Dietary crude protein concentrations, feed grains, and whey protein interactively influence apparent digestibility coefficients of amino acids, protein, starch, and performance of broiler chickens

Shiva Greenhalgh, Andreas Lemme, Juliano C. de Paula Dorigam, Peter V. Chrystal, Shemil P. Macelline, Sonia Yun Liu, and Peter H. Selle

Poultry Research Foundation within The University of Sydney, Camden, 2570 NSW, Australia; School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camperdown, 2006 NSW, Australia; Evonik Operations GmbH, Hanau-Wolfgang 63457, Germany; and Sydney School of Veterinary Science, The University of Sydney, Camden, 2570 NSW, Australia

ABSTRACT The present study was designed to investigate the impacts of dietary crude protein (CP) concentrations (220 and 180 g/kg) in either maize- or wheat-based diets, without or with 25 g/kg inclusions of whey powder (WP) concentrate on performance parameters and apparent amino acid digestibility coefficients in broiler chickens. The maize and wheat used in this study had CP levels of 84 and 119 g/kg, respectively. The 2 x 2 factorial array of 8 dietary treatments was offered to a total of 336 off-sex, male Ross 308 chicks from 7 to 35 d post-hatch with 7 replicate cages (6 birds per cage) per treatment. A treatment interaction (P = 0.016) between dietary CP and feed grains was detected for weight gains, where birds offered 180 g/kg maize-based diets displayed a weight gain advantage of 6.74% (2,628 vs. 2,462 g/bird) compared to their wheat-based counterparts. An interaction (P = 0.022) between feed grains and whey protein was observed for FCR as the addition of WP to maize-based diets improved FCR by 3.45% (1.314 vs. 1.361), but compromised FCR in wheat-based diets by 2.98% (1.415 vs. 1.374). A treatment interaction (P = 0.038) between dietary CP and feed grains was recorded for relative abdominal fat-pad weights weight gains as birds offered 180 g/kg CP maize-based diets had 43.4% (11.17 vs. 7.79 g/kg) heavier fat-pads than their wheat-based counterparts. Following the reduction in dietary-CP, apparent amino acid digestibility coefficients were depressed to greater extents in wheat-based diets. However, significant interactions between CP and feed grains were found in 14 of the 16 amino acids assessed and significant interactions between CP and WP were observed for 15 amino acids. Maize was the more suitable feed grain in terms of weight gain and FCR in 180 g/kg CP diets despite causing greater fat deposition. The inclusion of WP in reduced-CP diets did not enhance bird performance. Data generated indicate concentrations of microbial amino acids in distal ileal digesta were depressing apparent amino acid digestibility coefficients, which was more evident in wheat-based diets. Higher gut viscosities in birds offered wheat-based diets may have facilitated the proliferation of microbiota along the small intestine.

Key words: amino acids, apparent digestibilities, crude protein, feed grains, whey protein

INTRODUCTION

Maize and wheat are the two most commonly used feed grains in chicken-meat production globally and maize is dominant in this respect. However, in the quest to develop reduced-crude protein (CP) diets for broiler-chickens, it is becoming evident that maize is more...
intestinal uptakes of non-bound amino acids (Wu, 2009).

Reductions in dietary CP generate perturbations in apparent amino acid digestibility coefficients, as reviewed by Liu et al. (2021), and were very evident in Chrystal et al. (2021). These researchers reported that reductions in dietary CP significantly enhanced apparent ileal digestibilities of 7 amino acids in birds offered maize- or wheat-based diets but significantly depressed digestibilities of another 7 amino acids. Only alanine digestibility was not influenced by treatment. The underlying causes for these fluctuations in apparent amino acid digestibility coefficients are almost certainly complex and foil the identification of appropriate amino acid ratios for reduced-CP broiler diets.

The digestive dynamics of starch/glucose and protein/amino acids impact on broiler performance (Liu and Selle, 2015) and this impact may be amplified in the context of reduced-CP diets (Liu and Selle, 2017). Whey protein (WP) is recognised as a rapidly digested protein source. A whey hydrolysate was shown to stimulate muscle protein synthesis to greater extents than a soy protein isolate or casein, which was attributed to the more rapid digestion of whey protein (Tang et al., 2009).

In broiler chickens, inclusions of a WP concentrate at 8 and 32 g/kg in standard maize-wheat diets were evaluated by Szczurek et al. (2013) from 1 to 42 days post-hatch. The 8 g/kg WP inclusion improved weight gain by 8.67% (2,130 vs. 1,960 g/bird) and FCR by 8.33% (1.76 vs. 1.92). The 32 g/kg WP inclusion improved weight gain by 12.8% (2,210 vs. 1,960 g/bird) and FCR by 11.5% (1.70 vs. 1.92). Also, the higher WP inclusion significantly improved ileal protein (N) digestibility by 7.31% (0.851 vs. 0.793). Pineda-Quiroga et al. (2017) concluded that dry whey powder and whey protein concentrate held promise as a feed ingredient for broiler chickens. Therefore, the potential of moderate WP inclusions in broiler diets as an alternative source of protein-bound amino acids is of interest in this context.

The purpose of comparing maize vs. wheat in diets with standard or reduced-CP concentrations was to verify the outcomes of the Chrystal et al. (2021) study in which maize clearly outperformed wheat. This was consistent with the Greenhalgh et al. (2020) study where birds offered reduced-CP, wheat-based diets performed remarkably poorly. Alternatively, in another study (Yin et al., 2020), the performance of birds offered 165 g/kg CP, wheat-based diets was relatively satisfactory, although the transition from 215 to 165 g/kg CP diets noticeably compromised FCR by 5.99% (1.576 vs. 1.487). That intestinal uptakes of non-bound amino acids are more rapid than protein-bound amino acids in poultry was demonstrated by Liu et al. (2013). It may be deduced from this study that the mean digestion rate constant of non-bound amino lysine and methionine was 8.64 × 10⁻² min⁻¹ in standard sorghum-based broiler diets as opposed to 2.35 × 10⁻² min⁻¹ for protein-bound amino acids. The rationale for including WP in the present study was to provide a highly and rapidly digested source of protein and amino acids as an alternative to both non-bound amino acids and protein-bound amino acids in soybean meal. Given the anticipated fluctuations in apparent amino acid digestibilities following dietary CP reductions, relative proportions of dietary, endogenous and microbial amino acids in distal ileal digesta were estimated by the Duvaux et al. (1990) mathematical model. Thus, the present study was designed to investigate the impacts of dietary CP concentrations in either maize- or wheat-based diets, without or with WP, on apparent amino acid digestibility coefficients and performance parameters in broiler chickens.

### MATERIALS AND METHODS

This feeding study fully complied with specific guidelines approved by the Research Integrity and Ethics Administration of The University of Sydney (Project number 2019/1497).

#### Experimental Design

The trial design comprised a 2²×2 factorial array of dietary treatments which were offered to birds from 7 to 35 d post-hatch. The diets were formulated to contain two CP concentrations of either 220 or 180 g/kg and were based on either maize or wheat, without and with 25 g/kg WP inclusions.

#### Diet Preparation

The diets were formulated based on evaluations of maize, wheat, soybean meal and full-fat soy by near-infrared spectroscopy (NIR) and the AMINO.NIR Advanced program (Evonik Operations GmbH. Hanau, Germany). WPC was analysed for amino acids concentrations and by size-exclusion chromatography as shown in Table 1. The total amino acid concentration in WP was 769.3 g/kg and 97% of protein was in the form of large polypeptides with molecular weights greater than 10,000 Daltons. The compositions and nutrient specifications of the experimental diets are shown in Tables 2 and 3, respectively. Maize and wheat were coarsely ground (6.0-mm hammer-mill screen) prior to incorporation into the complete diets, which were cold-pelleted at an approximate temperature of 65°C. Acid insoluble ash (Celite, Celite Corporation. Lompoc, CA) was included in diets at 20 g/kg as an inert dietary marker to determine apparent jejunal and ileal digestibility coefficients of starch, protein (N) and amino acids. All diets were formulated to 11.50 g/kg digestible lysine with an energy density of 12.97 MJ/kg, a concentration of 13.77 g/kg digestible glycine equivalents and a dietary electrolyte balance of 250 mEq/kg. Phytate- and non-starch polysaccharide-degrading feed enzymes were included across all diets. Soybean meal (505 g/kg protein) and full-fat soy (362 g/kg protein) were both sourced from the same manufacturer (Soon Soon Oil-mills Sdn Bhd. Perai, Malaysia). The analyzed starch,
crude protein, and amino acid concentrations of the experimental diets are shown in Table 4.

Bird Management

A total of 336 off-sex, male Ross 308 chicks (parent line) were procured from a commercial hatchery and were initially offered a proprietary starter diet. At 7 d post-hatch, birds were individually identified (wing-tags) and allocated into bioassay cages on the basis of body-weights so that their mean body-weights and variations between cages were statistically identical. Average weight of broiler chickens at day 7 was 177 g/bird with a standard deviation of ±1.15. Each of the dietary treatments was offered to 7 replicate cages (6 birds per cage) from 7 to 35 d post-hatch. Cage dimensions were 75 cm in width and depth and 50 cm in height. Broilers had unlimited access to water and feed under 23 h illumination for the first three days followed by 16 h illumination for the remainder of the study. There was an initial room temperature of 32°C, which was gradually

Table 2. Composition of experimental diets.

| Ingredient (g/kg) | 1A | 2B | 3C | 4D | 5E | 6F | 7G | 8H |
|------------------|----|----|----|----|----|----|----|----|
| Maize            | 565| 592| 686| 716| 587| 608| 702| 731|
| Wheat            | 25.0| 25.0| 25.0| 25.0| 25.0| 25.0| 25.0| 25.0|
| Soybean meal     | 254| 209| 132| 81.1| 223| 182| 33.5|
| Full-fat soy     | 100| 100| 100| 100| 100| 100| 100| 100|
| Soy oil          | 28.4| 22.0| 7.6| 1.2| 37.4| 32.7| 10.1| 22.8|
| Lysine HCl       | 2.28| 4.01| 1.58| 5.45| 2.94| 2.08| 8.60| 7.67|
| Methionine       | 3.28| 2.95| 4.31| 4.02| 3.16| 2.77| 4.65| 4.38|
| Threonine        | 1.11| 0.25| 0.62| 2.34| 1.40| 0.84| 3.93| 3.40|
| Arginine         | 0.87| 3.49| 4.52| 0.19| 0.90| 5.39| 6.12|
| Histidine        | 0.12| 0.18| 1.37| 0.47| 0.28| 0.15| 0.69| 0.86|
| Isoleucine       | 1.99| 1.81| 0.31| 0.20| 0.31| 0.20| 0.31| 0.20|
| Leucine          | 4.16| 3.42| 3.42| 3.42| 3.42| 3.42| 3.42| 3.42|
| Tryptophan       | 0.29| 0.23| 0.23| 0.23| 0.23| 0.23| 0.23| 0.23|
| Valine           | 2.20| 2.04| 0.26| 0.26| 0.26| 0.26| 0.26| 0.26|
| Glycine          | 0.72| 3.66| 4.11| 1.03| 4.68| 4.99|
| Limestone        | 13.5| 13.6| 13.8| 13.9| 13.6| 13.7| 14.1| 14.1|
| Dicalcium phosphate | 5.3| 5.9| 6.8| 7.5| 5.6| 6.1| 7.9| 8.5|
| Sodium chloride  | 3.08| 1.86| 1.86| 1.86| 1.86| 1.86| 1.86| 1.86|
| Sodium bicarbonate | 1.81| 4.58| 4.61| 4.61| 4.61| 4.61| 4.61| 4.61|
| Potassium carbonate | 2.08| 3.77| 3.77| 3.77| 3.77| 3.77| 3.77| 3.77|
| Phytase (Axtra Phy) | 0.10| 0.10| 0.10| 0.10| 0.10| 0.10| 0.10| 0.10|
| Xylanase (Danisco Xylanase) | 0.10| 0.10| 0.10| 0.10| 0.10| 0.10| 0.10| 0.10|
| Vitamin-mineral premix | 2.00| 2.00| 2.00| 2.00| 2.00| 2.00| 2.00| 2.00|
| Sand             | 27.7|
| Cellite          | 20.0| 20.0| 20.0| 20.0| 20.0| 20.0| 20.0| 20.0|
| Non-bound amino acids | 6.17| 7.92| 17.79| 23.44| 7.30| 6.26| 36.82| 35.04|
| Intact protein (%) | 97.1| 96.3| 10.2| 86.4| 96.4| 97.1| 78.8| 79.9|

1Feed enzymes from Danisco Animal Nutrition and Health. Phytase 1,000 FTU/kg, Xylanase 4,000 U/kg.
2Vitamin-trace mineral premix supplies in MIU/kg or mg/kg of diet: [MIU] retinol 12, cholecalciferol 5, [μg] 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.
3Approximate proportion of analysed dietary CP derived from intact protein.
decreased to 22°C by the end of the feeding study. Body weights and feed intakes were monitored from which feed conversion ratios (FCR) were calculated. The incidence of dead or culled birds was recorded daily and their body weights used to adjust feed intakes per cage and correct FCR calculations.

Sample Collection and Chemical Analysis

Total excreta were collected from 33 to 35 d post-hatch from each cage to determine parameters of nutrient utilization which included apparent metabolizable energy (AME), metabolizable energy to gross energy ratios (ME:GE), nitrogen (N) retention and N-corrected apparent metabolizable energy (AMEn). Excreta was dried in a forced air oven at 80°C for 24 h. The GE of diets and excreta was determined by bomb calorimetry using an adiabatic calorimeter (Parr 1281 bomb calorimeter, Parr Instruments Co., Moline, IL).

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

$$\text{AMEdiet} = \frac{\text{feedintake}}{C_2 \times \text{GEdiet}} - \frac{\text{excretaoutput}}{C_2 \times \text{GEexcreta}}$$

ME:GE ratios were calculated by dividing AME by the GE of the appropriate diets. N contents of diets and

| Item (g/kg) | 1A | 2B | 3C | 4D | 5E | 6F | 7G | 8H |
|------------|----|----|----|----|----|----|----|----|
| Protein (N) | 214 | 212 | 181 | 172 | 213 | 217 | 174 | 174 |
| Starch | 279 | 263 | 323 | 410 | 263 | 284 | 283 | 330 |
| Dietary starch:protein ratio | 1.30 | 1.74 | 1.78 | 2.38 | 1.23 | 1.31 | 1.63 | 1.90 |
| Arginine | 14.36 | 13.70 | 12.79 | 12.42 | 13.62 | 13.45 | 13.77 | 13.77 |
| Histidine | 5.42 | 5.31 | 4.21 | 4.05 | 5.16 | 5.00 | 5.00 | 5.00 |
| Isoleucine | 8.84 | 9.26 | 8.20 | 8.45 | 9.00 | 8.93 | 7.92 | 8.27 |
| Leucine | 18.08 | 18.57 | 15.14 | 15.36 | 15.17 | 15.38 | 13.04 | 13.34 |
| Lysine | 12.90 | 12.96 | 12.03 | 11.81 | 12.93 | 12.72 | 11.42 | 11.78 |
| Methionine | 6.08 | 6.02 | 6.25 | 6.25 | 5.84 | 5.64 | 6.12 | 6.33 |
| Phenylalanine | 10.33 | 9.98 | 7.95 | 7.32 | 10.20 | 9.74 | 6.47 | 6.21 |
| Threonine | 9.05 | 9.21 | 8.40 | 8.33 | 8.85 | 8.88 | 7.72 | 8.19 |
| Valine | 9.78 | 9.97 | 9.15 | 9.33 | 9.95 | 9.61 | 9.18 | 9.94 |
| Alanine | 10.62 | 10.84 | 8.97 | 8.94 | 8.63 | 8.73 | 5.42 | 5.62 |
| Aspartic acid | 21.36 | 21.43 | 15.52 | 14.51 | 19.97 | 19.51 | 10.31 | 10.65 |
| Cysteine | 3.01 | 3.26 | 2.47 | 2.57 | 3.44 | 2.66 | 2.46 | 2.66 |
| Glutamic acid | 38.02 | 37.85 | 29.59 | 28.27 | 45.42 | 45.60 | 34.50 | 34.31 |
| Glycine | 8.82 | 8.73 | 9.70 | 9.34 | 8.85 | 8.50 | 9.67 | 9.88 |
| Proline | 11.98 | 12.50 | 10.26 | 10.31 | 13.62 | 13.60 | 10.74 | 10.96 |
| Serine | 10.52 | 10.48 | 8.07 | 7.42 | 10.25 | 10.46 | 6.31 | 6.44 |
| Total | 199.17 | 200.07 | 168.70 | 164.68 | 200.71 | 199.19 | 157.33 | 160.79 |
| Glycine equivalents | 16.33 | 16.22 | 15.46 | 14.64 | 16.17 | 15.97 | 14.18 | 14.48 |
excreta were determined using a nitrogen determinator (Leco Corporation, St Joseph, MI) and N retentions calculated from the following equation:

\[ \text{Nretention}_{\%} = \frac{\text{feedintake} \times N_{\text{diet}} - \text{excretaoutput} \times N_{\text{excreta}}}{\text{feedintake} \times N_{\text{diet}}} \times 100 \]

N-corrected AME values on a dry matter basis 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 35 d post-hatch, birds were euthanized by intravenous sodium pentobarbitone injection. Abdominal cavities were opened and fat-pads dissected out and their weights recorded; absolute weights were matched with final body-weights of birds to calculate relative abdominal fat-pad weights. The small intestine was removed and digesta samples were collected in their entirety from the distal jejunum and distal ileum. The distal jejunum was demarcated by the mid-point between the end of the duodenal loop and Meckel’s diverticulum and the distal ileum by the mid-point between Meckel’s diverticulum and the ileo-cecal junction. Digesta were manually gently expressed from the distal half of the jejunum and ileum. Digesta samples from each cage were pooled, homogenized, freeze-dried, and ground through 0.5 mm screen. The samples were then analysed for concentrations of starch, protein (N), and amino acids. Starch concentrations were determined by a procedure based on dimethyl sulfoxide, \( \alpha \)-amylase and amyloglucosidase, as described in Mahasukhonthachat et al. (2010). Amino acid concentrations of diets and digesta were determined following 24-h liquid hydrolysis at 110°C in 6 M HCl and acid concentrations of diets and digesta were determined using a nitrogen determinator (1993). Apparent digestibility coefficients (ADC) from the following equation:

\[ \text{ADC} = \frac{\langle \text{nutrient/AIA}\rangle_{\text{diet}} - \langle \text{nutrient/AIA}\rangle_{\text{digesta}}}{\langle \text{Nutrient/AIA}\rangle_{\text{diet}}} \]

Disappearance rates (g/bird/day) of starch and protein (N) were calculated from daily feed intakes, dietary nutrient concentrations, and apparent digestibility coefficients (ADC) from the following equation:

\[ \text{Disappearance rate} = \frac{\text{dailyfeedintake(g/bird) \times dietarynutrient(g/kg)} \times \text{ADC}}{\text{AIA}} \]

\[ \text{Statistical Analysis} \]

Experimental data were analysed by analyses of variance using the IBM SPSS Statistics Version 27 program (IBM Corporation. New York, NY). Linear and quadratic regressions, Pearson correlations and Tukey’s range test were performed when considered relevant. Experimental units were the cage means and a probability level of less than 5% was considered statistically significant.

The relative proportions of dietary, endogenous and microbial amino acids in the amino acid pool in distal ileal digesta (Y) were estimated using the mathematical model developed by Duvaux et al. (1990). These estimates were based on the analysed dietary amino acid profile (X1), endogenous amino acids (X2) were based on the amino acid profile of avian mucin reported by Fang et al. (1993) and microbial amino acids (X3) were based on the amino acid profile of Lactobacillus bulgaricus reported by Xia et al. (2007). The model estimates the proportions of different protein sources in a mixture from their amino acid profiles on percentage basis. The multiple linear regression for any one amino acid in each of the 2 components where b is the coefficient of each component is as follows:

\[ Y_i/\sqrt{Y_i} = b_1X_{1,i}/\sqrt{Y_i} + b_2X_{2,i}/\sqrt{Y_i} + b_3X_{3,i}/\sqrt{Y_i} \]

The multiple linear regression for 16 amino acids in each of the 3 components is as follows:

\[ \Sigma Y_{i,k}/\sqrt{Y_{i,k}} = b_1\Sigma X_{1,i,k}/\sqrt{Y_{i,k}} + b_2\Sigma X_{2,i,k}/\sqrt{Y_{i,k}} + b_3\Sigma X_{3,i,k}/\sqrt{Y_{i,k}} \]

The multiple-linear regression (without intercept) between amino acid profiles in a protein mixture (Y) and amino acid profiles of different protein components in the mixture (X1, X2, X3) is used in this model where the percentage values of coefficients (b%) of independent variables represents their estimated proportions in the mixture.

\[ \text{RESULTS} \]

The effects of dietary treatments on growth performance from 7 to 35 days post-hatch and relative abdominal fat-pad weights are shown in Table 5. The average weight of birds at the commencement of the feeding study was 195 g. A treatment interaction \( (P = 0.016) \) between dietary CP and feed grain was observed for weight gain. Weight gains of birds offered 220 g/kg CP diets were comparable with diets based on maize (2,713 g/bird) and wheat (2,705 g/bird); however, there was an advantage of 6.74% (2628 vs. 2462 g/bird). A treatment interaction \( (P = 0.002) \) between feed grains and WP was found for FCR as the addition of WP to maize-based diets improved FCR by 3.45% (1.314 vs. 1.361). Conversely, WP inclusion in wheat-based diets compromised FCR by 2.98% (1.314 vs. 1.361). As a main effect, reducing dietary CP compromised FCR by 7.28% (1.414 vs. 1.318). A treatment interaction \( (P = 0.038) \) between dietary CP and feed grains was observed for relative fat-pad weights. Following the dietary CP reduction, fat-pad weights in birds offered maize-based diets increased by 27.1% (10.45 vs. 8.22 g/kg) as opposed to the more modest increase of
11.2% (7.07 vs. 6.36 g/kg) in birds offered wheat-based diets. There were no treatment effects on feed intake (\( P > 0.15 \)) nor mortality rate (\( P > 0.30 \)).

The effects of dietary treatments on nutrient utilization parameters and N concentrations in excreta are shown in Table 6. Maize-based diets supported higher AME values by 1.14 MJ (13.70 vs. 12.56 MJ/kg; \( P < 0.001 \)) than wheat-based diets. Diets with 180 g/kg CP generated a 4.62% increase in ME:GE ratios (0.815 vs. 0.779; \( P < 0.001 \)) in comparison to 220 g/kg CP diets.

### Table 5. Effects of dietary treatments on growth performance and relative abdominal fat-pad weights from 7 to 35 d post-hatch

| Treatment | Growth performance | Weight gain (g/bird) | Feed intake (g/bird) | FCR (g/g) | Mortality rates (%) | Relative fat-pad weights (g/kg) |
|-----------|--------------------|----------------------|----------------------|-----------|---------------------|-------------------------------|
| Crude protein (g/kg) | Feed grain | Whey protein (g/kg) | | | | |
| 220 Maize 0 | 2,690 | 3,583 | 1.338 | 2.38 | 8.02 |
| 220 Wheat 0 | 2,736 | 3,474 | 1.268 | 2.38 | 8.41 |
| 180 Maize 0 | 2,598 | 3,586 | 1.314 | 2.38 | 6.19 |
| 180 Wheat 0 | 2,658 | 3,609 | 1.360 | 2.38 | 6.53 |
| SEM Maize 0 | 2,414 | 3,570 | 1.479 | 0.00 | 7.78 |
| SEM Wheat 0 | 2,510 | 3,595 | 1.433 | 0.00 | 7.79 |

Main effects: Crude protein
- 220 2,709 3,565 1.318 a 1.79 7.29
- 180 2,545 3,590 1.414 b 0.60 9.48
Feed grain
- Maize 2,671 3,563 1.338 1.79 9.69
- Wheat 2,545 3,592 1.394 0.60 7.08
Whey protein
- 0 2,671 3,585 1.368 1.19 8.04
- 25 2,583 3,571 1.364 1.19 8.73

Significance (\( P = \))
- Crude protein (CP) \(< 0.001 \)< 0.001 < 0.001 0.024 < 0.001
- Feed grain (FG) \(< 0.001 \)< 0.001 < 0.001 < 0.001 < 0.001 < 0.001
- Whey protein concentrate (WP) 0.870 0.732 0.864 1.000 0.061
- CP \(\times\) FG interaction 0.016 0.279 0.151 1.000 0.038
- CP \(\times\) WP interaction 0.689 0.755 0.468 0.322 0.369
- FG \(\times\) WP interaction 0.074 0.475 0.022 0.322 0.157
- CP \(\times\) FG \(\times\) WP interaction 0.536 0.190 0.655 1.000 0.178

abMeans within columns not sharing a common suffix are significantly different at the 5% level of probability.

### Table 6. Effects of dietary treatments on parameters of nutrient utilization and excreta nitrogen concentrations.

| Treatment | Nutrient utilization | AME (MJ/kg DM) | ME:GE ratio (MJ/MJ) | N retention (%) | AMEn (MJ/kg DM) | Excreta N (mg/g) |
|-----------|----------------------|-----------------|---------------------|----------------|----------------|----------------|
| Crude protein (g/kg) | Feed grain | Whey protein (g/kg) | | | | |
| 220 Maize 0 | 13.62 | 0.820 | 82.88 | 12.19 | 4.62 |
| 220 Wheat 0 | 12.58 | 0.741 | 78.62 | 11.20 | 4.15 |
| 180 Maize 0 | 13.71 | 0.847 | 87.01 | 12.34 | 3.52 |
| 180 Wheat 0 | 13.74 | 0.859 | 87.15 | 12.46 | 3.56 |
| SEM Maize 0 | 12.56 | 0.770 | 84.30 | 11.28 | 3.07 |
| SEM Wheat 0 | 12.70 | 0.785 | 84.01 | 11.44 | 2.82 |

Main effects: Crude protein
- 220 13.09 0.779 a 80.49a 11.67a 4.43a
- 180 13.18 0.813 a 85.40b 11.88b 3.24a
Feed grain
- Maize 13.12 0.795 82.98 11.75 3.84
- Wheat 13.15 0.799 82.90 11.80 3.83
Whey protein
- 0 13.12 0.795 82.98 11.75 3.84
- 25 13.15 0.799 82.90 11.80 3.83

Significance (\( P = \))
- Crude protein (CP) 0.314 < 0.001 < 0.001 < 0.001 < 0.001
- Feed grain (FG) < 0.001 < 0.001 < 0.001 < 0.001 < 0.001
- Whey protein concentrate (WP) 0.699 0.413 0.821 0.628 0.906
- CP \(\times\) FG interaction 0.610 0.341 0.178 0.526 0.454
- CP \(\times\) WP interaction 0.522 0.108 0.217 0.291 0.170
- FG \(\times\) WP interaction 0.633 0.657 0.850 0.402 0.258
- CP \(\times\) FG \(\times\) WP interaction 0.249 0.477 0.637 0.313 0.384

abMeans within columns not sharing a common suffix are significantly different at the 5% level of probability.

Mean feed intake over total excreta collection period was 324 g/bird which did not vary between treatments (\( P = 0.122 \)).
Maize-based diets supported 10.6% higher ME:GE ratios (0.837 vs. 0.757; \( P < 0.001 \)) than wheat-based diets. N retention increased by 4.91 percentage units (85.40 vs. 80.49; \( P < 0.001 \)) following the dietary CP reduction and N retention in maize — was superior to wheat-based diets by 3.88 percentage units (84.88 vs. 81.00%; \( P < 0.001 \)). The dietary CP reduction increased AMEn by 0.21 MJ (11.88 vs. 11.67 MJ/kg; \( P = 0.024 \)) and maize was superior to wheat by 1.09 MJ (12.32 vs. 11.23 MJ/kg; \( P < 0.001 \)). The dietary CP reduction decreased N concentrations in excreta by 26.9% (3.24 vs. 4.43 mg/g; \( P < 0.001 \)). N concentrations in excreta were higher in birds offered maize-based diets by 15.1% (4.11 vs. 3.57 mg/g; \( P < 0.001 \)) in comparison to wheat-based diets.

Treatment effects on jejunal and ileal apparent starch digestibility coefficients and disappearance rates are shown in Table 7, where three-way treatment interactions (\( P < 0.008 \)) were observed for all parameters. The only significant difference in jejunal starch digestibility coefficients was the inclusion of WP in 180 g/kg CP diets, wheat-based diets which depressed digestibility coefficients by 4.33% (0.905 vs. 0.946; \( P = 0.004 \)). The more noticeable interactions in jejunal starch disappearance rates were observed with the 180 g/kg CP diets. WP inclusions in maize-based diets accelerated starch disappearance rates by 31.1% (67.11 vs. 51.18 g/bird/day), but retarded starch disappearance rates by 17.4% (51.01 vs. 61.74 g/bird/day) in wheat-based diets. WP inclusions did not influence ileal starch digestibility coefficients except following its inclusion in 180 g/kg CP, wheat-based diets where a reduction of 0.80% (0.991 vs. 0.999; \( P = 0.007 \)) was observed. Again, the more noticeable interactions in ileal starch disappearance rates were observed with the 180 g/kg CP diets. WP inclusions in maize-based diets accelerated starch disappearance rates by 29.9% (69.94 vs. 53.83 g/bird/day), but retarded starch disappearance rates by 14.3% (55.90 vs. 65.19 g/bird/day) in wheat-based diets.

Jejunal and ileal apparent protein (N) digestibility coefficients and disappearance rates in response to dietary treatments are shown in Table 8. Interactions (\( P < 0.001 \)) between CP and WP were observed in jejunum for both digestibility coefficients and disappearance rates. Whey protein inclusion in 220 g/kg CP diets increased protein (N) digestibility coefficients by 8.15% (0.756 vs. 0.843), but WP addition to 180 g/kg CP diets decreased digestibility by 6.35% (0.738 vs. 0.788). Similarly, WP inclusion in 220 g/kg CP diets accelerated protein (N) disappearance rates by 7.87% (20.56 vs. 19.06 g/bird/day); whereas, WP addition to 180 g/kg CP diets retarded disappearance rates by 8.49% (16.39 vs. 17.91 g/bird/day). Treatment interactions (\( P = 0.031 \)) between CP and WP were observed in ileum for protein (N) digestibility coefficients. Whey protein inclusion in 220 g/kg CP diets increased protein (N) digestibility coefficients by 2.25% (0.862 vs. 0.843), but WP addition to 180 g/kg CP diets decreased digestibility by 1.86% (0.842 vs. 0.858). As a main effect, maize-based diets supported higher digestibility coefficients by 1.90% (0.859 vs. 0.843; \( P = 0.042 \)) than wheat-based diets. Treatment interactions (\( P = 0.027 \)) between CP and feed grains were observed for ileal protein (N) disappearance rates, dietary CP by WP (\( P = 0.041 \), and feed
Table 8. Effects of dietary treatments on apparent protein (N) digestibility coefficients and disappearance rates (g/bird/day) and starch:protein disappearance rate ratios in distal jejunum and distal ileum at 35 d post-hatch.

| Treatment | Jejunum | Ileum | S:P disappearance ratios |
|-----------|---------|-------|--------------------------|
| Crude protein (g/kg) | Digestibility coefficient | Disappearance rate | Digestibility coefficient | Disappearance rate | Jejunum | Ileum |
| Wheat 0 | 0.707 | 19.35 | 0.849 | 23.23 | 2.33<sup>a</sup> | 2.04<sup>b</sup> |
| Wheat 25 | 0.768 | 20.20 | 0.863 | 22.72 | 2.13<sup>c</sup> | 2.00<sup>d</sup> |
| Wheat 45 | 0.740 | 18.77 | 0.838 | 22.78 | 2.32<sup>e</sup> | 2.02<sup>f</sup> |
| Wheat 60 | 0.747 | 20.91 | 0.860 | 24.19 | 2.17<sup>g</sup> | 1.94<sup>h</sup> |
| Wheat 80 | 0.784 | 18.16 | 0.869 | 20.14 | 2.83<sup>i</sup> | 2.68<sup>j</sup> |
| Wheat 100 | 0.742 | 16.36 | 0.856 | 18.98 | 4.09<sup>k</sup> | 3.69<sup>l</sup> |
| Wheat 120 | 0.734 | 16.32 | 0.828 | 18.36 | 3.16<sup>m</sup> | 3.07<sup>n</sup> |
| SEM | 0.0214 | 0.0391 | 0.0111 | 0.4403 | 0.0918 | 0.0665 |

Main effects: Crude protein

- 220: Maize 0.727, Wheat 0.763
- 180: Maize 0.750, Wheat 0.740

Feed grain

- Maize: 0.740, 0.750
- Wheat: 0.742, 0.747

Whey protein

- 0: 0.743, 0.747
- 25: 0.734, 0.747

Significance (P =)

- Crude protein (CP): 0.023
- Feed grain (FG): 0.149
- Whey protein concentrate (WP): 0.796
- CP × FG interaction: 0.509
- CP × WP interaction: <0.001
- FG × WP interaction: 0.714
- CP × FG × WP interaction: 0.907

Note: SEM = Standard Error of the Mean; CP = Crude Protein; WP = Whey Protein; FG = Feed Grain.

Grain by WP (P = 0.018). In 220 g/kg CP diets protein (N) disappearance rates were faster by 2.18% (23.48 vs. 22.98 g/bird/day) in birds offered wheat-base diets, but slower by 4.94% (18.64 vs. 19.56 g/bird/day) with 180 g/kg CP diets. Whey protein inclusions in 220 g/kg CP diets accelerated disappearance rates by 1.91% (23.45 vs. 23.01 g/bird/day) but retarded disappearance rates by 4.40% (18.67 vs. 19.53 g/bird/day) in 180 g/kg CP diets. Whey protein inclusions in maize-based diets retarded disappearance rates by 3.87% (20.85 vs. 21.69 g/bird/day) but accelerated disappearance rates in wheat-based diets by 2.01% (21.27 vs. 20.85 g/bird/day).

Jejunal and ileal starch:protein disappearance rate ratios in response to dietary treatments are also shown in Table 8 and three-way treatment interactions (P < 0.001) were observed in both intestinal segments. In the ileum, WP inclusions in maize-based diets significantly increased ratios from 2.83 to 4.09; in contrast, WP decreased disappearance rate ratios from 3.51 to 3.16 in wheat-based diets. A similar pattern was recorded in the ileum with an increase from 2.68 to 3.69 in maize-based diets but a decrease in starch:protein disappearance rate ratios from 3.45 to 3.07 in wheat-based diets.

Treatments effects on apparent amino acid digestibility coefficients in the distal jejunum are displayed in Tables 9 and 10. A treatment interaction (P = 0.004) between CP and WP was observed for aspartic acid. Addition of whey protein to 220 g/kg CP diets increased digestibility by 8.49% (0.767 vs. 0.707); whereas, whey protein addition to 180 g/kg CP diets decreased digestibility by 5.68% (0.714 vs. 0.757). As main effects, the transition from 220 to 180 g/kg CP diets increased digestibility of methionine by 2.61% (0.930 vs. 0.880) and glycine by 7.08% (0.786 vs. 0.734) but depressed digestibility of phenylalanine by 8.25% and serine by 10.2% (0.681 vs. 0.758). Maize-based diets supported significantly higher digestibility coefficients than wheat-based diets for 14 of the 16 amino acids assessed; the exceptions were glutamic acid (P = 0.053) and proline (P = 0.182). The average digestibility coefficients of the 14 amino acids in birds offered maize-based diets exceeded their wheat-based counterparts by 10.2% (0.801 vs. 0.727).

Treatments effects on apparent amino acid digestibility coefficients in the distal ileum are shown in Table 11 and 12. Significant treatment interactions between CP and feed grains were observed for arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, valine, alanine, aspartic acid, cysteine, glutamic acid, proline and serine or 14 of the 16 amino acids assessed. The exceptions were methionine (P = 0.061) and glycine (P = 0.052). Significant treatment interactions between CP and WP were observed for 15 of the 16 amino acids; the exception was aspartic acid (P = 0.139). Robust CP-feed grain interactions (P < 0.001) and CP-WP protein interactions (P = 0.008) were observed for phenylalanine, so this amino acid is taken as the example and the balance of interactions followed similar patterns. The CP-feed grain interaction was because phenylalanine digestibility was marginally higher in 220 g/kg CP, maize-based diets than wheat-based diets by 2.02% (0.909 vs. 0.891) but this advantage was a more...
pronounced 13.7% increase (0.904 vs. 0.795) in 180 g/kg CP diets. The CP-WP interaction stemmed from WP addition to 220 g/kg CP diets fractionally increased phenylalanine digestibility by 0.11% (0.907 vs. 0.906); in contrast, WP addition to 180 g/kg CP diets depressed phenylalanine digestibility by 4.97% (0.822 vs. 0.865).

The effects of dietary treatments on estimated proportions of dietary, endogenous, and microbial amino acids in distal ileal digesta are shown in Table 13. Treatment had relatively little effect on endogenous amino flows as reflected in the low 7.2% coefficient of variation. Variations were greater for dietary amino acids (19.8%) and

Table 9. Effects of dietary treatments on apparent amino acid digestibility coefficients in distal jejunum.

| Treatment | Arginine | Histidine | Isoleucine | Leucine | Lysine | Methionine | Phenylalanine | Threonine |
|-----------|----------|-----------|------------|---------|--------|------------|--------------|----------|
| 220 Maize | 0.845    | 0.793     | 0.771      | 0.796   | 0.814  | 0.889      | 0.798        | 0.740    |
| 220 Wheat | 0.866    | 0.831     | 0.823      | 0.842   | 0.854  | 0.912      | 0.828        | 0.798    |
| 180 Maize | 0.867    | 0.800     | 0.810      | 0.813   | 0.851  | 0.919      | 0.797        | 0.789    |
| 180 Wheat | 0.856    | 0.772     | 0.805      | 0.798   | 0.839  | 0.912      | 0.765        | 0.710    |

SEM: 0.0219 0.0303 0.0315 0.0341 0.0270 0.0152 0.0348 0.0347

Main effects: Crude protein
220 0.828 0.781 0.768 0.775 0.804 0.880 0.788 0.734
180 0.835 0.747 0.777 0.764 0.817 0.903 0.723 0.740

Significance (P =)
Crude protein (CP) 0.636 0.110 0.677 0.648 0.032 0.012 0.825
Feed grain (FG) <0.001 0.002 0.010 <0.001 0.004 0.003 0.001 0.004
Whey protein concentrate (WP) 0.833 0.810 0.652 0.682 0.845 0.638 0.649
CP £ FG interaction 0.941 0.680 0.950 0.922 0.839 0.908 0.797 0.774
CP £ WP interaction 0.463 0.216 0.363 0.257 0.875 0.714 0.700 0.740
FG £ WP interaction 0.560 0.653 0.548 0.830 0.569 0.710 0.693 0.661
CP £ FG £ WP interaction 0.765 0.761 0.722 0.894 0.726 0.689 0.850 0.661

abMeans within columns not sharing a common suffix are significantly different at the 5% level of probability.

Aspartic acid is the sum of asparagine and aspartic acid. Glutamic acid is the sum of glutamine and glutamic acid.

Table 10. Effects of dietary treatments on apparent amino acid digestibility coefficients in distal jejunum.

| Treatment | Valine | Alanine | Aspartic acid | Cysteine | Glutamic acid | Glycine | Proline | Serine |
|-----------|--------|---------|---------------|----------|---------------|---------|---------|--------|
| 220 Maize | 0.752  | 0.772   | 0.727         | 0.691    | 0.831          | 0.743   | 0.788   | 0.765  |
| 220 Wheat | 0.805  | 0.823   | 0.794         | 0.759    | 0.856          | 0.790   | 0.834   | 0.819  |
| 180 Maize | 0.712  | 0.670   | 0.687         | 0.668    | 0.833          | 0.700   | 0.783   | 0.718  |
| 180 Wheat | 0.718  | 0.704   | 0.741         | 0.694    | 0.837          | 0.701   | 0.800   | 0.739  |

SEM: 0.0319 0.0417 0.0245 0.0377 0.0206 0.0288 0.0256 0.0370

Main effects: Crude protein
220 0.747 0.742 0.737 0.703 0.839 0.734 0.801 0.758
180 0.764 0.684 0.736 0.655 0.810 0.786 0.777 0.681

Significance (P =)
Crude protein (CP) 0.444 0.054 0.935 0.077 0.053 0.014 0.182 0.005
Feed grain (FG) 0.009 <0.001 0.001 0.008 0.283 0.004 0.084 <0.001
Whey protein concentrate (WP) 0.768 0.524 0.625 0.599 0.824 0.579 0.733 0.905
CP £ FG interaction 0.918 0.115 0.440 0.274 0.621 0.841 0.477 0.157
CP £ WP interaction 0.312 0.421 0.004 0.218 0.224 0.257 0.171 0.285
FG £ WP interaction 0.522 0.897 0.931 0.625 0.681 0.521 0.710 0.973
CP £ FG £ WP interaction 0.690 0.675 0.786 0.778 0.759 0.640 0.687 0.600

abMeans within columns not sharing a common suffix are significantly different at the 5% level of probability.

Aspartic acid is the sum of asparagine and aspartic acid. Glutamic acid is the sum of glutamine and glutamic acid.
greater again for microbial amino acids (31.2%). It may be deduced from Table 13 that the reduction in dietary CP increased the average proportion of microbial amino acids from 23.4 to 32.4%. Moreover, 180 g/kg CP wheat-based diets generated an average proportion of 40.6%; substantially more than the 24.2% proportion found in birds offered maize-based diets. Instructively, there were highly significant Pearson correlations between proportions of microbial amino acids in distal ileal digesta and apparent ileal digestibility coefficients of the 16 amino acids assessed, as shown in Table 14. Alanine (r = \(-0.833\); P < 0.001) was the most strongly related amino acid and proline was the least (r = \(-0.421\); P = 0.001). There is a quadratic

| Treatment | Arginine | Histidine | Isoleucine | Leucine | Lysine | Methionine | Phenylalanine | Threonine |
|-----------|----------|-----------|------------|---------|--------|------------|---------------|----------|
| 220 Maize | 0.923    | 0.889     | 0.881      | 0.900   | 0.897  | 0.939      | 0.903         | 0.837    |
| 25        | 0.933    | 0.905     | 0.905      | 0.918   | 0.919  | 0.951      | 0.915         | 0.868    |
| Wheat     | 0.905    | 0.866     | 0.866      | 0.802   | 0.881  | 0.924      | 0.888         | 0.800    |
| 25        | 0.907    | 0.877     | 0.882      | 0.887   | 0.895  | 0.936      | 0.895         | 0.829    |
| 180 Maize | 0.940    | 0.989     | 0.909      | 0.914   | 0.923  | 0.960      | 0.910         | 0.871    |
| 25        | 0.934    | 0.887     | 0.907      | 0.911   | 0.917  | 0.957      | 0.898         | 0.863    |
| Wheat     | 0.904    | 0.840     | 0.866      | 0.857   | 0.883  | 0.940      | 0.841         | 0.803    |
| 25        | 0.874    | 0.763     | 0.804      | 0.791   | 0.827  | 0.913      | 0.749         | 0.703    |

**Table 11. Effects of dietary treatments on apparent amino acid digestibility coefficients in distal ileum.**

**Table 12. Effects of dietary treatments on apparent amino acid digestibility coefficients in distal ileum.**

| Treatment | Valine | Alanine | Aspartic acid | Cysteine | Glutamic acid | Glycine | Proline | Serine |
|-----------|--------|---------|---------------|----------|---------------|---------|---------|--------|
| 220 Maize | 0.864  | 0.860  | 0.875         | 0.807    | 0.913         | 0.848   | 0.857   | 0.856  |
| 25        | 0.888  | 0.894  | 0.896         | 0.848    | 0.924         | 0.871   | 0.890   | 0.895  |
| Wheat     | 0.844  | 0.816  | 0.849         | 0.784    | 0.915         | 0.829   | 0.877   | 0.849  |
| 25        | 0.859  | 0.847  | 0.861         | 0.818    | 0.921         | 0.837   | 0.890   | 0.869  |
| 180 Maize | 0.895  | 0.889  | 0.880         | 0.826    | 0.920         | 0.901   | 0.889   | 0.880  |
| 25        | 0.893  | 0.883  | 0.863         | 0.829    | 0.909         | 0.891   | 0.879   | 0.862  |
| Wheat     | 0.850  | 0.734  | 0.742         | 0.753    | 0.896         | 0.854   | 0.885   | 0.784  |
| 25        | 0.784  | 0.665  | 0.707         | 0.674    | 0.847         | 0.803   | 0.838   | 0.707  |

**abMeans within columns not sharing a common suffix are significantly different at the 5% level of probability.**

Aspartic acid is the sum of asparagine and aspartic acid. Glutamic acid is the sum of glutamine and glutamic acid.
relationship \( (r = 0.851; \ P < 0.001) \) between microbial amino acids proportions and apparent ileal alanine digestibility coefficients, as shown in Figure 1. The regression equation predicts that the 0.901 maximum alanine digestibility coefficient corresponds to a 12.64% proportion of microbial amino acids but alanine digestibilities are depressed in a quadratic manner as this proportion increases.

**DISCUSSION**

Overall growth performance of broiler chickens in the present study surpassed 2019 Aviagen performance objectives for Ross 308 male birds by 21.1% (2,627 vs. 2,169 g/bird) in weight gain, 8.10% (3,578 vs. 3310 g/bird) in feed intake and by 10.5% (1.366 vs. 1.526) in FCR from 7 to 35 d post-hatch. This growth performance, coupled with an acceptably low 1.19% mortality rate, is of a high order. An unusually high number of significant treatment interactions were observed in the present study; however, they are instructive.

Maize-based diets supported 6.74% superior weight gains in comparison to wheat-based diets in birds offered 180 g/kg CP diets, despite generating heavier relative fat-pad weights by 47.8% (10.45 vs. 7.07 g/kg) following the dietary CP reduction in the present study. This outcome is consistent with the Chrystal et al. (2021)

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**Table 13.** Effects of dietary treatment on estimated proportions of dietary, endogenous, and microbial amino acids in distal ileal digesta.

| Treatment | CP (g/kg) | Feed grain | WP (g/kg) | Dietary (%) | Endogenous (%) | Microbial (%) |
|-----------|-----------|------------|-----------|-------------|----------------|--------------|
| 220       | Maize     | 0          | 56.6      | 21.3        | 22.1           |
| 180       | Wheat     | 25         | 63.0      | 20.4        | 16.5           |
|           | Maize     | 0          | 46.4      | 24.3        | 29.2           |
|           | Wheat     | 25         | 51.4      | 22.8        | 25.7           |
|           | 0         | 52.1       | 24.6      | 24.4        |
|           | 25        | 51.7       | 24.4      | 23.9        |
|           | 0         | 36.4       | 25.0      | 38.6        |
|           | 25        | 34.2       | 23.2      | 42.6        |

Mean standard deviation 49.0 ± 9.72
Coefficient of variation 19.8% 7.2% 31.2%

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**Table 14.** Pearson correlations between proportions of microbial amino acids in distal ileal digesta and apparent ileal digestibility coefficients of 16 amino acids.

| Amino acid | Correlation coefficient | Significance | Amino acid | Correlation coefficient | Significance |
|------------|-------------------------|--------------|------------|-------------------------|--------------|
| Arginine   | r = −0.657              | \( P < 0.001 \) | Valine     | r = −0.584              | \( P < 0.001 \) |
| Histidine  | r = −0.734              | \( P < 0.001 \) | Alanine    | r = −0.833              | \( P < 0.001 \) |
| Isoleucine | r = −0.588              | \( P < 0.001 \) | Aspartic acid | r = −0.779          | \( P < 0.001 \) |
| Leucine    | r = −0.683              | \( P < 0.001 \) | Cysteine   | r = −0.672              | \( P < 0.001 \) |
| Lysine     | r = −0.623              | \( P < 0.001 \) | Glutamic acid | r = −0.638          | \( P < 0.001 \) |
| Methionine | r = −0.563              | \( P < 0.001 \) | Glycine    | r = −0.426              | \( P = 0.001 \) |
| Phenylalanine | r = −0.733         | \( P < 0.001 \) | Proline    | r = −0.421              | \( P = 0.001 \) |
| Threonine  | r = −0.612              | \( P < 0.001 \) | Serine     | r = −0.795              | \( P < 0.001 \) |

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**Figure 1.** Quadratic relationship \( (r = 0.851; \ P < 0.001) \) between proportions of microbial amino acids (MAA) in distal ileal digesta and apparent ileal alanine digestibility coefficients where \( y = 0.8596 + 0.0066*\text{MAA} - 0.000261*\text{MAA}^2 \).
the present study 180 g/kg CP, maize-based diets contained an average of 20.62 g/kg non-bound amino acids as opposed to 35.93 g/kg in wheat-based diets. Overall, non-bound amino acid inclusions depressed weight gains (r = 0.634; P < 0.001) and compromised FCR (r = 0.605; P < 0.001) in a quadratic manner in the present study. While these relationships are not conclusive, they suggest that non-bound amino acid inclusions in reduced-CP broiler diets may become excessive. Interestingly, non-bound amino acids may be more susceptible to postprandial oxidation because of their rapid absorption, which results in post-enteral amino acid imbalances (Nolles et al., 2009). Intestinal uptakes of non-bound amino acids are more rapid than their protein-bound counterparts, which was demonstrated by Liu et al. (2013) in broilers offered sorghum-based diets. This dichotomy may promote post-enteral amino acid imbalances and deamination of surplus amino acids. Deamination of amino acids generates ammonia (NH₃), which is inherently toxic (Stern and Mozdziak, 2019); thus, high levels of deamination coupled with inadequate NH₃ detoxification could result in ‘NH₃ overload’. Indeed, several studies (Namroud et al., 2008; Ospina–Rojas et al., 2013, 2014) have associated elevated NH₃ plasma concentrations with depressed growth performance. It is then noteworthy that Wilson et al. (1968) found that the intravenous LD₅₀ of ammonium acetate in broiler chickens is half that of mice (2.72 vs. 5.64 mmol/kg). This illustrates the potential of ‘NH₃ overload’ to compromise the performance of broilers offered reduced-CP diets.

Increased fat deposition, monitored by relative abdominal fat-pad weights, following CP reductions in maize-based diets is a typical outcome. In Chrystal et al. (2020a), dietary CP reductions from 210 to 165 g/kg CP increased relative fat-pad weights by 69.2% (14.62 vs. 8.64 g/kg), which were quadratically (r = 0.606; P = 0.003) associated with compromised FCR. In Yin et al. (2020), a CP reduction from 215 to 165 g/kg in wheat-based diets increased fat-pad weights by a relatively moderate, but significant, 12.2% (8.02 vs. 7.15 g/kg). In the present study, the CP reduction from 220 to 180 g/kg caused an increase in fat-pad weights of 27.1% in birds offered maize-based diets but a more modest increase of 11.2% in wheat-based diets. Thus, in a paradoxeffect, maize-based diets support better growth performance than wheat-based diets, despite the fact they generate more fat deposition in the context of reduced-CP diets.

Starch, the major energy source in broiler diets, is absorbed as glucose and post-prandial plasma glucose levels in birds offered maize-, wheat- or rice-based diets did not vary depending on starch source with an overall mean concentration of 12.72 mmol/L (Li et al., 2019). Thus, glucose homeostasis is maintained in poultry, despite relatively high plasma glucose levels. The metabolic disposal of glucose comprises direct oxidation in various tissues, glycogen synthesis in liver and skeletal muscles and hepatic de novo lipogenesis (Jequier, 1994). Glucose can be stored as glycogen but carbohydrate overfeeding in humans has been shown to trigger de novo lipogenesis once glycogen stores in liver and skeletal muscle have been saturated (Acheson et al., 1988). The liver is the main site of de novo lipogenesis in avian species, glucose is catabolized to acetyl-CoA which is converted into fatty acids and cholesterol. Cholesterol and triacylglycerol are incorporated into very low-density lipoproteins and transported to adipose via the circulation (Wang et al., 2017). Thus, de novo lipogenesis is a complex metabolic pathway in which excess carbohydrate is converted into fatty acids that are then esterified to storage triacylglycerols (Ameer et al., 2014). Rapidly digestible starch generates greater and more rapid changes in blood glucose, insulin, and non-esterified fatty acid concentrations than slowly digestible starch in humans (Lehmann and Robín, 2007). The extent to which human outcomes apply to poultry is problematic; nevertheless, it is possible that sustained glucose and insulin blood levels generated by maize-based diets is promoting more de novo lipogenesis than in birds offered wheat-based diets containing more rapidly digestible starch. It may be that glucose derived from rapidly digestible wheat starch is directly catabolized for energy provision; whereas, glucose from slowly digestible starch is being converted to glycogen and then fat via de novo lipogenesis to greater extents.

The feed grain by WP interaction for FCR (P = 0.022) was not anticipated. The inclusion of WP in 220 g/kg CP, maize-based diets improved FCR by 5.23% (1.268 vs. 1.338); in contrast, WP in 180 g/kg CP, wheat-based diets compromised FCR by 3.21% (1.479 vs. 1.433). It is then relevant that WP inclusion in 180 g/kg CP, wheat-based diets significantly depressed jejunal starch digestibility by 4.33% (0.905 vs. 0.946). The effects of WP on the in vitro digestibility of potato starch were investigated by Liu et al. (2022). Whey protein reduced potato starch digestibility, which was attributed to a combination of hydrophobic interactions and hydrogen bonding and compromised efficacy of starch-degrading enzyme activity. Similarly, negative impacts of WP on in vitro digestibility of maize starch (Zhang et al., 2022) and wheat starch (Yang et al., 2013) have been reported.

The rate of wheat starch digestion (0.035/min) is more rapid than maize starch (0.017/min) under in vitro conditions (Ginberti et al., 2012). This applies in vivo as starch digestion in wheat-based diets (0.311/min) was faster than in maize-based diets (0.087/min) offered to in broiler chickens (Selle et al., 2021). WP is a rapidly digestible source of protein (Dangin et al., 2003) and it appears that WP has the potential to interfere with starch digestion. That this was evident in wheat- than maize-based diets may be because both wheat starch and WP are digested and absorbed proximally in the small intestine, whereas the site of maize-starch digestion is more distally located. This was more apparent in 180 g/kg diets and is probably related to higher starch
concentrations in the lower CP diets. The addition of WP to 180 g/kg CP, wheat-based diets negatively influenced jejunal and ileal starch digestibility in the present study (Table 7).

Reductions in dietary CP enhanced nutrient utilisation including ME:GE ratios, N retention and AMEn with a reduction of 26.9% (3.24 vs. 4.43 mg/g) in N excreta concentrations. In relation to feed grains, maize-based diets significantly outperformed wheat-based diets with a substantial improvement of 1.14 MJ (13.70 vs. 12.56 MJ/kg) in AME. This is consistent with previous outcomes that prompted Chrystal et al. (2020b) to suggest that reduced-CP diets have an energy sparing effect. The genesis of this energy sparing effect probably stems from reductions in thermogenesis or heat increment (Musharaf and Latshaw, 1999) triggered by the 40 g/kg reduction in dietary CP in the present study. In addition, dietary CP reductions diminish the need for birds to synthesise and excrete N as uric acid and this process attains an energy cost of 60.7 kJ/g of uric acid N (Van Milgen, 2021).

The relevance of starch-protein digestive dynamics in birds offered reduced-CP diets has been reviewed by Liu and Selle (2017). This applies to the present study as jejunal starch:protein disappearance rate ratios were quadratically related to weight gain (0.415; P = 0.001), FCR (0.494; P < 0.001) and relative fat-pad weights (r = 0.538; P < 0.001). Similarly, ileal disappearance rate ratios were quadratically related to weight gain (r = 0.584; P < 0.001), FCR (r = 0.471; P = 0.001), and fat-pad weights (r = 0.520; P < 0.001). In all instances, decreasing disappearance rate ratios were associated with better outcomes.

Significant treatment interactions for distal ileal apparent digestibility coefficients were observed across all 16 amino acids. However, it is instructive to make a direct comparison between amino acid digestibility coefficients in either the maize- or wheat-based 180 g/kg CP diets without WP additions. The mean digestibility coefficient of 16 amino acids in birds offered wheat-based diets was inferior by 6.67% (0.840 vs. 0.900) in comparison to their maize-based counterparts. As mentioned, reductions in dietary CP generate perturbations in apparent amino acid digestibility coefficients. When the dietary CP main effect is considered in the present study, the CP reduction depressed average ileal digestibility coefficients of all 16 amino acids by 2.96% (0.853 vs. 0.879). However, the average digestibility of 7 amino acids that were present in diets exclusively as protein-bound entities was depressed to a greater extent, by 5.84% (0.826 vs. 0.874).

Increases in digestibilities of certain amino acids (particularly lysine, methionine, threonine, arginine) may be attributed to the notional 100% digestibility of non-bound amino acids (Lemme et al., 2005) coupled with their high inclusions in reduced-CP diets. Also, diminished endogenous amino acid flows would enhance amino acid digestibilities and reductions in dietary CP may attenuate endogenous flows (Ravindran et al., 2009). Decreases in amino acid digestibilities following reductions in dietary CP are less readily explained; however, it appears that increases in microbial amino acids in distal ileal digesta may be contributing to this negative impact.

By definition, the relative proportions of amino acids in distal ileal digesta calculated by the Duvaux et al. (1990) model are estimates; nevertheless, our contention is that they are indicative. The amino acid profile of avian mucin (Fang et al., 1993) was taken to represent endogenous amino acids to simplify the approach. It follows that amino acids derived from mucin are a dominant constituent of endogenous amino acids because mucin essentially remains undigested; whereas other endogenous amino acids may be re-absorbed (Lien et al., 1997; Ravindran, 2021). The amino acid profile of Lactobacillus bulgaricus (Xia et al., 2007) was taken to represent microbial amino acids because Lactobacilli are dominant in the small intestine of poultry making up approximately 83% of the total gut microbiota and Lactobacilli can assimilate from 3 to 6% of dietary protein (Apajalaiti and Vienola, 2016; Munyaka et al., 2016).

The most noticeable shift in amino acid proportions was the increase in microbial amino acids in birds offered the two 180 g/kg wheat-based as opposed to the two 180 g/kg maize-based diets. There were substantially higher proportions of microbial amino acids in ileal digesta of birds offered wheat-based diets than maize-based diets by 16.4 percentage units (mean values: 40.6 vs. 24.2%) or a factor of 1.68. Increased gut viscosities in birds offered wheat-based diets, triggered by soluble non-starch polysaccharides (NSP), may have facilitated proliferation of small intestinal gut microbiota. An NSP-degrading enzyme was included across all diets in the present study; however, wheat-based diets would still have generated higher gut viscosities than in birds offered maize-based diets based on data generated by Munyaka et al. (2016). These researchers reported that wheat-based diets (3.08 Mpa) supported higher average gut viscosities than maize-based diets (1.91 Mpa), without or with the addition of an NSP-degrading enzyme. The proposal that increased gut viscosities are associated with increases in gut microbiota is supported by Wagner and Thomas (1977), who reported that broilers offered rye-based diets, the more ‘viscous’ cereal grain, had greater ileal anaerobe counts than maize-based diets by 2 or 3 logarithmic cycles. Also, Choc et al. (1996) found that soluble NSP increased small intestinal fermentation and this is partly responsible for the anti-nutritive effects soluble NSP. Accordingly, Hibener et al. (2002) found that cereals producing high intestinal viscosities cause increased bacterial activity in the small intestine. Thus, the proposal that increased gut viscosities triggered by soluble NSP in wheat are associated with the proliferation of the gut microbiota in the small intestine and, if so, the transition from 220 to 180 g/kg CP diets would amplify this effect as dietary wheat inclusions increased from 598 to 747 g/kg, which would increase soluble NSP to a corresponding extent.
Dietary inclusions of WP had little impact on levels of non-bound amino acid inclusions in dietary treatments (Table 2) in the present study. Inclusions of WP did not generate any significant responses as main effects but were involved in numerous treatment interactions. The capacity of WP to interact with starch and negatively impact starch digestibility coefficients, which was evident in 180 g/kg CP, wheat-based diets, may have contributed to these interactions. Interestingly, WP was mainly composed of large polypeptides with molecular weights greater than 10,000 Daltons (Table 1), but, presumably, they are readily converted into oligopeptides and rapidly absorbed (Daniel, 2004). The possibility is that rapid intestinal uptakes of WP oligopeptides contributed to the treatment interactions observed in the present study.

CONCLUSIONS

In conclusion, the present study confirmed that maize is the more suitable feed grain than wheat in the context of reduced-CP diets. The lower inclusions of non-bound amino acids in reduced-CP, maize-based diets appear to be a contributing factor, possibly because the occurrence of postprandial oxidation of amino acids is limited and there is an attenuation of post-enteral amino acid imbalances and the deamination of surplus amino acids. Paradoxically, wheat-based, reduced-CP diets do not drive the same level of fat deposition. This may be because rapidly digestible wheat starch is more readily oxidized, whereas slowly digestible maize starch is more likely to trigger de novo lipogenesis. Whey protein inclusions generated numerous treatment interactions with both dietary CP and/or feed grain. However, the WP amino acid profile is not suitable to meet amino acid requirements and, consequently, WP had little effect on non-bound amino acid concentrations in the dietary treatments. Moreover, that WP depressed jejunal and ileal starch digestibilities in 180 g/kg CP, wheat-based diets and it appears that WP inclusions in reduced-CP diets is not appropriate. Essentially, reductions in dietary CP compromised apparent ileal amino acid digestibility coefficients and this was more evident in wheat-based diets. It appears that this was caused by a proliferation of microbial amino acids in distal ileal digesta, which was probably facilitated by higher gut viscosities in birds offered wheat-based diets due to the greater presence of soluble NSP in wheat. It follows that the transition to reduced-CP diets would exacerbate this effect simply because such diets contain higher levels of wheat. In the present study, the transition from 220 to 180 g/kg CP diets resulted in a 24.9% (747 vs. 598 g/kg) increase in wheat inclusions.

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Authors’ contributions: SG supervised the overall completion of the feeding study. The concept of the trial design was agreed between SYL, AL, JCdePD and PHS. SPM completed the Duvaux et al. (1990) estimations, PVC formulated the dietary treatments and SPM and PHS conducted the statistical analyses. The documentation of the feeding study was completed by SG, SYL, SPM and PHS. All authors have read and approved the final manuscript.

DISCLOSURES

The authors declare that they have no known competing interests that could have influenced the work reported in this paper. A. Lemme and J. C. de Paula Dorigam are employed by Evonik Operations GmbH.

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