Effects of reduced crude protein levels, dietary electrolyte balance, and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein, and amino acid metabolism

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ABSTRACT The crude protein (**CP**) content of 4 isoenergetic, maize-based diets containing 11.00 g/kg digestible lysine was reduced in gradations from 200 to 156 g/kg with increasing inclusions of synthetic, or unbound, essential amino acids. A constant dietary electrolyte balance (**DEB**) of 230 mEq/kg was maintained, but a second 156 g/kg CP diet had a DEB of 120 mEq/kg, and energy densities of the 156 g/kg CP diet were reduced in the sixth and seventh treatments. Each of the 7 dietary treatments were offered to 7 replicate cages (6 birds/cage) or a total of 294 Ross 308 off-sex male broilers from 14 to 35 D posthatch. Reductions in CP from 200 to 156 g/kg did not influence weight gain but quadratically increased feed conversion ratio (**FCR**) and linearly increased relative abdominal fat-pad weights and feed intakes. The reduction in DEB did not influence growth performance but did adversely influence some amino acid digestibilities. Reducing energy density by 100 kcal/kg did not influence growth performance of birds offered the 156 g/kg CP diet but numerically reduced fat-pad weights. The transition from 200 to 156 g/kg CP diets generally enhanced jejunal and ileal amino acid digestibility coefficients but had diverse effects on free amino acid concentrations in systemic plasma with a remarkable 116% increase in threonine. Starch:protein disappearance rate ratios linearly increased in the jejunum and the ileum following the same transition, and these expanding ratios were related to heavier fat-pads and compromised FCR. This study indicates that reductions in dietary CP from 200 to 172 g/kg supported by inclusions of unbound essential amino acids do not compromise growth performance, but a further reduction to 156 g/kg CP significantly increased FCR. Both heavier relative fat-pad weights and inferior FCR were related to expanding starch:protein disappearance rate ratios, which suggests condensed dietary starch:protein ratios may advantage birds offered reduced CP diets.

Key words: amino acids, broiler chickens, dietary electrolyte balance, reduced crude protein, starch

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INTRODUCTION

To meet the increasing demand for chicken meat, global production may need to double by the year 2050. Consequently, there is considerable interest in the successful development of reduced crude protein (**CP**) diets for broiler chickens to promote sustainable production. An attenuation of the soybean meal demand is just one reason why reduced-CP diets would be advantageous. However, tangible reductions in dietary CP usually compromise broiler performance despite inclusions of unbound (crystalline or synthetic) amino acids to meet requirements. For example, dietary CP reductions from 210 to 195 and 180 g/kg did not statistically influence feed conversion ratio (**FCR**) from 14 to 35 D posthatch, but the further reduction from 180 to 165 g/kg CP compromised FCR by 4.55% in a previous study (Chrystal et al., 2019). In addition, dietary CP reductions from 210 to 165 g/kg linearly increased relative fat-pad weights, and fat-pad weights were linearly related to FCR, thus dietary CP reductions compromise

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FCR with associated increases in fat deposition. The implication is that broilers fed reduced CP diets consume relatively more energy than protein and deposit this excess of energy as lipid (Gous et al., 1990).

Waldroup (2007) listed several reasons for the poor performance of broilers that were offered reduced CP diets, including an insufficient nitrogen pool for synthesis of nonessential amino acids, imbalances among amino acids, and an inadequate knowledge of amino acid requirements. This researcher also suggested that decreases in dietary electrolyte balance (**DEB**) associated with reduced CP diets could be a contributing factor. According to Johnson and Karunajeewa (1985), a DEB between 250 and 300 mEq/kg is ideal for broiler diets, but weight gains will be compromised by a DEB of less than 180 mEq/kg. Therefore, the influence of DEB on performance is investigated in this study by offering broilers a 156 g/kg CP diet with either adjusted or non-adjusted DEB of 230 or 120 mEq/kg, respectively.

Reductions in dietary CP from 236 to 190 g/kg generated increased fat deposition by 32.9% in broilers offered iso-energetic diets across 3 experiments in Bregendahl et al. (2002). The concept of maintaining constant energy:protein ratios in reduced CP broiler diets has been investigated by Kamran et al. (2008). It follows that discounts in energy density of reduced CP diets could negate increased fat deposition which would, in turn, benefit FCR. In this experiment, the impact of reducing metabolizable energy densities of the 156 g/kg CP diet from 3,071 to 2,971 and 2,870 kcal/kg is investigated, and it is relevant that these energy density reductions would be economically advantageous.

The quantity of starch relative to protein is axiomatically greater in reduced CP diets than conventional diets. This impacts on their digestive dynamics including starch and protein digestion, absorption of glucose and amino acids along the small intestine, and the transition of glucose and amino acids across enterocytes to enter the portal circulation (Liu and Selle, 2017). The metabolic fates of glucose and amino acids in the gut mucosa finally determine their postenteral bioavailability (Selle and Liu, 2019). The bilateral bioavailability of glucose and amino acids, expressed as starch:protein disappearance rate ratios, have been shown to be related to broiler performance (Liu et al., 2015; Sydenham et al., 2017).

Thus, the objectives of this experiment are to offer broiler chickens maize-based diets with declining CP concentrations of 200, 188, 172, and 156 g/kg, which amounts to a "dose titration study" with a focus on starch-protein digestive dynamics. Therefore, apparent digestibility coefficients and disappearance rates of starch and protein (N) and their disappearance rate ratios in 2 small intestinal segments are determined. In addition, apparent jejunal and ileal amino acid digestibility coefficients and concentrations of free amino acids in systemic plasma are monitored. The impacts of manipulating DEB and energy density of the 156 g/kg CP diet, as outlined, are determined to ascertain if these manipulations will improve performance of birds fed a tangibly reduced CP diet.

MATERIALS AND METHODS

The present study is one of a series to investigate the influence of reduced CP diets on growth performance and nutrient utilization in broiler chickens. Therefore, details of bird management, diet manufacturing, and sample collection were similar to methodology described in Moss et al. (2018) and Chrystal et al. (2019).

Experimental Design

The present study included 7 dietary treatments as outlined in Table 1. CP contents of the first 4 treatments were decreased in a step-wise manner from 200 to 156 g/kg and maintained at 156 g/kg in the final 3 treatments. The inclusions of synthetic or unbound essential amino acids increased from 6.0 to 24.9 g/kg to meet requirements. The DEB was maintained at 230 mEq/kg in all diets with the exception of Diet 5 which had an "unadjusted" DEB of 120 mEq/kg. The DEB was calculated from dietary concentrations and molecular weight of sodium (**Na**), potassium (**K**), and chlorine (**Cl**) by the following equation (Ahmad and Sarwar, 2006):

$$\begin{aligned}\mathrm{DEB(mEq/kg)} = {} & \mathrm{Na(g/kg)} \times 1{,}000/23.0 + \mathrm{K(g/kg)} \\ & \times 1{,}000/39.1 - \mathrm{Cl\,(g/kg)} \\ & \times 1{,}000/35.5.\end{aligned}$$

The DEB was adjusted by dietary inclusions of sodium chloride, sodium bicarbonate, and potassium carbonate as shown in Table 2. Diet 6 and 7 contained reduced metabolizable energy levels of 2,971 and 2,870 kcal/kg, respectively, as opposed to 3,071 kcal/kg

Table 1. Outline of dietary treatments.

		Specified levels			Analyzed concentrations		
Diet	Unbound amino acids (g/kg)	Protein (g/kg)	DEB (mEq/kg)	AME (kcal/kg)	Starch (g/kg)	Protein (g/kg)	Ratio (g/g)
1	6.0	200	230	3,071	302.5	204.1	1.48
2	6.8	188	230	3,071	322.2	183.3	1.76
3	11.7	172	230	3,071	356.0	173.7	2.05
4	24.9	156	230	3,071	398.5	156.8	2.54
5	24.9	156	120	3,071	407.9	156.5	2.61
6	24.9	156	230	2,971	339.0	155.0	2.19
7	24.9	156	230	2,870	308.0	160.5	1.92

Abbreviation: AME, apparent metabolizable energy; DEB, dietary electrolyte balance.

Table 2. Composition of experimental diets.

Feed ingredient (g/kg)	1	2	3	4	5	6	7
Maize	560	602	659	719	727	741	614
Soybean meal	329	289	233	171	170	168	165
Oats	-	-	-	-	-	-	131
Vegetable oil	49.7	42.7	32.8	22.4	19.4	3.82	-
Lysine HCl	1.622	2.850	4.558	6.454	6.476	6.509	6.507
d,*l*-methionine	2.897	3.249	3.742	4.296	4.288	4.275	4.305
l-threonine	0.974	1.533	2.311	3.178	3.181	3.185	3.202
l-tryptophan	-	-	0.202	0.533	0.537	0.542	0.497
l-valine	0.673	1.364	2.326	3.400	3.401	3.403	3.333
l-arginine	-	0.454	2.080	3.886	3.903	3.929	3.737
l-isoleucine	0.235	0.930	1.898	2.974	2.982	2.992	2.957
l-leucine	-	-	-	1.239	1.220	1.193	1.454
l-histidine	-	-	-	0.319	0.317	0.314	0.375
Sodium chloride	4.009	2.426	0.222	-	4.253	-	4.086
Sodium bicarbonate	0.010	2.401	5.730	6.187	-	6.194	-
Potassium carbonate	-	-	-	2.615	-	2.655	7.406
Limestone	7.25	7.17	7.06	6.93	6.94	6.96	7.14
Dicalcium phosphate	20.29	20.91	21.77	22.75	22.73	22.70	22.32
Choline chloride (60%)	0.900	0.900	0.900	0.900	0.900	0.900	0.900
Celite	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin-mineral premix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00

[1] The vitamin-mineral premix supplied per tonne of feed: [MIU] retinol 12, cholecalciferol 5, [g] tocopherol 50, menadione 3, thiamine 3, riboflavin 9, pyridoxine 5, cobalamin 0.025, niacin 50, pantothenate 18, folate 2, biotin 0.2, copper 20, iron 40, manganese 110, cobalt 0.25, iodine 1, molybdenum 2, zinc 90, selenium 0.3.

in the balance of the treatments. Diet 7 included 131 g/kg oats to facilitate this further reduction in energy density. Dietary starch:protein ratios expanded from a minimum of 1.48 in Diet 1 to a maximum of 2.61 in Diet 5 based on analyzed starch and protein concentrations.

Diet Preparation

Before diet formulation, soybean meal and maize were characterized by near-infrared spectroscopy (Evonik Nutrition and Care GmbH, Hanau, Germany). Maize was coarsely ground (6.0 mm hammer-mill screen) before incorporation into the complete diets. All diets were steam-pelleted using a Palmer PP330 pellet press (Palmer Milling Engineering, Griffith, NSW, Australia) at a conditioning temperature of 80°C with a residence time of 14 s. Acid insoluble ash (Celite, Celite Corporation. Lompoc, CA) was included in diets at 20 g/kg as an inert dietary marker to determine starch, protein (N), and amino acid digestibility coefficients.

Importantly, diets were formulated so that standardized ileal digestible lysine concentrations remained constant at 11.0 g/kg across all dietary treatments. Minimum "ideal protein ratios", as recommended by Wu (2014), remained constant for essential amino acids relative to lysine. The diet compositions and nutrient specifications are shown in Table 2 and Table 3, respectively and analyzed amino acid concentrations of the experimental diets are shown in Table 4.

Bird Management

This feeding study was approved by the Research Integrity and Ethics Administration of The University of Sydney (Project number 2016/973). A total of 336 off-sex, male Ross 308 chicks were procured from a commercial hatchery and were initially offered a standard starter diet. At 14 D posthatch, birds were individually identified (wing-tags) and allocated into bioassay cages on the basis of BW. There was no statistical difference on the average BW for each cage at the commencement of the study. Each of the dietary treatments was offered to 7 replicate cages (6 birds/cage) or a total of 294 chicks from 14 to 35 D post-hatch. Broilers had unlimited access to water and feed under a "23-h-on-1-h-off" lighting regime for the first 3 D and then under a "16-h-on-8-h-off" lighting regime for the remainder of the study. Room temperature was maintained at 32°C for the first week, then gradually decreased to 22°C by the end of the third week and maintained at the same temperature until the end of the feeding study. BW and feed intakes were recorded from 14 to 35 D posthatch from which FCR were calculated. The incidence of dead or culled birds was recorded daily, and their BW was used to correct feed intakes and adjust FCR calculations.

Sample Collection and Chemical Analysis

Total excreta were collected from 31 to 33 D posthatch from each cage to determine parameters of nutrient utilization which included apparent metabolizable energy (**AME**), metabolizable energy to gross energy (**GE**) ratios (**ME:GE**), nitrogen retention, and N-corrected apparent metabolizable energy (**AMEn**). Excreta were dried in a forced-air oven at 80°C for 24 h, and GE of diets and excreta were determined by bomb calorimetry using an adiabatic calorimeter (Parr 1281 bomb calorimeter, Parr Instruments Co., Moline, IL).

Table 3. Nutrient specifications of experimental diets.

Nutrient (g/kg)	1	2	3	4	5	6	7
Metabolizable energy (kcal/kg)	3,071	3,071	3,071	3,071	3,071	2,971	2,870
Crude protein	200	188	172	156	156	156	156
True protein	182	171	157	143	143	143	143
Fat	73.5	67.1	57.9	48.1	45.4	30.4	31.0
Crude fibre	21.6	21.2	20.5	19.8	19.9	20.1	33.7
Acid detergent fibre	33.7	33.1	32.2	31.0	31.2	31.6	47.8
Neutral detergent fibre	84.5	85.0	85.1	84.9	85.6	86.7	113.3
Calcium	8.25	8.25	8.25	8.25	8.25	8.25	8.25
Total phosphorus	7.35	7.29	7.20	7.09	7.11	7.13	7.01
Available phosphorus	4.13	4.13	4.13	4.13	4.13	4.13	4.13
Sodium	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Potassium	9.30	8.53	7.45	7.72	6.24	7.74	10.49
Chloride	3.06	2.35	1.37	1.62	4.18	1.64	4.13
DEB (mEq/kg)	230	230	230	230	120	230	230
Digestible amino acids							
Lysine	11.0	11.00	11.00	11.00	11.00	11.00	11.00
Methionine	5.51	5.68	5.91	6.17	6.17	6.16	6.18
Methionine + cysteine	8.14	8.14	8.14	8.14	8.14	8.14	8.14
Threonine	7.37	7.37	7.37	7.37	7.37	7.37	7.37
Tryptophan	2.12	1.91	1.82	1.82	1.82	1.82	1.82
Isoleucine	7.70	7.70	7.70	7.70	7.70	7.70	7.70
Leucine	14.52	13.52	12.12	11.77	11.77	11.77	11.77
Arginine	12.15	11.44	11.44	11.44	11.44	11.44	11.44
Valine	8.80	8.80	8.80	8.80	8.80	8.80	8.80
Histidine	4.80	4.42	3.90	3.63	3.63	3.63	3.63

Abbreviation: DEB, dietary electrolyte balance.

The AME values were calculated on a DM basis from the following equation:

$$\mathrm{AME_{diet}} = \frac{(\text{Feed intake } \times \mathrm{GE_{diet}}) - (\text{Excreta output } \times \mathrm{GE_{excreta}})}{(\text{Feed intake})}$$

ME:GE ratios were calculated by dividing AME by the GE of the appropriate diets. N contents of diets and excreta were determined using a nitrogen analyser (Leco Corporation, St Joseph, MI), and N retentions were calculated from the following equation:

$$\text{Retention } (\%) = \frac{(\text{Feed intake } \times \text{Nutrient}_{\text{diet}}) - (\text{Excreta output } \times \text{Nutrient}_{\text{excreta}})}{(\text{Feed intake} \times \text{Nutrient}_{\text{diet}})} \times 100$$

N-corrected AME (AMEn MJ/kg DM basis) values were calculated by correcting N retention to zero using the factor of 8.73 kcal/g N retained in the body (Hill and Anderson, 1958).

At 34 D posthatch, 3 birds at random were selected from each cage, and blood samples were taken from the brachial vein before euthanasia. Blood samples were then pooled and centrifuged, and the decanted plasma samples were then kept at −80°C before analysis. Concentrations of 20 proteinogenic amino acids in plasma taken from the brachial and anterior mesenteric veins were determined using precolumn derivatization amino acid analysis with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters AccQTag Ultra; Waters Corporation, Milford, MA) followed by separation of the derivatives and quantification by reversed phase ultra-performance liquid chromatography (Cohen and Michaud, 1993).

At day 35, birds were euthanized by intravenous injections of sodium pentobarbitone, and abdominal cavities were opened. The small intestine was removed, and digesta samples were collected in their entirety from the distal jejunum and distal ileum. The jejunum was demarcated by the end of the duodenal loop and Meckel's diverticulum and the ileum by Meckel's diverticulum and the ileo-cecal junction. Digesta was taken from the segment posterior to the respective midpoints. Digesta samples from birds within a cage were pooled, homogenized, freeze-dried, and ground through 0.5 mm screen. Then, the samples were analyzed for the content of starch, protein, and amino acids. Starch concentration was determined by a procedure based on dimethyl sulfoxide, α-amylase, and amyloglucosidase, as described in Mahasukhonthachat et al., 2010. Nitrogen concentrations were determined as outlined by Siriwan et al. (1993). Amino acid concentrations of diets and digesta were determined following 24-h liquid hydrolysis at 110°C in 6 mol HCl, and then 16 amino acids are analyzed using the Waters AccQTag Ultra chemistry

Table 4. Analyzed amino acid concentrations of experimental diets.

Amino acid (g/kg)	1	2	3	4	5	6	7
Arginine	13.21	11.75	11.78	11.29	11.23	11.13	11.65
Histidine	5.28	4.61	4.28	3.76	3.80	3.75	3.88
Isoleucine	9.02	8.45	8.42	8.40	8.15	8.08	8.22
Leucine	16.91	14.86	14.32	12.83	13.14	13.11	13.26
Lysine	12.52	11.64	11.68	11.37	11.17	11.01	11.52
Methionine	5.33	5.54	5.49	6.24	5.73	5.67	5.58
Phenylalanine	10.03	8.70	8.13	6.39	6.54	6.44	6.76
Threonine	8.43	7.86	7.91	7.74	7.62	7.41	7.67
Valine	10.44	9.78	9.79	9.59	9.50	9.44	9.68
Alanine	10.01	8.84	8.51	7.08	7.29	7.25	7.30
Aspartic acid	20.97	17.66	16.17	12.37	12.47	12.37	13.15
Cysteine	3.22	2.88	2.75	2.32	2.36	2.31	2.51
Glutamic acid	35.69	30.66	28.73	22.70	23.09	23.18	24.13
Glycine	8.39	7.21	6.66	5.33	5.35	5.34	5.76
Proline	11.25	9.52	9.64	8.05	8.00	8.09	8.05
Serine	9.94	8.44	8.05	6.29	6.44	6.33	6.72
Total amino acids	190.6	168.4	162.3	141.8	141.9	140.9	145.8

(Waters) on a Waters Acquity UPLC. Acid insoluble ash (**AIA**) was included in the diets at 20 g/kg as an inert marker. Apparent digestibility coefficients of starch, protein (N), and amino acids were calculated by the following equation:

$$\text{Digestibility Coefficient} = \frac{(\text{Nutrient}/\text{AIA})_{\text{diet}} - (\text{Nutrient}/\text{AIA})_{\text{digesta}}}{(\text{Nutrient}/\text{AIA})_{\text{diet}}}$$

Starch and protein (N) disappearance rates (g/bird/D) were deduced from feed intakes over the final phase of the feeding period from the following equation:

$$\text{Disappearance rate}_{(\text{g/bird/day})} = \text{Feed intake}_{\ (\text{g/bird})} \times \text{Dietary nutrient}_{(\text{g/kg})} \times \text{Digestibility coefficient.}$$

Statistical Analysis

Experimental data were subject to one-way analyses of variance, including pair-wise comparisons, using the IBM SPSS Statistics, Version 24 program (IBM Corporation. Somers, NY). Selected experimental data were subject to linear regressions, when appropriate, including feed intakes, relative fat-pad weights, parameters of nutrient utilization, starch disappearance rates, N digestibility coefficients and disappearance rates, starch:protein disappearance rate ratios, and amino acid digestibility coefficients. The FCR outcomes were subject to quadratic regressions. Multiple linear regressions were used to relate starch:protein disappearance rate ratios to FCR and relative fat-pad weights. Cage means were the experimental units and a probability level of less than 5% was considered statistically significant.

RESULTS

The effects of dietary treatments on growth performance and relative abdominal fat-pad weights are shown in Table 5. The transition from 200 to 156 g/kg CP diets increased feed intakes by 5.12%, from 2,888 to 3,036 g/bird, in a linear manner ($r = -0.433$; $P < 0.025$) and compromised FCR by 8.96%, from 1.495 to 1.629. Reduced CP diets quadratically compromised FCR ($r = 0.821$; $P < 0.001$) as the transition from 172 to 156 g/kg CP increased FCR by 7.03% from 1.522 to 1.629, which was significant ($P < 0.001$) based on a pair-wise comparison. Weight gain and FCR of birds offered Diet 7 were significantly inferior to their Diet 4 counterparts by 6.33 and 8.35%, respectively. The transition from 200 to 156 g/kg CP diets increased relative fat-pad weights by 70.8%, from 7.26 to 12.40 g/kg, which was a linear increase ($r = -0.840$; $P < 0.001$). Adjusting DEB did not significantly influence growth performance or relative fat-pad weights when Diets 4 and 5 are

Table 5. Effect of dietary treatments on growth performance, relative abdominal fat-pad weights, and mortality rates from 14 to 35 D posthatch.

	Growth performance				
Treatment	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Abdominal fat-pad (g/kg)	Mortality rate (%)
1. 200 g/kg CP	$1{,}934^{b}$	$2{,}888^{a}$	1.495^{a}	7.26^{a}	7.1
2. 188 g/kg CP	$1{,}931^{b}$	$2{,}896^{a,b}$	1.500^{a}	8.49^{a}	7.1
3. 172 g/kg CP	$1{,}912^{b}$	$2{,}907^{a,b}$	1.522^{a}	10.13^{b}	2.4
4. 156 g/kg CP	$1{,}879^{b}$	$3{,}036^{b,c}$	1.629^{b}	12.40^{c}	7.1
5. 120 mEq/kg DEB	$1{,}869^{b}$	$3{,}027^{a,b,c}$	1.621^{b}	12.20^{c}	9.5
6. 2,971 kcal/kg	$1{,}864^{b}$	$3{,}096^{c}$	1.648^{b}	$11.44^{b,c}$	9.5
7. 2,870 kcal/kg	$1{,}760^{a}$	$3{,}107^{c}$	1.765^{c}	10.10^{b}	4.8
SEM	36.00	50.45	0.0169	0.5117	3.378
Significance (P=)	0.024	0.006	<0.001	<0.001	0.667
LSD ($P < 0.05$)	102.7	144.0	0.0482	1.460	-
Linear/quadratic	$r = 0.282$[1]	$r = -0.433$[1]	$r = 0.821$[2]	$r = -0.840$[1]	$r = 0.063$[1]
Effect Diets 1 to 4	$P = 0.146$	$P = 0.021$	$P < 0.001$	$P < 0.001$	$P = 0.750$

[a-c] Means within columns not sharing common suffixes are significantly different at the 5% level of probability.

Abbreviations: DEB, dietary electrolyte balance; FCR, feed conversion ratio; LSD, low stocking density.

[1] Linear.

[2] Quadratic.

Table 6. Effect of dietary treatments on nutrient utilization [apparent metabolizable energy (AME), metabolizable:gross energy ratio (ME:GE), nitrogen (N) retention, N-corrected AME (AMEn)] from 31 to 33 D posthatch.

Treatment	AME (kcal/kg DM)	ME:GE ratio	N retention (%)	AMEn (kcal/kg DM)
1. 200 g/kg CP	3,099^{b}	0.769^{a}	66.10^{a}	2,851^{b,c}
2. 188 g/kg CP	3,159^{b}	0.795^{a,b,c}	71.11^{b}	2,893^{c,d}
3. 172 g/kg CP	3,196^{b}	0.816^{c,d,e}	75.32^{c,d}	2,909^{c,d}
4. 156 g/kg CP	3,162^{b}	0.823^{d,e}	76.55^{d}	2,914^{c,d}
5. 120 mEq/kg DEB	3,182^{b}	0.829^{e}	75.10^{c,d}	2,953^{d}
6. 2,971 kcal/kg	2,980^{a}	0.802b^{c,d}	72.73^{b,c}	2,766^{a,b}
7. 2,870 kcal/kg	2,953^{a}	0.789^{a,b}	75.48^{c,d}	2,695^{a}
SEM	34.42	0.0091	1.3139	29.80
Significance (P=)	<0.001	<0.001	<0.001	<0.001
LSD ($P < 0.05$)	98.2	0.0261	3.750	85.1
Linear effect	$r = -0.384$	$r = -0.813$	$r = -0.841$	$r = -0.364$
Diets 1 to 4	$P = 0.044$	$P < 0.001$	$P < 0.001$	$P = 0.057$

$^{a\text{-}e}$Means within columns not sharing common suffixes are significantly different at the 5% level of probability.

Abbreviations: DEB, dietary electrolyte balance; LSD, low stocking density.

compared. The overall 6.8% mortality rate was not related ($P > 0.65$) to treatment.

The outcomes for parameters of nutrient utilization are shown in Table 6. The transition from 200 to 156 g/kg CP diets linearly increased AME ($r = -0.384$; $P < 0.05$), ME:GE ratios ($r = -0.813$; $P < 0.001$), N retention ($r = -0.841$; $P < 0.001$), and tended to increase AMEn ($r = -0.364$; $P = 0.057$) in a linear manner. The 100 kcal reduction in dietary energy density (Diets 6 vs. 4) significantly reduced AME by 182 kcal, N retention by 3.82 percentage units, and AMEn by 148 kcal. The 201 kcal reduction in dietary energy density (Diets 7 vs. 4) significantly reduced AME by 209 kcal, ME:GE ratios by 4.13%, and AMEn by 219 kcal. Dietary electrolyte balance (Diets 4 vs. 5) did not significantly influence nutrient utilization parameters.

The effects of dietary treatments on starch digestibility coefficients and disappearance rates are shown in Table 7. Both jejunal (0.919) and ileal (0.998) starch digestibility coefficients were uniformly of a high order and not influenced by treatment. However, the transition from 200 to 156 g/kg protein diets linearly increased starch disappearance rates in both the jejunum ($r = -0.993$; $P < 0.001$) and ileum ($r = -0.950$; $P < 0.001$). Starch disappearance rates were increased by 41.1% (53.34 vs. 37.81 g/bird/D) in the jejunum and by 38.7% (57.54 vs. 41.48 g/bird/D) in the ileum when birds offered Diets 1 and 4 are compared. The DEB did not influence any starch parameters, but starch disappearance rates significantly declined in birds offered reduced energy density diets.

Table 8 shows treatment effects on protein (N) digestibility coefficients and disappearance rates and starch:protein disappearance rate ratios in 2 intestinal segments. The transition from 200 to 156 g/kg protein diets linearly increased protein (N) digestibility coefficients in the jejunum ($r = -0.678$; $P < 0.001$) and ileum ($r = -0.609$; $P < 0.001$). When Diets 1 and 4 are compared, protein (N) digestibility significantly increased by 11.9% (0.754 vs. 0.674) in the jejunum and by 10.3% (0.843 vs. 0.764) in the ileum. In comparison to Diet 4, jejunal and ileal protein (N) digestibility coefficients were not influenced by Diets 5 and 6 but were significantly lower in Diet 7. In contrast to digestibilities, protein (N) disappearance rates linearly declined in the jejunum ($r = 0.382$; $P < 0.05$) and ileum ($r = 0.514$; $P < 0.01$), as a result of lower dietary protein

Table 7. Effect of dietary treatments on apparent starch digestibility coefficients and starch disappearance rates (g/bird/D) in the distal jejunum and distal ileum at 35 D posthatch.

Dietary treatment	Distal jejunum		Distal ileum	
	Digestibility	Disappearance	Digestibility	Disappearance
1. 200 g/kg CP	0.909	37.81^{a}	0.997	41.48^{a}
2. 188 g/kg CP	0.910	40.41^{a}	0.997	44.32^{b}
3. 172 g/kg CP	0.920	45.33^{b,c}	0.998	49.20^{c}
4. 156 g/kg CP	0.926	53.34^{d}	0.999	57.54^{d}
5. 120 mEq/kg DEB	0.901	52.90^{d}	0.998	58.69^{d}
6. 2,971 kcal/kg	0.920	45.97^{c}	0.998	49.90^{c}
7. 2,870 kcal/kg	0.945	43.08^{b}	0.999	45.52^{b}
SEM	0.0110	0.9178	0.0004	0.8833
Significance (P=)	0.137	<0.001	0.150	<0.001
LSD ($P < 0.05$)	-	2.619	-	2.521
Linear effect	$r = -0.227$	$r = -0.993$	$r = -0.432$	$r = -0.950$
Diets 1 to 4	$P = 0.245$	$P < 0.001$	$P = 0.022$	$P < 0.001$

$^{a\text{-}d}$Means within columns not sharing common suffixes are significantly different at the 5% level of probability.

Abbreviations: LSD, low stocking density; DEB, dietary electrolyte balance.

Table 8. Effect of dietary treatments on apparent protein (N) digestibility coefficients and disappearance rates (g/bird/D) in the distal jejunum and distal ileum and starch:protein disappearance rate ratios in distal jejunum and distal ileum at 35 D posthatch.

	Distal jejunum		Distal ileum		Starch:protein (N) disappearance rate ratio	
Treatment	Digestibility	Disappearance	Digestibility	Disappearance	Distal jejunum	Distal jejunum
1. 200 g/kg CP	0.674^{a}	$18.30^{b,c}$	0.764^{a}	20.65^{b}	2.08^{a}	2.01^{a}
2. 188 g/kg CP	0.716^{b}	18.91^{c}	$0.823^{b,c}$	21.72^{b}	2.14^{a}	2.04^{a}
3. 172 g/kg CP	$0.740^{c,d}$	18.54^{c}	$0.833^{b,c}$	20.87^{b}	2.45^{b}	2.36^{b}
4. 156 g/kg CP	0.754^{d}	16.85^{a}	0.843^{c}	18.84^{a}	3.17^{d}	3.06^{d}
5. 120 mEq/kg DEB	$0.724^{b,c,d}$	16.11^{a}	$0.825^{b,c}$	18.38^{a}	3.28^{d}	3.19^{e}
6. 2,971 kcal/kg	$0.740^{c,d}$	$17.01^{a,b}$	$0.825^{b,c}$	18.97^{a}	2.70^{c}	2.63^{c}
7. 2,870 kcal/kg	0.700^{a}	16.57^{b}	0.804^{b}	19.04^{a}	2.61^{c}	2.39^{b}
SEM	0.0116	0.4537	0.0111	0.4147	0.0507	0.0316
Significance (P=)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD ($P < 0.05$)	0.0332	1.295	0.0318	1.184	0.145	0.090
Linear effect	$r = -0.678$	$r = 0.382$	$r = -0.609$	$r = 0.514$	$r = -0.908$	$r = -0.918$
Diets 1 to 4	$P < 0.001$	$P = 0.045$	$P < 0.001$	$P = 0.005$	$P < 0.001$	$P < 0.001$

[a-e]Means within columns not sharing common suffixes are significantly different at the 5% level of probability.
Abbreviations: LSD, low stocking density; DEB, dietary electrolyte balance.

(N) concentrations. Protein (N) disappearance rates significantly decreased by 7.92% (16.85 vs. 18.30 g/bird/D) in the jejunum and by 8.77% (18.84 vs. 20.65 g/bird/D) in the ileum when diets 1 and 4 are compared. As a consequence, the transition from 200 to 156 g/kg protein diets linearly increased starch:protein disappearance rate ratios in the jejunum ($r = -0.908$; $P < 0.001$) and ileum ($r = -0.918$; $P < 0.001$). Jejunal starch:protein disappearance rate ratios increased from 2.08 in birds offered 200 g/kg CP diets to 3.17 in their 156 g/kg CP counterparts, and the corresponding increase in the ileum was from 2.01 to 3.06.

The effects of dietary treatments on jejunal amino acid digestibility coefficients are shown in Table 9, where significant impacts were observed for 14 of the total of 16 amino acids assessed, with phenylalanine and aspartic acid being the 2 exceptions. Jejunal digestibilities of 14 amino acids were linearly increased to significant extents pursuant to the transition from 200 to 156 g/kg CP diets. The mean jejunal amino acid digestibility coefficient from the 156 g/kg CP diet was 9.38% superior (0.758 vs. 0.693) to birds offered the 200 g/kg CP diet, and a linear increase was observed ($r = -0.685$; $P < 0.001$). The 110 mEq/kg reduction in DEB (Diets 5 vs. 4) significantly depressed the jejunal digestibilities of histidine (4.62%), isoleucine (3.49%), leucine (4.17%), lysine (1.77%), methionine (5.53%), valine (3.55%), glutamic acid (3.03%), glycine (5.13%), and proline (6.80%), where the percentage reductions are in

Table 9. Effect of dietary treatments on apparent digestibility coefficients of amino acids in distal jejunum at 35 D posthatch.

Treatment	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine
1	0.798^{a}	0.738	0.719^{a}	0.739^{a}	0.766^{a}	0.563^{a}	0.728	0.636^{a}
2	0.809^{a}	0.748	0.750^{b}	$0.742^{a,b}$	$0.790^{a,b}$	0.646^{b}	0.731	0.677^{b}
3	0.835^{b}	0.759	0.780^{c}	$0.763^{b,c}$	$0.816^{b,c}$	$0.680^{b,c}$	0.752	$0.726^{c,d}$
4	0.869^{d}	0.779	0.832^{e}	0.792^{d}	0.848^{d}	0.760^{e}	0.756	0.763^{d}
5	0.855^{c}	0.743	$0.803^{c,d}$	$0.759^{a,b,c}$	$0.833^{c,d}$	$0.718^{c,d}$	0.732	$0.736^{c,d}$
6	$0.850^{b,c}$	0.758	$0.811^{d,e}$	$0.777^{c,d}$	$0.830^{c,d}$	0.720^{d}	0.742	$0.742^{c,d}$
7	$0.841^{b,c}$	0.743	$0.791^{c,d}$	0.768^{c}	$0.807^{b,c}$	0.676^{b}	0.725	0.714^{c}
SEM	0.0066	0.0095	0.0090	0.0098	0.0094	0.0137	0.0096	0.0013
Significance (P=)	<0.001	0.064	<0.001	0.006	<0.001	<0.001	0.182	<0.001
LSD ($P < 0.05$)	0.0109	-	0.0257	0.0230	0.02629	0.0392	-	0.0324
Linear effect	$r = -0.845$	$r = -0.526$	$r = -0.860$	$r = -0.636$	$r = -0.769$	$r = -0.857$	$r = -0.439$	$r = -0.826$
Diets 1 to 4	$P < 0.001$	$P = 0.005$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.022$	$P < 0.001$

Treatment	Valine	Alanine	Aspartic acid	Cysteine	Glutamic acid	Glycine	Proline	Serine
1	0.709^{a}	0.696^{a}	0.689	0.519	0.776	0.638	$0.696^{a,b,c}$	$0.673^{a,b}$
2	0.740^{b}	0.698^{a}	0.700	0.556	0.775	0.652	$0.691^{a,b}$	$0.676^{a,b}$
3	0.770^{c}	$0.735^{b,c}$	0.727	0.582	0.79	0.669	$0.726^{a.b,d}$	0.714^{c}
4	0.816^{e}	0.750^{c}	0.719	0.574	0.791	0.663	$0.721^{c,d}$	$0.701^{b,c}$
5	$0.787^{c,d}$	$0.722^{a,b,c}$	0.695	0.559	0.767	0.629	0.672^{a}	$0.676^{a,b}$
6	$0.800^{d,e}$	0.739^{c}	0.703	0.559	0.781	0.643	$0.707^{b,c,d}$	$0.678^{a,b}$
7	$0.778^{c,d}$	$0.705^{a,b}$	0.690	0.537	0.778	0.620	$0.688^{a,b}$	0.661^{a}
SEM	0.0095	0.0109	0.0099	0.0153	0.0079	0.0122	0.0105	0.0108
Significance (P=)	<0.001	0.0044	0.069	0.097	0.283	0.074	0.011	0.019
LSD ($P < 0.05$)	0.0270	0.0310	-	-	-	-	0.0292	0.0308
Linear effect	$r = -0.830$	$r = -0.612$	$r = -0.443$	$r = -0.424$	$r = -0.344$	$r = -0.289$	$r = -0.395$	$r = -0.409$
Diets 1 to 4	$P < 0.001$	$P = 0.001$	$P = 0.021$	$P = 0.027$	$P = 0.079$	$P = 0.143$	$P = 0.042$	$P = 0.034$

[a-e]Means within columns not sharing common suffixes are significantly different at the 5% level of probability.
Abbreviation: LSD, low stocking density.

Table 10. Effect of dietary treatments on apparent digestibility coefficients of amino acids in distal ileum at 35 D posthatch.

Treatment	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine
1	0.857^{a}	0.793	0.797^{a}	0.802	0.808^{a}	0.669^{a}	0.787	0.665^{a}
2	0.900^{b}	0.810	0.831^{a,b}	0.818	0.833^{a,b}	0.739^{b,c}	0.806	0.711^{b}
3	0.906^{b,c}	0.811	0.832^{a,b}	0.821	0.847^{b}	0.738^{b}	0.802	0.735^{b,c}
4	0.925^{d}	0.820	0.866^{b}	0.837	0.858^{b}	0.811^{d}	0.800	0.770^{c}
5	0.919^{c,d}	0.801	0.837^{b}	0.825	0.849^{b}	0.769^{b,c,d}	0.783	0.738^{b,c}
6	0.915^{b,c,d}	0.823	0.861^{b}	0.841	0.855^{b}	0.784^{c,d}	0.798	0.760^{c}
7	0.908^{b,c}	0.806	0.847^{b}	0.832	0.840^{b}	0.769^{b,c,d}	0.786	0.737^{b,c}
SEM	0.0055	0.0120	0.0137	0.0127	0.0103	0.0158	0.0123	0.0142
Significance (P=)	<0.001	0.625	0.021	0.377	0.026	$P < 0.001$	0.772	<0.001
LSD ($P < 0.05$)	0.0157	-	0.0390	-	0.0294	0.0452	-	0.0406
Linear effect	$r = -0.727$	$r = -0.288$	$r = -0.523$	$r = -0.361$	$r = -0.566$	$r = -0.710$	$r = -0.118$	$r = -0.700$
Diets 1 to 4	$P < 0.001$	$P = 0.137$	$P = 0.004$	$P = 0.059$	$P = 0.002$	$P < 0.001$	$P = 0.549$	$P < 0.001$

Treatment	Valine	Alanine	Aspartic aci^{d}	Cysteine	Glutamic aci^{d}	Glycine	Proline	Serine
1	0.763^{a}	0.753	0.733	0.599^{a}	0.826	0.694	0.756	0.730^{a,b}
2	0.798^{a,b}	0.765	0.749	0.651^{b}	0.839	0.714	0.764	0.746^{a,b,c}
3	0.811^{b,c}	0.783	0.753	0.666^{b}	0.840	0.716	0.780	0.752^{b,c}
4	0.838^{c}	0.781	0.736	0.666^{b}	0.833	0.691	0.779	0.738^{a,b}
5	0.821^{b,c}	0.766	0.708	0.633^{a,b}	0.819	0.661	0.747	0.710^{a}
6	0.838^{c,d}	0.789	0.742	0.667^{b}	0.840	0.694	0.779	0.739^{a,b}
7	0.819^{b,c}	0.760	0.733	0.619^{a,b}	0.833	0.675	0.758	0.719^{a,b}
SEM	0.0122	0.0145	0.0132	0.0175	0.0100	0.0148	0.0126	0.0144
Significance (P=)	0.001	0.541	0.318	0.049	0.715	0.129	0.364	0.453
LSD ($P < 0.05$)	0.0349	-	-	0.0500	-	-	-	-
Linear effect	$r = -0.633$	$r = -0.301$	$r = -0.033$	$r = -0.436$	$r = -0.092$	$r = 0.034$	$r = -0.294$	$r = -0.079$
Diets 1 to 4	$P < 0.001$	$P = 0.120$	$P = 0.866$	$P = 0.020$	$P = 0.641$	$P = 0.865$	$P = 0.129$	$P = 0.691$

$^{a\text{-}d}$Means within columns not sharing common suffixes are significantly different at the 5% level of probability.
Abbreviation: LSD, low stocking density.

parentheses. The DEB reduction significantly depressed the mean jejunal amino acid digestibility coefficient by 3.69% (0.730 vs. 0.758).

Treatment effects on ileal amino acid digestibility coefficients are shown in Table 10, where significant effects for a total of 7 amino acids were observed. Across the individual amino acids, linear increases were observed for the digestibilities of arginine (7.93%), isoleucine (8.66%), lysine (6.19%), methionine (21.2%), threonine (15.8%), valine (9.83%), and cysteine (11.2%), where the percentage increases in digestibility coefficients of the 156 g/kg vs. the 200 g/kg CP diets are shown in parentheses. The 110 mEq/kg difference in DEB did not impact on ileal amino acid digestibility coefficients nor did reductions in energy density.

Table 11 shows the treatment effects on concentrations of free amino acids in systemic plasma. Total amino acid concentrations in birds offered the 156 g/kg CP diet were significantly higher than their counterparts that were offered 200 g/kg CP by 10.2% (5,563 vs. 5,046 μmol/L; $P < 0.05$). However, there is a real dichotomy in outcomes for individual amino acids when the same comparisons are drawn. The 156 g/kg CP diet generated significantly higher concentrations of lysine (85.9%), methionine (70.0%), threonine (116.4%), and valine (27.3%). In contrast, the 156 g/kg CP diet generated significantly lower concentrations of arginine (30.8%), histidine (30.2%), leucine (17.2%), phenylalanine (17.9%), glycine (38.5%), and serine (21.9%). There were no significant differences for the remaining 6 amino acids, but the magnitude of the increases was such that there was an overall 10.2% increase in total free amino acids in systemic plasma in birds offered the 156 g/kg CP diet.

DISCUSSION

The overall growth performance of birds from 14 to 35 D posthatch in the present study exceeded Ross 308 growth performance guidelines for weight gain (1,879 vs. 1,795 g/bird), feed intake (2,994 vs. 2,964 g/bird), and FCR (1.597 vs. 1.652). Indeed, birds offered the 200 g/kg CP diet outperformed the guidelines with a weight gain of 1,934 g/bird and an FCR of 1.495, which represent advantages of 7.74 and 3.33%, respectively.

In the present study, the reduction in dietary CP from 172 g/kg (Diet 3) to 156 g/kg (Diet 4) compromised FCR by 7.03% (1.629 vs. 1.495). The reduction in CP from 200 to 156 g/kg increased relative fat-pad weights by 69.9% in the present study, which is remarkably similar to the 69.8% increase reported in Chrystal et al. (2019) study, where CP was reduced from 210 to 165 g/kg. The diets in both studies are reasonably alike, and they indicate that while moderate reductions in CP are feasible, further reductions are not because feed conversion efficiency is compromised. A range of what could be described as amino acid or nonamino acid limiting factors have been nominated as possible causes for compromised efficiency of feed conversion and increased fat deposition. Given that the responsible limiting factors can be identified, it well may be possible to rectify them. Based on data generated by Dean et al. (2006) and some subsequent studies, it could be argued that inclusions of synthetic or unbound glycine and serine in the 156 g/kg CP diet may have enhanced bird performance.

Table 11. Effect of dietary treatments on plasma concentrations (μmol/L) of free amino acids in the systemic circulation (brachial vein) at 34 D posthatch.

Treatment	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine
1	412^{a}	63^{d}	111	203^{b}	290^{a}	100^{a}	140^{c}	505^{a}
2	324^{b}	49^{c}	121	203^{b}	275^{a}	119^{a}	140^{c}	$631^{a,b}$
3	286^{b}	$38^{a,b}$	110	158^{a}	318^{a}	123^{a}	120^{b}	715^{b}
4	285^{b}	$44^{b,c}$	128	168^{a}	539^{b}	$170^{b,c}$	$115^{a,b}$	$1,093^{c}$
5	287^{b}	35^{a}	123	161^{a}	563^{b}	187^{c}	103^{a}	$1,086^{c}$
6	313^{b}	$41^{a,b}$	128	172^{a}	525^{b}	$182^{b,c}$	117^{b}	$1,141^{c}$
7	308^{b}	$44^{a,b}$	136	179^{a}	555^{b}	161^{b}	118^{b}	$1,090^{c}$
SEM	20.85	2.50	6.45	7.66	30.05	8.10	4.77	57.25
Significance (P=)	0.001	<0.001	0.077	<0.001	<0.001	<0.001	<0.001	<0.001
LSD ($P < 0.05$)	59.5	7.1	-	22.6	85.7	23.1	13.6	163.4
Linear effect	$r = 0.602$	$r = 0.648$	$r = 0.266$	$r = 0.579$	$r = -0.751$	$r = -0.790$	$r = 0.626$	$r = -0.836$
Diets 1 to 4	$P = 0.001$	$P < 0.001$	$P = 0.172$	$P = 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Treatment	Tryptophan	Valine	Alanine	Aspartate + Asparagine	Glutamic aci^{d}	Glycine	Serine	Tyrosine
1	77	227^{a}	961	$234^{c,d}$	259	675^{d}	575	$211^{a,b,c}$
2	73	246^{a}	1,145	265^{d}	260	581^{c}	508	235^{c}
3	71	237^{a}	1,081	$231^{b,c}$	252	484^{b}	477	$223^{b,c}$
4	81	289^{b}	1,147	$207^{a,b,c}$	250	415^{a}	449	$182^{a,b}$
5	84	287^{b}	1,204	$207^{a,b,c}$	265	$429^{a,b}$	499	165^{a}
6	79	290^{b}	1,182	190^{a}	255	417^{a}	465	173^{a}
7	81	300^{b}	1,116	$199^{a,b}$	244	$475^{a,b}$	511	$183^{a,b}$
SEM	5.18	12.76	66.65	11.74	16.10	23.56	29.48	16.57
Significance (P=)	0.615	<0.001	0.213	0.001	0.976	<0.001	0.092	0.029
LSD ($P < 0.05$)	-	36.4	-	33.5	-	67.2	-	47.3
Linear effect	$r = 0.072$	$r = -0.527$	$r = -0.302$	$r = 0.348$	$r = 0.101$	$r = 0.880$	$r = 0.580$	$r = 0.273$
Diets 1 to 4	$P = 0.717$	$P = 0.004$	$P = 0.118$	$P = 0.070$	$P = 0.608$	$P < 0.001$	$P = 0.001$	$P = 0.160$

$^{a\text{-}d}$Means within columns not sharing common suffixes are significantly different at the 5% level of probability.

Abbreviation: LSD, low stocking density.

However, this approach was deliberately not followed because a study that is focussing on the impact of glycine equivalents has been planned for the near future.

The concept of starch-protein digestive dynamics in the context of reduced CP diets has been considered (Liu and Selle, 2017; Selle et al., 2019), and the validity of starch:protein disappearance rate ratios in relation to broiler performance has been demonstrated by Sydenham et al. (2017). In the present study, the analyzed dietary starch:protein ratios increased from 1.48 in the 200 g/kg CP diet to 2.54 in the 156 g/kg CP diet. Largely as a consequence of this fundamental difference, starch:protein disappearance rate ratios increased from 2.08 to 3.17 in the jejunum and from 2.01 to 3.06 in the ileum in birds offered the corresponding diets. However, the jejunal starch:protein disappearance rate ratios across Diets 1 through 4 were linearly related to both relative fat-pad weights ($r = 0.784$, $P < 0.001$) and FCR ($r = 0\ 792$; $P < 0.001$). Ileal starch:protein disappearance rate ratios across Diets 1 through 4 were also linearly related to both relative fat-pad weights ($r = 0.745$, $P < 0.001$) and FCR ($r = 0\ 767$; $P < 0.001$). Moreover, relative fat-pad weights and FCR were themselves related ($r = 0.605$; $P < 0.001$). Thus, increasing starch:protein disappearance rate ratios are associated with both deteriorating feed efficiency and greater fat deposition.

It is instructive to examine the impacts of jejunal starch and protein disappearance rates in relation to FCR and relative fat-pad weights across all 7 dietary treatments. The relationships between jejunal starch and protein disappearance rates with both FCR ($r = 0.552$; $P < 0.001$) and fat-pad weights ($r = 0.772$; $P < 0.001$) are significant, and the multiple linear regression equations are as follows:

$$\mathrm{y(FCR)} = 2.005 + 0.003 \times \mathrm{starch}_{(\mathrm{g/bird/D})} - 0.032 \times \mathrm{protein}_{(\mathrm{g/bird/D})}$$

$$\mathrm{y}_{(\mathrm{fat-pad\ weights})} = 1.505 + 0.267 \times \mathrm{starch}_{(\mathrm{g/bird/D})} - 0.194 \times \mathrm{protein}_{(\mathrm{g/bird/D})}$$

Thus, an increase in protein disappearance rates combined with a decrease in starch digestion rates, or effectively a greater absorption of amino acids relative to glucose, could both enhance FCR and attenuate fat deposition. These outcomes suggest that higher starch concentrations are impeding the successful development of reduced CP diets and that better growth performance outcomes may result if dietary starch:protein ratios were not permitted to increase to similar extents as was the case in the present and previous studies. One possible means to achieve this objective would be to replace soybean meal with alternative protein sources with lower protein concentrations to reduce dietary CP rather than increase maize. This would restrict increases in maize, and therefore starch, inclusions and limit the expansion of dietary starch:protein ratios.

The DEB of soybean meal is inherently greater than that of maize (Sumanthi et al., 2015); therefore, reducing dietary CP by increasing maize inclusions at the expense of soybean meal will result in a DEB reduction. Borges et al. (2011) found that a reduction in DEB from 240 to 140 mEq/kg depressed 21-D weight gains in broiler chickens, and when left unadjusted, DEB declined from 230 to 120 mEq/kg in the present study. Thus, it was anticipated that this reduction in DEB would compromise growth performance. However, the present study demonstrated that this DEB differential did not impact growth performance in the context of reduced CP diets. Nevertheless, the 110 mEq/kg DEB reduction significantly depressed jejunal digestibilities of 9 amino acids with a 3.69% reduction in the mean digestibility coefficient of 16 amino acids. Ravindran et al. (2008) reported that DEB impacts on the digestibility of amino acids in typical broiler diets, and Adedokun et al. (2017) found that reducing DEB from 208 to 106 mEq/kg generated a 5.24% increase in endogenous amino acid flows in protein-free diets. Thus, reductions in amino acid digestibilities observed in the present study may have stemmed from increased endogenous amino acid flows pursuant to the DEB reduction. Therefore, DEB should be considered in reduced CP diet feeding studies, and it would be prudent to adjust dietary electrolyte levels to maintain a constant DEB across experimental diets given their potential impact on amino acid digestibilities.

The transition from 200 to 156 g/kg CP diets linearly increased AME and ME:GE ratios, which suggests that reduced CP diets have an energy sparing effect. This is plausible as less intact protein requires digestion, and less uric acid needs to be synthesized for N excretion. Increasing dietary energy levels of broiler diets have been shown to increase abdominal fat and carcass fat (Kassim and Suwanpradit, 1996). In the present study, Diet 6 had a lower energy density than Diet 4 by 100 kcal/kg, and this translated to a reduction of 182 kcal/kg AME in birds. Nevertheless, there were no significant differences in growth performance between birds offered Diets 4 and 6, but Diet 6 generated a numerical reduction of 7.74% in relative fat-pad weights. However, growth performance was compromised in birds offered Diet 7, which had a 201 kcal/kg lower energy density relative to Diet 4. Interestingly, Kamran et al. (2008) offered birds maize-based diets from 27 to 35 D posthatch with graded reductions in CP from 200 to 170 g/kg in which the energy-to-protein ratio was maintained constant. This resulted in depressed weight gains and compromised FCR, but abdominal fat deposition was not influenced. Nevertheless, it may be feasible to apply lower energy densities in reduced CP diets without compromising growth performance, and given the potential economic advantages, this possibility should be further investigated.

The dietary transition from 200 to 156 g/kg CP linearly increased the mean jejunal digestibility coefficient of 16 amino acids by 9.38% (0.758 vs. 0.693), which is not as robust as the corresponding increase of 29.4% (0.594 vs. 0.459) reported by Chrystal et al. (2019). Nevertheless, ileal amino acid digestibilities in the 2 studies were similar. Chrystal et al. (2019) observed an increase of 6.18% (0.790 vs. 0.744) in mean ileal digestibility coefficients; whereas, the corresponding increase in the present study was 5.84% (0.797 vs. 0.753). Moreover, these increases in amino acid digestibilities are also similar to those reported by Awad et al. (2016). The proportions of synthetic or unbound or amino acids of total digestible amino acids in the 156 g/kg CP are substantial for methionine (70%), threonine (43%), isoleucine (39%), valine (39%), and lysine (46%) as shown in parentheses. The average amino acid digestibility coefficient was 5.84% higher in the 156 g/kg CP in comparison to the 200 g/kg CP diet; however, this increase was exceeded by the nominated amino acids with increases of 21.2% for methionine, 15.8% for threonine, 9.83% for valine, 8.66% for isoleucine, and 6.19% for lysine. Superficially, it would appear that the notional 100% digestibility of unbound amino acids (Izquierdo et al., 1988) is contributing to the observed increases in amino acid digestibilities.

The impacts of graded reductions in dietary CP on concentrations of free amino acids in systemic plasma in the present study are similar to those observed in Chrystal et al. (2019). Concentrations of lysine, methionine, threonine, and valine increased, and alanine increased numerically, but the free concentrations of most amino acids decreased in both studies. Remarkably, threonine increased by 116.4% (1,093 vs. 505 μmol/L) in the present study with a corresponding increase of 65.9% (1,027 vs. 619 μmol/L) in Chrystal et al. (2019) study. This is not without precedent as Fancher and Jensen (1989) reported an increase of 86.6% in free threonine plasma concentrations following a dietary CP reduction from 183 to 159 g/kg. This raises the possibility that reductions in dietary protein downregulate hepatic threonine dehydrogenase activity (Juan and Austic, 2001; Hilliar et al., 2019), which may account for the observed increases. Endogenous flows of threonine in broiler chickens are substantial (Angkanaporn et al., 1994), and threonine is the dominant amino acid in porcine mucin (Lien et al., 1997). Therefore, the high threonine plasma levels may be indicative of threonine being recycled to the gut mucosa via the systemic circulation to be incorporated into endogenous protein secretions. Additionally, increases in lysine, methionine, threonine, and valine concentrations observed may indicate that they were not being fully utilized for either skeletal muscle accretion or as functional amino acids (Wu, 2009). In addition, the increased lysine concentration may indicate that another amino acid has become limiting and that dietary amino acid profiles may require adjustments.

In conclusion, this study indicates that reductions in dietary CP from 200 to 172 g/kg supported by inclusions of unbound essential amino acids do not compromise growth performance but a further reduction to 156 g/kg CP significantly increased FCR. However, fat deposition was linearly increased by the transition from 200 to 156 g/kg CP diets, and relative fat-pad weights were related to FCR. Both heavier relative fat-pad weights and inferior FCR were related to expanding starch:protein disappearance rate ratios. This suggests that a condensation of dietary starch:protein ratios may advantage birds offered reduced CP diets. An unadjusted decline in DEB from 230 to 120 mEq/kg did not influence growth performance of birds offered 156 g/kg CP diets. It may be feasible to reduce the energy density of reduced CP diets, but this possibility requires further investigation. Clearly, more investigations into the impacts of reduced CP diets in digestibilities and plasma concentrations of amino acids are required given the conflicting responses observed in the present study.

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