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

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A R T I C L E   I N F O

Article history:
Received 11 September 2019
Received in revised form 7 January 2020
Accepted 12 January 2020
Available online 23 January 2020

Keywords:
Amino acids
Broiler chicken
Crude protein
Feathering
Starch:protein ratio
Wheat

A B S T R A C T

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.

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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).

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.
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 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 medium-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 then cooled.

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, Hanau, 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 medium-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 then cooled.

2.2. Diet preparation

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 gain, 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.
The appropriate diets. Nitrogen contents of diets and excreta were corrected N retention to zero using the factor of 36.54 kJ/g N calculated from the following equation:

\[ \text{AME} = \left( \frac{\text{Nutrient/AIA}}{\text{diet}} \right) \times \left( \frac{\text{Excreta output}}{\text{GE excreta}} \right) \times \frac{\text{Feed intake}}{\text{Feed intake}}. \]

The ME:GE ratios were calculated by dividing AME by the GE of the diets on a dry matter basis were calculated by methods described in Siriwan et al. (1993). Amino acid digestibilities were determined in birds offered diets 1A, 2B and 5F. Apparent digestibility coefficients were calculated by the following equation:

\[ \text{Digestibility coefficient} = \left( \frac{\text{Nutrient/AIA}}{\text{diet}} - \frac{\text{Nutrient/AIA}}{\text{digesta}} \right) \times \frac{\text{Feed intake}}{\text{Feed intake}}. \]

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 compositions of 7 experimental diets (g/kg, as-is basis).
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

### 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, mg/kg                | 250   | 250   | 250   | 250   | 250   | 250   | 250   |
| Crude fat                 | 60.0  | 51.2  | 38.4  | 29.7  | 81.0  | 78.5  | 91.7  |
| Crude fibre               | 27.0  | 26.5  | 25.1  | 23.2  | 24.9  | 22.4  | 21.0  |

**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  | 8.6  | 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  | 4.9  | 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|

dEB = dietary electrolyte balance.
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 than the corresponding modified diets 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

| Item                          | Weight gain, g/bird | Feed intake, g/bird | FCR, g/g | Mortalities1, % | 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               | 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               | 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               | 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

| Crude protein (CP) | Feed intake, g/bird | Mortalities1, % | Fat-pad weights, g/kg | Feather scores |
|--------------------|---------------------|----------------|-----------------------|---------------|
| 197.5              | 2.063c              | 3.389c         | 1.650a                | 8.26          | 1.9a          |
| 180.0              | 1.444b              | 2.840b         | 1.972b                | 8.41          | 2.9b          |
| 162.5              | 1.008a              | 2.482a         | 2.490c                | 8.26          | 3.1c          |

Mode

| Standard          | Feed intake, g/bird | Mortalities1, % | Fat-pad weights, g/kg | Feather scores |
|-------------------|---------------------|----------------|-----------------------|---------------|
| 1.471             | 2.812a              | 1.996          | 0.70                  | 7.77          | 2.9           |
| Modified          | 2.076               | 3.47          | 3.47                  | 8.84          | 2.4           |

Significance (P-value)

| Crude protein (CP) | Feed intake, g/bird | Mortalities1, % | Fat-pad weights, g/kg | Feather scores |
|--------------------|---------------------|----------------|-----------------------|---------------|
| <0.001             | 0.014               | 0.014         | 0.014                 | 0.006         | 0.230         |

Pair-wise comparisons (P-value)

| Diet 1A versus 2B | Feed intake, g/bird | Mortalities1, % | Fat-pad weights, g/kg | Feather scores |
|-------------------|---------------------|----------------|-----------------------|---------------|
| 0.010             | 0.017               | 0.027          | 0.005                 | 0.091         |
| 1A versus 5E      | 0.096               | 0.959         | 0.910                 | 0.012         | 0.842         |
| 2B versus 5E      | 0.009               | 0.019         | 0.456                 | 0.725         | 0.949         |

**Note**: 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).
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 mg/mL in birds offered diet 2B, 251 mg/mL in diet 1A and 267 μg/mL in diet 5E. Total concentrations followed the same pattern, 796, 843 and 857 mg/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 6
Effects of dietary treatments on parameters of nutrient utilisation and excreta dry matter from 32 to 34 d post–hatch.

| Item | Crude protein, g/kg | Mode | AME, MJ/kg DM | ME:GE ratio, MJ:1MJ | N retention, % | AMEn, MJ/kg DM | Excreta dry matter, g/kg |
|------|---------------------|------|---------------|---------------------|----------------|---------------|------------------------|
| 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<sup>b</sup> | 255<sup>b</sup> |
| 5E | 197.5 | Modified | 12.53 | 0.767 | 62.31 | 12.00<sup>b</sup> | 279<sup>b</sup> |
| 3C | 180.0 | Standard | 12.67 | 0.783 | 62.74 | 12.23<sup>b</sup> | 237<sup>b</sup> |
| 6F | 180.0 | Modified | 12.25 | 0.766 | 58.84 | 11.90<sup>c</sup> | 291<sup>c</sup> |
| 4D | 162.5 | Standard | 12.83 | 0.808 | 60.64 | 12.53<sup>c</sup> | 223<sup>c</sup> |
| 7G | 162.5 | Mode | 12.14 | 0.774 | 58.09 | 11.82<sup>a</sup> | 288<sup>a</sup> |
| SEM | 0.1301 | 0.0084 | 1.0747 | 0.1006 | 7.912 |

Main effect: Crude protein

197.5 | 12.48 | 0.765<sup>a</sup> | 63.28<sup>b</sup> | 11.99 | 267 |
180.0 | 12.46 | 0.775<sup>b</sup> | 60.79<sup>a</sup> | 12.07 | 264 |
162.5 | 12.57 | 0.791<sup>c</sup> | 59.63<sup>a</sup> | 12.17 | 255 |

Mode

Standard | 12.70<sup>b</sup> | 0.785<sup>b</sup> | 62.54<sup>b</sup> | 12.25 | 238 |
Modified | 12.31<sup*c</sup> | 0.769<sup*a</sup> | 59.75<sup>a</sup> | 11.91 | 286 |

Significance (P-value)

Crude protein (CP) | 0.674 | 0.012 | 0.03 | 0.192 | 0.332 |
Mode (M) | <0.001 | 0.028 | 0.03 | <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.

<sup>a, b, c</sup>Within a column, 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 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 x 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 Standard 60.8 64.4 26.2 32.4 2.34 2.01 |
| 2B 197.5 Standard 58.5b 62.0f 23.6 30.9 2.53cd 2.01h |
| 5E 197.5 Modified 51.2e 55.9b 22.7 32.8 2.31e 1.71h |
| 3C 180.0 Standard 51.9b 55.1b 19.1 23.1 2.73e 2.36e |
| 6F 180.0 Modified 42.5b 48.6b 18.6 25.2 2.02b 1.70b |
| 4D 162.5 Standard 61.4d 63.8c 16.5 20.2 3.78b 3.17f |
| 7G 162.5 Modified 29.8a 38.2a 15.6 19.4 1.95b 1.99b |
| SEM 2.125 1.900 1.382 1.278 0.1270 0.0500 |

Main effect: Crude protein

| CP | Mode | Significance (P-value) | Pair-wise comparisons (P-value) |
|----|------|------------------------|--------------------------------|
| 197.5 | Standard | <0.001 | <0.001 | <0.001 | <0.001 |
| 180.0 | Standard | <0.001 | 0.503 | 0.309 | <0.001 |
| 162.5 | Standard | <0.001 | 0.985 | 0.438 | <0.001 |

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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 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            | 2.42b     | 187a| 2.30b   | 0.0775        | < 0.001        | 0.228         |
| Histidine           | 0.92b     | 0.77a| 0.82b   | 0.0335        | 0.014          | 0.099         |
| Isoleucine          | 1.52b     | 1.34a| 1.58b   | 0.0581        | 0.024          | 0.171         |
| Leucine             | 2.65b     | 2.18a| 2.55b   | 0.0975        | 0.007          | 0.287         |
| Lysine              | 1.97b     | 1.69a| 1.85b   | 0.0758        | 0.049          | 0.223         |
| Methionine          | 1.77b     | 1.45a| 1.63b   | 0.0632        | 0.007          | 0.186         |
| Phenylalanine       | 1.77b     | 1.45a| 1.63b   | 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        | 0.006          | –             |
| Alanine             | 1.31b     | 1.07a| 1.24b   | 0.0536        | 0.017          | 0.158         |
| Aspartic acid       | 3.15b     | 2.44a| 2.86b   | 0.1167        | 0.001          | 0.343         |
| Glutamic acid       | 7.98b     | 6.92a| 7.46b   | 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              | 1.96b     | 1.39a| 1.58b   | 0.0652        | 0.023          | 0.792         |
| Tyrosine            | 0.85      | 0.61a| 0.73b   | 0.0316        | < 0.001        | 0.093         |

PC = positive control; SEM = standard error of the mean; LSD = least significant difference.

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, mg/mL | Treatment | SEM | P-value | LSD (P < 0.05) |
|-------------|-----------|-----|---------|---------------|
|             | 1A (PC)   | 2B  | 5E      |               |
| Arginine    | 79b       | 63a | 78b     | 3.17          | 0.003          | 9.3           |
| Histidine   | 16b       | 11a | 13ab    | 1.08          | 0.014          | 4.9           |
| Isoleucine  | 17        | 17  | 19      | 0.72          | 0.158          | –             |
| Leucine     | 27        | 23  | 25      | 1.07          | 0.105          | –             |
| Lysine      | 26b       | 17a | 20ab    | 2.51          | 0.046          | 7.4           |
| Methionine  | 11b       | 17a | 20b     | 0.54          | < 0.001        | 1.6           |
| Phenylalanine| 24       | 23  | 23      | 0.61          | 0.264          | –             |
| Threonine   | 78a       | 78a | 97b     | 4.52          | 0.009          | 13.2          |
| Tryptophan  | 7         | 6   | 6       | 0.28          | 0.823          | –             |
| Valine      | 33a       | 36b | 38b     | 1.39          | 0.034          | 4.1           |
| Alanine     | 68        | 61  | 64      | 3.19          | 0.537          | –             |
| Aspartate   | 8a        | 0b  | 7ab     | 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.

Within a row, means not sharing a common superscript are significantly different at P < 0.05.
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 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 body-weight 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. Birki 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: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

### Table 12

| 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    | 65.9          | 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 |                     |
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.

Acknowledgements

The authors wish to acknowledge the guidance and financial support provided by Agri Futures Chicken-meat. This experiment was part of project PRJ-010623 entitled “Utilisation of synthetic amino acids by poultry”. Also, we would very much thank Ms Joy Gill, Ms Kylee Warr and Mr Duwei Chen of the Poultry Research Foundation for their invaluable technical support.

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