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Original Research Article

Capping dietary starch:protein ratios in moderately reduced crude protein, wheat-based diets showed promise but further reductions generated inferior growth performance in broiler chickens

Shiva Greenhalgh [a], Bernard V. McInerney [b], Leon R. McQuade [b], Peter V. Chrystal [a, c], Ali Khoddami [a, d], Molly A.M. Zhuang [a], Sonia Y. Liu [a, d], Peter H. Selle [a, *]

[a] Poultry Research Foundation, The University of Sydney, Camden, NSW, Australia
[b] Australian Proteome Analysis Facility, Macquarie University, Sydney, NSW, Australia
[c] Baiada Poultry Pty Limited, Pendle Hill, NSW, Australia
[d] School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Sydney, NSW, Australia

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ABSTRACT

The hypothesis that capping dietary starch:protein ratios would enhance the performance of broiler chickens offered reduced-crude protein (CP) diets was tested in this experiment. A total of 432 off-sex, male Ross 308 chicks were allocated to 7 dietary treatments from 7 to 35 d post-hatch. The experimental design consisted of a 3 × 2 factorial array of treatments with the seventh treatment serving as a positive control. Three levels of dietary CP (197.5, 180.0 and 162.5 g/kg) with either uncapped or capped dietary starch:protein ratios constituted the factorial array of treatments, whilst the positive control diet contained 215.0 g/kg CP. The positive control diet had an analysed dietary starch:protein ratio of 1.50 as opposed to a ratio of 1.68 in the uncapped 197.5 g/kg CP diet and 1.41 in the corresponding capped diet and the capped 197.5 g/kg CP diet displayed promise. The growth performance this diet matched the positive control but outperformed the uncapped 197.5 g/kg CP diet by 10.4% (2,161 vs. 1,958; P = 0.009) in weight gain, by 3.10% (3,492 vs. 3,387; P = 0.019) in feed intake on the basis of pair-wise comparisons and numerically improved FCR by 4.04% (1.616 vs. 1.684). However, the growth performance of birds offered the 180.0 and 162.5 g/kg CP dietary treatments was remarkably inferior, irrespective of dietary starch:protein ratios. This inferior growth performance was associated with poor feathering and even feather-pecking and significant linear relationships between feather scores and parameters of growth performance were observed. The amino acid profile of feathers was determined where cysteine, glutamic acid, glycine, proline and serine were dominant in a crude protein content of 931 g/kg. Presumably, the feathering issues observed were manifestations of amino acid inadequacies or imbalances in the more reduced-CP diets and consideration is given to the implications of these outcomes.

1. Introduction

The interest in successfully developing reduced crude protein (CP) diets is widespread with a focus on alternative dietary strategies to reduce CP levels whilst maintaining acceptable growth performance. Reduced CP diets have the potential to provide advantages in respect of the environment, from attenuated outputs of nitrogen (N) and ammonia (Nahm, 2007), bird welfare, from enhanced litter quality and lower incidences of foot-pad dermatitis (Dunlop et al., 2016), and flock health, from less undigested protein entering the large intestine to fuel the proliferation of potential pathogens including *Clostridium perfringens* (Wilkie et al., 2015). Moreover, there is the promise of economic advantages from reductions in feed ingredients costs. Modest reductions in CP are already being realised by inclusions of unbound (synthetic or crystalline) methionine, lysine and threonine, which have been routine in chicken-meat production for decades (Kidd et al., 2013).

* Corresponding author.
E-mail address: peter.selle@sydney.edu.au (P.H. Selle).
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Moderate reductions in dietary CP levels can be achieved without compromised broiler performance when coupled with judicious inclusions of unbound amino acids. Nevertheless, there appears to be a threshold where tangible CP reductions negatively influence growth performance, especially FCR and this is associated with increased fat deposition (Belloir et al., 2017).

Reductions in dietary CP levels are almost invariably achieved by increasing feed grain inclusions at the expense of soybean meal with additions of essential, and even non-essential, unbound amino acids. This approach automatically increases dietary starch:protein ratios and consequently impacts on starch-protein digestive dynamics in broiler chickens (Liu and Selle, 2017; Selle and Liu, 2019). In a recent study, CP was reduced from 200 to 156 g/kg in maize-based diets that were offered to male broilers from 14 to 35 d post–hatch (Selle et al., 2019). This was achieved by increasing maize inclusions from 560 to 718 g/kg, decreasing soybean meal from 329 to 171 g/kg and increasing unbound amino acid additions from 6.0 to 24.9 g/kg. Consequently, analysed dietary starch:protein ratios increased from 1.55 to 2.57. This transition significantly compromised FCR by 8.96% (1.629 vs. 1.495), increased relative abdominal fat-pad weights by 70.8% (12.40 vs. 7.26 g/kg) and expanded jejunal starch:protein disappearance rate ratios from 2.08 to 3.17. However, there were quadratic relationships between disappearance rate ratios with both FCR ($r = 0.838$; $P < 0.001$) and relative fat-pad weights ($r = 0.786$; $P < 0.001$) where both parameters increased with expanding disappearance rate ratios. Although these significant relationships are not conclusive, the implication is that tangible increases in digestion/absorption of starch/glucose relative to that of protein/amino acids is compromising feed conversion efficiency and generating heavier fat-pads. These outcomes suggest that CP reductions in broiler diets may be better achieved by more modest feed grain increases in the formulation which would be facilitated by the partial substitution of soybean meal with protein sources with lower CP contents.

The performance of pigs (Gloaguen et al., 2014) offered reduced CP diets appears to be superior to poultry and this may be partially because there is insufficient consideration of the amino acid requirements for feathering in broiler chickens. However, as Van Emous and Van Krimpen (2019) concluded that there is no consistent, recent data in relation to the amino acid composition of feathers it was decided to determine the amino acid composition of feathers in the present study.

The primary objective of this study was to test the outlined hypothesis that condensing or capping dietary starch:protein ratios will enhance the performance of broiler chickens offered reduced CP diets. The partial substitution of soybean meal (475 g/kg CP) with notional full-fat soy (360 g/kg CP) permits reductions in dietary CP without increasing feed grain and starch contents. In the present study the notional full-fat soy was actually an appropriate blend of soybean meal and soy oil. This approach was adopted to avoid the potential confounding effect of comparing imported soybean meal with full-fat soy derived from locally grown soybeans.

2. Materials and methods

This study fully complied with the guidelines (2017/1252) specifically approved by the Research Integrity and Ethics Administration of The University of Sydney.

2.1. Experimental design

The experimental design consisted of a positive control plus a 3 × 2 factorial array of dietary treatments. Three levels of dietary CP (197.5, 180.0 and 162.5 g/kg) with either uncapped (1.97, 2.42, 2.91) or capped (1.63, 1.63, 1.92) dietary starch:protein ratios constituted the factorial array of treatments as outlined in Table 1. The positive control diet contained 215.0 g/kg CP with a dietary starch:protein ratio of 1.63. All 7 diets contained 11.00 g/kg digestible lysine with an energy density of 12.60 MJ/kg ME and dietary electrolyte balance (dEB) was maintained constant at 250 mEq/kg by manipulating dietary inclusions of sodium chloride, sodium bicarbonate and potassium bicarbonate.

2.2. Diet preparation

The diets were formulated on the basis of near-infrared spectroscopy (NIR) of wheat and soybean meal using the AMINOIR Advanced program (Evonik Nutrition & Care GmbH, Hanua, Germany). A positive control, wheat-soybean meal diet (1A) was formulated to meet standard recommendations with the composition and nutrient specifications shown in Tables 2 and 3, respectively. The CP of diets 2B, 3C and 4D were reduced to 197.5, 180.0 and 162.5 g/kg, respectively, by increasing wheat, decreasing soybean meal and elevating inclusions of unbound, essential amino acids in order to meet requirements. As a result, the analysed dietary starch:protein ratios increased from 1.68 to 1.99 and 2.60 for diets 2B, 3C and 4D, respectively, as shown in Table 4. The CP of diets 5E, 6F and 7G were similarly reduced to 197.5, 180.0 and 162.5 g/kg essentially by substituting soybean meal with “full fat soy” (a blend of 748 g/kg soybean meal and 252 g/kg soy oil) and wheat inclusions remained relatively static with a mean inclusion level of 483 g/kg. Consequently, analysed dietary starch:protein ratios remained relatively constant at 1.41, 1.42 and 1.56 in diets 5E, 6F and 7G, respectively. The analysed amino acid concentrations for the 7 dietary treatments are shown in Table 4. Wheat was mediumly ground (4.0 mm hammer-mill screen) prior to being blended into the complete diets which were steam-pelleted through a Palmer PP330 pellet press (Palmer Milling Engineering, Griffith, NSW, Australia) at a conditioning temperature of 80 °C with a conditioner residence time of 14 s and were then cooled.

2.3. Bird management

A total of 432 male Ross 308 chicks were procured from a commercial hatchery and were initially offered a standard starter diet. At 7 d post–hatch, birds were individually identified (wing-tags) and allocated into bioassay cages on the basis of bodyweights so that mean weights and variations within cages were nearly identical. Each of 7 dietary treatments was offered to 8 replicate cages (6 birds per cage) from 7 to 35 d post–hatch. Birds had unrestricted access to feed and water in an environmentally controlled facility, which remained illuminated for 18 h daily. An initial room temperature of 32 °C was maintained for the first week, which was gradually decreased to 22 °C by the end of the third week.

2.4. Data and sample collection, chemical analyses, calculations

Growth performance (weight again, feed intake, and FCR) was determined at 28 (data not shown) and 35 d post–hatch. Weight gains and feed intakes were monitored over both experimental periods, and the bodyweights of any dead or culled birds were recorded on a daily basis to correct feed intakes and adjust FCR calculations.

Total excreta outputs and feed intakes were recorded from 32 to 34 d post–hatch to determine parameters of nutrient utilisation by the classical approach. These parameters included apparent metabolizable energy (AME), metabolizable-to-gross energy (ME:GE) ratios, N retention and N-corrected AME (AMEn). Excreta were weighed before and after drying in a forced-air oven at 80 °C

Table 1
Outline of dietary treatments based on nutrient specifications.

Item	1A	2B	3C	4D	5E	6F	7G
Crude protein, g/kg	215.0	197.5	180.0	162.5	197.5	180.0	162.5
Dietary starch:protein ratio	1.63	1.97	2.42	2.91	1.63	1.63	1.92
Metabolizable energy, MJ/kg	12.60	12.60	12.60	12.60	12.60	12.60	12.60
Digestible lysine, g/kg	11.00	11.00	11.00	11.00	11.00	11.00	11.00
dEB, mEq/kg	250	250	250	250	250	250	250
Crude fat, g/kg	60.0	51.2	38.4	29.7	81.0	87.5	91.7
Starch, g/kg	351	389	436	472	323	294	313

dEB = dietary electrolyte balance.

Table 2
Composition of 7 experimental diets (g/kg, as-is basis).

Ingredient	1A	2B	3C	4D	5E	6F	7G
Wheat	547	607	680	738	502	458	488
Soybean meal	294	236	174	113	253	209	148
Soy oil	13.7	4.8	–	–	44.3	53.6	56.2
Canola seed	70.0	70.0	50.5	30.0	70.0	70.0	70.0
Dextrose	25.0	25.0	25.0	25.0	25.0	59.9	50.0
L-lysine HCl	1.51	3.19	5.20	7.24	2.74	4.26	6.20
DL-methionine	2.28	2.71	3.29	3.92	2.82	3.46	4.06
L-threonine	0.63	1.38	2.28	3.22	1.27	2.05	2.95
L-tryptophan	–	–	–	0.25	–	–	0.18
L-valine	–	0.92	2.03	3.19	0.86	1.90	3.05
L-arginine	–	0.42	2.31	4.25	0.09	1.64	3.54
L-isoleucine	–	0.75	1.84	2.97	0.61	1.57	2.70
L-leucine	–	–	1.17	3.04	–	0.88	2.75
L-histidine	–	–	0.03	0.69	–	–	0.55
Sodium chloride	2.02	–	–	–	0.30	–	
Sodium bicarbonate	2.01	5.48	5.67	5.67	4.57	5.79	5.80
Potassium carbonate	–	–	3.11	6.44	–	1.55	4.78
Limestone	8.88	8.93	9.12	9.27	8.70	8.49	8.45
Dicalcium phosphate	10.26	10.95	11.76	12.66	11.14	12.19	13.16
Xylanase	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride (60%)	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Sand	–	–	–	8.55	49.5	82.3	106
Celite	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin-mineral premix[1]	2.0	2.0	2.0	2.0	2.0	2.0	2.0

[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.

for 24 h to determine excreta dry matter and the gross energy (GE) of excreta, and diets were determined using an adiabatic bomb calorimeter. The AME values of the diets on a dry matter basis were calculated from the following equation:

$$\text{AME}_{\text{diet}}\,(\text{MJ/kg DM}) = [(\text{Feed intake} \times \text{GE}_{\text{diet}}) - (\text{Excreta output} \times \text{GE}_{\text{excreta}})]/\text{Feed intake}.$$

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

$$\text{N retention}\,(\%) = [(\text{Feed intake} \times \text{N}_{\text{diet}}) - (\text{Excreta output} \times \text{N}_{\text{excreta}})]/(\text{Feed intake} \times \text{N}_{\text{diet}}) \times 100.$$

The N-corrected AME (MJ/kg DM) values were calculated by correcting N retention to zero using the factor of 36.54 kJ/g N retained in the body (Hill and Anderson, 1958).

At 34 d post–hatch, blood samples were taken from the brachial vein of 3 birds in each replicate cage that were offered diets 1A, 2B and 5F to determine free amino acid concentrations in systemic plasma. Blood samples were centrifuged, and decanted plasma samples were then kept at −80 °C prior to analysis. Concentrations of 20 proteinogenic amino acids in plasma taken from the brachial vein were determined using precolumn derivatisation amino acid analysis with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC; Waters AccQTag Ultra; Waters Australia PL; www.waters.com) followed by separation of the derivatives and quantification by reversed phase ultra-performance liquid chromatography (RP-UPLC). All amino acids were detected by UV absorbance and this procedure is fully described in Selle et al. (2016).

At 35 d post–hatch, birds were euthanised by an intravenous injection of sodium pentobarbitone, the abdominal cavities opened and abdominal fat-pads dissected out and weighed. The small intestine was removed and digesta was gently expressed in its entirety from the distal half of the jejunum and ileum and pooled by cage, homogenised, freeze dried and weighed to determine the apparent digestibility coefficients of starch, protein (N) and amino acids. Starch concentration were determined by a procedure based on dimethyl sulfoxide, α-amylase and amyloglucosidase, as described in Mahasukhonthachat et al. (2010). Acid insoluble ash (AIA) (Celite, Celite Corporation, Lompoc, CA) was included in diets at 20 g/kg as a dietary marker and AIA and protein (N) concentrations were determined by methods described in Siriwan et al. (1993). Amino acid concentrations of diets and digesta were determined via 24 h liquid hydrolysis at 110 °C in 6 mol/L HCl followed by analysis of 16 amino acids using the Walters AccQTag Ultra chemistry on a Waters Acquity UPLC. Amino acid digestibility determinations were completed in birds offered diets 1A, 2B and 5F. Apparent digestibility coefficients were calculated by the following equation:

$$\text{Digestibility coefficient} = [(\text{Nutrient/AIA})_{\text{diet}} - (\text{Nutrient/AIA})_{\text{digesta}}]/(\text{Nutrient/AIA})_{\text{diet}}$$

Disappearance rates (g/bird per d) of starch, protein (N) and amino acids in the distal jejunum and distal ileum were calculated from the product of dietary concentrations of nutrient (g/kg), daily feed intake (g/d) from 28 to 35 d post–hatch and the relevant apparent jejunal and ileal digestibility coefficients. Feather-pecking was observed during the course of the feeding study; therefore, a "feather score" on a scale from 1 (good) to 5 (poor) was recorded for each bird by 2 technicians independently as an assessment of feathering.

Cysteine and tryptophan were analysed separately to determine the amino acid profile of feathers. A standard procedure to oxidize both cysteine and cystine to cysteic acid prior to acid hydrolysis was adopted (AOAC, 2006). Tryptophan was quantified using NaOH hydrolysis again by a standard procedure (AOAC, 1995).

2.5. Statistical analysis

The experimental data, as a 2 × 3 factorial array, was subject to analyses of variance using the IBM SPSS Statistics 24 program (IBM Corporation, Somers, NY, USA). Experimental units were cage

Table 3
Nutrient specifications of 7 experimental diets (g/kg, as-is basis).

Item	1A	2B	3C	4D	5E	6F	7G
Metabolizable energy, MJ/kg	12.60	12.60	12.60	12.60	12.60	12.60	12.60
Crude protein	215.0	197.5	180.0	162.5	197.5	180.0	162.5
Starch	351	389	436	472	323	294	313
Calcium	8.25	8.25	8.25	8.25	8.25	8.25	8.25
Total phosphorus	5.20	5.09	4.92	4.73	5.03	4.83	4.67
Available phosphorus	4.13	4.13	4.13	4.13	4.13	4.13	4.13
Sodium	1.80	1.94	2.00	2.00	1.80	2.00	2.00
Potassium	9.12	7.95	8.32	8.79	8.20	7.95	8.39
Chloride	2.18	1.34	1.77	2.19	1.35	1.43	1.83
dEB, mEq/kg	250	250	250	250	250	250	250
Crude fat	60.0	51.2	38.4	29.7	81.0	87.5	91.7
Crude fibre	27.0	26.5	25.1	23.2	24.9	22.4	21.0
Digestible amino acids							
Lysine	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Methionine	5.01	5.19	5.43	5.71	5.29	5.61	5.87
Methionine + Cysteine	8.14	8.14	8.14	8.14	8.14	8.14	8.14
Threonine	7.15	7.15	7.15	7.15	7.15	7.15	7.15
Tryptophan	2.51	2.24	1.91	1.82	2.27	1.97	1.82
Isoleucine	7.88	7.70	7.70	7.70	7.70	7.70	7.70
Leucine	13.88	12.39	11.77	11.77	12.53	11.77	11.77
Arginine	12.61	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	4.74	4.23	3.63	3.63	4.28	3.72	3.63
Phenylalanine	9.18	8.16	6.98	5.75	8.20	7.15	5.90

dEB = dietary electrolyte balance.

Table 4
Analysed starch, crude protein and amino acid concentrations of experimental diets (g/kg).

Item	1A	2B	3C	4D	5E	6F	7G
Starch	332	347	373	427	288	259	272
Crude protein (6.25 × N)	222	206	187	164	204	173	174
Dietary starch:protein ratio	1.50	1.68	1.99	2.60	1.41	1.42	1.56
Arginine	13.7	11.2	10.9	10.1	12.1	11.3	11.1
Histidine	5.8	4.9	4.4	4.1	5.1	4.4	4.2
Isoleucine	9.6	8.6	8.3	8.2	9.1	86	8.8
Leucine	16.6	14.0	13.2	12.5	14.8	13.5	13.6
Lysine	12.4	11.1	10.2	8.9	11.2	9.9	9.4
Methionine	3.7	3.8	4.1	4.5	4.4	4.9	4.9
Phenylalanine	10.8	9.1	8.1	6.7	9.3	8.1	7.1
Threonine	8.7	7.9	7.5	7.0	8.3	8.0	7.8
Valine	10.5	9.7	9.7	9.6	10.1	9.6	9.7
Alanine	8.9	7.4	6.5	5.3	7.6	6.6	5.8
Aspartic acid	20.6	16.5	13.8	10.6	17.5	14.9	12.6
Glutamic acid	45.9	41.0	37.6	34.1	40.7	35.6	31.4
Glycine	9.5	8.1	7.2	6.1	8.3	7.2	6.4
Proline	13.8	12.6	11.8	10.8	12.5	10.9	9.7
Serine	10.9	9.2	8.2	6.8	9.4	8.2	7.2
Tyrosine	5.3	4.0	3.5	2.9	4.4	4.0	3.5
Total amino acids	206.7	179.1	165.0	148.2	184.8	165.7	153.2

means (8 replicate cages of 6 birds per dietary treatment) and a probability level of less than 5% was considered statistically significant. In addition, one-way analyses of variance of all 7 dietary treatments were completed in order to compare treatments 1A, 2B and 5E on the basis of pair-wise comparisons. Data for amino acid digestibility coefficients and plasma concentrations of free amino acids were analysed as a one-way ANOVA of the 3 relevant treatments (diets 1A, 2B and 5E). Pearson correlations, linear and quadratic regressions and were examined when considered appropriate.

3. Results

The effects of dietary treatments on growth performance from 7 to 35 d post–hatch, mortality rates, relative fat-pad weights and feather scores are shown in Table 5. The 'capped starch' 197.5 g/kg CP diet supported almost identical weight gains to the positive control diet but outperformed the 'uncapped starch' 197.5 g/kg CP diet with a weight gain advantage of 10.4% (2,161 vs. 1,958 g/bird; P = 0.009). However, reducing dietary CP from 197.5 to 180.0 g/kg depressed weight gain by 30.0% (1,444 vs. 2,063 g/bird) and from 197.5 to 162.5 g/kg by 51.1% (1,008 vs. 2,063 g/bird) to remarkable and significant extents. Feed intake results followed a similar pattern except, as a main effect, modified or capped diets supported higher feed intakes than standard diets by 6.51% (2,995 vs. 2,812 g/bird; $P < 0.001$). Crude protein reductions compromised FCR ($P < 0.001$) to substantial extents; the transition from 197.5 to 180.0 g/kg compromised FCR by 19.5% (1.972 vs. 1.650) and from 197.5 to 162.5 g/kg by 50.9% (2.490 vs. 1.650). There was a treatment interaction ($P = 0.005$) for relative fat-pad weights because they were

significantly heavier in birds offered the modified 7G diet than the standard 4D diet; in contrast, there were no significant differences at higher CP levels. Instructively, feather scores significantly deteriorated ($P < 0.05$) from 1.9 to 2.9 and 3.1 as dietary CP levels were reduced. The overall mortality rate of 2.98% was not related to treatment ($P > 0.09$).

The outcomes for parameters of nutrient utilisation in response to dietary treatments are shown in Table 6. The standard diets supported a higher AME than the modified diets by 0.39 MJ/kg DM (12.70 vs. 12.31 MJ/kg DM; $P < 0.001$) and a better ME:GE ratio by 2.08% (0.785 vs. 0.769; $P = 0.028$). Also, reducing dietary CP from 197.5 to 162.5 g/kg significantly increased ME:GE ratios by 3.40% (0.791 vs. 0.765). Reducing dietary CP from 197.5 to 162.5 g/kg significantly decreased N retention by 3.65% (59.63% vs. 63.28%; P = 0.003) and standard diets supported higher N retention by 2.79% (62.54% vs. 59.75%; $P = 0.003$) than modified diets. There was a treatment interaction ($P = 0.003$) for AMEn as these values were similar in birds offered 197.5 g/kg CP diets but the standard 180.0 and 162.5 g/kg CP diets supported significantly higher AMEn than the corresponding modified diets. There was a treatment interaction ($P < 0.001$) for excreta dry matter; higher dry matter contents were recorded for modified diets at all 3 CP levels but the difference was less pronounced with 197.5 g/kg CP diets. As a main effect, modified diets increased excreta dry matter by an average of 48 g/kg or 20.2% (286 vs. 238 g/kg).

The effects of dietary treatments on jejunal and ileal starch and protein (N) digestibility coefficients are shown in Table 7. Treatment interactions ($P < 0.01$) were observed for starch digestibility as the capped starch diets were significantly inferior to the standard diets for 180.0 g/kg CP diets in the jejunum and were significantly inferior to the standard diets in both intestinal segments in birds offered the 162.5 g/kg CP diets. Modified diets supported lower jejunal protein (N) digestibility by 10.5% (0.557 vs. 0.622; $P = 0.012$) than standard diets. Ileal protein (N) digestibility was superior to the positive control in birds offered diet 2B by 12.8% (0.774 vs. 0.686; $P = 0.001$) and diet 5E by 10.5% (0.758 vs. 0.686; $P = 0.002$).

Outcomes for starch and protein (N) disappearance rates and starch:protein disappearance rate ratios in jejunum and ileum appear in Table 8. Treatment interactions ($P < 0.001$) were observed for starch disappearance rates in both segments because slower rates were recorded for modified than standard diets. The starch disappearance rates in birds offered diet 1A was more rapid than diet 5E by 18.8% (60.8 vs. 51.2 g/bird per d; $P = 0.002$) in jejunum and by 15.2% (64.4 vs. 55.9 g/bird per d; $P = 0.004$) in ileum. Protein (N) disappearance rates were retarded ($P < 0.001$) by up to 30.7% in jejunum and 37.7% in ileum by declining dietary CP levels. Treatment interactions ($P < 0.001$) were observed for starch:protein disappearance rate ratios in both intestinal segments; nevertheless, the modified diets had condensed ratios in the jejunum (2.09 vs. 3.02) and ileum (1.80 vs. 2.53).

Effects of 3 dietary treatments on apparent ileal amino acid digestibility coefficients are shown in Table 9. Reducing dietary CP from 215.0 to 197.5 g/kg in the “capped starch” diet significantly increased digestibility coefficients of histidine (9.14%), isoleucine (9.96%), leucine (8.56%), phenylalanine (7.67%), threonine (15.8%), valine (13.1%), alanine (11.3%), glutamic acid (5.75%), glycine (12.2%), proline (9.91%) and serine (10.8%) where the percentage increase are stated in parentheses. Increases of similar magnitudes were observed in birds offered the standard 197.5 g/kg diet.

Table 10 shows the effects of the same 3 dietary treatments on amino acid disappearance rates (g/bird per d). Birds offered the 197.5 g/kg “capped starch” diet had significantly faster disappearance rates than their counterparts offered the standard 197.5 g/kg diet for 12 of 16 amino acids assessed where threonine, valine, glycine and proline were the 4 exceptions. It may be deduced that total amino acid disappearance rate was 34.30 g/bird per d in birds offered the positive control diet were statistically similar with 32.76 g/bird per d for the ‘capped starch’ diet but

Table 5
Effects of dietary treatments on growth performance from 7 to 35 d post−hatch, mortality rates, relative fat-pad weights and feather scores.

Item			Weight gain, g/bird	Feed intake, g/bird	FCR, g/g	Mortalities[1], %	Fat-pad weights, g/kg	Feather scores
Diet	Crude protein, g/kg	Mode						
1A (PC)	215.0		2,159	3,496	1.627	8.33	6.50	1.8
2B	197.5	Standard	1,958	3,387	1.684	2.08	8.37bc	1.9
5E	197.5	Modified	2,161	3,492	1.616	6.25	8.15b	1.9
3C	180.0	Standard	1,451	2,717	1.878	0.00	8.14b	3.1
6F	180.0	Modified	1,437	2,963	2.066	4.17	8.74bc	2.8
4D	162.5	Standard	1,010	2,433	2.426	0.00	6.79a	3.7
7G	162.5	Modified	1,005	2,532	2.554	0.00	9.63c	2.6
SEM			48.18	59.25	0.0671	1.875	0.4562	0.476
Main effect: Crude protein								
197.5			2,063c	3,389c	1.650a	4.17	8.26	1.9a
180.0			1,444b	2,840b	1.972b	2.08	8.41	2.9b
162.5			1,008a	2,482a	2.490c	0.00	8.26	3.1c
Mode								
Standard			1,471	2,812a	1.996	0.70	7.77	2.9
Modified			1,535	2,995b	2.076	3.47	8.84	2.4
Significance (P-value)								
Crude protein (CP)			<0.001	<0.001	<0.001	0.097	0.866	0.031
Mode (M)			0.140	<0.001	0.140	0.077	0.006	0.230
CP × M interaction			0.059	0.448	0.150	0.446	0.005	0.450
Pair-wise comparisons (P-value)								
1A versus 2B			0.010	0.017	0.527	0.074	0.005	0.891
1A versus 5E			0.969	0.959	0.910	0.546	0.012	0.842
2B versus 5E			0.009	0.019	0.456	0.229	0.725	0.949

PC = positive control.
[a, b, c] Within a column, means not sharing a common superscript are significantly different at $P < 0.05$.
[1] Overall mortality rate: 2.98% (SEM = 2.417; $P = 0.092$).

Table 6
Effects of dietary treatments on parameters of nutrient utilisation and excreta dry matter from 32 to 34 d post–hatch.

Item			AME, MJ/kg DM	ME:GE ratio, MJ/MJ	N retention, %	AMEn, MJ/kg DM	Excreta dry matter, g/kg
Diet	Crude protein, g/kg	Mode					
1A (PC)	215.0		12.65	0.753	61.80	11.98	268
2B	197.5	Standard	12.60	0.763	64.25	11.98^{ab}	255^{b}
5E	197.5	Modified	12.53	0.767	62.31	12.00^{ab}	279^{c}
3C	180.0	Standard	12.67	0.783	62.74	12.23^{b}	237^{ab}
6F	180.0	Modified	12.25	0.766	58.84	11.90^{a}	291^{c}
4D	162.5	Standard	12.83	0.808	60.64	12.53^{c}	223^{a}
7G	162.5	Modified	12.14	0.774	58.09	11.82^{a}	288^{c}
SEM	0.1301	0.0084	1.0747	0.1006	7.912		
Main effect: Crude protein							
197.5			12.48	0.765^{a}	63.28^{b}	11.99	267
180.0			12.46	0.775^{ab}	60.79^{a}	12.07	264
162.5			12.57	0.791^{b}	59.63^{a}	12.17	255
Mode							
Standard			12.70^{b}	0.785^{b}	62.54^{b}	12.25	238
Modified			12.31^{a}	0.769^{a}	59.75^{a}	11.91	286
Significance (*P*-value)							
Crude protein (CP)			0.674	0.012	0.003	0.192	0.332
Mode (M)			<0.001	0.028	0.003	<0.001	<0.001
CP × M interaction			0.061	0.073	0.645	0.003	0.035
Pair-wise comparisons (*P*-value)							
1A versus 2B			0.795	0.448	0.099	0.971	0.253
1A versus 5E			0.554	0.262	0.725	0.913	0.358
2B versus 5E			0.739	0.714	0.190	0.885	0.042

AME = apparent metabolizable energy; GE = gross energy; N = nitrogen; AMEn = N-corrected AME; PC = positive control.
[a, b, c] Within a column, means not sharing a common superscript are significantly different at $P < 0.05$.

Table 7
Effects of dietary treatments on apparent starch and protein (N) digestibility coefficients in distal jejunum and distal ileum at 35 d post–hatch.

Item			Starch digestibility		Protein digestibility (N × 6.25)	
Diet	CP, g/kg	Mode	Jejunum	Ileum	Jejunum	Ileum
1A (PC)	215.0		0.863	0.912	0.599	0.686
2B	197.5	Standard	0.865^{c}	0.917^{b}	0.613	0.774
5E	197.5	Modified	0.836^{bc}	0.916^{b}	0.526	0.758
3C	180.0	Standard	0.865^{c}	0.918^{b}	0.637	0.770
6F	180.0	Modified	0.790^{b}	0.919^{b}	0.561	0.760
4D	162.5	Standard	0.889^{c}	0.921^{c}	0.613	0.758
7G	162.5	Modified	0.711^{a}	0.910^{a}	0.526	0.722
SEM			0.0224	0.0020	0.0296	0.0562
Main effect: CP						
197.5			0.854	0.917	0.601	0.766
180.0			0.827	0.919	0.599	0.769
162.5			0.800	0.916	0.569	0.740
Mode						
Standard			0.873	0.919	0.622^{b}	0.767
Modified			0.779	0.915	0.557^{a}	0.749
Significance (*P*-value)						
CP			0.099	0.274	0.519	0.211
Mode (M)			<0.001	0.019	0.012	0.212
CP × M interaction			0.008	0.006	0.651	0.641
Pair-wise comparisons (*P*-value)						
1A versus 2B			0.949	0.077	0.768	0.001
1A versus 5E			0.722	0.182	0.121	0.002
2B versus 5E			0.387	0.654	0.067	0.883

N = nitrogen; CP = crude protein; PC = positive control.
[a, b, c] Within a column, means not sharing a common superscript are significantly different at $P < 0.05$.

significantly faster than 28.99 g/bird per d for birds offered the 'uncapped starch' diet.

The effects of dietary treatments 1A, 2B and 5E on concentrations of 19 free amino acids in plasma taken from the brachial vein are shown in Table 11. Significant differences were observed for 7 amino acids: arginine, histidine, lysine, methionine, threonine, valine and aspartic acid. The sum of the concentrations of these 7 amino acids was 221 μg/mL in birds offered diet 2B, 251 μg/mL in diet 1A and 267 μg/mL in diet 5E. Total concentrations followed the same pattern, 796, 843 and 857 μg/mL, respectively, but the differences were not significant ($P > 0.15$).

The amino acid profile of feathers is shown in Table 12. The total concentration of 18 amino acids was 928.4 mg/g, where serine (112.9 mg/g), proline (95.9 mg/g) and glutamic acid (94.9 mg/g) were the dominant amino acids. The CP content of feathers (analysis in triplicate) was 931.0 g/kg.

Table 8
Effects of dietary treatments on apparent starch and protein (N) disappearance rates and starch:protein disappearance rate ratios in distal jejunum and distal ileum at 35 d post–hatch.

Item			Starch disappearance rate, g/bird per d		Protein disappearance rate (N × 6.25), g/bird per d		Starch:protein disappearance rate ratio	
Diet	CP, g/kg	Mode	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
1A (PC)	215.0		60.8	64.4	26.2	32.4	2.34	2.01
2B	197.5	Standard	58.5^{b}	62.0^{c}	23.6	30.9	2.53^{bc}	2.01^{b}
5E	197.5	Modified	51.2^{c}	55.9^{b}	22.7	32.8	2.31^{ab}	1.71^{a}
3C	180.0	Standard	51.9^{c}	55.1^{b}	19.1	23.1	2.73^{c}	2.36^{c}
6F	180.0	Modified	36.4^{b}	42.5^{a}	18.6	25.2	2.02^{a}	1.70^{a}
4D	162.5	Standard	61.4^{d}	63.8^{c}	16.5	20.2	3.78^{c}	3.17^{d}
7G	162.5	Modified	29.8^{a}	38.2^{a}	15.6	19.4	1.95^{a}	1.99^{b}
SEM			2.125	1.900	1.382	1.278	0.1270	0.0500
Main effect: Crude protein								
197.5			54.8	59.0	23.1^{c}	31.8^{c}	2.87	2.58
180.0			41.2	48.8	18.9^{b}	24.1^{b}	2.38	2.04
162.5			45.6	51.0	16.0^{a}	19.8^{a}	2.42	1.86
Mode								
Standard			57.3	60.3	19.7	24.7	3.02	2.53
Modified			39.1	45.5	19.0	25.8	2.09	1.80
Significance (*P*-value)								
CP			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Mode (M)			<0.001	<0.001	0.503	0.309	<0.001	<0.001
CP × M interaction			<0.001	<0.001	0.985	0.438	<0.001	<0.001
Pair-wise comparisons (*P*-value)								
1A versus 2B			0.455	0.382	0.183	0.406	0.269	0.922
1A versus 5E			0.002	0.004	0.077	0.838	0.849	<0.001
2B versus 5E			0.017	0.035	0.653	0.302	0.197	<0.001

N = nitrogen; CP = crude protein; PC = positive control.
[a, b, c, d] Within a column, means not sharing a common superscript are significantly different at $P < 0.05$.

Table 9
Effects of 3 dietary treatments on apparent ileal amino acid digestibility coefficients at 35 d post-hatch.

Item	Treatment			SEM	*P*-value	LSD ($P < 0.05$)
	1A (PC)	2B	5E			
Arginine	0.829	0.857	0.86	0.0127	0.197	–
Histidine	0.744^{a}	0.804^{b}	0.812^{b}	0.0166	0.017	0.0488
Isoleucine	0.743^{a}	0.801^{b}	0.817^{b}	0.0158	0.008	0.0464
Leucine	0.748^{a}	0.800^{b}	0.812^{b}	0.0165	0.029	0.0485
Lysine	0.746	0.781	0.78	0.016	0.236	–
Methionine	0.796	0.834	0.827	0.0146	0.176	–
Phenylalanine	0.769^{a}	0.819^{b}	0.828^{b}	0.0159	0.035	0.0469
Threonine	0.640^{a}	0.731^{b}	0.741^{b}	0.0209	0.004	0.6615
Valine	0710^{a}	0.787^{b}	0.803^{b}	0.0174	0.002	0.0511
Alanine	0.689^{a}	0.743^{ab}	0.767^{b}	0.019	0.026	0.0559
Aspartic acid	0.718	0.76	0.77	0.017	0.096	–
Glutamic acid	0.817^{a}	0.867^{b}	0.864^{b}	0.0124	0.016	0.0363
Glycine	0.674^{a}	0.746^{b}	0.756^{b}	0.0192	0.003	0.0566
Proline	0.757^{a}	0.831^{b}	0.832^{b}	0.0157	0.003	0.0462
Serine	0.714^{a}	0.774^{b}	0.791^{b}	0.0176	0.013	0.0517
Tyrosine	0.753	0.777	0.805	0.0178	0.179	–

PC = positive control; SEM = standard error of the mean; LSD = least significant difference.
[a, b] Within a row, means not sharing a common superscript are significantly different at $P < 0.05$.

4. Discussion

The remarkable outcome of the present study was the distinctly inferior growth performance of birds offered 180.0 and 162.5 g/kg CP wheat-based diets. On average, these 4 diets supported a weight gain of 1,226 g/bird, which was 43.2% less that the 2,159 g/bird weight gain supported by the positive control diet from 7 to 35 d post–hatch. This outcome was not anticipated as it was in direct contrast to the performance and birds offered wheat-based diets with similar CP reductions as reported by Yin et al. (2020) and birds offered maize-based diets as reported by Chrystal et al. (2019). Effectively, however, there was a precedent for this remarkable outcome in an as yet unpublished feeding study. In this study, the transition from 222 to 165 g/kg CP wheat-based diets compromised 7 to 35 d weight gains by 35.5%; whereas, instructively, the same transition led to a 7.05% increase in weigh gains in birds offered maize-based diets.

One possible explanation for this extraordinary outcome is that amino acid imbalances in the 180.0 and 162.5 g/kg CP diets led to catabolism of excess amino acids and the accumulation of ammonia, which is toxic (Stern and Mozdziak, 2019). Plasma ammonia concentrations in broiler chickens were significantly increased by 7.94% (0.68 vs. 0.63 mg/dL) following a reduction in dietary CP from 230 to 170 g/kg as reported by Namroud et al. (2008). Moreover, it may be deduced from this study that plasma ammonia concentrations compromised FCR in a linear

Table 10
Effects of 3 dietary treatments on apparent distal ileal amino acid disappearance rates (g/bird per day) at 35 d post-hatch.

Item, g/bird per day	Treatment			SEM	*P*-value	LSD ($P < 0.05$)
	1A (PC)	2B	5E			
Arginine	2.42[b]	187[a]	2.20[b]	0.0775	< 0.001	0.228
Histidine	0.92[b]	0.77[a]	0.88[b]	0.0335	0.014	0.099
Isoleucine	1.52[b]	1.34[a]	1.58[b]	0.0581	0.024	0.171
Leucine	2.65[b]	2.18[a]	2.55[b]	0.0975	0.007	0.287
Lysine	1.97[b]	1.69[a]	1.85[ab]	0.0758	0.049	0.223
Methionine	1.77[b]	1.45[a]	1.63[ab]	0.0632	0.007	0.186
Phenylalanine	1.77[b]	1.45[a]	1.63[ab]	0.063	0.007	0.185
Threonine	1.19	1.13	1.31	0.057	0.1	–
Valine	1.59	1.48	1.72	0.0652	0.06	–
Alanine	1.31[b]	1.07[a]	1.24[b]	0.0536	0.017	0.158
Aspartic acid	3.15[b]	2.44[a]	2.86[b]	0.1167	0.001	0.343
Glutamic acid	7.98[b]	6.92[a]	7.46[ab]	0.2713	0.037	0.798
Glycine	1.37	1.18	1.33	0.057	0.066	–
Proline	2.22	2.04	2.21	0.0844	0.26	–
Serine	1.66[b]	1.39[a]	1.58[ab]	0.0652	0.023	0.792
Tyrosine	0.85[c]	0.61[a]	0.75[b]	0.0316	< 0.001	0.093

PC = positive control; SEM = standard error of the mean; LSD = least significant difference.
[a, b, c] Within a row, means not sharing a common superscript are significantly different at $P < 0.05$.

Table 11
Effects of 3 dietary treatments on free amino acid concentrations in systemic plasma (brachial vein) at 34 d post-hatch.

Item, μg/mL	Treatment			SEM	*P*-value	LSD ($P < 0.05$)
	1A (PC)	2B	5E			
Arginine	79[b]	63[a]	78[b]	3.17	0.003	9.3
Histidine	16[b]	11[a]	13[ab]	1.08	0.014	4.9
Isoleucine	17	17	19	0.72	0.158	–
Leucine	27	23	25	1.07	0.105	–
Lysine	26[b]	17[a]	20[ab]	2.51	0.046	7.4
Methionine	11[a]	17[b]	20[ab]	0.54	< 0.001	1.6
Phenylalanine	24	23	23	0.61	0.264	–
Threonine	78[a]	78[a]	97[b]	4.52	0.009	13.2
Tryptophan	7	6	6	0.28	0.823	–
Valine	33[a]	36[ab]	38[b]	1.39	0.034	4.1
Alanine	66	61	64	3.19	0.537	–
Aspartate	8[b]	6[a]	7[ab]	0.51	0.045	1.5
Asparagine	34	32	33	1.77	0.752	–
Cysteine	17	17	18	0.51	0.393	–
Glutamate	19	18	19	0.69	0.714	–
Glutamine	170	175	173	5.78	0.857	–
Glycine	57	53	55	1.5	0.171	–
Proline	60	53	53	2.1	0.064	–
Serine	63	63	63	1.48	0.983	–
Tyrosine	33	35	40	2.2	0.121	–
Total	843	796	857	23.31	0.177	-

PC = positive control; SEM = standard error of the mean; LSD = least significant difference.
[a, b] Within a row, means not sharing a common superscript are significantly different at $P < 0.05$.

manner ($r = 0.720$; $P < 0.05$). In addition, Ospina-Rojas et al. (2014) found that a 220 to 190 g/kg reduction in dietary CP increased plasma ammonia concentrations by 59.4% (7.27 vs. 4.56 mg/dL). Instructively, plasma ammonia concentrations compromised weight gains in a quadratic manner ($r = 0.780$; $P < 0.025$) in birds from 1 to 21 d post–hatch. Thus, in both the Namroud et al. (2008) and Ospina-Rojas et al. (2014) studies it appears that an accumulation of ammonia or, effectively, ammonia toxicity was negatively impacting on the performance of birds offered reduced CP diets, which may have been the case in the present study.

Amino acid imbalances prompt degradation of excess amino acids which principally involves hepatic oxidative deamination with the generation of ammonia. Glutamine synthetase is pivotal to ammonia detoxification as it catalyses the condensation of ammonia plus glutamate to glutamine (Hakvoort et al., 2017). Glutamine then enters the Krebs uric acid cycle which generates uric acid and N is excreted as uric acid (Salway, 2018). However, there is a requisite input for glycine into this cycle and both serine, and possibly threonine, may serve as glycine precursors. Reduced CP diets will often contain less glutamic acid, glycine and serine than conventional diets, and in the present study, when analysed values in the positive control diet are compared with the mean values in the 180.0 and 162.5 g/kg CP diets, there are declines in glutamic acid of 24.4% (34.7 vs. 45.9 g/kg), in glycine of 30.3% (7.6 vs. 10.9 g/kg) and in serine of 29.5% (6.7 vs. 9.5 g/kg). Thus, one implication is that inadequate glutamic acid, glycine and serine concentrations may be impeding ammonia detoxification and uric acid excretion compounding the increase in deamination of amino acids. In mammalian models, inadequate ammonia detoxification and increased circulating ammonia concentration, termed hyperammonaemia, leads to severe skeletal muscle atrophy, increased apoptosis and reduced protein synthesis (Stern and Mozdziak, 2019). Therefore, it is plausible that amino acid imbalances in reduced CP diets results in deamination of surplus amino acids and, without adequate detoxification, leads to

Table 12
Concentrations of amino acids and crude protein in feathers of male broiler chickens offered the positive control (PC) diet at 35 d post–hatch.

Item	Content, g/kg	Content, mol/100 mol
Arginine	64.4	4.7
Histidine	4.2	0.3
Isoleucine	44.3	4.3
Leucine	75.4	7.3
Lysine	13.2	1.1
Methionine	3.8	0.3
Phenylalanine	44.7	3.5
Threonine	44.2	4.7
Tryptophan	4.3	1.3
Valine	72.5	7.9
Alanine	37.0	5.3
Aspartic acid	58.0	5.6
Cysteine	69.4	8.2
Glutamic acid	94.9	8.2
Glycine	66.9	11.4
Proline	95.9	10.6
Serine	112.9	13.7
Tyrosine	22.4	1.6
Total amino acids	928.4	100.0
Crude protein (N × 6.25)	931.0	

excessive accumulation of ammonia in birds with negative impacts on growth performance.

An important caveat is that systemic portal ammonia concentrations are volatile over time in poultry as reported by Okumura and Tasaki (1968). Thus, excess ammonia levels appear to be an important factor in birds offered reduced CP diets but may be difficult to demonstrate because of the volatility of plasma ammonia concentrations.

In the present study, there was a quadratic relationship ($r = 0.443$; $P < 0.005$) between feather scores and 7 to 35 d weight gain across all 7 treatments as shown in Fig. 1. It may be deduced from the regression equation that the maximum weight gain of 1,769 g/bird was associated with a low feather score of 1.48 but higher, or poorer, feather scores were associated with marked deteriorations in weight gain. Factors influencing feathering in poultry have been reviewed by Leeson and Walsh (2004) and these researchers noted that diets with less than 160 g/kg CP can trigger poor feathering in young broiler breeder chicks but that this is not corrected by

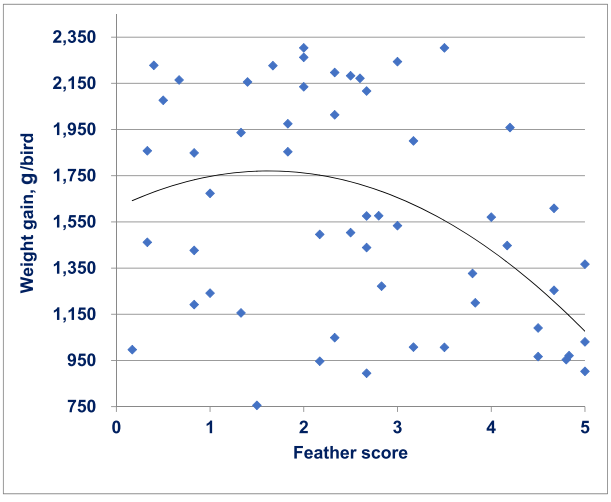

Fig. 1. The quadratic relationship ($r = 0.443$; $P < 0.005$) between feather scores (FS) and weight gain (WG) from 7 to 35 d post-hatch across all 7 treatments where $WG = 1{,}610 + 198 \times FS - 61 \times FS^2$.

relatively high dietary levels of unbound amino acids. This suggests that there may be either a requirement for protein *per se* or that the optimum balance of amino acids for adequate feathering has not been correctly established (Urdaneta-Rincon and Leeson, 2004). Feathers of male birds at 21 d of age have a CP content of 921 g/kg (Fisher et al., 1981). and Si et al. (2004) reported that male broiler chickens had feather weights of 75.3 g/bird or 9.7% of their bodyweight at 21 d of age. Thus, feathering requires tangible amino acid inputs and the inferior growth performance observed in the present study was associated with poor feather scores.

The amino acid profiles in feathers from broiler chickens in the present study are in reasonable agreement with previously documented assays (Fisher et al., 1981; Stilborn et al., 1997); Adler et al. (2018); Wecke et al. (2018). Feather-pecking is a prevalent cause of mortality in commercial laying hens, but it is not usually observed in broiler chickens as was the case in the present study. Birkl et al. (2017) suggested that feather-pecking may be triggered by inadequate plasma levels of aromatic amino acids including tryptophan, the precursor of serotonin, and phenylalanine and tyrosine, precursors of dopamine. Deficient levels of the neurotransmitters, serotonin and dopamine, within the brain have been associated with aggressive behaviour such as feather-pecking (De Hass and Van der Eijk, 2018). The likelihood is that the feathering issues and inferior growth performance observed in the present study are both manifestations of amino acid inadequacies or imbalances in wheat-based, reduced CP diets.

It is conceded that capping starch:protein ratios in the 180.0 and 162.5 g/kg CP diets was not an effective strategy. However, the growth performance of birds offered these diets was not acceptable which may have been a consequence of hyperammonaemia. In contrast, the performance of birds offered the 215.0 and 197.5 g/kg CP diets was acceptable; therefore, it is reasonable to consider the first 3 diets separately in relation to capping dietary starch:protein ratios in reduced CP broiler diets. The performance generated by the capped 197.5 g/kg CP diet did not differ ($P > 0.90$) from the 215.0 g/kg CP diet in terms of weight gain, feed intake and FCR. Moreover, the capped diet outperformed the uncapped 197.5 g/kg CP diet by 10.4% (2,161 vs. 1,958; $P = 0.009$) in weight gain, by 3.10% (3,492 vs. 3,387; $P = 0.019$) in feed intake on the basis of pair-wise comparisons and supported a numeric improvement in FCR of 4.04% (1.616 vs. 1.684). Additionally, there was a significant negative linear relationship ($r = -0.443$; $P < 0.05$) between distal jejunal starch: protein disappearance rate ratios and weight gain across the 3 specified dietary treatments as shown in Fig. 2.

These outcomes support the proposal, advanced by Selle et al. (2019), that capping dietary starch-to-protein ratios in reduced CP broiler diets should be beneficial. This proposal was based on empirical evidence that expanding dietary starch:protein ratios translate to expanding starch:protein disappearance rate ratios in birds which are statistically related to both less efficient FCR and heavier relative abdominal fat-pad weights. Effectively, this indicates that greater intestinal uptakes of amino acids relative to glucose are advantageous. This is not surprising in the context of reduced CP diets and emphasises the relevance of starch-protein digestive dynamics (Selle and Liu, 2019). However, dietary lipid levels have been shown to modify responses to dietary protein levels (Liu et al., 2017) and starch to lipid ratios have been shown to influence growth performance (Khoddami et al., 2018). In the present study the positive control diet contained 60 g/kg fat (specified) in comparison to 51.2 g/kg in the uncapped 197.5 g/kg CP diet but 81.0 g/kg fat in the capped 197.5 g/kg CP diet. Also, the specified starch to lipid ratios varied from 5.85 to 7.60 and 3.99 in the 3 respective diets. These differences in fat levels and starch to lipid ratios may have been contributing to the better growth performance of birds offered the capped diet. It is also possible that

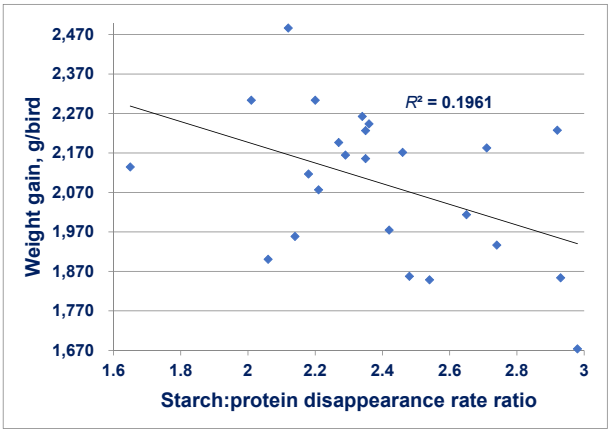

Fig. 2. The linear relationship ($r = -0.443$; $P = 0.030$) between distal jejunal starch:protein disappearance rate ratios and weight gain (g/bird) in birds offered diets 1A, 2B, 5F where Weight gain = 2,721 − 262 × Disappearance rate ratio.

there was less competition between glucose and amino acids for intestinal uptakes of amino acids via their respective Na^+-dependent transport systems (Vinardell, 1990). This possibility is supported by the ileal amino acid disappearance rates. The capped 197.5 g/kg CP diet supported numerically faster disappearance rates for nine amino acids and statistically more rapid disappearance rates for 7 amino acids including arginine, histidine, isoleucine, leucine, alanine aspartic acid and tyrosine than the uncapped 197.5 g/kg CP diet.

5. Conclusion

In conclusion, the hypothesis that capping dietary starch:protein ratios in reduced CP broiler diets is advantageous was established, albeit on a limited basis. Further evaluations of this approach are clearly justified. The possibility that accumulation of ammonia in birds offered reduced CP diets is having negative impacts on growth performance also merits more investigations.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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