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ANIMAL PRODUCTION SCIENCE

Supplementation of reduced protein diets with L-arginine and L-citrulline for broilers challenged with subclinical necrotic enteritis. 1. Growth, carcass yield, and intestinal lesion scores

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ABSTRACT

Context. Improving immune status through nutritional adjustments may be part of an effective strategy to reduce reliance on antibiotic growth promoters for controlling necrotic enteritis (NE) in broiler chickens. **Aims.** This study examined the effect of dietary protein level and the replacement of crystalline L-arginine (Arg) with L-citrulline (Cit) in the reduced-protein diet on the performance of broilers challenged with subclinical NE. **Methods.** Ross 308 cockerels (n = 720) were randomly allocated to six dietary treatments, with eight replicates of 15 birds per pen, during a 35-day feeding experiment. The treatments were as follows: standard protein without NE challenge (SP−); standard protein with NE challenge (SP+); reduced protein (two percentage points lower crude protein) without NE challenge (RP−); reduced protein with NE challenge (RP+); RP+ plus added Arg (103% of RP, RPA+) and RPC+ where supplemental Arg in RPA+ was replaced with Cit. The first four treatments were considered as a 2 × 2 factorial arrangement, with factors being NE (− or +) and protein level (SP or RP). Treatments SP+, RP+, RPA+, and RPC+ were analysed by one-way ANOVA. **Key results.** Subclinical NE challenge reduced feed intake (FI), reduced body weight gain (BWG) and increased feed to gain ratio (FCR) from Day 0 to Day 35, increased intestinal lesion scores on Day 16, and reduced relative breast yield on Day 35 ($P < 0.05$). Feeding RP diets increased FI ($P < 0.001$), increased BWG ($P < 0.01$) and reduced FCR ($P < 0.01$) during the grower phase compared with SP diets when birds were challenged with NE. Birds in the RPC+ treatment had a lower overall FCR than did those in the SP+ treatment ($P < 0.001$). Birds in the RPA+ treatment had similar FI, BWG and FCR to those in the RP+ treatment ($P > 0.05$). **Conclusions.** Collectively, the results showed protective effects of replacing the supplemental Arg with Cit against NE in RP diets, as indicated by higher performance during and after the challenge. **Implications.** Feeding the RP diets supplemented with Cit may be part of an effective strategy to reduce reliance on antibiotic growth promoters for controlling NE in broiler chickens.

Keywords: arginine, carcass yield, citrulline, liver, low protein, meat chicken, necrotic enteritis, uric acid.

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Introduction

Economic losses from necrotic enteritis (NE) have increased in the broiler industry with the removal of in-feed antibiotic growth promoters (Widyaratne 2012). The disease is caused by *Clostridium* (*C.*) *perfringens*, a Gram-positive, rod-shaped, spore-forming, and non-motile bacteria (Wilson *et al.* 2005). In clinical form, *C. perfringens* may overgrow in the small intestine, causing mucosal necrosis, acute diarrhoea, and high mortality rates (up to 40%, Ross 1999). Coccidiosis predisposes birds to NE (Rodgers *et al.* 2015). The subclinical form of NE is more economically devastating than is the acute form as it reduces growth performance in a greater number of birds, and increases carcass condemnation at slaughter (Løvland and Kaldhusdal 1999; Lovland and Kaldhusdal 2001). Subclinical NE

may also increase feed cost as birds are kept for a longer time to achieve their target bodyweight (BW). Losses in the global broiler industry due to NE have been estimated to be USD6 billion annually (Wade and Keyburn 2015). Furthermore, the presence of *C. perfringens* in poultry meat has raised public health concerns as it is considered a factor in causing food-born infections (Immerseel *et al.* 2004). Incorporating antibiotics in broiler diets has been used as a common approach to control NE in birds (Widyaratne 2012). However, a ban on in-feed antibiotic growth promoters (AGP) in animal production in several countries with increasing consumer demand for AGP-free poultry products has resulted in an increased incidence of NE worldwide (Kaldhusdal and Lovland 2000). Mwangi *et al.* (2019) recently reported that confounding factors such as bird health before the proliferation of virulent *C. perfringens* are crucial for NE development in broiler chickens. Thus, improving immune status through nutritional adjustments may be part of an effective strategy to reduce reliance on AGP for controlling NE in broiler chickens.

Dietary protein concentration and amino acid (AA) balance play a critical role in the development of *C. perfringens* in the gastrointestinal tract (Drew *et al.* 2004). Diets with a high protein level, particularly animal-derived proteins, have been shown to facilitate the proliferation of *C. perfringens* and are considered a predisposing factor for NE (Drew *et al.* 2004; Wilkie *et al.* 2005; Liu *et al.* 2017). A high concentration of protein-bound AA from high crude protein (CP) diets and the use of poorly digested protein sources increase undigested protein reaching the hindgut (Hilliar *et al.* 2019). This material can then provide a substrate for the proliferation of gut-specific pathogens, including *C. perfringens*, that may reduce gut health and growth performance (Lan *et al.* 2004; Mcdevitt *et al.* 2006) and increase losses from NE. Thus, the use of reduced-protein diets with greater protein digestibility may be beneficial for chickens subjected to NE challenge.

Arginine (Arg) has been known to have a direct immunomodulatory effect through its metabolic pathways involving the production of ornithine and nitric oxide (NO, Le Floc'h *et al.* 2004). It has been hypothesised that the demand for Arg would increase in birds exposed to inflammation or disease stress (Kidd 2004; Le Floc'h *et al.* 2004; Li *et al.* 2007). Furthermore, Arg has been demonstrated to effectively compensate for reduced growth performance in birds inoculated with infectious bronchitis virus (Lee *et al.* 2002) and alleviate gut injury and normalise ileal microbiota population in *C. perfringens*-challenged birds (Zhang *et al.* 2018). Citrulline (Cit), a metabolite of Arg, can be recycled to Arg and has been reported to be more effective than dietary Arg in increasing blood Arg concentrations and NO production in mammals (Schwedhelm *et al.* 2008; Lassala *et al.* 2009; Wijnands *et al.* 2012). There are few if any reports on Arg and/or Cit supplementation in broilers fed reduced-protein (RP) diets. Thus, this study was designed to investigate the effects of Arg and Cit supplementation to RP diets on growth performance, carcass traits, internal organ weights, serum uric acid, and intestinal lesion score of broilers growing under NE challenge. The results of this study may provide valuable information to control and/or mitigate the effects of NE in AGP-free poultry production.

Materials and methods

Experimental design and diets

The study was implemented at the Centre of Animal Research and Teaching at the University of New England, Armidale, New South Wales, Australia, approved by its Animal Ethics Committee (Approval number: AEC19-119), and met the requirements of the Australian code of practice to care and use of animals for scientific purposes (NHMRC 2013). Day-old Ross 308 cockerels ($n = 720$) were assigned to 48 equal-sized floor pens (120 × 80 cm), with 15 birds per pen and eight replicates per treatment. Starting pen weights were similar across treatments. Birds were grown to mimic commercial conditions with hardwood shavings as bedding material in environmentally controlled rooms. Feed and water were provided *ad libitum* throughout the 35-day feeding study. The temperature, lighting and ventilation conditions followed Ross 308 recommendations (Aviagen 2014*a*). Six treatments were used in this study, with eight replicate pens per treatment. Feed was provided as crumbles for starter (Days 0–10), and pellets for grower (Days 10–24) and finisher (Days 24–35) phases. Feed was pelleted at a temperature of 65°C. The treatments were as follows: standard protein diet without NE challenge (SP−); SP with NE challenge (SP+); reduced protein balanced with crystalline AA without NE challenge (RP−); RP with NE challenge (RP+); RP diet supplemented with additional Arg to 103% of the requirement (equal to 15% additional supplemental crystalline Arg) with NE challenge (RPA+); and RP with Cit replacing all supplemental Arg in previous treatment with NE challenge (RPC+). The levels of essential AA in the RP diet were equivalent to those in the SP diet and in accordance with Ross 308 broiler nutrition specifications (Aviagen 2014*b*). Concentrations of added crystalline Arg in the RP treatments in starter, grower and finisher phases were 0.217%, 0.213% and 0.212% respectively. Concentrations of added crystalline Arg in the RPA+ treatment in starter, grower and finisher phases were 0.249%, 0.245%, and 0.244% respectively. Concentrations of Cit in the RPC+ treatment were equivalent to Arg concentrations in the RPA+ treatment. Details on diet composition and nutrient contents are presented in Tables 1 and 2. Arg and Cit were supplemented in the RP diets at the expense of wheat. The nutritional compositions of wheat, sorghum and soybean meal were analysed before diet formulation. Crude protein, crude fat,

dry matter and ash content of ingredients were measured using AOAC methods (AOAC 1994) and metabolisable energy, and total and digestible AA were estimated using near-infrared reflectance spectroscopy (Foss NIR 6500, Denmark) and standardised with the Evonik AMINONIR® Advanced calibration. There was a two percentage point difference in crude protein content between SP and RP diets for all feeding phases.

Necrotic enteritis challenge

Subclinical NE was established following procedures previously described by Rodgers *et al.* (2015). On Day 9, birds in RPA+ and RPC+ treatments and half of the birds in SP and RP treatments (challenged group) were orally inoculated with 1 mL of sterile phosphate-buffered solution (PBS) containing a vaccine strain of *Eimeria* with 5000 sporulated oocysts of *Eimeria acervulina*, 5000 sporulated oocysts of *Eimeria maxima*, and 2500 sporulated oocysts of *Eimeria brunetti* (Eimeria Pty Ltd, Ringwood, Victoria, Australia). The remaining birds in SP and RP treatments (eight replicates for each) were given 1 mL of sterile PBS as a sham treatment on Day 9 (unchallenged control groups). On Day 14, challenged birds were orally inoculated with 1 mL of *C. perfringens* with an approximate concentration of 10^8 CFU (EHE-NE18 strain, Commonwealth Scientific and Industrial Research Organization, Geelong, Victoria, Australia) in a starch thioglycollate broth. Birds in the unchallenged groups were orally inoculated with 1 mL of sterile thioglycollate broth media as a sham treatment. Necropsy was performed on the victims to determine the cause of death.

Data collection

Bodyweight and feed consumption were measured per pen on Days 8, 10, 14, 16, 24 and 35 of the study. Bodyweight gain (BWG) and feed conversion ratio (expressed as feed:gain, FCR) were then calculated accordingly. The FCR was corrected for mortality by adding the weight gain of dead birds to live birds for each period. Feed intake (FI) was calculated as the corrected FCR multiplied by BWG. On Day 16, three birds per pen were randomly collected, weighed, electrically stunned (MEFE CAT 44N, Mitchell Engineering Food Equipment, Clontarf, Queensland, Australia), and euthanised by decapitation for collection of blood (from a jugular vein) and small intestine for serum uric acid measurement and lesion scoring respectively. Blood samples were collected in vacutainers (Becton, Dickinson UK Ltd, Plymouth, UK) containing spray-coated silica and a polymer gel and centrifuged at 3000*g* at 4°C for 10 min to separate the serum. Serum samples were stored at −20°C until further analysis. Serum uric acid concentration was quantified using an integrated chemistry analyser (Siemens Dimension Xpand Plus, Siemens Healthcare, Newark, NJ, USA) following the manufacturer's instructions (URCA Uric Acid, reference number: DF77). Lesion scoring (scored from 0 to 6 on lesion severity) was performed in the duodenum, jejunum and ileum samples on Day 16 by experienced personnel blind to the experimental design following criteria described by Keyburn *et al.* (2006). Weights of internal organs (liver, spleen, bursa of Fabricius) were also collected on Day 16. On Day 35, two birds per pen were randomly selected and euthanised using similar procedures as described for Day 16 sampling. After birds were dissected, weights of different carcass cuts (breast, thigh and drumstick, abdominal fat) and internal organs (liver, spleen, bursa of Fabricius) were determined. Weights of breast, thigh and drumstick, abdominal fat, and internal organs on Day 16 and Day 35 were expressed as relative weights per unit of live BW.

Feed analysis

The nutrient composition including CP, dry matter, crude fiber, ash contents, and AA profiles of diets were analysed by standard methods (AOAC 1994). Added Cit in RPC diets were quantified using the Waters AccQTag amino acid analysis methodology (Cohen 2001), but adapted to run on an ultra-performance liquid chromatography system as described by Wheat *et al.* (2008). Specifically, samples (100–130 mg) were weighed in duplicate into hydrolysis vials and 5 mL of 20% HCl was added. The samples were then incubated at 110°C for 24 h. After hydrolysis, the samples were derivatised using AccQTag reagents (Waters Corporation, Milford, MA, USA). Then, samples were analysed using a high-resolution reversed-phase column (BEH C18, 2.1 × 100 mm; 1.7 μm) on an ultra-performance liquid chromatography system with a 12-min run time. The column temperature, detection wavelength, and flow rate employed were 57°C, 260 nm and 0.55 mL/min respectively.

Data analyses

R Commander (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria) was used to analyse data. All data were tested for normality and variance homogeneity before analysis. First, two-way ANOVA was used to test the interaction between NE challenge (no or yes) and protein level (SP or RP) in four treatments, including SP−, SP+, RP− and RP+ (2 × 2 factorial arrangement of treatments). The results were used to evaluate the successful implementation of the NE model and the interactions between NE and protein levels. Then, one-way ANOVA was used to test statistical differences between the four NE-challenged treatments (SP+, RP+, RPA+, and RPC+) that were then employed to evaluate the effects of Arg and Cit supplementation to the RP diets during the NE challenge. Tukey's *post hoc* test was used to identify pairwise differences between the treatments from significant ANOVA

Table 1. Diet composition for normal and reduced-protein diets (as-fed basis).

Parameter	Starter		Grower		Finisher	
	SP	RP	SP	RP	SP	RP
Ingredient (%)						
Wheat	39.85	47.84	35.22	43.07	40.19	47.96
Sorghum	20.00	20.00	30.00	30.00	30.00	30.00
Soybean meal	34.15	26.32	29.20	21.49	24.12	16.46
Canola oil	2.45	1.37	2.51	1.51	2.96	1.98
Calcium carbonate	1.31	1.33	1.21	1.22	1.13	1.15
Dicalcium phosphate	0.89	0.93	0.67	0.72	0.49	0.54
Sodium chloride	0.25	0.16	0.21	0.15	0.21	0.10
Sodium bicarbonate	0.11	0.23	0.10	0.18	0.10	0.25
Choline chloride 70%	0.04	0.06	0.04	0.07	0.04	0.06
L-lysine HCl[A]	0.23	0.46	0.22	0.45	0.20	0.43
D,L-methionine	0.36	0.41	0.31	0.36	0.28	0.33
L-threonine	0.15	0.25	0.12	0.21	0.09	0.19
Xylanase[B]	0.01	0.01	0.01	0.01	0.01	0.01
Phytase[C]	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin premix[D]	0.09	0.09	0.08	0.08	0.08	0.08
Mineral premix[E]	0.11	0.11	0.10	0.10	0.10	0.10
L-valine	–	0.11	–	0.09	–	0.07
L-arginine	–	0.22	–	0.21	–	0.21
L-isoleucine	–	0.10	–	0.08	–	0.08
Calculated composition (%)						
AMEn[F] (kcal/kg)	3000	3000	3075	3075	3150	3150
Crude protein	23.20	21.20	21.38	19.38	19.44	17.44
Crude fat	4.47	3.46	4.65	3.71	5.13	4.22
Crude fiber	2.91	2.74	2.80	2.63	2.68	2.52
Dig. arginine	1.37	1.37	1.23	1.23	1.09	1.09
Dig. lysine	1.28	1.28	1.15	1.15	1.02	1.02
Dig. methionine	0.65	0.67	0.59	0.61	0.53	0.55
Dig. cysteine	0.30	0.28	0.29	0.26	0.27	0.25
Dig. M + C[G]	0.95	0.95	0.87	0.87	0.80	0.80
Dig. tryptophan	0.28	0.24	0.26	0.22	0.23	0.20
Dig. histidine	0.51	0.44	0.47	0.40	0.42	0.35
Dig. phenylalanine	1.00	0.87	0.93	0.79	0.84	0.71
Dig. leucine	1.67	1.47	1.62	1.43	1.49	1.30
Dig. isoleucine	0.88	0.86	0.82	0.78	0.74	0.70
Dig. threonine	0.86	0.86	0.77	0.77	0.68	0.68
Dig. valine	0.97	0.96	0.91	0.87	0.83	0.78
Dig. glycine	0.77	0.67	0.70	0.60	0.63	0.53
Calcium	0.96	0.96	0.86	0.86	0.78	0.78
Available phosphorus	0.48	0.48	0.43	0.43	0.39	0.39
Sodium	0.20	0.20	0.18	0.18	0.18	0.18
Potassium	1.01	0.88	0.92	0.79	0.84	0.70

(*Continued on next page*)

Table 1. (*Continued*).

Parameter	Starter		Grower		Finisher	
	SP	RP	SP	RP	SP	RP
Chloride	0.25	0.25	0.23	0.24	0.22	0.21
Linoleic acid	1.56	1.29	1.63	1.38	1.74	1.49

[A]The supplemental amino acids contained the following energy (AME), crude protein (CP), and amino acid: L-lysine HCl: 4063 kcal/kg AME, 95% CP, 78% digestible lysine; D,L-methionine: 4635 kcal/kg AME, 58.7% CP, 99% digestible methionine; L-threonine: 3560 kcal/kg AME, 73.5% CP, 98% digestible threonine; L-valine: 5255 kcal/kg AME, 72.1% CP, 96.5% digestible valine; L-arginine: 2940 kcal/kg AME, 201% CP, 99% digestible arginine; L-isoleucine: 5617 kcal/kg AME, 66.0% CP, 99% digestible isoleucine.
[B]Econase XT, 25 (AB Vista, 16 000 BXU/kg of diet).
[C]Quantum Blue, 5G (AB Vista, 500 FTU/kg of diet).
[D]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.
[E]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.
[F]AMEn, apparent metabolisable energy corrected to zero N retention.
[G]Methionine + cysteine.
SP, diet contained standard protein concentrations at 23.2%, 21.4% and 19.4% crude protein for starter, grower and finisher phases respectively. RP, diet had reduced protein contents, with two percentage points lower crude protein than in the SP diets in all feeding phases. Dig, standard ileal digestible amino acid coefficients as determined by near-infra red spectroscopy (Foss NIR 6500, Denmark) standardised with Evonik AMINONIR® Advanced calibration.

results. As data on livability and intestinal lesion scores were found to be not normally distributed, they were tested for significance using the Kruskal–Wallis non-parametric test and were not subjected to two-way ANOVA. The *P*-value of <0.05 was considered significant, and a tendency was considered at $0.05 \leq P \leq 0.10$.

Results

Diet and growth performance

Generally, the final diets satisfied formulation objectives in terms of achieving reduced CP diets with lower CP concentrations than in the SP diets. The analysed CP concentrations of all starter diets were higher than expected, with only a one-percentage point difference between the SP and RP diet; however, those of grower and finisher diets were as expected, with a two-percentage point difference between the SP and RP diets. The analysed AA contents of the diets were generally consistent with the calculated values (Tables 1, 2). The analysed lysine concentrations of the RP diets were lower than the recommended total lysine concentrations for Ross 308 broilers (Aviagen 2014*b*), but similar to those of the SP diets.

Performance results are presented in Tables 3 and 4. The NE × protein interactions were not observed for performance parameters from Day 0 to Day 10 ($P > 0.05$; Table 3). From Day 10 to Day 24, NE × protein interactions were detected for FI ($P < 0.001$), BWG ($P < 0.01$) and FCR ($P < 0.01$), indicating higher performance with the RP diet than with the SP diet in NE-challenged birds (Table 3). Under NE-challenge conditions, birds fed the RP had higher FI ($P < 0.001$), higher BWG ($P < 0.001$) and lower FCR ($P < 0.001$) than did those fed the SP diet from Day 10 to Day 24. The NE × protein interactions were also observed for FI ($P < 0.05$) and FCR ($P < 0.001$) in the overall period (Days 0–35; Table 4). Birds fed the RP diet had similar FI ($P > 0.05$) but lower FCR ($P < 0.001$) than did those fed the SP diet only when the birds were not challenged with NE from Day 0 to Day 35. In NE-challenged birds, FI and FCR were not different between birds fed the RP and SP diets from 0 to 35 days ($P > 0.05$). NE challenge reduced FI in birds fed the SP diets but not in birds fed the RP diets from Day 0 to Day 35.

Necrotic-enteritis challenge as the main effect did not affect growth performance during the starter phase (Days 0–10) as expected, as *Eimeria* gavaging was not applied until Day 9, but the challenge decreased ($P < 0.05$) FI and BWG and increased ($P < 0.05$) FCR during the grower (Days 10–24), finisher (Days 24–35), and overall periods (Tables 3, 4). Birds challenged with NE had 12% lower FI (1186 g vs 1348 g), 23% lower BWG (796 g vs 1037 g), and 19 points higher FCR (1.492 vs 1.300) than did unchallenged birds during the grower phase ($P < 0.001$; Table 3). Birds fed the RP diet had a higher FI and BWG than did those offered the SP diet during the starter phase ($P < 0.05$), as shown by the main effect of protein concentration. Protein concentration as the main effect did not affect FI, BWG and FCR during the finisher phase ($P > 0.05$). Neither NE challenge nor protein concentration affected livability ($P > 0.05$; Tables 3, 4).

Birds in the RP+, RPA+ and RPC+ treatments had a higher BWG and lower FCR than did those in the SP+ treatment in the grower phase ($P < 0.001$; Table 3). Additionally, birds in the RP+ and RPC+ treatments had a higher FI than did those in the SP+ treatment in the grower phase ($P < 0.001$; Table 3).

Table 2. Analysed nutrient values of experimental diets (as-fed basis).

Nutrient composition (%)	Starter				Grower				Finisher			
	SP	RP	RPA	RPC	SP	RP	RPA	RPC	SP	RP	RPA	RPC
Dry matter	87.2	87.6	87.6	87.9	87.7	87.4	87.7	87.5	86.7	87.7	87.2	87.1
Gross energy (kcal/kg)	3979	3956	3938	3958	4004	3940	3939	3934	3972	3959	3948	3952
Crude protein	24.30	23.12	23.35	23.44	20.35	19.03	18.51	18.58	18.95	16.70	16.41	16.57
Crude fiber	2.86	2.64	2.89	2.94	2.71	2.80	2.52	2.48	3.19	2.74	3.21	2.68
Ash	4.98	4.90	4.85	4.85	4.42	4.61	4.39	4.26	4.15	4.04	3.77	3.82
Arginine	1.41	1.44	1.48	1.23	1.19	1.19	1.22	0.96	1.09	1.06	1.09	0.85
Citrulline	–	–	–	0.23	–	–	–	0.26	–	–	–	0.24
Lysine	1.32	1.33	1.32	1.34	1.12	1.13	1.14	1.14	1.05	0.95	0.95	1.01
Methionine	0.55	0.65	0.63	0.48	0.48	0.57	0.54	0.45	0.44	0.47	0.47	0.47
Histidine	0.61	0.55	0.55	0.55	0.51	0.44	0.45	0.44	0.48	0.38	0.39	0.39
Phenylalanine	1.19	1.07	1.09	1.10	1.01	0.86	0.87	0.87	0.94	0.74	0.75	0.78
Leucine	1.88	1.69	1.72	1.76	1.68	1.44	1.46	1.49	1.57	1.25	1.29	1.34
Isoleucine	1.00	0.97	0.97	0.99	0.85	0.79	0.80	0.80	0.79	0.67	0.68	0.71
Threonine	0.99	0.97	0.98	0.99	0.83	0.82	0.81	0.82	0.78	0.69	0.70	0.73
Valine	1.12	1.11	1.12	1.13	0.97	0.91	0.91	0.91	0.90	0.77	0.79	0.81
Glycine	0.98	0.89	0.88	0.91	0.82	0.70	0.71	0.72	0.77	0.60	0.62	0.65
Serine	1.17	1.06	1.06	1.09	0.98	0.83	0.84	0.85	0.91	0.71	0.73	0.77
Glutamic acid	5.13	4.91	5.02	5.02	4.13	3.63	3.72	3.70	3.88	3.22	3.33	3.45
Proline	1.66	1.61	1.64	1.64	1.33	1.21	1.23	1.22	1.25	1.09	1.12	1.15
Alanine	1.03	0.93	0.94	0.95	0.96	0.83	0.84	0.84	0.90	0.72	0.74	0.76
Tyrosine	0.61	0.56	0.55	0.46	0.51	0.45	0.46	0.34	0.48	0.38	0.40	0.34
Aspartic acid	2.14	1.86	1.85	1.89	1.85	1.47	1.51	1.51	1.70	1.22	1.23	1.28

Values of all amino acids presented are total amino acids. SP, the diet containing standard protein concentrations at 23.2%, 21.4% and 19.4% crude protein for starter, grower and finisher phases respectively. RP, the diet containing reduced protein concentrations, with two percentage points lower crude protein than in the SP diets in all feeding phases. RPA, the RPA diet was created by adding L-arginine on top of the RP diet at the level of 0.03% in all feeding phases. Level of calculated supplemental L-arginine in RPA diet in starter, grower and finisher was 0.25%, 0.25% and 0.24% respectively. RPC, the RPC diet was created by replacing all supplemental L-arginine in RPA diet with L-citrulline.

During the finisher phase, FI, BWG and FCR were not different among the SP+, RP+, RPA+ and RPC+ treatments ($P > 0.05$; Table 4). Birds in the RPA+ treatment had 7% lower FI (3107 g vs 3348 g, $P = 0.01$), 7% lower BWG (2088 g vs 2247 g, $P < 0.01$), but similar FCR ($P > 0.05$) to those in the RP+ treatment from Day 0 to Day 35. Birds in the RPC+ treatment had lower FCR (approximately three points) than did birds in the SP+ treatment from Day 0 to Day 35 (1.469 vs 1.497, $P < 0.05$).

Serum uric acid concentration and intestinal lesion score

A NE × protein interaction was detected for serum uric acid concentration on Day 16 ($P < 0.01$; Table 5), indicating that serum uric acid was decreased in birds fed the SP diet when they were challenged with NE. The one-way ANOVA results showed that birds in the RP+, RPA+ and RPC+ treatments had lower serum uric acid concentration on Day 16 than did those in the SP+ treatment ($P < 0.01$; Table 5). Necrotic-enteritis challenge as the main effect increased lesion scores of both duodenum ($P < 0.01$), jejunum ($P < 0.001$) and ileum ($P < 0.001$) on Day 16 (Fig. 1). Intestinal lesion scores on Day 16 were not different between birds fed SP or RP diets ($P > 0.05$; Fig. 1). The one-way ANOVA results showed that intestinal lesion scores on Day 16 were not different among the SP+, RP+, RPA+ and RPC+ treatments ($P > 0.05$; Fig. 1).

Carcass traits and internal organ weights

Results on relative weights of the carcass on Day 35 as well as relative weights of liver, spleen and bursa of Fabricius on Day 16 and Day 35 are shown in Tables 5 and 6. No NE × protein interactions were observed for relative weights of breast, thigh and drumstick, and fat pad on Day 35 ($P > 0.05$; Table 5). Necrotic-enteritis challenge as the main effect reduced relative weights of the breast ($P < 0.01$), increased relative

Table 3. Growth performance and livability in starter (Days 0–10) and grower (Days 10–24) phases.

Effect	Days 0–10				Days 10–24			
	FI (g)	BWG (g)	FCR	Livability (%)	FI (g)	BWG (g)	FCR	Livability (%)
Two-way ANOVA results (2 × 2 factorial arrangement of treatments)								
Treatment								
SP–	331	310	1.069	99.2	1403c	1024c	1.369b	98.3
RP–	341	319	1.068	98.3	1294b	1051c	1.231a	100.0
SP+	328	305	1.075	98.3	1136a	750a	1.514d	95.8
RP+	337	318	1.061	99.2	1275b	876b	1.455c	96.6
NE								
No	336	315	1.069	98.8	1348b	1037b	1.300a	99.2
Yes	332	311	1.069	98.7	1186a	796a	1.492b	96.2
Protein								
SP	330a	308a	1.072	98.7	1269	887	1.442a	97.1
RP	339b	319b	1.065	98.8	1287	987	1.313b	98.3
s.e.m.	2	2	0.003	0.5	23	27	0.023	0.8
P-value								
NE	0.328	0.337	0.930	0.934	<0.001	<0.001	<0.001	0.082
Protein	0.031	0.008	0.188	0.934	0.784	0.063	0.003	0.502
NE × protein	0.902	0.690	0.285	NA	<0.001	0.002	0.006	NA
One-way ANOVA results (four NE-challenged treatments)								
Treatment								
SP+	328	305	1.075	98.3	1136a	750a	1.514b	95.8
RP+	337	318	1.061	99.2	1275b	876b	1.455a	96.6
RPA+	334	311	1.075	99.2	1194ab	828b	1.442a	99.2
RPC+	327	310	1.056	98.3	1228b	861b	1.426a	99.1
s.e.m.	2	2	0.003	0.5	13	11	0.009	0.8
P-value	0.382	0.355	0.119	0.824	<0.001	<0.001	<0.001	0.440

Symbols – and + indicate absence or presence respectively, of necrotic-enteritis challenge in the treatments. Two-way ANOVA presents results of the 2 × 2 factorial arrangement of treatments, with the main factors being necrotic-enteritis challenge (NE, yes or no) and protein concentration (SP or RP). Different letters within a column indicate significant differences between the means. NA, *P*-values for two-way ANOVA analysis were not available for livability as the data were not normally distributed. SP, the diet containing standard protein concentrations at 23.2%, 21.4% and 19.4% crude protein for starter, grower and finisher phases respectively. RP, the diet containing reduced protein concentrations, with two percentage points lower crude protein than in SP diets in all feeding phases. RPA, the RPA diet was created by adding L-arginine on top of the RP diet at the level of 0.03% in all feeding phases. Level of calculated supplemental L-arginine in RPA diet in starter, grower and finisher was 0.25%, 0.25% and 0.24% respectively. RPC, the RPC diet was created by replacing all supplemental L-arginine in RPA diet with L-citrulline.

weight of thigh and drumstick ($P < 0.05$), and tended to increase relative fat pad weight on Day 35 ($P = 0.079$; Table 5). Protein concentration as the main effect did not affect relative weights of breast, thigh and drumstick, and fat pad on Day 35 ($P > 0.05$; Table 5). The relative breast weight of birds in the RPC+ treatment was higher than that of SP+ birds on Day 35 ($P < 0.001$; Table 5).

No NE × protein interactions were detected for relative weights of the liver, spleen, and bursa of Fabricius on day 16 and day 35 ($P > 0.05$; Table 6). Necrotic enteritis challenge as the main effect increased relative weight of the liver on day 35 ($P < 0.05$; Table 6). Birds fed the RP diet tended to have lower relative bursa of Fabricius weight on day 16 ($P = 0.062$), and higher relative liver weight on day 35 ($P < 0.05$) compared to those of SP fed birds regardless of the NE challenge. Arginine or Cit supplementation to the RP+ diet did not affect relative weights of liver, spleen, and bursa of Fabricus in the respective groups on day 16 and day 35 ($P > 0.05$; Table 6).

Discussion

The NE challenge established in the current study was effective in decreasing FI and BWG and increasing FCR in grower, finisher and overall growth periods without affecting livability. These results are in agreement with previous reports (Sharma *et al.* 2018; Hilliar *et al.* 2020;

Table 4. Growth performance and livability in finisher phase (Days 24–35) and in the overall period (Days 0–35).

Effect	Days 24–35				Days 0–35			
	FI (g)	BWG (g)	FCR	Livability (%)	FI (g)	BWG (g)	FCR	Livability (%)
Two-way ANOVA results (2 × 2 factorial arrangement of treatments)								
Treatment								
SP–	1798	1086	1.656	100.0	3454b	2408	1.434b	97.5
RP–	1813	1086	1.670	99.0	3374b	2461	1.374a	97.5
SP+	1714	1016	1.688	100.0	3135a	2095	1.497c	94.1
RP+	1769	1033	1.713	98.9	3348ab	2247	1.490c	95.0
NE								
No	1807b	1086b	1.664a	99.5	3414b	2437b	1.404a	97.5
Yes	1740a	1024a	1.700b	99.4	3242a	2171a	1.493b	94.6
Protein								
SP	1750	1046	1.674	100.0	3326	2283	1.459	95.8
RP	1793	1062	1.690	98.9	3364	2383	1.420	96.3
s.e.m.	16	11	0.005	0.4	37	36	0.012	1.0
P-value								
NE	0.038	0.004	<0.001	0.964	0.017	<0.001	<0.001	0.267
Protein	0.190	0.496	0.153	0.151	0.624	0.162	0.097	0.781
NE × protein	0.522	0.678	0.527	NA	0.022	0.216	<0.001	NA
One-way ANOVA results (four NE-challenged treatments)								
Treatment								
SP+	1714	1016	1.688	100.0	3135a	2095a	1.497b	94.1
RP+	1769	1033	1.713	98.9	3348b	2247b	1.490ab	95.0
RPA+	1687	988	1.708	97.7	3107a	2088a	1.488ab	96.7
RPC+	1750	1036	1.689	97.9	3237ab	2204ab	1.469a	95.8
s.e.m.	15	10	0.006	0.6	29	21	0.004	1.1
P-value	0.187	0.280	0.348	0.485	0.010	0.008	0.032	0.848

Symbols – and + indicate absence or presence respectively, of necrotic-enteritis challenge in the treatments. Two-way ANOVA presents results of the 2 × 2 factorial arrangement of treatments, with the main factors being necrotic-enteritis challenge (NE, yes or no) and protein concentration (SP or RP). Different letters within a column indicate significant differences between the means. NA, *P*-values for two-way ANOVA analysis were not available for livability as the data were not normally distributed. SP, the diet containing standard protein concentrations at 23.2%, 21.4% and 19.4% crude protein for starter, grower and finisher phases respectively. RP, the diet containing reduced protein concentrations, with two percentage points lower crude protein than in the SP diets in all feeding phases. RPA, the RPA diet was created by adding L-arginine on top of the RP diet at the level of 0.03% in all feeding phases. Level of calculated supplemental L-arginine in RPA diet in starter, grower and finisher was 0.25%, 0.25% and 0.24% respectively. RPC, the RPC diet was created by replacing all supplemental L-arginine in RPA diet with L-citrulline.

Zanu *et al.* 2020*a*). Damaged intestinal epithelial cells, reduced nutrient absorption, and loss of appetite have been considered as the main reasons for reduced growth performance in NE-challenged birds (Cooper *et al.* 2013; Amerah and Ravindran 2015; Kraieski *et al.* 2017). This was confirmed in the current study, as shown by increased intestinal lesion scores in the grower phase due to the NE challenge. Furthermore, the results of the current study showed that NE challenge increased the relative weight of liver on Day 35. A similar result was observed by Ugwuoke and Pewan (2020) in *Eimeria*-challenged birds. The increased relative liver weight in NE-challenged birds in the current study might be attributed to an increase in metabolic activities as a result of bacterial infection (Ogbe *et al.* 2008; Mustapha *et al.* 2017). This process needs a certain amount of time that might explain the absence of difference in liver weight between NE-challenged and non-challenged groups on Day 16 in the current study. All changes observed in NE-challenged birds suggest that subclinical NE was successfully established in the current study.

Subclinical NE can cause more economic problems to poultry producers than does the acute form as it reduces the meat yield in a greater number of birds and increases carcass condemnation at slaughter (Løvland and Kaldhusdal 1999; Lovland and Kaldhusdal 2001). In the current study, the subclinical NE challenge reduced the relative weights of the breast, increased the relative weight of thigh and

Table 5. Serum uric acid concentration on Day 16, and relative carcass weights per unit of bodyweight on Day 35.

Effect	Serum uric acid concentration (mg/dL)	Relative carcass weight (g/kg bodyweight)		
		Breast	Thigh and drumstick	Abdominal fat
Two-way ANOVA results (2 × 2 factorial arrangement of treatments)				
Treatment				
SP–	10.09c	193	198	9.22
RP–	5.68ab	192	198	10.54
SP+	6.78b	174	205	10.93
RP+	4.89a	185	204	11.20
NE				
No	7.89b	192b	198a	9.88
Yes	5.84a	180a	205b	11.06
Protein				
SP	8.44b	184	202	10.08
RP	5.29a	188	201	10.87
s.e.m.	0.40	2	1	0.34
P-value				
NE	0.012	0.002	0.015	0.079
Protein	<0.001	0.298	0.809	0.247
NE × protein	0.003	0.101	0.926	0.426
One-way ANOVA results (four NE-challenged treatments)				
Treatment				
SP+	6.78b	174a	205	10.93
RP+	4.89a	185ab	204	11.20
RPA+	4.21a	179ab	210	11.82
RPC+	4.36a	192b	209	10.78
s.e.m.	0.26	2	2	0.28
P-value	0.002	0.007	0.522	0.603

Symbols − and + indicate the absence or presence of necrotic enteritis challenge in the treatments. Two-way ANOVA presents results of the 2 × 2 factorial arrangement of treatments, with the main factors being necrotic-enteritis challenge (NE, yes or no) and protein concentration (SP or RP). Different letters within a column indicate significant differences between the means. SP, the diet containing standard protein concentrations at 23.2%, 21.4% and 19.4% crude protein for starter, grower and finisher phases respectively. RP, the diet containing reduced protein concentrations, with two percentage points lower crude protein than in the SP diets in all feeding phases. RPA, the RPA diet was created by adding L-arginine on top of the RP diet at the level of 0.03% in all feeding phases. Level of calculated supplemental L-arginine in RPA diet in starter, grower and finisher was 0.25%, 0.25% and 0.24% respectively. RPC, the RPC diet was created by replacing all supplemental L-arginine in RPA diet with L-citrulline.

drumstick, and tended to increase tje relative fat pad weight on Day 35. As relative carcass weights were calculated by dividing the absolute weights by the BW and the absolute weights of both breast, thigh and drumstick in NE-challenged birds were lower than those in the unchallenged birds ($P < 0.001$, data no presented), these results reflect that the weight loss might occur more severely and rapidly in the breast and other parts of the body than the in the thigh and drumstick in the NE-challenged birds. Xue *et al.* (2017) reported reduced relative weights of breast, thigh and drumstick but no difference in relative fat pad weight as a result of NE challenge compared with unchallenged controls. A reduction in feed consumption results in a decreasing glucose production that may induce skeletal muscle to catabolise gluconeogenic AA to provide energy for maintenance and this may reduce muscle protein accretion (Wu *et al.* 1991). The latter phenomena were observed in the current study, as illustrated by the reduced FI, with the subsequent effects on reduced BWG, and relative breast yield in NE-challenged birds. Additionally, literature evidence has shown that the fat accumulation in the liver and adipose tissues is regulated by the concentration of serum lipids such as cholesterol and triglyceride and lipoproteins (Mossab *et al.* 2002; Afsharmanesh *et al.* 2013). Thus, lowering total cholesterol and triglyceride concentrations has been considered the most effective way to decrease fat accumulation (Yang *et al.* 2014). Previous studies have shown that NE challenge increases concentrations of total cholesterol

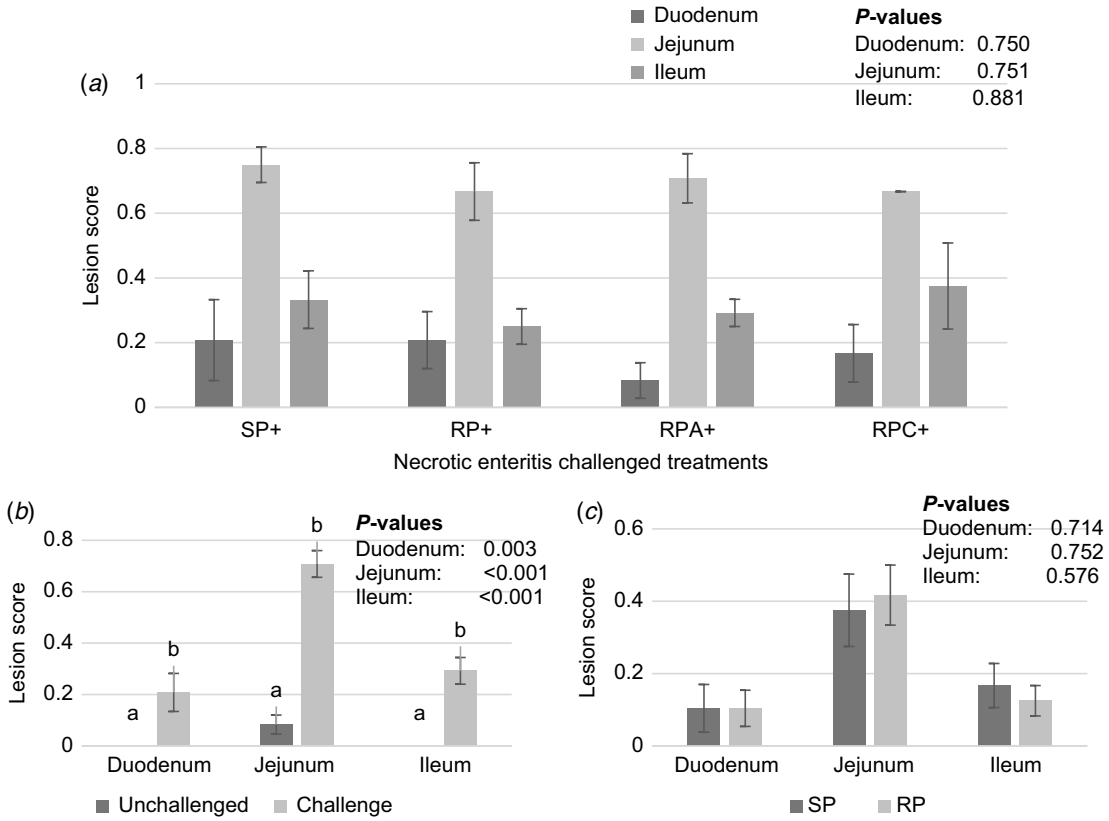

Fig. 1. Effects of necrotic-enteritis challenge and dietary treatments on intestinal lesion scores on Day 16. The bars represent means and error bars present standard errors of the means. (*a*) Intestinal lesion scores of necrotic enteritis-challenged treatments. (*b*) The main effect of necrotic-enteritis challenge on intestinal lesion scores. (*c*) The main effect of dietary protein concentration on intestinal lesion scores. SP, the diet containing standard protein concentrations at 23.2%, 21.4% and 19.4% crude protein for starter, grower and finisher phases respectively; RP, the diet containing reduced protein concentrations, with two percentage points lower crude protein than in the SP diets in all feeding phases. RPA, the RPA diet was created by adding L-arginine on top of the RP diet at the level of 0.03% in all feeding phases. Level of calculated supplemental L-arginine in RPA diet in starter, grower and finisher was 0.25%, 0.25% and 0.24% respectively. RPC, the RPC diet was created by replacing all supplemental L-arginine in RPA diet with L-citrulline.

and low-density lipoprotein cholesterol and downregulates gene expression of lipoprotein lipase and peroxisome proliferator-activated receptor γ that mediates adipocyte differentiation and maturation, resulting in an increased rate of abdominal fat in the respective group (Zhou *et al.* 2016; Qing *et al.* 2017). These facts may explain the increased relative fat pad weight in NE-challenged birds compared with the unchallenged group in the current study.

The NE × protein interactions observed in the current study indicated the beneficial effects of dietary protein reductions on FI, BWG and FCR in birds challenged with NE. Feeding the RP diets increased FI and BWG during the starter phase and better prepared birds for NE challenge, with increased FI, increased BWG, and lower FCR being observed during the main course of NE challenge (grower phase), than in those fed the higher protein levels. These findings confirmed the tested hypothesis in the present study. Broiler diets with high protein concentrations have been shown to facilitate the proliferation of *C. perfringens*, the causative agent of NE (Drew *et al.* 2004; Wilkie *et al.* 2005; Liu *et al.* 2017). High-CP diets are likely to be less digestible than are reduced-protein diets, as the latter have higher additions of crystalline AA (Hilliar *et al.* 2019; Dao *et al.* 2021*a*, 2021*b*). Undigested AA from the small intestine may accumulate in the hindgut, providing nutrients for the proliferation of pathogenic microbes such as *C. perfringens* that may, consequently, influence gut health and growth performance in birds (Lan *et al.* 2004; McDevitt *et al.* 2006). As *C. perfringens* lack the genes responsible for the synthesis of Arg, lysine, methionine, threonine, serine, histidine and branched-chain AA (Shimizu *et al.* 2002), they are highly dependent on feed materials for these nutrients. In contrast to the current findings, Hilliar *et al.* (2020) found a reduced growth rate in NE-challenged birds fed RP diets, with 4.5

Table 6. Relative weights of internal organs on Day 16 and Day 35 as per unit of bodyweight (g/kg).

Effect	Day 16			Day 35		
	Liver	Bursa of Fabricius	Spleen	Liver	Bursa of Fabricius	Spleen
Two-way ANOVA results (2 × 2 factorial arrangement of treatments)						
Treatment						
SP–	29.02	2.12	0.69	22.03	1.49	0.77
RP–	30.48	2.06	0.73	23.17	1.52	0.84
SP+	28.80	2.37	0.76	23.27	1.42	0.81
RP+	29.22	2.08	0.68	25.56	1.39	0.80
NE						
No	29.70	2.09	0.71	22.60a	1.51	0.81
Yes	29.01	2.22	0.72	24.49b	1.41	0.80
Protein						
SP	28.91	2.24	0.73	22.61a	1.45	0.79
RP	29.81	2.07	0.71	24.36b	1.46	0.82
s.e.m.	0.30	0.05	0.02	0.44	0.06	0.02
P-value						
NE	0.266	0.148	0.795	0.027	0.428	0.906
Protein	0.141	0.062	0.634	0.042	0.457	0.498
NE × protein	0.392	0.210	0.133	0.464	0.821	0.367
One-way ANOVA results (four NE-challenged treatments)						
Treatment						
SP+	28.80	2.37	0.76	23.27	1.42	0.81
RP+	29.22	2.08	0.68	25.56	1.39	0.80
RPA+	30.32	2.08	0.79	26.03	1.43	0.92
RPC+	29.89	2.13	0.75	24.38	1.68	0.86
s.e.m.	0.30	0.05	0.02	0.44	0.06	0.02
P-value	0.302	0.134	0.350	0.119	0.232	0.151

Symbols – and + indicate absence or presence of necrotic-enteritis challenge in the treatments. Two-way ANOVA presents results of the 2 × 2 factorial arrangement of treatments with the main factors were necrotic-enteritis challenge (NE, yes or no) and protein concentration (SP or RP). Different letters within a column indicate significant differences between the means. SP, the diet containing standard protein concentrations at 23.2%, 21.4% and 19.4% crude protein for starter, grower and finisher phases respectively. RP, the diet containing reduced protein concentratoins, with two percentage points lower crude protein than in the SP diets in all feeding phases. RPA, the RPA diet was created by adding L-arginine on top of the RP diet at the level of 0.03% in all feeding phases. Level of calculated supplemental L-arginine in RPA diet in starter, grower and finisher was 0.25%, 0.25% and 0.24% respectively. RPC, the RPC diet was created by replacing all supplemental L-arginine in RPA diet with L-citrulline.

and 4 percentage points lower CP concentrations in the grower and finisher phases respectively, than in those offered SP diets. In the current study, CP concentrations of the RP diets were only two percentage points lower than were thos of the SP diets in all feeding phases. The difference in protein reductions best explains these dichotomous results between the studies. The NE × protein interaction on serum uric acid in the current study showed that serum uric acid was decreased in birds fed the SP diet only when they were challenged with NE. The decrease in FI in the challenged SP fed birds compared to the unchallenged SP group might reduce substrate for uric acid production, then serum uric acid concentration as a consequence. Furthermore, it may be worth noting that NE-challenged birds fed RP diet or RP diet supplemented with Arg or Cit had lower serum uric acid concentrations than did those offered the SP diet in the current study. As serum uric acid is a product of AA degradation (Namroud *et al.* 2008); this finding may re-affirm the higher protein utilisation efficiency in birds fed the RP diet than in those fed the SP diet. Besides, the lower relative bursa of Fabricius weight observed on Day 16 and the higher relative liver weight on Day 35 in RP-fed birds than in those offered the SP diet in the current study may suggest differential effects of dietary protein level on internal organ weights in birds.

The main objective of the current study was to determine whether feeding RP diets supplemented with Arg or Cit would

improve the growth performance and gut health of birds experimentally induced with subclinical NE, compared with the birds fed SP diets. Dietary Arg supplementation has been shown to reduce growth loss and alleviate gut damage in chickens challenged with *C. perfringens*, infectious bronchitis virus and *Eimeria* (Tan *et al.* 2014; Laika and Jahanian 2017; Zhang *et al.* 2018). The results of the current study showed that Cit supplementation to the RP diet further increased the beneficial effects of the RP diet on the growth performance and promoted recovery in NE-challenged birds. The same supplementation with Arg had only minor effects. During the experimental period, feeding-challenged birds with the RPC diet reduced the FCR compared with those fed the SP diets. Additional supplementation of Arg to the RP diet reduced FI and BWG in the challenged birds by 7%, compared with challenged birds fed the RP diet from Day 0 to Day 35. No differences in FI, BWG and FCR were observed by Hilliar *et al.* (2020) when a reduced-CP diet with either 115% essential AA, 100%, or 115% of both essential and non-essential AA were fed to NE-challenged birds. Thus, the lack of response to additional supplementation of Arg in the current study might be attributed to reduced digestion and absorption capacity in NE-challenged birds, and/or low levels of Arg supplementation in the RPA diet. Subclinical NE is likely to alter the requirement for Arg (and other essential AA) relative to breeder company recommendations targeted at healthy flocks. Kidd and Tillman (2016) pointed out that the threonine requirement was greatly increased in broilers with subclinical enteric infection. Deficiencies of aspartate, asparagine and alanine have been reported to increase the requirements of Arg, choline, methionine, threonine and branched-chain AA in NE-challenged birds in an attempt to maintain muscle accretion (Wu 2014). Supplemental Arg is known to be degraded by arginase in the intestinal mucosa, thereby limiting its presence in plasma while Cit can escape this degradation and, once absorbed, Cit may be converted to Arg in the kidney, thereby increasing plasma Arg concentration (McCarty 2010). Studies on mammals have shown that dietary Cit supplementation results in a higher plasma Arg and NO production than does supplementation with Arg at the same dose (Schwedhelm *et al.* 2008; Lassala *et al.* 2009; Wijnands *et al.* 2012). These facts may explain the positive effects of Cit over Arg supplementation on the growth performance in challenged birds in the current study.

It has been reported that supplementation of either meat and bone meal, antibiotic, phytase, or phytogenic feed additives does not affect relative weights of the breast, leg, and fat pad in NE-challenged birds (Cho *et al.* 2014; Zanu *et al.* 2020*b*). In the current study, Cit supplementation in the RP diet was effective in increasing breast meat yield in NE-challenged birds. This information may be important to the poultry producers who may want to reduce the economic loss caused by subclinical NE infection in the flock where the disease symptoms are not easy to diagnose. Besides, dietary Arg supplementation has been reported to reduce *C. perfringens* count, intestinal lesion scores, and mucosal damage in *C. perfringens* challenged birds by modulating innate immune responses, enhancing gut integrity, and promote NO production (Zhang *et al.* 2017, 2018). However, the beneficial effects of either Arg or Cit supplementation on gut NE lesion scores were not observed in the current study. The differences in experimental design, diet composition, dosage use, and chicken age may explain the dichotomous results between the studies.

Conclusions

An advantage of Cit over Arg supplementation was demonstrated and it is likely to be attributed to its escape from degradation by arginase present in enterocytes. It can be concluded that feeding the RP diet supplemented with Cit was beneficial in promoting gut health and recovery from the NE challenge. Further work is warranted to determine optimal levels of Cit to support growth and immune status in birds subjected to disease stress such as the NE challenge.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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