the growth performance and meat quality of ducks.
- Adding GAA and betaine accelerated the energy metabolism of ducks.
- There was a synergistic effect on non-esterified fatty acid, lactic acid and uric acid concentrations in the plasma of ducks between GAA and betaine.

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## Introduction

Guanidinoacetic acid (GAA) is the immediate precursor for creatine (Cr) synthesis. Creatine is critical in energy metabolism as a carrier and reservoir of phosphate for adenosine triphosphate (ATP) formation. To meet the bird's requirement for creatine, especially in diets with reduced animal protein, GAA may be supplemented into diets as a source of creatine. GAA can not only improve the concentration of Cr and phosphocreatine (PCr) in the muscle but also help to convert adenosine diphosphate (ADP) into ATP when energy is insufficient (Portocarero and Ulrike 2021), and also delay the accumulation of lactic acid produced by glycolysis (Wolf 2000), thereby improving the water-holding capacity and muscle tenderness and ultimately improving meat quality (Lindahl et al. 2006; Schoch et al. 2006). Compared with Cr, GAA is more stable and less expensive, which has led to the idea that perhaps GAA could be a substitute for dietary Cr (Liu et al. 2015a).

Betaine, a trimethyl derivative of the amino acid glycine, contains three chemically reactive methyl groups, which can be used in transmethylation reactions for the synthesis of many substances such as Cr (Chen et al. 2018). When demethylated, betaine becomes glycine and shows the function of the amino acid glycine as well, and participate in protein

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andenergy metabolism and act as an organic osmolyte to protect cells under stress (Saunderson and Mackinlay 1990). Thus, the methyl group of betaines can be used in transmethylation reactions for the synthesis of creatine and may spare methyl group donors such as S-adenosyl methionine, methionine, and choline (Siljander-Rasi et al. 2003). Some studies showed that betaine could improve the growth performance and muscle percentage in broilers (Zhan et al. 2006; Rao et al. 2011). It also has been reported that betaine, when used as an additive to broilers diets, could not only decrease feed:gain ratio and abdominal fat weight but also improve the body weight gain and breast muscle yield in broilers (Chen et al. 2018).

Since GAA requires methyl when Cr is formed in vivo, it was assumed in this study that after GAA was added, there was insufficient methyl donor in ducks, and it was required additional methyl donor supplementation. Because betaine is a trimethyl donor with good stability (Sales 2011), 2.0 g/Kg betaine was added to meet the needs of GAA, prompting GAA to synthesise Cr in vivo. This experiment was designed to study the effects of GAA alone or GAA combined with betaine on growth performance, muscle energy metabolism and meat quality of ducks.

## Materials and methods

### Animals and diets

A total of 384-one-day-old Cherry Valley meat ducks (55.75 ± 0.55 g) were randomly allotted to 4 treatments with 6 replicates and 16 birds per replicate. Ducks were housed in an environmentally controlled room and allowed ad libitum access to diets and water. The 4 diets included a basal diet and 3 experimental diets supplemented with either 0.6 g guanidinoacetic acid (GAA)/kg, 2.0 g betaine/kg, or 0.6 g GAA/kg + 2.0 g betaine/kg in the basal diet. The dose rate of GAA addition was based on Cordova-Noboa et al. (2018) and Majdeddin et al. (2017). The basal diets for the starter (days 1–14) and the grower (days 15–35) phases (Table 1) were formulated according to the nutrient requirements for meat-type ducks published by the Ministry of Agriculture of the People's Republic of China (2012). GAA and betain were from Beijing Gendone Agricultural Technology Co., Ltd and Beijing Challenge Biotechnology Co., Ltd, respectively.

### Sampling

On days 14 and 35 following 12 h of fasting, all ducks were weighed, and feed intake was measured on a per cage basis. The average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio (F/G) were calculated. At 35 d of age, one duck of average body weight (BW) from each replicate cage was chosen, weighed, stunned, exsanguinated, and scalded. Blood samples were withdrawn by cardiac puncture into EDTA anticoagulated tubes, centrifuged at 1 300 × *g* for 10 min at 4 °C, and stored at −20 °C to determine plasma biochemical indices. The weight of the carcase, eviscerated, breast meat, deboned thigh, and abdominal fat were recorded. The muscle from the left breast was sampled and stored at −80 °C for analysis of the free amino acid, Cr, adenylate, and activities of key enzymes, and the right one for water-holding capacity, pH and meat colour determination.

**Table 1.** Composition and nutrient contents of basal diets, % on dry matter or as fed basis.

| Items | Starter phase (1–14 d) | Grower phase (15–35 d) |
|---|---|---|
| Ingredients | | |
| Corn | 51.82 | 58.80 |
| Soybean meal | 28.09 | 17.78 |
| Soybean oil | 3.66 | 3.40 |
| Defatted rice bran | 4.00 | 5.00 |
| Cottonseed meal | 3.00 | 4.00 |
| Rapeseed meal | 3.00 | 4.00 |
| Corn distillers dried grains with soluble | 2.00 | 3.00 |
| Limestone | 1.34 | 1.44 |
| Calcium hydrophosphate | 1.66 | 1.16 |
| NaCl | 0.30 | 0.30 |
| Methionine | 0.13 | 0.12 |
| Vitamin premix[A] | 0.03 | 0.03 |
| Trace mineral premix[B] | 0.10 | 0.10 |
| Zeolite powder | 0.87 | 0.87 |
| Total | 100.00 | 100.00 |
| Nutrient level | | |
| Digestible energy, MJ/kg | 12.13 | 12.34 |
| Crude protein | 20.00 | 17.18 |
| Calcium | 1.00 | 0.90 |
| Total phosphorus | 0.72 | 0.64 |
| Available phosphorus | 0.50 | 0.42 |
| Lysine | 0.95 | 0.74 |
| Methionine | 0.45 | 0.40 |
| Methionine + cystine | 0.80 | 0.71 |

[A]Vitamin premix provided per kg of diet as follows: 15,000 IU of vitamin A, 3900 IU of vitamin $D_3$, 30 IU of vitamin E, 3 mg of vitamin $K_3$, 12.4 mg Vitamin C, 29 mg of vitamin C, 4.5 mg of $C_6$, 0.021 mg of $C_{12}$, 30 mg of pantothenic acid, 45 mg of nicotinamide, 1.2 mg of folic acid and 0.18 mg of biotin.
[B]Trace minerals premix provided per kg of diet as follows: 8 mg of cuprum, 100 mg of manganese, 40 mg of zinc, 80 mg of ferric, 0.35 mg of iodine and 0.15 mg of selenium.
NaCl = sodium chloride

## Measurements

### Meat quality

The meat colour, pH value and shear force were measured according to Cai et al. (2018). The pH values of breast muscle were measured at 24 h post-mortem using a portable pH metre (Testo 205, Testo AG, Lenzkirch, Germany). The meat colour including the

values of lightness (L*), redness (a*), and yellowness (b*) was assessed at 24 h post-mortem by a Spectro photo colorimeter (Minolta CR-400, Konica Minolta Sensing, Osaka, Japan). Approximately 30 g of regular-shaped right breast muscle ($W_1$) was hung in an inverted wax paper cup within a zip-sealed plastic bag that was then filled with nitrogen and stored at 4 °C for 24 h, then removed to wipe off the surface juice and reweighed ($W_2$). Drip loss was calculated as: Drip loss(%) = $(W_1–W_2)/W_2 \times 100\%$. About 20 g of regular-shaped right breast muscle was removed from a refrigerator ($W_3$) at 72 h post-mortem. After 20 min of heating in a zip-sealed plastic bag in a water bath at 80 °C, then removed the meat samples and placed on filter paper for 30 min to cool to room temperature and reweighed($W_4$). Cooking loss was calculated as cooking loss (%) = $(W_3–W_4)/W_3 \times 100\%$ (Kin et al. 2009). One piece of the muscle of 1 cm (width) ×1 cm (thickness) ×2 cm (length) was taken from the muscles used for cooking loss analysis to determine the shear force using a Warner-Bratzler shear attachment (C-LM3B, Tevono, Harbin, China).

### *Plasma biochemical indices*

Plasma biochemical indices including glucose (GLU), total protein (TP), albumin, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), uric acid (UA), urea, Non-esterified fatty acid (NEFA), lactic acid (LA), creatine kinase (CK), and lactate dehydrogenase (LDH) were determined by an automatic biochemical analyser (Hitachi 7600, Hitachi, Tokyo, Japan). L-arginine: glycine amidino transferase (AGAT) was analysed by the ELISA method using a Multiskan MK3 (Thermo Fisher Scientific, Waltham, MA, USA). The Cr, ATP, ADP and adenosine monophosphate (AMP) contents in the breast muscle were determined using HPLC methods as reported by Zhang et al. (2019).

### *Free amino acid content*

The sampled breast meat was ground, stored at −80 °C and measured amino acid (AA) content by the method described by Henderson et al. (2010). Plasma was stored at −20 °C and analysed free AA by ion-exchange chromatography using a Technicon Sequential Multi-sample Amino Acid Analyser (Technicon Instruments Corporation, NY, USA).

**Table 2.** Effects of GAA and betaine supplementation on the growth performance of ducks.

| Items | Control | GAA | Betaine | GAA + betaine | SEM | *p*-value |
|---|---|---|---|---|---|---|
| Initial BW, g | 55.580 | 56.210 | 55.480 | 55.700 | 0.409 | .929 |
| D 1-14 | | | | | | |
| ADFI, g | 59.210 | 57.590 | 57.810 | 57.230 | 0.708 | .816 |
| ADG, g | 44.460 | 45.510 | 46.890 | 46.470 | 0.532 | .412 |
| F/G | 1.330[a] | 1.270[b] | 1.230[b] | 1.230[b] | 0.013 | .013 |
| D 14 | | | | | | |
| BW, g | 622.430 | 637.130 | 656.500 | 650.580 | 7.450 | .412 |
| D 15-35 | | | | | | |
| ADFI, g | 182.140 | 177.320 | 175.360 | 174.880 | 1.373 | .263 |
| ADG, g | 83.680 | 85.590 | 86.690 | 85.430 | 0.708 | .551 |
| F/G | 2.170 | 2.070 | 2.030 | 2.050 | 0.021 | .094 |
| D 35 | | | | | | |
| BW, kg | 2.340[a] | 2.440[b] | 2.480[b] | 2.430[b] | 0.018 | .041 |
| D 1-35 | | | | | | |
| ADFI, g | 132.970 | 129.430 | 128.340 | 127.820 | 0.933 | .237 |
| ADG, g | 66.370 | 67.960 | 69.190 | 68.250 | 0.479 | .231 |
| F/G | 1.990[a] | 1.910[b] | 1.860[b] | 1.870[b] | 0.018 | .018 |

Data are the means of 6 replicates per treatment with 16 ducks per pen.
BW: body weight; ADFI : average daily feed intake; ADG : average daily gain; F/G : feed/gain; GAA: guanidinoacetic acid; SEM: standard error of the mean.
[a–b]Means within a row with different superscripts differ significantly at $p < .05$.

## Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) procedure in the SPSS 19.0 software package for Windows (SPSS Inc. Chicago, IL, USA). Significant differences between treatment means were separated using Duncan's multiple range test. Differences were considered significant at $p < .05$.

## Results

### *Growth performance*

The growth performance parameters of ducks are shown in Table 2. Ducks in three experimental groups had lower F/G during periods from day 1 to 14 and 1 to 35, and higher BW at 35 d of age than the control group ($p < .05$). Supplementing GAA and/or betaine in diets did not affect the ADFI or ADG of ducks ($p > .05$). There was no synergistic effect between GAA and betaine ($p > .05$).

### *Carcass characteristics*

The effects of dietary betaine and/or GAA supplementation on the carcass characteristics of ducks are shown in Table 3. Compared with the control group, the GAA and GAA + betaine groups had a greater breast muscle percentage ($p < .05$). The abdominal fat percentage of ducks in the three experimental treatments was lower ($p < .05$). GAA and/or betaine supplementation did not affect other carcass traits of ducks

**Table 3.** Effects of GAA and betaine supplementation on the carcase traits and meat quality of ducks at 35 d of age.

| Items | Control | GAA | Betaine | GAA + betaine | SEM | *p*-value |
|---|---|---|---|---|---|---|
| Carcase traits, % | | | | | | |
| Dressing percentage | 81.440 | 81.600 | 80.960 | 82.050 | 0.516 | .917 |
| Semi-eviscerated percentage | 76.160 | 76.420 | 75.870 | 76.500 | 0.498 | .974 |
| Eviscerated percentage | 70.310 | 70.880 | 70.140 | 70.330 | 0.465 | .956 |
| breast muscle percentage | 6.790[a] | 8.610[b] | 7.640[ab] | 7.930[b] | 0.213 | .013 |
| Thigh muscle percentage | 12.000 | 12.710 | 12.880 | 12.440 | 0.167 | .278 |
| Abdominal fat percentage | 2.370[a] | 1.700[b] | 1.770[b] | 1.650[b] | 0.091 | .019 |
| Meat quality | | | | | | |
| L* | 50.240 | 50.280 | 49.610 | 49.180 | 0.867 | .970 |
| a* | 13.820 | 14.810 | 13.240 | 13.540 | 0.512 | .752 |
| b* | 10.130 | 11.010 | 10.560 | 10.010 | 0.455 | .890 |
| pH | 5.690 | 5.720 | 5.840 | 5.810 | 0.034 | .535 |
| Drip loss, % | 5.850[a] | 3.270[b] | 3.840[b] | 4.380[b] | 0.326 | .009 |
| Cooking loss, % | 36.590 | 30.900 | 29.540 | 30.980 | 1.087 | .091 |
| Shear force value, *N* | 33.390[a] | 25.930[b] | 25.040[b] | 27.210[b] | 1.192 | .044 |

Data are the means of 6 replicates per treatment with 16 ducks per pen.
GAA: guanidinoacetic acid; SEM: standard error of the mean.
L*= lightness; a*=redness; b*= yellowness.
[a–b]Means within a row with different superscripts differ significantly at $p < .05$.

($p > .05$). There was no synergistic effect between GAA and betaine ($p > .05$).

### Meat quality

As indicated in Table 3, the three experimental groups had lower drip loss and shear force value in the breast muscle of ducks compared with the control group ($p < .05$). There was no significant difference in pH, L*, a*, or b* value among the four groups ($p > .05$). No synergistic effect between GAA and betaine was observed ($p > .05$).

### Free amino acid content in breast muscle

As shown in Table 4, the concentration of free serine (Ser), methionine (Met), tryptophan (Trp), and total AA in muscle was decreased by supplementing GAA alone or together with betaine, compared to the control group ($p < .05$). Dietary betaine reduced the concentration of free Ser and Trp in the muscle of ducks ($p < .05$). There was no significant difference between the control and experimental groups in the concentration of other free AA in muscle ($p > .05$).

**Table 4.** Effects of GAA and betaine supplementation on the free amino acids in breast muscle of ducks at 35 d of age, ug/g.

| Items | Control | GAA | Betaine | GAA + betaine | SEM | *p*-value |
|---|---|---|---|---|---|---|
| Serine | 192.570[a] | 126.360[b] | 132.720[b] | 143.340[b] | 8.808 | .030 |
| Glycine | 373.420 | 250.230 | 369.020 | 337.880 | 29.875 | .493 |
| Histidine | 49.990 | 34.600 | 38.810 | 35.480 | 3.244 | .364 |
| Threonine | 109.970 | 85.320 | 88.700 | 90.420 | 6.319 | .574 |
| Glutamic acid | 109.660 | 77.160 | 86.060 | 92.620 | 5.268 | .198 |
| Glutamine | 363.440 | 235.200 | 287.150 | 298.610 | 16.808 | .068 |
| Aspartic acid | 26.510 | 23.980 | 38.070 | 24.600 | 2.481 | .123 |
| Asparagine | 41.250 | 28.800 | 35.720 | 39.530 | 2.591 | .388 |
| Alanine | 321.670 | 234.360 | 262.810 | 260.580 | 11.903 | .074 |
| Arginine | 189.590 | 114.700 | 131.230 | 149.090 | 10.509 | .078 |
| Proline | 79.980 | 62.050 | 69.460 | 62.390 | 3.044 | .142 |
| Cysteine | 13.530 | 9.520 | 8.990 | 9.200 | 0.868 | .230 |
| Lysine | 164.760 | 91.660 | 152.640 | 138.950 | 10.717 | .092 |
| Methionine | 39.860[a] | 30.370[b] | 34.850[ab] | 29.400[b] | 1.496 | .046 |
| Valine | 78.510 | 66.190 | 74.880 | 66.350 | 2.958 | .384 |
| Tyrosine | 86.170 | 61.970 | 78.350 | 68.340 | 3.931 | .154 |
| Isoleucine | 53.610 | 39.450 | 44.620 | 40.200 | 2.991 | .365 |
| Leucine | 91.200 | 64.610 | 83.320 | 71.940 | 5.060 | .294 |
| Phenylalanine | 78.070 | 51.440 | 67.550 | 58.670 | 3.862 | .093 |
| Tryptophan | 24.490[a] | 15.910[b] | 19.230[b] | 17.680[b] | 1.107 | .037 |
| Total | 2488.260[a] | 1743.910[b] | 2137.510[ab] | 2034.280[b] | 92.647 | .042 |

Data are the means of 6 replicates per treatment with 16 ducks per pen.
GAA: guanidinoacetic acid; SEM: standard error of the mean.
[a–b]Means within a row with different superscripts differ significantly at $p < .05$.

### Free amino acid content in plasma

As shown in Table 5, compared to the control group, the concentration of free Ser, aspartyl (Asn), phenylalanine (Phe), and tryptophan (Trp) in plasma of ducks in the GAA + betaine group was higher ($p < .05$). Adding betaine decreased free Ser by comparison with the control group ($p < .05$). There was no significant difference between the control and the experimental groups in the concentration of other free AA in plasma ($p > .05$).

### Biochemical parameters in plasma

The effects of dietary betaine and/or GAA supplementation on plasma biochemical parameters of ducks are shown in Table 6. Compared with the control group, adding betaine and GAA + betaine decreased NEFA content ($p < .05$). The three experimental groups had a lower concentration of GLU, TG, LA, and UA in the plasma of ducks ($p < .05$). There was a synergistic

**Table 5.** Effects of GAA and betaine supplementation on free amino acid contents in plasma of ducks at 35 d of age, ug/mL.

| Items | Control | GAA | Betaine | GAA + betaine | SEM | *p*-value |
|---|---|---|---|---|---|---|
| Serine | 56.840[a] | 65.770[ab] | 70.410[b] | 77.850[b] | 2.737 | .037 |
| Glycine | 72.790 | 67.280 | 62.950 | 67.650 | 3.179 | .793 |
| Histidine | 11.400 | 13.980 | 14.120 | 12.800 | 0.442 | .084 |
| Threonine | 34.330 | 35.080 | 35.760 | 38.700 | 1.910 | .878 |
| Glutamic acid | 28.520 | 28.520 | 30.630 | 29.300 | 1.123 | .912 |
| Glutamine | 59.000 | 72.730 | 67.060 | 64.350 | 4.119 | .722 |
| Aspartic acid | 9.130 | 9.520 | 9.690 | 9.640 | 0.347 | .949 |
| Asparagine | 3.540[a] | 3.100[ab] | 2.990[ab] | 4.810[b] | 0.233 | .019 |
| Alanine | 77.260 | 68.420 | 76.310 | 84.860 | 2.579 | .163 |
| Arginine | 82.220 | 93.780 | 85.620 | 82.160 | 3.482 | .634 |
| Proline | 29.690 | 29.880 | 35.690 | 36.750 | 1.755 | .349 |
| Cysteine | 20.700 | 21.640 | 19.450 | 19.430 | 0.722 | .674 |
| Lysine | 70.580 | 85.180 | 104.720 | 72.240 | 5.914 | .142 |
| Methionine | 12.130 | 13.840 | 14.760 | 17.300 | 0.817 | .152 |
| Valine | 33.620 | 35.110 | 34.890 | 39.190 | 1.119 | .338 |
| Tyrosine | 36.000 | 35.480 | 36.720 | 32.370 | 1.191 | .614 |
| Isoleucine | 15.820 | 16.790 | 17.120 | 16.610 | 0.603 | .903 |
| Leucine | 23.330 | 23.280 | 24.970 | 24.900 | 0.854 | .843 |
| Phenylalanine | 23.590[a] | 23.020[a] | 26.760[a] | 32.430[b] | 1.143 | .005 |
| Tryptophan | 7.880[a] | 8.090[a] | 8.640[a] | 16.070[b] | 0.901 | .001 |
| Total | 684.470 | 750.530 | 784.490 | 769.270 | 20.716 | .480 |

Data are the means of 6 replicates per treatment with 16 ducks per pen. GAA: guanidinoacetic acid; SEM: standard error of the mean.
[a–b]Means within a row with different superscripts differ significantly at $p < .05$.

**Table 6.** Effects of GAA and betaine supplementation on the biochemical indices in plasma of ducks at 35 d of age.

| Items | Control | GAA | Betaine | GAA + betaine | SEM | *p*-value |
|---|---|---|---|---|---|---|
| GLU, mmol/L | 7.550[a] | 5.000[b] | 5.090[b] | 6.230[b] | 0.362 | .031 |
| TP, g/L | 29.280 | 32.770 | 29.800 | 32.230 | 0.601 | .095 |
| ALB, g/L | 9.600 | 10.580 | 10.150 | 10.350 | 0.148 | .100 |
| HDL-C, mmol/L | 2.590 | 3.100 | 3.140 | 2.940 | 0.095 | .152 |
| LDL-C, mmol/L | 2.230 | 1.970 | 1.920 | 1.970 | 0.085 | .615 |
| TC, mmol/L | 6.120 | 5.600 | 5.610 | 5.660 | 0.133 | .475 |
| TG, mmol/L | 1.640[a] | 1.430[b] | 1.210[b] | 1.370[b] | 0.049 | .008 |
| NEFA, μmol/L | 650.670[a] | 626.880[ab] | 594.890[b] | 512.100[c] | 18.263 | .028 |
| LA, mmol/L | 12.220[a] | 9.460[b] | 9.950[b] | 7.900[c] | 0.571 | .049 |
| UA, μmol/L | 258.500[a] | 227.830[b] | 224.000[b] | 196.000[c] | 6.815 | .006 |
| Urea, mmol/L | 1.890 | 1.500 | 1.510 | 1.440 | 0.195 | .437 |

Data are the means of 6 replicates per treatment with 16 ducks per pen. GAA: guanidinoacetic acid; SEM: standard error of the mean; GLU: glucose; TP: total protein; ALB: albumin; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride; NEFA: Non-esterified fatty acid; LA: lactic acid; UA: uric acid.
[a–c]Means within a row with different superscripts differ significantly at $p < .05$.

effect on NEFA, LA and UA between GAA and betaine ($p < .05$).

### *Key enzyme activity in plasma*

The activities of the key enzyme in the plasma of ducks are shown in Table 7. The activity of CK in the plasma of ducks in GAA and GAA + betaine groups was higher, compared with the control group. Activities of AGAT and LDH in the plasma of ducks from the three experimental treatments were lower

**Table 7.** Effects of GAA and betaine supplementation on the activities of key enzymes in plasma of ducks, U/L at 35 d of age.

| Items | Control | GAA | Betaine | GAA + betaine | SEM | *p*-value |
|---|---|---|---|---|---|---|
| CK | 5141.230[c] | 5577.750[a] | 5246.980[bc] | 5447.130[ab] | 58.591 | .025 |
| LDH | 846.500[a] | 676.480[bc] | 731.220[b] | 552.980[c] | 30.451 | .002 |
| AGAT | 35.490[a] | 25.600[b] | 28.570[b] | 28.280[b] | 1.027 | .001 |

Data are the means of 6 replicates per treatment with 16 ducks per pen. GAA: guanidinoacetic acid; SEM: standard error of the mean; CK: creatine kinase; LDH: lactate dehydrogenase; AGAT: L-arginine: glycine amidino transferase.
[a–c]Means within a row with different superscripts differ significantly at $p < .05$.

**Table 8.** Effects of GAA and betaine supplementation on the creatine and adenylate in breast muscle of ducks at 35 d of age.

| Items | Control | GAA | Betaine | GAA + betaine | SEM | *p*-value |
|---|---|---|---|---|---|---|
| ATP, umol/g | 76.650[b] | 92.870[a] | 78.980[ab] | 86.800[a] | 2.392 | .049 |
| ADP, ug/g | 0.650 | 1.050 | 0.800 | 1.410 | 0.175 | .470 |
| AMP, ug/g | 0.040 | 0.010 | 0.010 | 0.010 | 0.046 | .073 |
| Cr, ug/mg | 3.950[b] | 4.680[a] | 4.460[ab] | 4.930[a] | 0.133 | .046 |

Data are the means of 6 replicates per treatment with 16 ducks per pen. GAA: guanidinoacetic acid; SEM: standard error of the mean; ATP: Adenosine triphosphate; ADP: Adenosine diphosphate; AMP: Adenosine monophosphate adenylic acid; Cr: Creatine.
[a–b]Means within a row with different superscripts differ significantly at $p < .05$.

($p < .05$). There was no synergistic effect between betaine and GAA ($p > .05$).

### *Creatine and adenylate contents in breast muscle*

The effects of dietary betaine and GAA supplementation on the concentration of Cr and adenylate in the breast muscle of ducks are presented in Table 8. In comparison to the control group, supplementing GAA or GAA + betaine significantly increased the concentration of ATP and Cr in the breast muscle ($p < .05$), no synergistic effect between betaine and GAA ($p > .05$).

## Discussion

As a feed additive, many researchers have focussed on the potential use of GAA in animal production in recent years. In the present study, the GAA improved FCR and BW of ducks, which is in agreement with the results of Ringel et al. (2007) and Lemme et al. (2007) in broilers. Similar results were also reported in finishing pigs and newly weaned piglets (Wang et al. 2012; Teixeira et al. 2017). Guanidinoacetic acid exerts a growth effect through its primary physiological fate to form Cr and additionally spares dietary arginine from GAA synthesis so as to accelerate energy metabolism and protein deposition (Portocarero and Ulrike 2021). In previous studies, betaine added in a methionine

deficient diet could significantly improve the growth rate and feed utilisation rate of animals. In this experiment, the methionine level in the basal diet basically met the growing needs of ducks. While the supplementation of 2 g/kg betaine significantly improved the FCR and BW of ducks, possibly because betaine tastes sweet can stimulate the animal's sense of smell, and the exogenous betaine make ducks make full use of the active methyl in betaine, to ensure that the protein and the neurotransmitter synthesis, thereby promoting the growth of ducks. This finding was consistent with the data of Chen et al. (2018), who found that betaine could improve the growth performance and muscle growth of partridge shank broiler chickens via altering myogenic gene expression and insulin-like growth factor-1 signalling pathway. There was no synergistic effect on the growth performance of ducks between betaine and GAA in the present study, which indicated that methyl in diet may be sufficient for GAA to form Cr.

The present study manifested that GAA and betaine supplementation resulted in a decrease in the abdominal fat percentage and an increase in the breast muscle percentage of ducks, which is consistent with previous results (Michiels et al. 2012; Ahmadipour et al. 2018). Betaine is involved in the synthesis of methylated compounds choline such as carnitine and reducing the requirement for other methyl donors such as creatine (Zhan et al. 2006). Consequently, betaine is used as a carcase modifier to increase the muscle percent and decrease the fat percentage. The effect of GAA on muscle yield and fat content of ducks in this study possibly resulted from that GAA increased creatine and ATP concentration in muscle so as to enhance energy metabolism and protein deposition (He et al. 2018).

The basic assessment indicators of meat quality including colour, pH value, water-holding capacity, and tenderness. The present study showed that GAA supplementation decreased the drip loss and shear force value. It was proved that GAA can improve meat tenderness and water-holding capacity through changing muscle morphology traits and muscle fibre characteristics of muscle Zhu et al. (2020). Betaine has various functions as a feed additive in livestock, such as methyl donor and osmoregulation (Eklund et al. 2005). Betaine via osmoregulation roles could protect the cells from osmotic stressors, thereby increasing the water retention and tenderness of the muscles tissues (Wen et al. 2019).

The present study showed that GAA and betaine supplementation resulted in a decrease in the concentration of free AA in muscles including Ser, Met, Trp and total AA, and an increase in the concentration of free AA including Ser, Asn, Phe, and Trp in plasma. Blood in the body has the function of transporting and exchanging free AA. The increased AA content in plasma means the enhanced proteion anabolism. The free amino acids in the muscle are mainly used for energy storage and energy supply for body activity. Since the supplementation of GAA can increase Cr and ATP concentrations, we speculate that changes in the concentration of free AA may be due to sufficient energy supply in the body thereby reinforcing the synthesis of protein rather than decomposition, which is consistent with the improved BW and breast muscle percentage of ducks by adding GAA alone or together with betain in this study. The regulation of betaine on AA content was due to its methyl-supplying action to change amino acid metabolism. There was no synergistic effect between betaine and GAA.

In our study, GAA was added alone or together with betaine resulted in a reduction of GLU, TG, UA, LA and NEFA in plasma. The main function of blood glucose and TG are to provide energy for the body. LA is the end product of the glycolysis energy supply system, and too much LA will cause internal environment acid-base balance disorder. The decrease of GLU and LA in this study may be due to that adding GAA in diets improve the concentration of Cr and ATP concentration, reduced GLU and TG used for energy supply, simultaneously delay the accumulation of LA produced by glycolysis (Wolf 2000). Fat can be hydrolysed by lipase into NEFA and glycerol then released into the blood, oxidised and utilised by tissues (Zou 2005). In this study, the addition of GAA and betaine decreased the concentration of NEFA in the plasma, suggesting that GAA could reduce fat mobilisation in the body by increasing the body's energy storage materials. In brief, the addition of GAA and betaine weakens the metabolic processes for energy supply and reduces the consumption of energy substances by providing additional energy storage in the body. There was a synergistic effect on NEFA, LA and UA in plasma of ducks between GAA and betaine.

In the present study, GAA and betaine decreased activities of AGAT and LDH in plasma but increased CK activity in plasma and content of Cr and ATP in breast muscle, which is consistent with some previous reports (Ale Saheb Fosoul et al. 2019; He et al. 2018). In cells, Cr is phosphorylated by CK to form PCr, which subsequently transfers its high energy-yielding phosphoryl group to ADP to resynthesize ATP (Wallimann

et al. 2011; McKinnon et al. 2012), GAA is the precursor of creatine synthesis, and AGAT is the first rate-limiting enzyme in the whole creatine synthesis reaction and is regulated by the negative feedback of creatine synthesis concentration (Guthmiller et al. 1994; Zhang et al. 2019). In this study, the reason for the increase of creatine and ATP content resulted from that GAA provided sufficient precursor of creatine, enhanced Cr phosphorylated to PCr by increasing CK, down-regulated AGAT activity, the rate-limiting enzyme of creatine synthesis, and/or betaine supplied additionally methyl donor for creatine synthesis. Studies have shown that after animal slaughter, ATP is produced by anaerobic glycolysis along with lactic acid, which decreasing muscle pH and even affects the meat quality (Scheffler and Gerrard 2007). LA is the end product of the glycolysis energy supply system. LDH is one of the key restriction enzymes for anaerobic glycolysis, which catalyses the conversions of pyruvate to LA (Liu et al. 2015b). In the present study, the reduced activity of LDH in plasma is possibly related to the sufficient energy storage by the addition of GAA and betaine. It was consistent with the reduced lactic acid content and increased ATP content in plasma. In summary, the addition of GAA and betaine can improve energy metabolism in the body, delay the glycolysis process and improve the meat quality of ducks.

## Conclusion

GAA and betaine supplemented with diets can improve growth performance and meat quality of ducks through regulating energy metabolism by increasing precursor of creatine and changing key enzyme activities, so as to accelerate protein synthesis and decrease lipid synthesis and deposition in peripheral tissues. There was a synergistic effect on NEFA, LA and UA in the plasma of ducks between GAA and betaine. It is an effective way to improve the feed efficiency and meat quality of ducks by adding GAA and betaine in the feed.

## Ethical approval

All experimental procedures were approved by the Animal Ethics Committee of the Chinese Academy of Agricultural Sciences (AEC-CAAS-20191106).

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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