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Response of meat chickens to arginine in reduced protein diets

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Project Summary

Project Title	Response of meat chickens to arginine in reduced protein diets
Project No.	18-414
Date	Start: 02/05/2019 End: 28/03/2019
Project Leader(s)	Prof Robert A. Swick
Organisation	School of Environmental and Rural Science, University of New England, Armidale, NSW Australia 2351
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Project Aim	This study was conducted to investigate the response of broiler chickens to different sources of arginine in Australian practical reduced protein diets based on wheat, sorghum, meat and bone meal, soybean meal and canola meal.
Background	Male Ross 308 birds (n = 768) were assigned to 8 dietary treatment: normal protein diet, reduced protein diet deficient in arginine and diets added with 2 levels (low and high) of either arginine, guanidinoacetic acid (GAA) or citrulline. There were three feeding phases: starter, grower and finisher in the study with 6 replicates of 16 birds (starting) per treatment. Bird weight and feed intake were measured on the weekly basis throughout the 35 day feeding trial. The measurements included body weight gain, feed intake, feed conversion ratio, carcass yield (breast, thigh and drumstick), abdominal fat, liver weight, intestine weight, tibia ash, tibia diameters and tibia breaking strength.
Research Outcome	The results of the current study showed that reductions of 40 g/kg crude protein may be excessive in Australian diets based on wheat, sorghum, soybean meal, meat and bone meal, and canola meal with reduced performance even when all essential SID amino acids are supplied at the published Ross 308 nutrient requirements. It should be noted that the requirements for the essential amino acids phenylalanine, histidine and leucine are not listed in either the Ross 308 or Cobb 500 publications and with a 40 g/kg reduction in crude protein, phenylalanine begins to become limiting. When arginine was deficient there was a reduction in feed intake, body weight, FCR and breast meat yield. When different sources of arginine were added back to the diets, there was a large and significant response to the first level and much less if any to the second level. With the second level of GAA and arginine the response tended to be negative in young birds. This suggests that a deficiency of phenylalanine may be more pronounced in prestarter diets in reduced protein diets. Interestingly, citrulline had more of a positive effect than the other arginine sources at the higher level.
Impacts and Outcomes	There will likely be economic and welfare benefits to feeding reduced protein diets with added methionine, lysine, threonine, arginine, valine and isoleucine. Pending future reductions in manufacturing price, arginine, valine and isoleucine will be the next supplemented amino acids in broiler diets.
Publications	There is no paper or conference presentations that has been published from the results of this study yet.

Executive Summary

This study was conducted to investigate the response of broiler chickens to different sources of arginine in Australian practical reduced protein diets based on wheat, sorghum, meat and bone meal, soybean meal and canola meal. Pending future reductions in manufacturing price, arginine, valine and isoleucine will be the next supplemented amino acids in broiler diets. There will likely be economic and welfare benefits to feeding reduced protein diets with added methionine, lysine, threonine, arginine, valine and isoleucine. In this study, 768 male Ross 308 birds were assigned to 8 dietary treatment: normal protein diet, reduced protein diet deficient in arginine and diets added with 2 levels (low and high) of either arginine, guanidinoacetic acid (GAA) or citrulline. There were three feeding phases: starter, grower and finisher in the study with 6 replicates of 16 birds (starting) per treatment. Bird weight and feed intake were measured on the weekly basis throughout the 35 day feeding trial. The measurements included body weight gain, feed intake, feed conversion ratio, carcass yield (breast, thigh and drumstick), abdominal fat, liver weight, intestine weight, tibia ash, tibia diameters and tibia breaking strength.

There was a reduction in feed intake, weight gain and increase in FCR of birds fed arginine deficient reduced protein diets as compared to the normal protein and all diets with sources of arginine added (L-arginine, GAA or L-citrulline). Birds fed the normal protein diet had higher feed intake, body weight, body weight gain than those fed the other diets; however, their feed conversion efficiencies were comparable with birds fed high levels of arginine or citrulline. As a percent of BW, birds fed the normal protein diet had higher breast meat yield, lower abdominal fat but also lower thigh and drumstick meat yield than those fed with other diets. Interestingly, birds fed the arginine deficient diet had heavier relative liver weight and tended to have heavier intestine weight compared to those received diets with different sources of arginine added (L-arginine, GAA or L-citrulline) and normal protein diet. In addition, the results of the current study showed that L-arginine, GAA and citrulline were all effective in improving tibia shape (length, diameter) and quality (dry matter, ash, breaking strength) in reduced protein diets (arginine deficient) diets. The reduced protein diets were more expensive than the normal protein diets. Reduced protein diets used less soybean meal and canola oil but more wheat and crystalline amino acids. Thus, the decrease in prices of crystalline amino acids is essential to increase the use of reduced protein diets in the future.

Overall, the results of the current study showed that reductions of 40 g/kg crude protein may be excessive in Australian diets based on wheat, sorghum, soybean meal, meat and bone meal, and canola meal with reduced performance even when all essential SID amino acids are supplied at the published Ross 308 nutrient requirements. It should be noted that the requirements for the essential amino acids phenylalanine, histidine and leucine are listed in either the Ross 308 or Cobb 500 publications and with a 40 g/kg reduction in crude protein, phenylalanine begins to become limiting. When arginine was deficient there was a reduction in feed intake, body weight, FCR and breast meat yield. When different sources of arginine were added back to the diets, there was a large and significant response to the first level and much less if any to the second level. With the second level of GAA and arginine the response tended to be negative in young birds. This suggests that a deficiency of phenylalanine may be more pronounced in prestarter diets in reduced protein diets. Interestingly, citrulline had more of a positive effect than the other arginine sources at the higher level. This discovery is worthy of follow-up.

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Introduction

Global poultry production is forecast to double by 2030 to satisfy demand for human consumption of protein (Harlan, 2007). This increased demand for poultry products has required the industry to constantly look for strategies to produce products more efficiently within limited available resources. In poultry production, feed remains as the most significant portion in the total cost. Much of this cost is attributed to the protein/amino acid portion of the diet (DeGroot, 2014). Prices of plant origin protein sources such wheat and soybean meal have been generally increasing over time and food safety concerns exist over inclusion of meat by-products in poultry diets.

Supplementation with crystalline amino acids is an effective solution to satisfy at least part if the protein requirement in chickens. Key essential amino acids including methionine, lysine and threonine are commercially available and in widespread use but others including valine, isoleucine and arginine, while commercially available, are still too expensive for widespread use. As more is known how best to use these amino acids in formulation, demand will increase and cost of production will decrease. This study was focussed on arginine. Chickens have a high dietary arginine requirement compared to other animals as they lack a urea cycle and as such do not have much, if any, *de novo* synthesis. Research evidence indicates that young chicks fed diets lacking in lysine could survive longer and lose less weight than those with a similar deficiency of arginine (Ousterhout, 1960). Dietary lysine is known to be antagonistic to arginine metabolism in the chick (Jones et al., 1967) but the practical significance of this interaction is unknown. The demand for both lysine and arginine is greatest in rapidly growing chickens with high muscle protein deposition (Ball et al., 2007). The decreased use of meat and bone meal, use of sorghum as a grain source and a move to reduced crude protein formulations has led to higher arginine nutrient costs in broiler diets (DeGroot et al., 2018). Literature evidence has indicated that L-arginine, guanidinoacetic acid (GAA) and citrulline can all provide arginine activity in chicken diets and all are commercially available. No study has examined the additions of L-arginine, GAA or citrulline in practical Australian meat chicken diets. This study was designed to determine if an arginine deficiency can be demonstrated in reduced protein diets based on sorghum and wheat and the economics of adding it back as either L-arginine, GAA or citrulline.

Arginine is important for protein synthesis and also is involved in immunity being converted to nitric oxide at the macrophage level (Jahanian, 2009). Nitric oxide serves as a paracrine mediator for certain immunological functions. It is a cytotoxic product produced by activated avian macrophages (a form of blood monocytes) that are present in many tissues including osteoblasts in bones and liver Kupffer cells (Qureshi, 2003). The free radical nitric oxide is formed from the guanidine nitrogen of L-arginine via the enzyme nitric oxide synthase with L-citrulline is a co-product. As arginine is a limiting factor for nitric oxide synthesis, the increased availability of arginine following dietary supplementation of L-arginine, GAA or L-citrulline may improve the nitric oxide production and subsequent immune status in meat chickens. Such improvements on bird health may become useful for AGP free broiler production.

The American patent application US 2004/0235953 describes and claims a method for the prevention and therapeutic treatment of pathologies associated with decreased formation of nitric oxide, in particular sepsis (systemic infection), using the administration of a precursor of nitric oxide which can be citrulline (Summar et al., 1999). Patent US20100093863A1 describes the used of L-citrulline for preventing an increase in protein carbonylation and for treating diseases resulting therefrom (Moinard et al, 2007). To our knowledge, no study has reported on the arginine-sparing effect of citrulline in arginine-deficient practical diets fed to modern broiler strains.

Objectives

The overall objectives of this study were to determine if an arginine deficiency can be demonstrated in birds fed Australian practical reduced protein diets based on sorghum, wheat soybean meal and canola meal and the economics of adding it back as either L-arginine, GAA or L-citrulline.

Methodology

In this study, eight dietary treatments were used as follows:

1. Control reduced protein, no added L-Arg (deficient, RP)
2. (1) plus L-arg low level (0.238%) (RP + low Arg)
3. (1) plus L-arg high level (0.476%) (RP + high Arg)
4. (1) plus GAA low level (molar equivalent to L-Arg, 0.309%) (RP + low GAA)
5. (1) plus GAA high level (molar equivalent to L-Arg, 0.618%) (RP + high GAA)
6. (1) plus citrulline low level (molar equivalent to L-Arg, 0.238%) (RP + low Cit)
7. (1) plus citrulline high level (molar equivalent to L-Arg, 0.476%) (RP + high Cit)
8. Normal protein diet (NP)

The experiment was approved by UNE's animal ethics committee (Authority number: AEC18-066) and met the requirements of the Australian Code of practice to care and use of animals for scientific purposes (NHMRC, 2013). Ross 308 day-old males birds (n=768) were assigned to the 8 dietary treatments above in a completely randomised design. There were 16 birds per pen, with 6 replicates per treatment. Birds were grown under commercial conditions with wood shavings as bedding material during 35 day-experiment. The 2 weakest birds per pens were culled immediately after the 7 day weighing. Feed was provided as crumbles for starter, and pellets for grower and finisher. Birds and feed intake were weighed on days 7, 14, 21, 28 and 35. Measurements were: body weight (BW), gain (BWG), feed intake (FI), feed conversion ratio (FCR), carcass yield (breast, thigh and drumstick), abdominal fat, liver weight and intestine weight (at day 35). On day 23 and again on day 35, 2 birds per pen were randomly collected, weighed and euthanized for tibia collection. The right legs of birds were used for analysing tibia quality (dry matter (DM), ash, length and diameter, tibia breaking strength). After soft tissue removal from tibias, samples were air dried in a fume hood for 72 hours. Initial weights of tibia and sampled birds were recorded. Tibia length and diameter (middle point of the tibia) were measured using an electronic calliper while tibia breaking strength was measured using a Lloyd Testing Instrument (model 1000R, Lloyd Instruments Ltd., Fareham, Hampshire, UK). Tibia samples were further oven dried at 105ºC for 24 hours and ashed at 600ºC for 13 hours for DM and ash measurement.

The diets used in this study were formulated in consulting with national nutrition manager of Inghams Group Limited. The nutritional composition (crude protein, crude fat, dry matter, and ash content) of major ingredients including wheat, sorghum, meat and bone meal, soybean meal and canola meal was analysed before starting the experiment. Total and digestible amino acids used to formulate the diets were obtained from NIR results. Cost-benefit analysis was also be measured. The study was conducted in UNEs Animal House facility, Armidale, New South Wales, Australia. All data analysis was carried out in R Commander (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria). Either one-way ANOVA or the non-parametric ANOVA (Kruskal–Wallis test) was employed to test statistical differences between the treatments. Then, Tukey's post-hoc test was used to identify pairwise differences between the groups.

Table 1. Ingredient composition of normal and reduced protein diets, as is basis

Ingredient	**Starter normal CP**	**Starter reduced CP**	**Grower normal CP**	**Grower reduced CP**	**Finisher normal CP**	**Finisher reduced CP**
Wheat	31.78	50.01	21.85	40.10	28.19	46.60
Sorghum	25.00	25.00	35.00	35.00	35.00	35.00
Meat and bone meal	6.29	6.60	5.45	5.76	4.68	5.17
Soybean meal	26.44	9.24	23.37	6.14	17.91	0.45
Canola meal	5.00	5.00	8.00	8.00	8.00	8.00
Canola oil	3.43	0.75	4.21	1.54	4.69	1.99
Limestone	0.36	0.36	0.36	0.36	0.37	0.31
Xylanase Econase	0.01	0.01	0.01	0.01	0.01	0.01
Salt	0.20	0.19	0.19	0.18	0.20	0.18
Na bicarb	0.100	0.100	0.100	0.100	0.100	0.100
TiO2	-	-	0.50	0.50	-	-
Premix vitamin	0.09	0.09	0.05	0.05	0.05	0.05
Premix mineral	0.12	0.12	0.08	0.08	0.08	0.08
Choline Cl 60%	0.08	0.14	0.08	0.14	0.07	0.13
L-lysine	0.37	0.85	0.28	0.76	0.27	0.76
DL-methionine	0.39	0.48	0.33	0.41	0.27	0.35
L-threonine	0.20	0.40	0.12	0.32	0.10	0.31
L-tryptophan	-	0.04	-	0.02	-	0.02
L-isoleucine	0.05	0.32	-	0.27	-	0.27
L-arginine	0.04	-	-	-	-	-
L-valine	0.03	0.29	-	0.22	-	0.20
Albac 150 (ZnBac)	0.03	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100	100

Arg, GAA and Cit were added at 2 levels for reduced protein diets. Essential SID AAs equivalent in normal protein and reduced protein + high levels of Arg sources

Table 2. Calculated nutrient composition of normal and reduced protein diets, as is basis

Nutrient Name	**Units**	**Starter normal CP**	**Starter reduced CP**	**Grower normal CP**	**Grower reduced CP**	**Finisher normal CP**	**Finisher reduced CP**
Dry Matter	%	90.77	91.10	90.58	90.91	90.66	91.00
AMEn Poultry	kcal/kg	3100	3100	3150	3150	3250	3250
Crude Protein	%	24.90	20.90	23.67	19.70	21.38	17.40
Crude fat	%	6.58	4.12	7.80	5.36	8.28	5.83
Ash	%	5.43	4.67	5.61	4.85	4.59	3.88
SID arginine	%	1.42	0.94	1.30	0.86	1.141	0.70
SID lysine	%	1.32	1.32	1.19	1.19	1.05	1.05
SID methionine	%	0.70	0.72	0.63	0.65	0.54	0.57
SID cysteine	%	0.31	0.27	0.30	0.26	0.29	0.25
SID M + C	%	0.98	0.98	0.90	0.90	0.81	0.81
SID tryptophan	%	0.25	0.21	0.24	0.19	0.219	0.16
SID isoleucine	%	0.89	0.89	0.81	0.81	0.72	0.72
SID threonine	%	0.89	0.89	0.79	0.79	0.70	0.70
SID valine	%	0.99	0.99	0.93	0.90	0.84	0.79
SID glycine	%	1.06	0.86	0.98	0.79	0.88	0.69
Calcium	%	0.99	0.99	0.90	0.90	0.80	0.80
Phosphorus avail	%	0.50	0.50	0.45	0.45	0.40	0.40
Sodium	%	0.18	0.18	0.17	0.17	0.17	0.17
Chloride	%	0.28	0.38	0.26	0.35	0.26	0.35
Choline	mg/kg	175	1757	1652	1652	1523	1523
Linoleic 18:2	%	2.03	1.47	2.40	1.84	2.56	1.99

Arg, GAA and Cit were added at 2 levels for reduced protein diets. Essential SID AAs equivalent in normal protein and reduced protein + high levels of Arg sources
SID = standard ileal digestibility using coefficients from Amino Dat 5.0 (Evonik, Frankfurt, Germany

Discussion of Results

There was a reduction in FI, BWG and increase in FCR of birds fed arginine deficient diets (RP) as compared to the normal protein (NP) and all diets with sources of arginine added (L-arginine, GAA or L-citrulline) (Table 3, 4, 5). Although livability was not affected by the dietary treatments (table 3, 4 and 5), birds fed with arginine deficient and normal protein diets tended to have lower livability than those on the other diets especially at the later stage of the growout (from day 14 thereafter). Birds fed the normal protein diet had higher FI, BW, BWG than those fed the other diets ($P < 0.05$); however, their feed conversion efficiency were comparable with those of birds fed with high levels of arginine or citrulline ($P > 0.05$).

Table 3. Feed intake, body weight gain, FCR and livability from day 0 to 7

Treatment	Initial weight, g	FI, g	BWG, g	FCR, g/g	Livability, %
NP	45	176	170^{d}	1.039^{a}	99
LP	44	158	116^{ab}	1.367^{ab}	100
LP + low Arg	44	175	143^{ad}	1.220^{ab}	99
LP + high Arg	45	170	119^{abc}	1.474^{b}	100
LP + low GAA	45	169	142^{ad}	1.198^{ab}	99
LP + high GAA	45	153	114^{a}	1.465^{b}	100
LP + low Cit	44	172	153^{cd}	1.145^{ab}	100
LP + high Cit	44	169	151^{bd}	1.128^{ab}	100
SEM	0.12	2.12	3.82	0.038	0.22
P value	NS	0.077	<0.001	0.011	NS

Low = 0.238% Arg, 0.309%GAA, 0.238% Cit (equivalent on molar basis); High = 0.476% Arg, 0.618%GAA, 0.476% Cit (equivalent on molar basis)

Corzo and Kidd (2003) examined the response of broiler chickens to graded level of L-arginine (from 0.95% to 1.55%) supplemented in titration diets based on corn-soybean meal. No significant difference in the weight gain and feed efficiency was observed between different dietary treatments for broiler females from 21 to 35 days of age. Meanwhile, a quadratic increase in the weight gain and gain:feed ratio were obtained when levels of L-arginine were increased in diets fed to broiler chicks from 0 to 18 days of age (Corzo and Kidd, 2003). Citrulline has also been previously found to have an arginine-sparing effect in broilers. Based on the findings from *in vitro* and *in vivo* experiments, Tamir and Ratner (1963) demonstrated the capacity of chicks to convert citrulline to arginine and that the process takes place in kidney and extrahepatic tissues via argininosuccinate formation. More recently, Su and Austic (1999) provided evidence that macrophages convert citrulline to arginine in arginine insufficient diets. Importantly, citrulline has been demonstrated to spare arginine in meat chickens (Klose and Almquist, 1940; Tamir and Ratner, 1963). In this study, the improvement in feed intake, body weight gain and feed conversion efficiency were observed at all growth stages when different sources of arginine (L-arginine, GAA, citrulline) were added to arginine deficient diets. These findings demonstrate that L-arginine, GAA and citrulline were all effective in arginine-deficient diets. However weight gain in younger birds (0 to 7 d) was reduced in birds fed the higher level of L-arginine or GAA compared to the lower level. Weight of birds fed the high level were not different to the RP diet without arginine ($P > 0.05$) while weight of those fed the lower levels of L-arginine, and GAA or both low or high citrulline were not different to birds fed the normal protein (NP) control ($P > 0.05$). From 0 to 35 d, birds fed the RP diet without arginine had lower weight gain ($P < 0.05$), and higher FCR compared to NP fed birds. Additions of L-arginine, GAA or citrulline improved growth and reduced FCR but were still numerically poorer than those fed the NP diets.

Table 4. Feed intake, body weight gain, FCR and livability from day 0 to 21

Treatment	**FI, g**	**BWG, g**	**FCR, g/g**	**Livability, %**
NP	1370^{c}	1165^{d}	1.177^{a}	96
LP	1024^{a}	697^{a}	1.488^{c}	93
LP + low Arg	1222^{bc}	936^{c}	1.306^{ab}	99
LP + high Arg	1154^{ab}	909^{bc}	1.272^{ab}	98
LP + low GAA	1117^{ab}	841^{ac}	1.346^{bc}	97
LP + high GAA	974^{a}	725^{ab}	1.360^{bc}	99
LP + low Cit	1255^{bc}	1015^{cd}	1.237^{ab}	100
LP + high Cit	1254^{bc}	1023^{cd}	1.227^{ab}	97
SEM	22.79	25.83	0.017	0.69
P value	<0.001	<0.001	<0.001	NS

Low = 0.238% Arg, 0.309%GAA, 0.238% Cit (equivalent on molar basis); High = 0.476% Arg, 0.618%GAA, 0.476% Cit (equivalent on molar basis)

The FI, BWG, FCR and livability of birds fed on two levels of arginine and citrulline were comparable. However birds fed with low level of GAA had higher BWG and lower FCR than those fed the high GAA level suggesting the possibility of toxicity of this source of arginine at the higher level. The higher level of GAA was based on the molar equivalent level of L-arginine and it was expected to give even higher performance due to an energy component. GAA is a natural precursor of creatine, an important constituent involved in energy metabolism. GAA is formed from arginine and glycine in the kidney via activity of the enzyme arginine:glycine amidinotransferase (Wu and Morris, 1998). Thereafter, GAA is methylated at the amidino group by *S*-adenosyl-methionine in the liver to form creatine. This process is catalyzed by guanidinoacetic acid methyltransferase (Brosnan et al., 2009; Ostojic et al., 2013). Synthesized creatine is transported from liver to different organs, primarily tissues that have a high and fluctuating energy demand such as skeletal muscle, heart and brain, where creatine is phosphorylated to create phosphocreatine. Phosphocreatine plays an important role in physiological functions as it not only an essential component of cellular energy transport system but also help to reserve high-energy phosphate groups to reinstate adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Because of this crucial role in transferring cell energy, high concentrations of creatine are observed in muscle tissues (DeGroot et al., 2018). Dilger et al. (2013) demonstrated the potential for GAA to replace the dietary arginine in young chicks (8-17 days old) and showed similar gain:feed ratios in chicks fed either 0.12% GAA, 0.25% arginine, or 0.15% creatine in arginine-deficient practical diets. Similar increases in weight gain and gain:feed ratios were also obtained when 0.12% GAA and 0.12% L-arginine were supplemented to semi-purified arginine-deficient diets for young chicks at the same ages (Dilger et al., 2013). However, the efficacy of GAA might depend on growing periods of birds, dosages used and nutrition density in the diets. Mousavi et al. (2013) indicated that supplementation with GAA at 0.06% improved feed conversion ratio of Cobb 500 broilers from 23 to 40 days and 0 to 40 days of age ($P \leq 0.05$). However, no significant response on body weight gain was found in this study. Similarly, a study by Tossenberger et al. (2016) showed that addition of 0.06% GAA did not affect growth performance while super-level of GAA (0.6%) significantly reduced feed intake and weight gain of Ross 308 male broilers. The decrease in feed intake, weight gain and carcass yield were also observed in this study when high levels of GAA was fed to Arginine-deficient birds.

Table 5. Feed intake, body weight gain, FCR and livability from day 0 to 35

Treatment	**FI, g**	**BWG, g**	**FCR, g/g**	**Livability, %**
NP	3611^{d}	2607^{d}	1.386^{a}	96
LP	2607^{ab}	1520^{a}	1.701^{d}	92
LP + low Arg	3166^{cd}	2047^{bc}	1.547^{c}	98
LP + high Arg	3032^{bc}	2078^{bc}	1.459^{ab}	97
LP + low GAA	2879^{ac}	1844^{ab}	1.566^{c}	97
LP + high GAA	2517^{a}	1642^{a}	1.533^{bc}	98
LP + low Cit	3197^{cd}	2151^{bc}	1.486^{bc}	100
LP + high Cit	3165^{bcd}	2222^{c}	1.449^{ab}	97
SEM	62.54	52.46	0.015	0.78
P value	<0.001	<0.001	<0.001	NS

Low = 0.238% Arg, 0.309%GAA, 0.238% Cit (equivalent on molar basis); High = 0.476% Arg, 0.618%GAA, 0.476% Cit (equivalent on molar basis)

Table 6. Body weight by week

Treatment	**d0**	**d7**	**d14**	**d21**	**d28**	**d35**
NP	45	214^{c}	588^{d}	1208^{e}	1897^{c}	2646^{e}
LP	44	160^{a}	342^{a}	749^{a}	1082^{a}	1591^{a}
LP + low Arg	44	188^{ac}	492^{cd}	981^{c}	1514^{b}	2091^{c}
LP + high Arg	45	164^{ab}	455^{bc}	956^{c}	1494^{b}	2124^{cd}
LP + low GAA	45	187^{ac}	467^{bc}	887^{b}	1353^{ab}	1893^{b}
LP + high GAA	45	159^{a}	388^{ab}	769^{a}	1178^{a}	1686^{a}
LP + low Cit	44	197^{bc}	520^{cd}	1060^{d}	1583^{b}	2195^{cd}
LP + high Cit	44	195^{ac}	526^{cd}	1067^{d}	1619^{bc}	2263^{d}
SEM	0.12	3.82	12.85	25.84	40.97	52.47
P value	NS	<0.001	<0.001	<0.001	<0.001	<0.001

Low = 0.238% Arg, 0.309%GAA, 0.238% Cit (equivalent on molar basis); High = 0.476% Arg, 0.618%GAA, 0.476% Cit (equivalent on molar basis)

Regarding carcass traits, birds fed the NP diet had higher breast meat yield, lower abdominal fat but also lower thigh and drumstick meat yield than those fed other diets (Table 7). Interestingly, birds fed the RP diet without added arginine had heavier relative liver weights and tended to have heavier relative intestine weight compared to those received diets with different sources of arginine added (L-arginine, GAA or L-citrulline) and NP diet (Table 7). Among birds supplemented with the arginine sources, the highest relative liver and intestine weights were observed in birds fed with high levels of GAA. As high levels of energy are used to maintain metabolism in the intestine, this finding might suggest the reasons for poorer growth performance and possible toxicity in high-GAA-supplemented birds. Esser et al. (2017) reported that birds fed diets supplemented with L-arginine had heavier carcass weights, higher breast meat yields and lower abdominal fat than those fed on pure vegetable diets. Similar results were found in this study. Progressively increased breast meat yield have also been reported from increasing levels of GAA in broiler diets (Lemme, 2007; Michiels et al., 2012). Conversely, Ringel et al. (2007) observed no differences in breast meat yield when graded levels of GAA (from 0.031 to 0.126%) were supplemented to Ross 308 male broilers. Similar results were indicated by Mousavi et al. (2013) and Abudabos et al. (2014) as GAA supplementation did not affect overall carcass yield as well as yields of different commercial parts. No response to breast meat yield was also observed in the current study when different levels of GAA were added in the RP diet without added arginine however the levels used were much higher than previously reported. Importantly, Mousavi et al. (2013) found a significant decrease in the liver percentage in GAA-supplemented birds (0.6%) as compared to the non-supplemented group; however, the reasons for that were not mentioned. Similar results were found in this study.

Table 7. Relative carcass yield and internal organ weights (percent of body weight)

Treatment	Breast	Thigh and drum-stick	Abdominal fat	Liver	Duo-denum	Jej-unum	Ileum	Whole intes-tine
NP	16.44^{d}	17.86^{a}	0.97^{a}	1.98^{a}	0.79^{a}	1.10^{a}	0.89	2.78^{ab}
LP	10.52^{a}	18.56^{ab}	1.77^{d}	2.94^{d}	0.92^{ab}	1.20^{ab}	0.97	3.09^{ac}
LP + low Arg	12.86^{bc}	19.16^{bc}	1.53^{cd}	2.62^{bd}	0.81^{a}	1.07^{a}	0.85	2.73^{a}
LP + high Arg	11.27^{ab}	20.02^{c}	1.20^{ab}	2.34^{b}	0.86^{ab}	1.11^{a}	0.93	2.90^{ab}
LP + low GAA	11.98^{ac}	19.42^{bc}	1.47^{bd}	2.75^{cd}	0.93^{ab}	1.28^{ab}	1.02	3.22^{bc}
LP + high GAA	10.76^{a}	19.85^{c}	1.45^{bc}	2.64^{bd}	1.00^{b}	1.44^{b}	1.11	3.55^{c}
LP + low Cit	13.55^{c}	18.93^{ac}	1.45^{bc}	2.43^{bc}	0.78^{a}	1.09^{a}	0.90	2.77^{ab}
LP + high Cit	12.21^{ac}	19.95^{c}	1.25^{abc}	2.44^{bc}	0.83^{a}	1.18^{a}	0.84	2.84^{ab}
SEM	0.29	0.13	0.04	0.05	0.016	0.02	0.02	0.05
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.057	<0.001

Low = 0.238% Arg, 0.309%GAA, 0.238% Cit (equivalent on molar basis); High = 0.476% Arg, 0.618%GAA, 0.476% Cit (equivalent on molar basis)

Birds fed the RP diet without arginine had lower relative tibia weight, lower ash percentage but greater DM at both 23 and 35 day than NP fed birds ($P < 0.05$). Birds fed the RP diet also had higher relative tibia weight (Table 8 and 9). In this study, the improvement in tibia weight, tibia DM and ash were observed at both 23 and 35 day when L-arginine and citrulline levels were added to the RP diets without added arginine. The results on these bone parameters of birds fed either levels of L-arginine and citrulline were comparable with the NP while those fed with GAA were not different to the RP diet without added arginine (Table 8 and 9).

Regarding the bone shape and strength, birds fed the NP diet had significantly longer tibia, greater tibia diameter and greater tibia breaking strength than birds fed RP diet without added arginine at both 23 and 35 day (Table 10). When different sources of arginine were added back in the RP diet as either L-arginine, GAA or citrulline, there were improvements in tibia length, diameter and breaking strength comparable to NP fed birds. At day 23, the tibia breaking strength of birds fed either level of L-arginine and citrulline were not different to the NP while those fed with GAA were not different to the RP diet without added arginine. A similar trend was observed at day 35 except tibia breaking strength of birds fed with low level of GAA was comparable to those on NP diet.

Table 8. Tibia dry matter and ash at day 23

Treatment	Air dry tibia weight (g)	Air dry tibia weight as percentage of BW (%)	Weight after oven dry (g)	Oven dry DM (%)	Ash weight (g)	Relative ash (% air dry bone)	Relative ash (% oven dry bone)
NP	3.32^{d}	0.24^{a}	3.02^{d}	90.7^{a}	1.47^{d}	44.1^{b}	48.6^{b}
LP	2.21^{a}	0.27^{b}	2.02^{a}	91.7^{c}	0.89^{a}	40.2^{a}	43.8^{a}
LP + low Arg	2.85^{bcd}	0.25^{ab}	2.59^{bcd}	91.1^{ab}	1.23^{bcd}	43.1^{b}	47.3^{b}
LP + high Arg	2.81^{ad}	0.27^{ab}	2.56^{ad}	90.9^{ab}	1.21^{bcd}	42.8^{ab}	47.1^{ab}
LP + low GAA	2.61^{ac}	0.24^{ab}	2.38^{ac}	91.3^{bc}	1.11^{ac}	42.1^{ab}	46.1^{ab}
LP + high GAA	2.36^{ab}	0.26^{ab}	2.15^{ab}	91.3^{bc}	0.98^{ab}	41.5^{ab}	45.4^{ab}
LP + low Cit	3.11^{cd}	0.25^{ab}	2.83^{cd}	91.0^{ab}	1.34^{cd}	43.2^{b}	47.5^{b}
LP + high Cit	3.32^{d}	0.25^{ab}	3.03^{d}	91.1^{ab}	1.42^{d}	42.7^{ab}	46.9^{ab}
SEM	0.07	0.003	0.07	0.06	0.04	0.27	0.32
P value	< 0.001	0.014	< 0.001	< 0.001	< 0.001	0.004	0.002

Low = 0.238% Arg, 0.309%GAA, 0.238% Cit (equivalent on molar basis); High = 0.476% Arg, 0.618%GAA, 0.476% Cit (equivalent on molar basis)

Table 9. Tibia dry matter and ash at day 35

Treatment	Air dry tibia weight (g)	Air dry tibia weight as percentage of BW (%)	Weight after oven dry (g)	Oven dry DM (%)	Ash weight (g)	Relative ash (% air dry bone)	Relative ash (% oven dry bone)
NP	6.84^{d}	0.27^{a}	6.12^{c}	89.6^{a}	2.67^{d}	39.0^{b}	43.5^{b}
LP	5.09^{ab}	0.31^{b}	4.61^{ab}	90.6^{b}	1.86^{ab}	36.6^{a}	40.4^{a}
LP + low Arg	6.00^{bcd}	0.30^{ab}	5.42^{bc}	90.3^{ab}	2.28^{bcd}	38.0^{ab}	42.1^{ab}
LP + high Arg	6.21^{cd}	0.31^{b}	5.59^{c}	90.1^{ab}	2.40^{cd}	38.7^{ab}	42.9^{ab}
LP + low GAA	5.82^{ac}	0.29^{ab}	5.26^{ac}	90.4^{ab}	2.17^{ac}	37.3^{ab}	41.3^{ab}
LP + high GAA	4.84^{a}	0.30^{ab}	4.37^{a}	90.4^{ab}	1.77^{a}	36.7^{ab}	40.7^{ab}
LP + low Cit	6.21^{cd}	0.29^{ab}	5.60^{c}	90.1^{ab}	2.34^{cd}	37.7^{ab}	41.8^{ab}
LP + high Cit	6.32^{cd}	0.30^{ab}	5.69^{c}	89.9^{ab}	2.39^{cd}	37.7^{ab}	41.9^{ab}
SEM	0.12	0.004	0.10	0.08	0.05	0.20	0.25
P value	< 0.001	0.036	< 0.001	0.047	< 0.001	0.017	0.021

Low = 0.238% Arg, 0.309%GAA, 0.238% Cit (equivalent on molar basis); High = 0.476% Arg, 0.618%GAA, 0.476% Cit (equivalent on molar basis)

Table 10. Tibia length, diameter and breaking strength at day 23 and 35

Treatment	Day 23			Day 35		
	Tibia length (mm)	**Tibia diameter (mm)**	**Tibia breaking strength (N)**	**Tibia length (mm)**	**Tibia diameter (mm)**	**Tibia breaking strength (N)**
NP	71.80^{b}	7.06^{c}	298.55^{c}	90.16^{bc}	8.94^{b}	429.83^{b}
LP	66.92^{a}	5.41^{a}	172.14^{a}	87.31^{ab}	7.60^{a}	287.21^{a}
LP + low Arg	69.60^{ab}	6.50^{bc}	250.84^{bc}	90.21^{bc}	8.17^{ab}	332.73^{ab}
LP + high Arg	69.09^{ab}	6.43^{bc}	262.05^{bc}	89.39^{bc}	8.62^{b}	407.08^{b}
LP + low GAA	68.57^{ab}	6.27^{ac}	213.55^{ab}	90.81^{c}	8.17^{ab}	367.98^{ab}
LP + high GAA	68.05^{ab}	5.91^{ab}	204.32^{ab}	85.00^{a}	7.50^{a}	278.74^{a}
LP + low Cit	70.32^{ab}	6.53^{bc}	264.61^{bc}	90.73^{c}	8.33^{ab}	367.71^{ab}
LP + high Cit	72.17^{b}	6.82^{bc}	253.73^{bc}	89.48^{bc}	8.79^{b}	372.05^{ab}
SEM	0.39	0.10	7.29	0.36	0.10	10.18
P value	0.003	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Low = 0.238% Arg, 0.309%GAA, 0.238% Cit (equivalent on molar basis); High = 0.476% Arg, 0.618%GAA, 0.476% Cit (equivalent on molar basis)

The costs of experimental diets are presented in Table 11. Reduced protein diets used less soybean meal and canola oil but more wheat and crystalline amino acids than the NP diet. Some of these amino acids like lysine, methionine and threonine are commercially available and in widespread use but the others including valine, isoleucine, tryptophan and arginine while commercially available are still too expensive for widespread use. If RP diets are used, valine, isoleucine, tryptophan and arginine will be needed to meet the nutrient requirement of chickens. With the current prices of these four crystalline amino acids, the RP diets had higher cost more per ton than the NP diet (Table 11). Thus, the decrease in prices of crystalline amino acids is essential to increase the use of reduced protein diets in the future.

Table 11. Costs of experimental diets

Treatment	**Starter**		**Grower**		**Finisher**	
	Total cost ($/ton)	**Crude protein (%)**	**Total cost ($/ton)**	**Crude protein (%)**	**Total cost ($/ton)**	**Crude protein (%)**
NP	556	24.9	539	23.7	511	21.4
LP	554	20.9	538	19.7	508	17.4
LP + low Arg	585	20.9	568	19.7	570	17.4
LP + high Arg	615	20.9	599	19.7	539	17.4
LP + low GAA	584	20.9	597	19.7	568	17.4
LP + high GAA	613	20.9	567	19.7	538	17.4
LP + low Cit	585	20.9	568	19.7	570	17.4
LP + high Cit	615	20.9	599	19.7	539	17.4

Low = 0.238% Arg, 0.309%GAA, 0.238% Cit (equivalent on molar basis); High = 0.476% Arg, 0.618%GAA, 0.476% Cit (equivalent on molar basis)

Implications

The results of the current study show that reductions of 40 g/kg crude protein (4 percentage points) is excessive in Australian diets based on wheat, sorghum, soybean meal, meat and bone meal, and canola meal. Performance will suffer even when all Ross 308 recommended essential SID amino acids are supplied at requirement. When supplemental arginine was removed from the RP diet there was a reduction in FI, BW, BWG, FCR, breast meat yield and bone quality. When different sources of arginine were added back to the RP, there was a large response to the first level and much less if any to the second level and in some cases negative. This response tended to vary with chicken age and was most negative with the high level of GAA. This suggests that high levels of arginine in prestarter diets may be negative to performance especially if other amino acids such as phenylalanine are limiting. Citrulline had more of a positive effect than the other arginine sources at the higher level. The results suggests that citrulline may be a more effective source of arginine being less toxic or slow release for starting chicks. Guanidino-acetic acid (CreAmino or GAA) was obviously toxic at the high level of inclusion. The effect of the various sources on the potential for an arginine:lysine antagonism should be further examined at different lysine levels in young broilers.

Recommendations

Key essential amino acids including methionine, lysine and threonine are commercially available and in widespread use but others including valine, isoleucine and arginine while commercially available are still too expensive for widespread use. As more is known how best to use these amino acids in formulation, demand will increase and cost of production will decrease. The decrease in prices of crystalline amino acids is critical to increase the use of reduced protein diets in the future. As the price of arginine, valine and isoleucine are reduced in the marketplace, more attention to the requirements of phenylalanine, histidine, leucine and non-essential amino acids will be required.

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Media and Publications

There is no paper or conference presentations that has been published from the results of this study yet; however, a manuscript has been preparing and will be submitted to Poultry Science.

Intellectual Property Arising

There is no intellectual property arising from this study.

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Appendices

Prices of ingredients used in the study

Ingredient	Cost ($/ton)
Wheat	360
Sorghum	400
Meat and bone meal	500
Soybean meal	720
Canola meal	425
Canola oil	1150
Limestone	75
Xylanase Econase	20000
Salt	260
Na bicarb	560
TiO2	2000
Premix vitamin	5000
Premix mineral	5000
Choline Cl 60%	1440
L-lysine	1690
DL-methionine	3660
L-threonine	2190
L-tryptophan	3000
L-isoleucine	18900
L-arginine	13300
Creamino (GAA)	10000
L-citrulline	13300
L-valine	8500
Albac 150 (ZnBac)	5000