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Response of meat chickens to guanidinoacetic acid and betaine in reduced protein diet

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In submitting this report, the researcher has agreed to Poultry Hub Australia publishing this material in an edited form.

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Project Summary

Project Title	Response of meat chickens to guanidinoacetic acid and betaine in reduced protein diet
Project No.	20-225
Date	Start: 12/01/2021 End: 31/01/2022
Project Leader(s)	Nishchal K. Sharma
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Project Aim	This project aimed to determine the extent that guanidinoacetic acid (GAA) may spare arginine (Arg) in broilers offered low crude protein (CP) diets, and if supplemental betaine provides additional benefits on growth performance, carcass yield and meat quality.
Background	Nutritional strategies to improve the performance of broilers offered low CP diets supplemented with crystalline amino acids are of interest to the poultry industry. In low CP diets, dietary creatine (Cr) or its amino acid precursors Arg and glycine are naturally low as less protein meals and more crystalline amino acids are used. This puts burden on *de novo* Cr synthesis, which is not adequate to meet the birds' requirement of Cr and thus growth performance is compromised. In this project, GAA was used to maximise the benefits of a low CP feeding program. GAA is an immediate precursor of Cr and can be used to spare Arg for Cr synthesis. The methyl donors such as betaine will maximise the balance in metabolism, stopping the build-up of potentially toxic homocysteine, and sparing methionine and glycine. Thus, betaine together with GAA may be useful in a low CP feeding program for broilers.
Research Outcome	The addition of supplemental Arg to the Arg deficient low CP diet restored growth performance loss in broilers. When GAA spared Arg at 150%, feed intake, weight gain, FCR, breast yield, abdominal fat yield and breast meat creatine concentration became comparable to Arg sufficient low CP and normal CP treatments. When GAA spared Arg at 100% and 50%, FCR was 3 and 5 points better than the normal CP treatment. There was a linear increase in breast meat Cr concentration with increasing levels of GAA, and this translated to better FCR and lower abdominal fat pad. There were no benefits in adding betaine with GAA on the parameters measured but the results with GAA were consistent in the presence or absence of betaine.
Impacts and Outcomes	This work indicates that GAA can spare 150% Arg in low CP diets for comparable growth performance, meat quality and production of Cr. For enhanced feed efficiency and higher muscle Cr deposition, a higher replacement rate of 1:1 or 100% may be considered. Increased FCR and abdominal fat pad weight are major issues in broilers fed low CP diets but a partial replacement of dietary Arg with GAA may help to solve these issues.
Publications	The outcome of this project has been accepted for oral presentation at the 2022 Australian Poultry Science Symposium. A manuscript has been submitted to the Poultry Science journal.

Executive Summary

Arginine (Arg) concentration is naturally low in wheat-sorghum based low crude protein (CP) diets, which makes Arg a costly nutrient and contributes significantly to feed cost. Dietary guanidinoacetic acid (GAA) may be used as a partial replacement to Arg as it reduces the requirement of Arg for creatine (Cr) synthesis. Adding GAA produces more Cr in the muscle cells than adding a higher level of L-Arg as the latter may be metabolised quickly in the blood and may not be the most efficient way of maximising Cr. A sufficient level of dietary methyl donor is required to convert GAA to Cr, especially when the dietary level of GAA is higher. Thus, this project examined the rate at which GAA may replace supplemental L-Arg in moderately low CP diets with and without a methyl donor to maximise growth performance, breast meat yield and Cr synthesis in broilers.

The study demonstrated the magnitude of Arg deficiency in broilers as observed by lower weight gain, lower breast meat yield, lower breast meat Cr concentration, higher FCR and higher abdominal fat pad compared to Arg sufficient dietary treatment. The efficacy of adding Arg back to the Arg deficient low CP diet was also demonstrated in this study, as the addition of Arg restored growth performance loss and improved the shelf life of breast meat by lowering pH and drip loss and enhancing colour. The important research question of this study was whether the benefits of supplemental Arg in Arg deficient low CP diet could be maintained or further enhanced by partly replacing it with GAA at different rates. When GAA is used to spare Arg in broiler diets, Arg can be diverted for other metabolic functions such as muscle accretion, cell signalling, and hormone release rather than Cr formation for which GAA is more efficient and economic. In this study, 1 kg/Metric Tonne of added L-Arg in low CP diet was replaced with GAA at replacement rates of 50% (2.0 kg/MT), 100% (1.0 kg/MT) and 150% (0.067 kg/MT) with or without betaine. The growth performance, carcass yield and meat quality data demonstrated that GAA could spare 1 kg/MT of added L-Arg at 150% in low CP diets with an additional payback through 27 g increased breast meat yield. There were no benefits in adding betaine with GAA on the parameters measured but the results with GAA were consistent in the presence or absence of betaine. The efficiency at which Arg and GAA may deposit Cr in breast meat was also demonstrated in this study and dietary GAA was observed to be a more potent Cr source than Arg. The positive correlation between breast meat total Cr concentration and breast meat moisture observed in this study highlights the muscle Cr/phosphocreatine role to draw water into the muscle cells and possibly result in increased breast meat weight. The negative correlation between breast meat Cr concentration and the relative weight of the abdominal fat pad suggests that Cr improves energy efficiency and increases fat oxidation.

Increased FCR and abdominal fat pad weight are major issues in broilers fed wheat-sorghum based low CP diets. Thus, it may be efficient to partially replace added Arg with GAA for maximising Cr in muscles, which possibly increases energy and nutrients available for growth resulting in improved FCR and decreased abdominal fat pad. To what extent added Arg can be replaced with GAA in low CP diets should be explored in the future.

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Introduction

Creatine (Cr) plays a vital role in energy metabolism and the formation of muscles and other tissues in the body (Brosnan et al. 2009). A three-week-old broiler of 985 g average body weight requires 169 mg of Cr daily (Khajali et al. 2020) and this requirement increases with age. Only two-thirds of the daily Cr requirements may be synthesised endogenously and the rest should be supplemented through feed (Tossenberger et al. 2016). Animal derived feed ingredients such as meat, and bone meal and fish meal are rich sources of Cr (Wyss & Kaddurah-Daouk 2000) but processing and heat treatment significantly diminishes their Cr contents (Tossenberger et al. 2016) resulting in a big variation in Cr contents between batches. Vegetable protein sources and grains, on the other hand, have negligible concentrations of Cr (Khajali et al. 2020). Thus, dietary Cr or its precursors are naturally low in low crude protein (CP) broiler diets as low CP diets use less protein meals and more crystalline amino acids This puts an additional burden on *de novo* Cr synthesis, which is not adequate to meet the bird's requirement for Cr, and thus the growth performance of broilers is compromised.

Creatine is synthesised endogenously from the amino acids arginine (Arg) and glycine (Gly). When Gly receives a guanidine group from Arg in a reaction catalysed by the enzyme L-Arg:Gly amidinotransferase (AGAT) mainly in the kidney, guanidinoacetic acid (GAA) is produced. GAA is then transported to the liver where it is methylated by S-adenosyl methionine (SAM) in a reaction catalysed by the enzyme guanidinoacetate N-methyltransferase to form Cr. This Cr is transported from the liver to the cells with high energetic requirements such as skeletal muscles, heart and brain, and gets phosphorylated to form phosphocreatine. Phosphocreatine converts adenosine diphosphate (ADP) into adenosine triphosphate (ATP) during its dephosphorylation (Khajali et al. 2020; Portocarero & Braun 2021). Thus, cellular Cr serves as an energy storage molecule and can produce ATP on demand for energy supply (Wyss & Kaddurah-Daouk 2000). This is particularly important during periods of high energy demand, such as rapid muscle growth in broilers, and prevents the formation of reactive oxygen substances, which negatively affect performance. As described above, two amino acids Arg and Gly are the precursors for endogenous Cr synthesis both of which are limiting in low CP diets. The formation of GAA from Arg and Gly is regulated by a negative feedback mechanism that involves circulating Cr and ornithine concentrations on AGAT activity (Wyss & Kaddurah-Daouk 2000). Thus, Cr synthesis and its concentration in muscle tissues may not be increased beyond such regulatory levels by simply supplying Arg or Gly in low CP diets. Dietary GAA supplementation would bypass this rate regulating mechanism to increase Cr synthesis in the body (Khajali et al. 2020).

Creatine is not an effective feed additive for poultry because it is unstable and costly (Baker 2009). GAA is a direct precursor of Cr and is stable in aqueous solution and pellet feed processing (Vranes et al. 2017; Van der Poel et al. 2018). As a feed additive, GAA has shown benefits on growth performance and the breast meat yield of broilers when it was added either on top of a complete feed or as a replacement to Arg in feed (Tossenberger et al. 2016; DeGroot et al. 2018; DeGroot et al. 2019). Theoretically, GAA has an Arg sparing potential of up to 149% in broilers (Khajali et al. 2020), but a more conservative approach of using a 77% replacement rate without exceeding 0.12% inclusion rates is common in the poultry industry. While dietary supplementation of L-Arg has less effect on energy metabolism and muscle Cr concentrations, dietary supplementation of GAA has been shown to improve muscle Cr level, phosphocreatine to ATP ratio in muscles, and an increased serum Arg level (DeGroot et al. 2018; DeGroot et al. 2019). Dietary supplementation of GAA has also been shown to increase breast meat yield (Michiels et al. 2012) and reduce wooden breast severity in broilers (Córdova-Noboa et al. 2018). The Arg sparing potential of GAA would be of considerable interest in low CP diets as they have lower inclusions of protein meals, lower concentrations of Cr and a significant amount of supplemental L-Arg, which may be cleared quickly in the blood and not an efficient way of maximising muscle Cr formation and growth promotion.

A recent study (Dao et al. 2021) showed that the response of broilers to supplemental GAA in an Arg deficient low CP diet was minimal. This is possibly due to excessive dietary CP reduction, a high level of GAA supplementation, and insufficient methyl donors to convert GAA to Cr. The methyl group donated by SAM to GAA originates from methionine. The hepatic synthesis of Cr from GAA uses a significant proportion of the methyl group and this can limit the methionine available for protein synthesis. Adding in more methionine as a methyl donor will increase the potentially toxic homocysteine. Therefore betaine (trimethylglycine) may be a better supplement, as betaine converts homocysteine back to methionine, and once it has donated its methyl group, it gets converted to Gly. Betaine may thereby restore balance in metabolism, stopping the build-up of potentially toxic homocysteine, and sparing methionine and Gly (Ostojic et al. 2013; Maidin et al. 2021). Thus, betaine in combination with GAA may be useful in low CP diets for broilers.

Objectives

This study aimed to determine the extent to which GAA may spare Arg in broilers offered low CP diets based on wheat and sorghum, and if supplemental betaine provides additional benefits on growth performance, carcass yield and meat quality.

This study hypothesised that a low CP Arg deficient diet will have a higher requirement for not only Arg, but also Gly and methyl donors (e.g. SAM and betaine). The methyl group donated by SAM to GAA originates from methionine. The hepatic synthesis of Cr from GAA uses a significant proportion of the methyl group and this can limit methionine available for protein synthesis. Adding in more methionine as a methyl donor will increase the potentially toxic homocysteine, therefore betaine (trimethylglycine) may be a better supplement, as betaine converts homocysteine back to methionine, and once it has donated its methyl group, it gets converted to Gly.

Methodology

Animal ethics

This experiment was approved by the animal ethics committee of the University of New England, Australia (Authority No. AEC21-005). All broiler management procedures including health care, husbandry and use of laboratory animals fulfilled the requirements of the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC 2013).

Experimental design and bird management

A total of 720 d-old Ross 308 off-sex male broiler chicks were sourced from Baiada hatchery in Goulburn, New South Wales. On d 0, chicks were randomly assigned to 72 floor pens of equal size in an environmentally controlled poultry research facility. Each pen was equipped with a feeder and two nipple drinkers. The birds had *ad libitum* access to feed and water throughout the study. The pens were spread with hard wood shavings up to a depth of approximately 7 cm. The lighting and temperature followed the Ross 308 breed guidelines (Aviagen 2018). On d 10, all birds were weighed and reassigned to pens of approximately equal weight within 3% of the experiment mean for body weight, and checked for no significant differences in weight between treatments. The birds were assigned into nine treatments with eight replicates of 10 birds each. The treatments were as follows: a normal CP diet, a low CP diet (CP reduced by 15 g/kg) deficient in Arg (low CP - Arg), a low CP diet sufficient in Arg (low CP + Arg) and low CP diets with GAA (Creamino®, AlzChem, Germany) where 0.1% added L-Arg was spared by GAA at 50% (GAA inclusion rate - 0.2%), 100% (GAA inclusion rate - 0.1%) and 150% (GAA inclusion rate - 0.067%) with and without 0.1% betaine.

Diet

The diets were thoroughly mixed and pelleted at a temperature of 65°C at the University of New England, Australia. The basal diet contained wheat, sorghum and soybean meal as major ingredients. The ingredients were analysed for CP, metabolisable energy, total and digestible amino acids before diet formulation by using near-infrared spectroscopy (Foss NIR 6500, Denmark), standardised with Adisseo PNE advanced calibration.

The ingredient and calculated nutrient composition of the diets are presented in Tables 1 and 2, respectively. A standard starter crumble (2975 kcal/kg AMEn, 23.3% CP) was offered for the first 10 days post-hatch. The treatment grower and finisher diets were offered as 3 mm pellets during d 10 to 24 and d 24 to 42, respectively. The diets were formulated to meet the Ross 308 nutrient specifications (Aviagen 2019) and the ratio of d Arg to d Lysine (Lys) was set at 105% as per commercial practice. The CP contributions from supplemental AA were included in diet formulations. The normal CP diet was balanced for digestible AA by adding supplemental L-lysine, D,L-methionine, and L-threonine. The low CP diet contained 15 g/kg lower CP than the normal CP diet. The digestible AA in a low CP diet was balanced by adding L-valine, L-Arg, L-isoleucine and L-Gly in addition to L-Lys, D,L-methionine and L-threonine. Diets were supplemented with xylanase (Axtra XB 201 TPT, Dupont Animal Nutrition, UK) and phytase (Quantam Blue 5G, AB Vista Feed Ingredient, UK). Vitamin and mineral premixes were added to meet requirements following manufacturer recommendations (UNE VM, Rabar Pty Ltd., Australia; UNE TM, Rabar Pty Ltd., Australia).

The dry matter contents of finished feeds were determined by subjecting samples to a forced air oven at 105°C until constant weight. The nitrogen contents of feeds were determined on a 0.25 g sample with a combustion analyser (Leco model FP-2000 N analyser, Leco Corp., St. Joseph, MI) using EDTA as a calibration standard, with CP being calculated by multiplying percentage Nitrogen by a correction factor (6.25). The amino acid profiles of feeds were determined at the Australian Proteome Analysis Facility, Macquarie University, Sydney. The gross energy contents of feeds were determined on a 0.5 g sample using an adiabatic bomb calorimeter (IKA Werke, C7000, GMBH and Co., Staufen, Germany) with benzoic acid as standard.

Table 1 Ingredient composition of the experimental diets

Ingredients %	**Starter diet**	**Grower diet**			**Finisher diet**		
		Normal CP[1]	**Low CP - Arg**[2]	**Low CP + Arg**[3]	**Normal CP**	**Low CP - Arg**	**Low CP + Arg**
Wheat (11.4% CP)	32.18	36.65	43.88	43.88	41.13	48.26	48.26
Sorghum (10.5% CP)	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soybean meal (46.5% CP)	35.14	30.46	23.15	23.15	25.50	18.25	18.25
Canola oil	3.27	4.35	3.28	3.28	5.23	4.19	4.19
Limestone	1.064	0.831	0.805	0.805	0.674	0.648	0.648
Dicalcium phosphate	1.776	1.248	1.391	1.391	1.055	1.197	1.197
Sodium chloride	0.120	0.124	0.047	0.047	0.125	0	0
Sodium bicarbonate	0.356	0.352	0.464	0.464	0.351	0.534	0.534
Potassium carbonate	0	0	0	0	0	0.016	0.016
Choline chloride, 70%	0.032	0.054	0.089	0.089	0.079	0.113	0.113
L-lysine HCl	0.302	0.275	0.490	0.490	0.257	0.471	0.471
D,L-methionine	0.352	0.307	0.362	0.362	0.275	0.329	0.329
L-threonine	0.153	0.124	0.220	0.220	0.099	0.195	0.195
L-valine	0.023	0.005	0.120	0.120	0	0.108	0.108
L-arginine	0.009	0	0	0.200	0	0	0.198
Guanidinoacetic acid[4]	0	0	0	0	0	0	0
L-isoleucine	0	0	0.098	0.098	0	0.096	0.096
L-glycine	0	0	0.179	0.179	0	0.178	0.178
Xylanase[5]	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Phytase[6]	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Vitamin premix[7]	0.085	0.080	0.080	0.080	0.080	0.080	0.080
Mineral premix[8]	0.110	0.100	0.100	0.100	0.100	0.100	0.100

[1] Diet 1 - Normal crude protein diet.

[2] Diet 2 - Low crude protein diet deficient in Arginine.

[3] Diet 3 - Low crude protein diet sufficient in Arginine.

[4] Diets 4 to 9 - Low CP diets with GAA where 0.1% added L-Arg was spared by GAA at 50% (inclusion rate - 0.2%), 100% (inclusion rate - 0.1%) and 150% (inclusion rate - 0.067%) with and without 0.1% betaine.

[5] Axtra XB 201 TPT, Dupont Animal Nutrition, UK.

[6] Quantam Blue 5G, AB Vista Feed Ingredient, UK.

[7] Vitamin premix per kg diet (UNE VM, Rabar Pty Ltd): vitamin A, 12 MIU; vitamin D, 5 MIU; vitamin E, 75 mg; vitamin K, 3 mg; nicotinic acid, 55 mg; pantothenic acid, 13 mg; folic acid, 2 mg; riboflavin, 8 mg; cyanocobalamin, 0.016 mg; biotin, 0.25 mg; pyridoxine, 5 mg; thiamine, 3 mg; antioxidant, 50 mg.

[8] Mineral premix per kg diet (UNE TM, Rabar Pty Ltd): Cu, 16 mg as copper sulfate; Mn, 60 mg as manganese sulfate; Mn, 60 mg as manganous oxide; I, 0.125 mg as potassium iodide; Se, 0.3 mg; Fe, 40 mg, as iron sulfate; Zn, 50 mg as zinc oxide; Zn, 50 mg as zinc sulfate.

Table 2 Calculated nutrient composition of the experimental diets (as-fed basis)

Nutrient composition %	Starter diet	Grower diet			Finisher diet		
		Normal CP[1]	Low CP - Arg[2]	Low CP + Arg[3]	Normal CP	Low CP - Arg	Low CP + Arg
AMEn, kcal/kg	2975	3100	3100	3100	3200	3200	3200
CP	23.3	21.5	20.0	20.0	19.7	18.2	18.2
Crude fat	5.44	6.51	5.48	5.48	7.39	6.38	6.38
Crude fibre	2.41	2.36	2.29	2.29	2.30	2.24	2.24
Guanidinoacetic acid[4]	0	0	0	0	0	0	0
Dig Arg: Dig Lys	1.050	1.050	0.877	1.050	1.050	0.856	1.050
Dig Lys[5]	1.280	1.150	1.150	1.150	1.020	1.020	1.020
Dig Met	0.641	0.577	0.600	0.600	0.524	0.547	0.547
Dig M+C	0.950	0.870	0.870	0.870	0.800	0.800	0.800
Dig Thr	0.860	0.770	0.770	0.770	0.680	0.680	0.680
Dig Val	0.960	0.870	0.870	0.870	0.786	0.780	0.780
Dig Arg	1.344	1.208	1.008	1.208	1.071	0.873	1.071
Dig Ile	0.869	0.796	0.780	0.780	0.718	0.700	0.700
Dig Trp	0.264	0.243	0.211	0.211	0.221	0.188	0.188
Dig Gly_{eq}[6]	1.459	1.345	1.345	1.345	1.222	1.222	1.222
Calcium	1.09	0.87	0.87	0.87	0.75	0.75	0.75
Available P	0.52	0.44	0.44	0.44	0.39	0.39	0.39
Sodium	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Chloride	0.22	0.22	0.22	0.22	0.22	0.19	0.19

[1] Diet 1- Normal crude protein diet.

[2] Diet 2- Low crude protein diet deficient in Arginine.

[3] Diet 3- Low crude protein diet sufficient in Arginine.

[4] Diets 4 to 9 - Low CP diets with GAA where 0.1% added L-Arg was spared by GAA at 50% (GAA inclusion rate - 0.2%), 100% (GAA inclusion rate - 0.1%) and 150% (GAA inclusion rate - 0.067%) with and without 0.1% betaine.

[5] Digestible coefficients of AA for raw materials were determined using AMINODat 5.0 (Evonik Animal Nutrition).

[6] Digestible Gly_{eq} was calculated as follows: Dig Gly_{eq} = Dig Gly + (Dig Ser × 0.7143).

Growth performance

Birds and feed were weighed on arrival and d 10, 24, and 42, and mortality was recorded daily. Feed intake, weight gain, and mortality adjusted feed conversion ratio (FCR) were determined during the experimental periods.

Carcass cuts and internal organs

On d 42, four birds were sampled per pen, weighed and euthanised by an electrical stunner (MEFE CAT 44N, Mitchell Engineering Food Equipment, Clontarf, QLD, Australia) followed by cervical dislocation. Breast meat, leg piece (thigh and drumstick), liver and abdominal fat pad were collected. The relative weights of carcass cuts and internal organs were calculated as mass per unit of live body weight (g/kg of live body weight). The left toe was collected to measure toe ash percentage.

Creatine and GAA concentrations in tissues and finished feeds

Around 400 g of feed was collected from each treatment and shipped to the AlzChem Trostberg GmbH lab in Germany to measure GAA concentrations. On d 42, four birds were sampled per pen (32 birds/treatment) to measure Cr concentration in breast meat. For Cr measurement, around 80 g of breast meat was collected from the cranial portion of the right breast muscle and packed individually in freezer bags. The samples were stored frozen at -20°C and shipped on dry ice to the AlzChem Trostberg GmbH lab in Germany.

Meat quality

Meat quality parameters were measured on breast meat samples collected from four birds per pen (32 birds per treatment) by the following procedures.

White striping and wooden breast

The right breast meat was visually assessed for the presence or absence of woody breast according to the method described by Kuttappan et al. (2016). The data were recorded as the percentage of birds with wooden breasts. The white stripping severity was recorded on a four-point scale following the procedures of Bailey et al. (2015). In short, score 0 represented no white striping, score 1 was mild noticeable striping covering part of the breast, score 2 was moderate noticeable striping covering the breast surface extensively, and score 3 was severe and very thick striping covering the breast surface extensively.

Drip loss

The left breast meat was excised with bone, weighed and placed into individually labelled Ziploc bags. Then the samples were immediately stored at 4°C in a fridge hanging in the Ziploc bags. At 24 h post-mortem, the breast meat samples were reweighed to calculate drip loss by using the following equation.

Drip loss = {day 1 breast weight (g) - day 2 breast weight (g)}/day 1 breast weight (g) *100

After drip loss measurement, the breast meat was removed from the bone and cut into two portions. The cranial half portion was used for measuring colour and pH. The caudal half portion was used for measuring moisture, cooking loss and shear force.

Moisture

Around 60 g of breast meat samples were freeze-dried at -50°C until constant weight to calculate the moisture content of the sample by using the following equation.

Breast meat moisture, % = {moist breast weight (g) - dry breast weight (g)}/moist breast weight × 100

pH and colour

A hand-held pH meter (IJ44C probe, Ionode Pty Ltd., Tennyson, QLD, Australia) with an integrated temperature sensor (WP-80 Waterproof pH-mV-Temperature Meter, TPS, Brendale, NSW, Australia) was used to measure the pH of a cranial half portion of left breast meat at 24 h post-mortem. The pH meter was calibrated at room temperature using buffers at pH 4.0 and 6.9, and the measurements were made in duplicates by inserting the pH probe at approximately 2.5 cm from the top of the breast. After measuring pH, breast meat colour (lightness- L*, redness- a*, and yellowness- b*) was measured in triplicates using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Osaka, Japan). The instrument was calibrated using a white tile (Y = 93.3, x = 0.3135, y = 0.3198; Minolta Co., Ltd., Osaka, Japan) using illuminant D-65. Three readings were taken on each sample and averaged. Values of lightness (L*), redness (a*), and yellowness (b*) were recorded.

Cooking loss and shear force

Around 65 g of breast meat samples were individually vacuum packed in vacuum pouches and stored at -20°C until cooking loss and shear force were determined. The cooking loss and shear force measurements were performed based on the methods described by Hopkins et al. (2010). For cooking loss, the frozen samples were placed into a water bath (Model: BTC 9090, Thermoline, Sydney, NSW, Australia) set at 85°C for 25 min and then placed under cold running tap water for 30 min to stop the cooking process. Samples were then removed from the bag, wrapped in paper towels to remove the excess water, and weighed (cooked sample weight) for cooking loss determination. After cooking loss measurements, the samples were stored in individual Ziplocbags at 4°C overnight for subsequent shear force analysis. Shear force analysis was performed using a Lloyd Instruments LRX Materials Testing Machine fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire, UK). Briefly, five subsamples with a rectangular cross-section of 15 mm width and 6.66 mm depth (1 cm^2) were cut from each block, with fibre orientation parallel to the long axis, at right angles to the shearing surface. The force required to shear through the clamped subsample with a triangulated 0.64 mm thick blade pulled upward at a speed of 100 mm/min at a 90° angle to the fibre direction and was expressed as kg peak force. Values of the kg peak force were recorded, and the mean value obtained from the subsamples was converted to Newton values (N) (1 kg = 9.81 N) for statistical analysis.

Statistical analysis

The data were analysed by one-way ANOVA using JMP statistical software version 14 (SAS Institute Inc, Cary, NC). When one-way ANOVA showed significant ($P < 0.05$) differences among treatments, treatment means were compared using the post hoc Tukey test. The wooden breast severity data were not normally distributed and the non-parametric Kruskal-Wallis test was used to detect the significance. Pearson correlation coefficients and associated significance were generated using JMP software to determine the relationship between breast meat Cr concentration and breast meat moisture, pH, abdominal fat and FCR. The relationship between breast meat Cr concentration and FCR was investigated by linear regression.

Dr Peter Chrystal of the University of Sydney assisted in formulating diets. Dr David Cadogan and Dr Stuart Wilkinson of Feedworks Pty Ltd helped in the experimental design and interpretation of results.

Discussion of results

The analysed nutrient composition of starter, grower and finisher diets are presented in Tables 3 and 4. The analysed GAA contents in the diets were close to the calculated inclusion rates. On average, the analysed GAA concentrations in grower and finisher diets were lower than the calculated values by 6.4% and 3.9%, respectively, which suggests that Creamino® provided the desired levels of GAA in the diets considering a minimum of 96% GAA concentration in Creamino®. The analysed Cr concentrations in the diets were below the minimum reporting limit set in the assay (i.e. 20 mg/kg) and thus not reported.

Growth performance

The overall mortality in this experiment during the experimental period of d 10 to 42 was 2.85% and was not related to dietary treatment ($P > 0.05$). The performance of birds exceeded the Ross 308 performance standards (Aviagen 2019) for feed intake, weight gain and FCR (Table 5). During d 10 to 42, the average feed intake, weight gain and FCR of birds offered a normal CP diet were 4843 g, 3194 g and 1.517, respectively. Compared to the Aviagen performance standard for Ross 308, feed intake was higher by 2.98% (4843 g versus 4703 g), weight gain was higher by 13.5% (3194 g versus 2813 g), and FCR was lower by 15 points (1.517 versus 1.672) during d 10 to 42.

The effect of low CP diets with GAA and betaine on the growth performance of broilers is presented in Table 5. Dietary treatments had a significant effect on feed intake ($P < 0.01$) during d 10 to 24 and d 10 to 42. During d 10 to 24, the birds offered a low CP diet deficient in Arg had 2.60% lower feed intake as compared to those offered a normal CP diet. When Arg was added back, feed intake improved and became statistically similar to the normal CP treatment. When GAA spared Arg at 50%, 100%, and 150% without or with betaine, there was no effect on feed intake except that the birds offered a low CP diet with GAA 100 + betaine had lower feed intake compared to those offered a low CP diet with Arg or low CP diet with GAA 100. During d 10 to 42, the birds offered a low CP diet deficient in Arg or a low CP diet sufficient in Arg had a similar feed intake to those offered a normal CP diet. When GAA spared Arg at 50%, 100%, and 150% without or with betaine, there was no effect on feed intake compared to the low CP + Arg treatment. Betaine did not affect feed intake when it was added to each level of GAA. When GAA spared Arg at 50%, feed intake was lower compared to when GAA spared Arg at 150% and normal CP treatment. Dietary treatments tended ($P = 0.076$) to affect feed intake during d 24 to 42 and the highest feed intake during this period was observed in the birds offered a normal CP diet.

Dietary treatments had a significant effect on weight gain ($P < 0.001$) in all the phases. The birds offered a low CP diet deficient in Arg had around 7.8% lower weight gain as compared to those offered a normal CP diet in all the phases. When Arg was added back, weight gain increased in all the phases and became comparable to the normal CP treatment during d 24 to 42 and d 10 to 42. When GAA spared Arg at 50%, 100%, and 150% without or with betaine, weight gain was higher than the Arg deficient low CP treatment and comparable to the low CP + Arg treatment in all the phases. Betaine did not affect weight gain when it was added to each level of GAA.

Table 3 Analysed nutrient composition of starter and grower diets (as-fed basis)

Nutrient Composition %	Starter diet	Grower diets								
		Normal CP[1]	Low CP - Arg[2]	Low CP + Arg[3]	Low CP + GAA 50[4]	Low CP + GAA 100[5]	Low CP + GAA 150[6]	Low CP + GAA 50 + Betaine[7]	Low CP + GAA 100 + Betaine[8]	Low CP + GAA 150 + Betaine[9]
DM[10]	88.2	87.5	87.2	87.0	87.1	87.0	87.0	87.1	87.3	87.5
GE[11], kcal/kg	4063	4111	4049	4025	4028	4015	4018	4030	4032	4044
CP[12]	24.5	22.6	20.4	20.7	21.0	20.8	20.6	20.6	21.0	20.5
GAA[13]	< RL	< RL	< RL	< RL	0.165	0.101	0.072	0.179	0.104	0.064
Lys	1.42	1.28	1.28	1.23	1.23	1.24	1.25	1.25	1.27	1.24
Met	0.63	0.48	0.54	0.53	0.54	0.53	0.52	0.52	0.55	0.55
Thr	1.00	0.90	0.87	0.87	0.86	0.86	0.88	0.88	0.88	0.86
Val	1.12	1.04	1.01	1.00	1.00	0.98	1.02	1.01	1.02	0.99
Arg	1.46	1.32	1.09	1.27	1.19	1.16	1.21	1.22	1.17	1.17
Ile	1.02	0.96	0.91	0.90	0.89	0.88	0.91	0.91	0.89	0.88
Leu	1.88	1.79	1.56	1.55	1.54	1.50	1.57	1.57	1.52	1.54
Gly	0.96	0.89	0.95	0.94	0.93	0.92	0.95	0.95	0.97	0.94
Ser	1.15	1.07	0.92	0.92	0.92	0.89	0.93	0.93	0.90	0.91
Phe	1.17	1.10	0.95	0.95	0.94	0.92	0.96	0.96	0.93	0.92
His	0.59	0.55	0.47	0.47	0.46	0.46	0.48	0.47	0.46	0.47
Tyr	0.63	0.60	0.48	0.50	0.50	0.48	0.51	0.53	0.47	0.49
Pro	1.40	1.36	1.25	1.26	1.25	1.23	1.27	1.27	1.24	1.28
Ala	1.06	1.01	0.90	0.89	0.89	0.86	0.90	0.90	0.87	0.89
Asp	2.29	2.09	1.75	1.74	1.73	1.68	1.77	1.76	1.71	1.69
Glu	4.77	4.57	4.17	4.19	4.15	4.08	4.24	4.23	4.13	4.07

[1] Diet 1 - Normal crude protein diet. [2] Diet 2 - Low crude protein diet deficient in Arginine. [3] Diet 3 - Low crude protein diet sufficient in Arginine.

[4-9] Diets 4 to 9 - Low CP diets with GAA where 0.1% added L-Arg was spared by GAA at 50% (GAA inclusion rate - 0.2%), 100% (GAA inclusion rate - 0.1%) and 150% (GAA inclusion rate - 0.067%) with and without 0.1% betaine.

[10] Dry matter. [11] Gross energy. [12] Crude protein. [13] Guanidinoacetic acid levels in the diets.

RL denotes the minimum reporting limit which was set at 20 mg/kg.

Table 4 Analysed nutrient composition of finisher diets (as-fed basis)

Nutrient Composition %	Finisher diet								
	Normal CP[1]	Low CP - Arg[2]	Low CP + Arg[3]	Low CP + GAA 50[4]	Low CP + GAA 100[5]	Low CP + GAA 150[6]	Low CP + GAA 50 + Betaine[7]	Low CP + GAA 100 + Betaine[8]	Low CP + GAA 150 + Betaine[9]
DM[10]	87.0	87.0	87.0	87.0	87.5	87.0	87.1	87.2	87.4
GE[11], kcal/kg	4125	4075	4089	4071	4077	4063	4065	4084	4085
CP[12]	20.2	18.7	19.1	19.3	18.9	18.6	18.4	19.0	18.9
GAA[13]	< RL	< RL	< RL	0.183	0.098	0.067	0.192	0.099	0.066
Lys	1.10	1.11	1.10	1.10	1.10	1.07	1.09	1.09	1.06
Met	0.42	0.49	0.49	0.46	0.49	0.48	0.49	0.52	0.45
Thr	0.78	0.79	0.79	0.79	0.78	0.74	0.75	0.76	0.76
Val	0.91	0.96	0.93	0.94	0.95	0.88	0.88	0.89	0.88
Arg	1.11	0.99	1.16	1.06	1.06	1.00	0.99	1.04	1.02
Ile	0.83	0.85	0.83	0.85	0.83	0.78	0.78	0.79	0.78
Leu	1.56	1.48	1.46	1.49	1.48	1.39	1.34	1.38	1.34
Gly	0.79	0.91	0.89	0.89	0.88	0.84	0.85	0.87	0.83
Ser	0.93	0.88	0.87	0.87	0.87	0.80	0.78	0.81	0.80
Phe	0.95	0.90	0.90	0.91	0.90	0.82	0.81	0.83	0.82
His	0.48	0.45	0.45	0.45	0.45	0.42	0.41	0.42	0.42
Tyr	0.46	0.44	0.44	0.42	0.44	0.42	0.40	0.44	0.41
Pro	1.28	1.36	1.36	1.36	1.36	1.18	1.16	1.19	1.19
Ala	0.89	0.83	0.82	0.84	0.84	0.80	0.77	0.80	0.77
Asp	1.74	1.49	1.47	1.49	1.48	1.42	1.38	1.42	1.40
Glu	4.11	4.24	4.24	4.23	4.23	3.69	3.66	3.71	3.75

[1] Diet 1- Normal crude protein diet. [2] Diet 2 - Low crude protein diet deficient in Arginine. [3] Diet 3 - Low crude protein diet sufficient in Arginine.

[4-9] Diets 4 to 9 - Low CP diets with GAA where 0.1% added L-Arg was spared by GAA at 50% (GAA inclusion rate - 0.2%), 100% (GAA inclusion rate - 0.1%) and 150% (GAA inclusion rate - 0.067%) with and without 0.1% betaine.

[10] Dry matter. [11] Gross energy. [12] Crude protein. [13] Guanidinoacetic acid levels in the diets.

RL denotes the minimum reporting limit which was set at 20 mg/kg .

Table 5 Growth performance of broilers offered low crude protein diets with guanidinoacetic acid and betaine

Treatment	Feed intake (g/bird)			Weight gain (g/bird)			FCR (g/g)		
	d 10-24	d 24-42	d 10-42	d 10-24	d 24-42	d 10-42	d 10-24	d 24-42	d 10-42
Normal CP[1]	1499^{a}	3344	4843^{a}	1140^{a}	2053^{a}	3194^{a}	1.315^{bc}	1.629^{b}	1.517^{bc}
Low CP - Arg[2]	1460^{b}	3290	4749^{abc}	1052^{c}	1894^{b}	2946^{c}	1.388^{a}	1.737^{a}	1.612^{a}
Low CP + Arg[3]	1465^{ab}	3279	4744^{abc}	1095^{b}	2047^{a}	3142^{ab}	1.337^{b}	1.602^{bc}	1.510^{bcd}
Low CP + GAA 50[4]	1434^{bc}	3216	4650^{c}	1103^{b}	2076^{a}	3179^{ab}	1.301^{c}	1.549^{d}	1.463^{e}
Low CP + GAA 100[5]	1470^{ab}	3265	4735^{abc}	1117^{ab}	2067^{a}	3184^{ab}	1.316^{bc}	1.581^{cd}	1.488^{d}
Low CP + GAA 150[6]	1469^{ab}	3311	4781^{ab}	1100^{b}	2032^{a}	3132^{ab}	1.337^{b}	1.631^{b}	1.527^{b}
Low CP + GAA 50 + betaine[7]	1450^{bc}	3213	4663^{bc}	1107^{b}	2021^{a}	3129^{ab}	1.310^{bc}	1.590^{c}	1.491^{d}
Low CP + GAA 100 + betaine[8]	1421^{c}	3235	4645^{c}	1095^{b}	2012^{a}	3099^{b}	1.297^{c}	1.608^{bc}	1.499^{cd}
Low CP + GAA 150 + betaine[9]	1447^{bc}	3241	4689^{bc}	1090^{b}	2019^{a}	3109^{ab}	1.328^{b}	1.605^{bc}	1.508^{bcd}
SEM	4.47	11.29	14.14	4.04	9.51	12.07	0.004	0.007	0.005
P value	< 0.01	0.076	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[a-d] Within each treatment factor, means in the same column with a different superscript differ significantly ($P<0.05$).

[1] Diet 1 - Normal crude protein diet.

[2] Diet 2 - Low crude protein diet deficient in Arginine.

[3] Diet 3 - Low crude protein diet sufficient in Arginine.

[4-9] Diets 4 to 9 - Low CP diets with GAA where 0.1% added L-Arg was spared by GAA at 50% (GAA inclusion rate - 0.2%), 100% (GAA inclusion rate - 0.1%) and 150% (GAA inclusion rate - 0.067%) with and without 0.1% betaine.

Dietary treatments led to a significant difference in FCR (*P* < 0.001) in all the phases. The birds offered a low CP diet deficient in Arg had 7 points higher FCR in the grower phase and 10 points higher FCR in the finisher and overall phases as compared to those offered a normal CP diet. When Arg was added back, FCR decreased and became comparable to the normal CP treatment in all the phases. When GAA spared Arg at 100% and 150%, FCR was lower than the low CP - Arg treatment but comparable to the low CP + Arg treatment in all the phases. When GAA spared Arg at 50%, FCR was lower than the normal CP treatment by 8 points during d 24 to 42 and by 5 points during d 10 to 42. When GAA spared Arg at 100%, FCR was lower than the normal CP treatment by 5 points during d 24 to 42 and by 3 points during d 10 to 42. FCR increased when betaine was added to low CP + GAA 50 during d 24 to 42 and d 10 to 42 but did not change when it was added to low CP + GAA 100 and low CP + GAA 150 in any phases

Carcass cuts and internal organs

The effect of low CP diets with GAA and betaine on absolute and relative (g/kg live weight) organ weights and toe ash of broilers is presented in Table 6. Dietary treatments had significant effects (*P* < 0.001) on absolute and relative breast weights. The birds offered a low CP diet deficient in Arg had 13.1% lower absolute breast weight and 8.5% lower relative breast weight compared to those offered a normal CP diet. When Arg was added back, absolute and relative breast weights increased and relative breast weight became comparable to the normal CP treatment. When GAA spared Arg at 50%, 100%, and 150% without or with betaine, absolute and relative breast weights were not affected compared to the low CP + Arg treatment but were higher than the low CP - Arg treatment. Betaine did not affect absolute and relative breast weights when it was added to each level of GAA.

Dietary treatments had significant effects (*P* < 0.05) on the absolute and relative weights of the leg piece (thigh plus drumstick). The birds offered a low CP diet deficient in Arg had 5.6% lower absolute leg piece weight compared to those offered a normal CP diet. When Arg was added back, absolute leg piece weight increased and became comparable to the normal CP treatment. The deficiency of Arg did not affect the relative weight of the leg piece. When GAA spared Arg at 50%, 100%, and 150% without betaine, absolute and relative leg piece weights were not affected compared to the low CP + Arg treatment. Betaine did not affect absolute and relative leg piece weights when it was added to each level of GAA.

Dietary treatments had a significant effect (*P* < 0.001) on relative abdominal fat pad weight. The birds offered a low CP diet deficient in Arg had 30.4% higher relative abdominal fat pad weight compared to those offered a normal CP diet. When Arg was added back, relative abdominal fat pad weight decreased and became comparable to the normal CP treatment. When GAA spared Arg at 50%, 100%, and 150% without or with betaine, relative abdominal fat pad weight was lower than the low CP - Arg treatment and comparable to the low CP + Arg treatment. Betaine did not affect relative abdominal fat pad weight when it was added to each level of GAA. Dietary treatments had a significant effect (*P* < 0.01) on relative liver weight. The birds offered a low CP diet deficient in Arg or a low CP diet sufficient in Arg had higher relative liver weight compared to those offered a normal CP diet. When GAA spared Arg at 50%, 100%, and 150% without or with betaine, relative liver weight was not affected compared to the low CP + Arg treatment but was lower than the normal CP treatment. Betaine did not affect relative liver weight when it was added to each level of GAA. Dietary treatments had no effect (*P* > 0.05) on toe ash content.

Table 6 Absolute (g) and relative (g/kg live weight) organ weights and toe ash of broilers offered low crude protein diets with guanidinoacetic acid and betaine on day 42

Treatment	Breast meat (g)	Breast meat (g/kg)	Leg piece[10] (g)	Leg piece (g/kg)	Abdominal fat (g/kg)	Liver (g/kg)	Toe ash[11] (%)
Normal CP[1]	685.3^{a}	189.6^{a}	747.7^{a}	204.2^{abc}	9.2^{c}	15.2^{c}	11.2
Low CP - Arg[2]	595.6^{c}	173.5^{d}	706.0^{b}	205.8^{ab}	12.0^{a}	17.5^{ab}	11.1
Low CP + Arg[3]	647.7^{b}	183.8^{abc}	727.9^{ab}	206.5^{a}	9.9^{bc}	16.6^{ab}	11.4
Low CP + GAA 50[4]	649.0^{b}	179.1^{cd}	739.7^{a}	204.1^{abc}	10.2^{bc}	16.4^{b}	11.4
Low CP + GAA 100[5]	673.5^{ab}	187.1^{ab}	729.4^{ab}	200.6^{abc}	9.8^{bc}	16.6^{ab}	11.2
Low CP + GAA 150[6]	675.1^{ab}	187.0^{ab}	722.7^{ab}	200.5^{abc}	10.4^{bc}	17.7^{a}	11.6
Low CP + GAA 50 + betaine[7]	651.1^{b}	184.1^{abc}	722.4^{ab}	204.2^{abc}	9.7^{bc}	16.9^{ab}	10.8
Low CP + GAA 100 + betaine[8]	644.9^{b}	181.4^{bc}	705.4^{b}	198.5^{c}	10.1^{bc}	16.3^{b}	11.4
Low CP + GAA 150 + betaine[9]	653.0^{b}	183.6^{abc}	709.5^{b}	199.5^{c}	10.9^{abc}	17.3^{ab}	11.2
SEM	4.202	0.087	3.412	0.685	0.164	0.015	0.264
P value	< 0.001	< 0.001	< 0.05	< 0.05	< 0.001	< 0.01	0.206

[a-d] Within each treatment factor, means in the same column with a different superscript differ significantly ($P<0.05$).

[1] Diet 1 - Normal crude protein diet.

[2] Diet 2 - Low crude protein diet deficient in Arginine.

[3] Diet 3 - Low crude protein diet sufficient in Arginine.

[4-9] Diets 4 to 9 - Low CP diets with GAA where 0.1% added L-Arg was spared by GAA at 50% (GAA inclusion rate - 0.2%), 100% (GAA inclusion rate - 0.1%) and 150% (GAA inclusion rate - 0.067%) with and without 0.1% betaine.

[10] Leg piece includes combined weight of thigh and drumstick.

[11] Percentage ash of dried toe.

Breast meat creatine concentration

The effect of low CP diets with GAA and betaine on breast meat Cr concentration is presented in Figure 1. Dietary treatments had a significant effect ($P < 0.001$) on breast meat Cr concentration. The birds offered a low CP diet deficient in Arg had a 27.2% lower Cr concentration in breast meat compared to those offered a normal CP diet. When Arg was added back, breast meat Cr level increased by 30% and was comparable to the normal CP treatment. When GAA spared Arg at 150% without or with betaine, breast meat Cr level was higher than the low CP - Arg treatment and comparable to the low CP + Arg treatment. When GAA spared Arg at 100% without betaine, breast meat Cr level was higher than the low CP + Arg treatment by 27.5% and comparable to the normal CP treatment. When GAA spared Arg at 50% without betaine, breast meat Cr level was higher than the low CP + Arg treatment by 45.2% and the normal CP treatment by 37.3%. Betaine did not affect breast meat Cr concentration when it was added to each level of GAA.

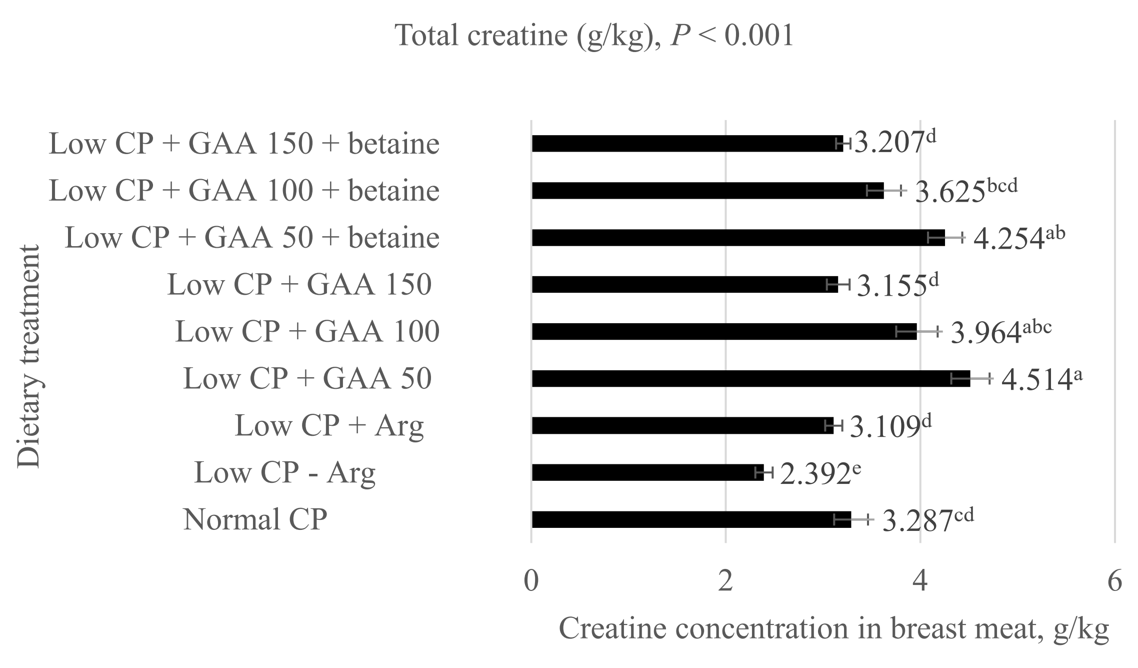

Figure 1 Effect of low crude protein diets with guanidinoacetic acid and betaine on creatine concentration in broiler breast meat at day 42

Meat quality

The effect of low CP diets with GAA and betaine on broiler breast meat drip loss, pH, moisture, cooking loss, shear force and colour (L*, a*, b*) values are presented in Table 7.

Drip loss

Dietary treatments led to a significant difference ($P < 0.05$) in breast meat drip loss. The birds offered a low CP diet sufficient in Arg had lower breast meat drip loss compared to those offered a low CP diet deficient in Arg. When GAA spared Arg at 50%, 100%, and 150% without or with betaine, drip loss was not affected compared to the low CP + Arg treatment. Betaine did not affect drip loss when it was added to each level of GAA.

Table 7 Meat quality of broilers offered low crude protein diets with guanidinoacetic acid and betaine on day 42

Treatment	Drip loss %	Cooking loss %	pH	Moisture %	Shear force N	Breast meat colour		
						L*	a*	b*
Normal CP[1]	1.45^{ab}	21.4^{cd}	5.96^{b}	74.8^{bcd}	19.55	53.4^{c}	4.8	1.9^{ab}
Low CP - Arg[2]	1.99^{a}	20.6^{d}	6.07^{a}	74.5^{d}	17.14	53.2^{c}	5.3	1.3^{b}
Low CP + Arg[3]	1.37^{b}	21.6^{bcd}	5.95^{b}	74.6^{cd}	18.19	55.8^{b}	4.9	2.2^{a}
Low CP + GAA 50[4]	1.41^{ab}	23.2^{a}	5.80^{de}	75.7^{a}	19.10	58.2^{a}	4.6	2.0^{ab}
Low CP + GAA 100[5]	1.36^{b}	21.5^{cd}	5.88^{bcd}	74.8^{bcd}	21.13	56.0^{b}	4.8	2.1^{a}
Low CP + GAA 150[6]	1.57^{ab}	22.8^{abc}	5.87^{bcd}	75.1^{abcd}	20.11	56.3^{ab}	4.9	2.7^{a}
Low CP + GAA 50 + betaine[7]	1.54^{ab}	23.1^{a}	5.79^{e}	75.4^{ab}	21.12	58.3^{a}	4.7	2.0^{ab}
Low CP + GAA 100 + betaine[8]	1.35^{b}	22.2^{abc}	5.86^{bcd}	75.0^{bcd}	20.99	56.8^{ab}	4.8	2.6^{a}
Low CP + GAA 150 + betaine[9]	1.61^{ab}	22.2^{abc}	5.90^{bc}	75.3^{abc}	19.26	56.0^{b}	5.0	2.5^{a}
SEM	0.127	0.485	0.029	0.209	1.528	0.688	0.232	0.251
P value	< 0.05	< 0.01	< 0.001	< 0.01	0.588	< 0.001	0.594	< 0.01

[a-e] Within each treatment factor, means in the same column with a different superscript differ significantly (*P*<0.05).

[1] Diet 1 - Normal crude protein diet.

[2] Diet 2 - Low crude protein diet deficient in Arginine.

[3] Diet 3 - Low crude protein diet sufficient in Arginine.

[4-9] Diets 4 to 9 - Low CP diets with GAA where 0.1% added L-Arg was spared by GAA at 50% (GAA inclusion rate - 0.2%), 100% (GAA inclusion rate - 0.1%) and 150% (GAA inclusion rate - 0.067%) with and without 0.1% betaine.

pH

Dietary treatments had a significant effect ($P < 0.001$) on breast meat pH. The birds offered a low CP diet deficient in Arg had higher breast meat pH compared to those offered a normal CP diet. When Arg was added back, pH decreased and became similar to the normal CP treatment. When GAA spared Arg at 50% without or with betaine, breast meat pH decreased compared to the Arg deficient and Arg sufficient low CP and normal CP treatments. When GAA spared Arg at 100% and 150% without or with betaine, breast meat pH was not affected compared to the low CP + Arg treatment. Betaine did not affect breast meat pH when it was added to each level of GAA. As shown in Table 8, the breast meat pH was negatively correlated with breast meat Cr concentration ($r = -0.49$, $P < 0.001$).

Moisture

Dietary treatments had a significant effect ($P < 0.01$) on the moisture content of breast meat. The birds offered a low CP diet deficient in Arg or a low CP diet sufficient in Arg **had no effect on** breast meat moisture compared to those offered a normal CP diet. When GAA spared Arg at 50% without or with betaine, breast meat moisture increased compared to the low CP + Arg and low CP - Arg treatments. When GAA spared Arg at 100% and 150% without or with betaine, breast meat moisture was not affected compared to the low CP + Arg treatment. Betaine did not affect breast meat moisture when it was added to each level of GAA. As shown in Table 8, the moisture content in breast meat was positively correlated with breast meat Cr concentration ($r = 0.33$, $P < 0.01$).

Shear force

Dietary treatments had no effect ($P > 0.05$) on the shear force of breast meat.

Cooking loss

Dietary treatments had a significant effect ($P < 0.01$) on cooking loss of breast meat. The birds offered a low CP diet deficient in Arg or a low CP diet sufficient in Arg did not affect cooking loss compared to those offered a normal CP diet. T When GAA spared Arg at 50% without or with betaine, cooking loss increased compared to the low CP + Arg, low CP - Arg and normal CP treatments. When GAA spared Arg at 100% and 150% without or with betaine, the cooking loss was not affected compared to the low CP + Arg treatment. Betaine did not affect cooking loss when it was added to each level of GAA.

Colour

Dietary treatments led to a significant difference in breast meat colour values for lightness (L^*, $P < 0.001$) and yellowness (b^*, $P < 0.01$) but not redness (a^*, $P > 0.05$). The birds offered a low CP diet deficient in Arg did not affect breast meat L*and b* values compared to those offered a normal CP diet. When Arg was added back, the L*value increased and became higher than the low CP - Arg and normal CP treatments, whereas the b*value increased and became similar to the normal CP treatment. When GAA spared Arg at 50% without or with betaine, breast meat L*values increased compared to the low CP - Arg, low CP + Arg and normal CP treatments. When GAA spared Arg at 100% and 150% without or with betaine, breast meat L*values were similar to the low CP + Arg treatment but higher than normal CP treatment. When GAA spared Arg at 50%, 100%, and 150% without or with betaine, the b*value was not affected compared to the low CP + Arg treatment. Betaine did not affect breast meat L*and b*values when it was added to each level of GAA.

Correlation between breast meat Cr concentration and performance parameters

The increase in breast meat Cr concentration improved FCR and the relationship was linear (Figure 2). The linear regression between FCR (d 10 to 42) and breast meat Cr concentration was FCR = 1.6612 - 0.0423 × Cr, g/kg ($r^2 = 0.51$, $P < 0.001$). The negative linear relationship between breast meat Cr concentration and relative weight of abdominal fat is shown in Figure 3. As shown in Table 8, breast meat Cr concentration was negatively correlated to FCR (r = -0.70, $P < 0.001$) and relative weight of abdominal fat (r = -0.37, $P < 0.01$).

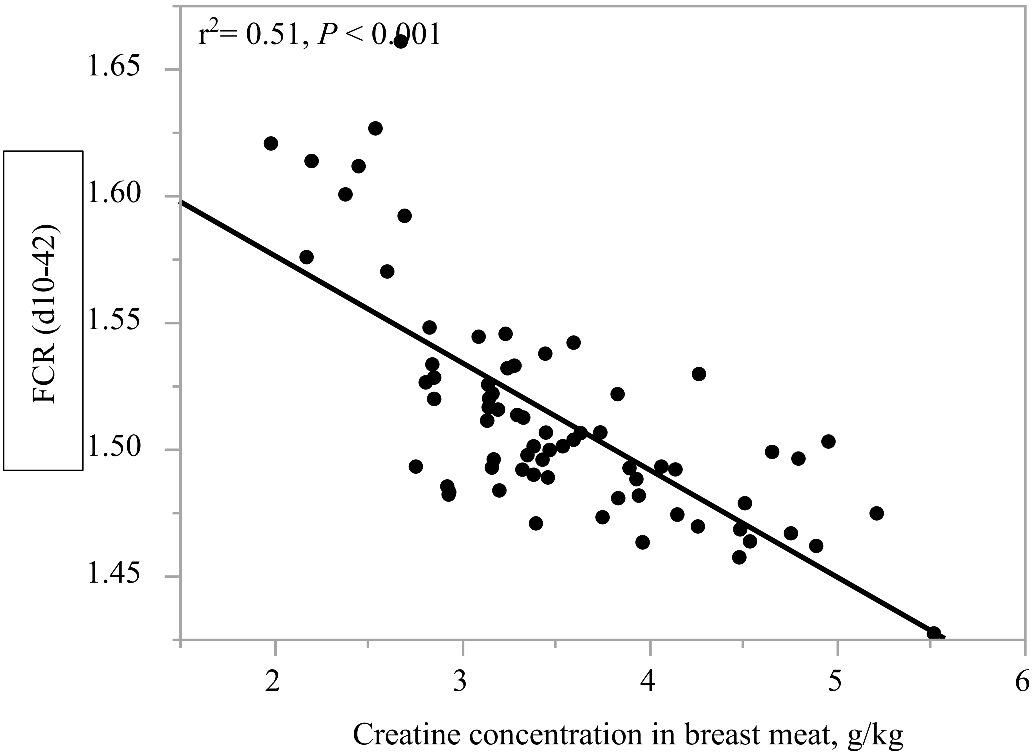

Figure 2 A linear relationship between breast meat creatine concentration and FCR (d10 to 42) of broilers offered low crude protein diets with guanidinoacetic acid and betaine

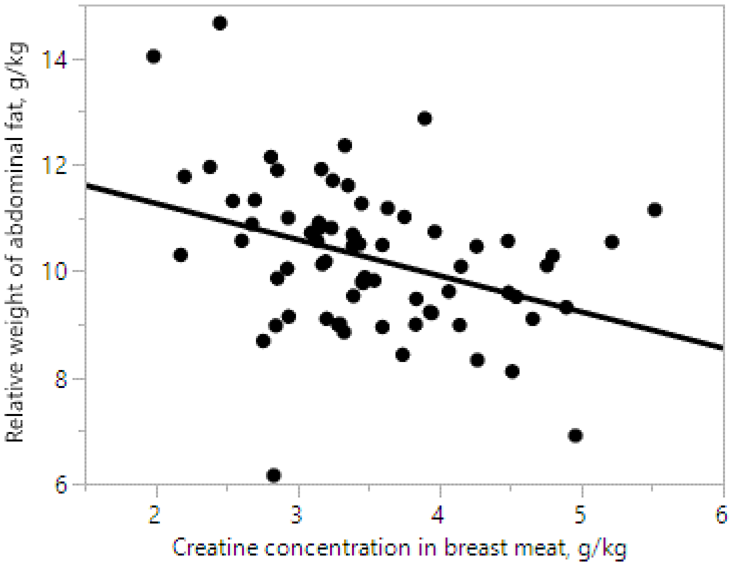

Figure 3 A linear relationship between breast meat creatine concentration and relative weight of abdominal fat at d 42 of broilers offered low crude protein diets with guanidinoacetic acid and betaine ($P < 0.01$)

Table 8 Pearson correlation coefficient (r) between breast meat creatine concentration and meat quality parameters

Parameter	**Breast meat moisture**	**Breast meat pH**	**Relative weight of abdominal fat**	**FCR (d10-42)**
Breast meat creatine concentration	0.33	-0.49	-0.37	-0.70
P value	< 0.01	< 0.001	< 0.01	< 0.001

White striping

The effect of low CP diets with GAA and betaine on white striping scores in the breast meat of broilers is presented in Figure 4. Dietary treatments had a significant effect ($P < 0.05$) on white striping scores in breast meat. The birds offered a low CP diet deficient in Arg had a lower white striping score in breast meat compared to those offered a normal CP diet. When Arg was added back, the white striping score increased and became similar to the birds offered a normal CP diet. When GAA spared Arg at 50%, 100%, and 150% without or with betaine, white striping scores in breast meat were not affected compared to the birds offered low CP diets sufficient in Arg. Betaine did not affect white striping scores when it was added to each level of GAA.

Wooden breast

The effect of low CP diets with GAA and betaine on wooden breast incidence of broilers is presented in Figure 5. Dietary treatments tended ($P = 0.065$) to affect wooden breast incidence with the lowest percentage being observed in the birds offered a low CP diet deficient in Arg.

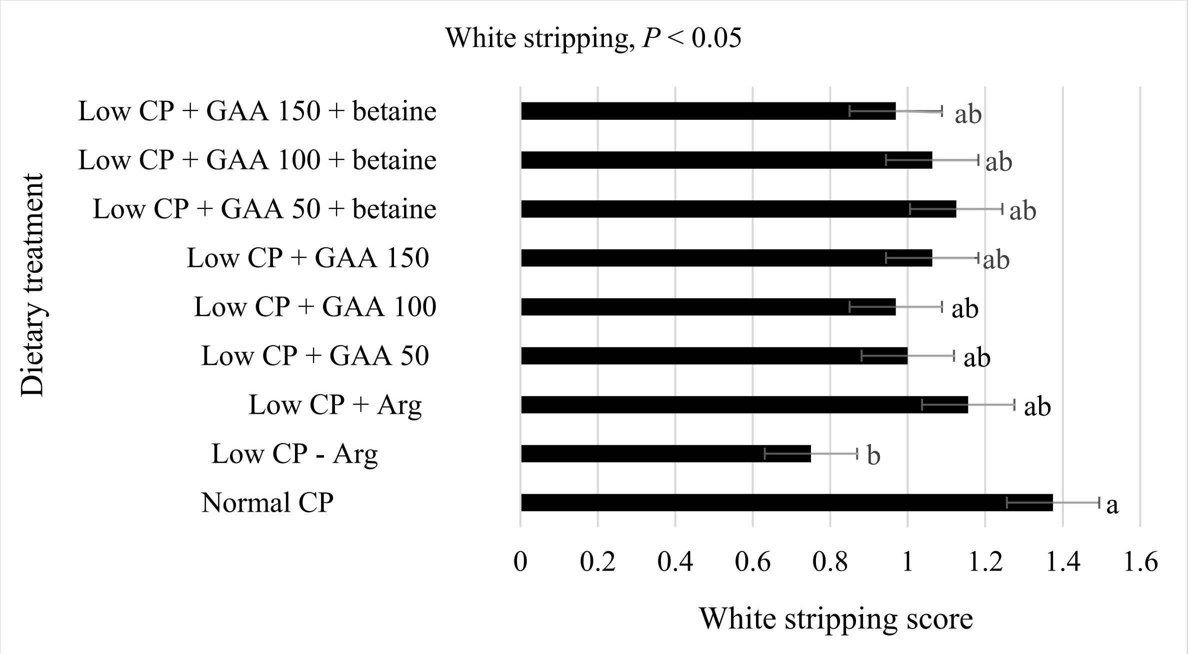

Figure 4 Effect of low crude protein diets with guanidinoacetic acid and betaine on white stripping score in broiler breast meat at day 42

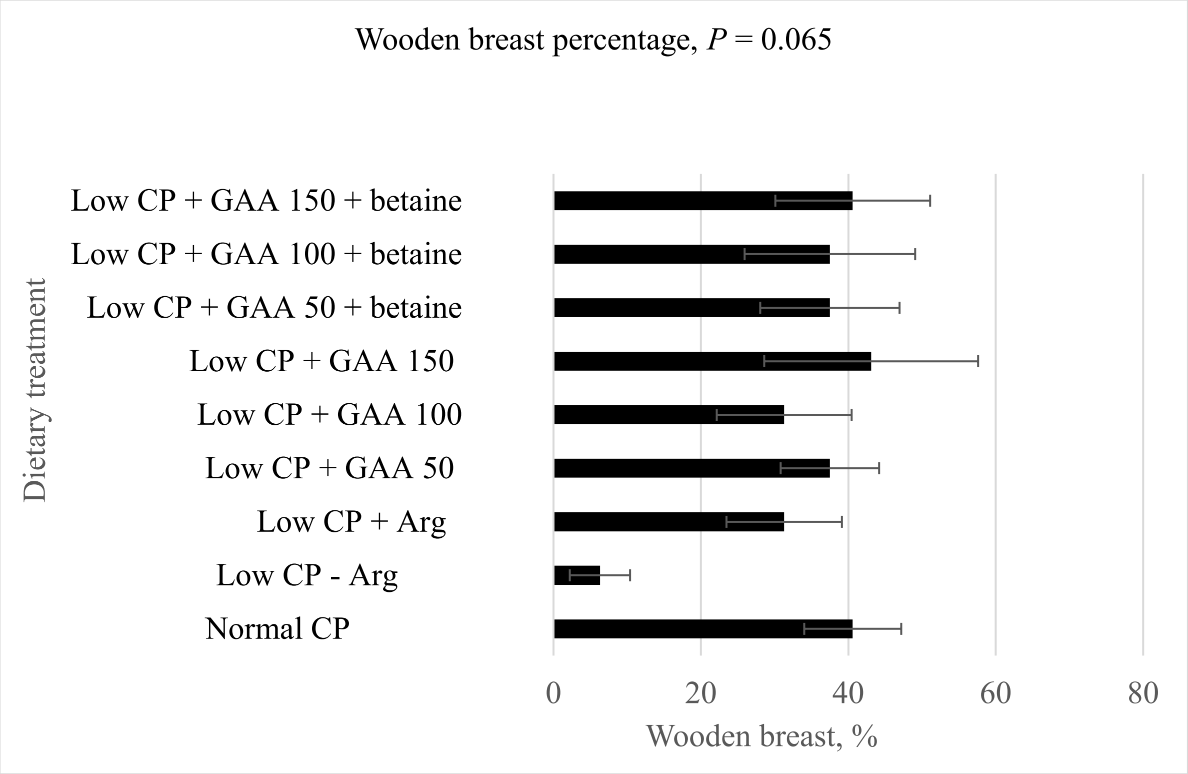

Figure 5 Effect of low crude protein diets with guanidinoacetic acid and betaine on wooden breast incidence in broilers at day 42

Implications

Recent Australian research has identified two major issues in feeding wheat based low CP diets for broilers: 1. a high FCR; and 2. a high abdominal fat pad. The findings of this study suggest that by partly replacing Arg with GAA in low CP diets, breast meat creatine concentration can be increased and FCR and abdominal fat pad can be decreased. GAA can be used to replace 150% of Arg in moderately low CP diets without affecting growth performance or meat quality, with an additional payback through 27 g increased breast meat yield. A higher replacement rate of 1:1 or 100% may be used to improve FCR by up to 3 points.

The outcomes of this project have been presented at the 2021 Poultry Hub Ideas Exchange program to disseminate the results to the industry. A paper has been accepted for oral presentation at the 33rd Australian Poultry Science Symposium in 2022 to convey the results to a wider audience. A manuscript has been submitted in the Poultry Science journal for the scientific community and industry worldwide.

Recommendations

Guanidinoacetic acid may allow the use of moderately reduced CP diets in the industry to maximise profitability. It may be used to spare Arg in low CP diets at 150% or at 100% for improving FCR. Further research is warranted to determine the maximum level of GAA that can be used to spare Arg in low CP diets, and the role of betaine in such cases should be explored.

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Media and Publications

1. Poultry Hub Ideas Exchange program – the results of this study were presented at the Poultry Hub Ideas Exchange program in 2021.
2. National Poultry Newspaper – some of the results of this study were published in the December 2021 issue of the National Poultry Newspaper.
3. Australian Poultry Science Symposium – a paper has been accepted for oral presentation at the 33rd Annual Australian Poultry Science Symposium in 2022. A 1-page abstract will be published in the proceedings.
4. Poultry Science journal – manuscript has been submitted for publication in the Poultry Science journal.

Intellectual Property Arising

There is no intellectual property related to this project as yet.

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