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[*,§,1]

[*]*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 × 2 × 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

2022 Poultry Science 101:102131
https://doi.org/10.1016/j.psj.2022.102131

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 suitable than wheat as the basis of such diets (Chrystal et al., 2021). This presents a problem in countries, including Australia, where wheat is the major feed grain. The factors contributing to the superiority of maize have yet to be clarified but probably include lower protein contents and slower starch digestion rates relative to wheat (Giuberti et al., 2012; Selle et al., 2021). The higher protein content of wheat results in higher inclusion levels of non-bound (synthetic, crystalline) amino acids and lower inclusions of soybean meal in wheat-based, reduced-CP diets in comparison to maize-based diets, which is evident in Table 2. This may generate post-enteral imbalances stemming from the more rapid

---

Received March 10, 2022.

Accepted August 6, 2022.

[1]Corresponding author: peter.selle@sydney.edu.au

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 $8.64 \times 10^{-2}\text{min}^{-1}$ in standard sorghum-based broiler diets as opposed to $2.35 \times 10^{-2}\text{min}^{-1}$ 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 2^x2^x2 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 Oilmills Sdn Bhd. Perai, Malaysia). The analyzed starch,

Table 1. Amino acid analysis and of size-exclusion chromatography of whey protein concentrate

	Amino acid analysis			Size-exclusion chromatography	
Amino acid	Concentration (mg/g)	Proportion (%)	Profile to lysine	Molecular weight (Daltons)	Area (%)
Arginine	20.4	2.65	30	> 10,000	97.0
Histidine	14.6	1.90	21	10,000−5,000	1.3
Isoleucine	53.2	6.92	78	5,000−2,000	0.6
Leucine	86.8	11.28	127	2,000−1,000	0.3
Lysine	68.2	8.87	100	1,000−500	0.3
Methionine	17.7	2.30	26	< 500	0.5
Phenylalanine	26.7	3.47	39		
Threonine	57.6	7.49	84		
Valine	48.4	6.29	71		
Alanine	39.3	5.10	58		
Aspartic acid	79.0	10.27	116		
Glutamic acid	132.3	17.20	194		
Glycine	15.2	1.98	22		
Proline	47.	6.11	70		
Serine	40.7	5.29	60		
Tyrosine	22.2	2.89	33		
Total	769.3	100			

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				
Wheat					587	608	762	731
Whey protein concentrate		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			0.79	0.92
Isoleucine			1.99	1.81			2.93	2.62
Leucine							4.16	3.42
Tryptophan			0.29	0.23			0.31	0.20
Valine			2.20	2.04	0.26		3.27	3.01
Glycine		0.72	3.66	4.11		0.13	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	0.58		
Sodium bicarbonate		1.81	4.58	4.61	1.81	3.30	4.15	4.20
Potassium carbonate			2.08	3.77			7.03	8.18
Phytase (Axtra Phy)[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Xylanase (Danisco Xylanase)[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin-mineral premix[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sand								27.7
Celite	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 (%)[3]	97.1	96.3	90.2	86.4	96.4	97.1	78.8	79.9

[1] Feed enzymes from Danisco Animal Nutrition and Health. Phytase 1,000 FTU/kg, Xylanase 4,000 U/kg.

[2] Vitamin-trace mineral premix supplies in MIU/kg or mg/kg of diet: [MIU] retinol 12, cholecalciferol 5, [mg] 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.

[3] Approximate proportion of analysed dietary CP derived from intact protein.

Table 3. Nutrient specifications of experimental diets (standardized digestible amino acids).

Item (g/kg)	1A	2B	3C	4D	5E	6F	7G	8H
Metabolizable energy (MJ/kg)	12.97	12.97	12.97	12.97	12.97	12.97	12.97	12.97
Crude protein	220	220	180	180	220	220	180	180
Starch	368	385	447	466	369	382	479	459
Dietary starch:protein ratio	1.67	1.75	2.48	2.59	1.68	1.74	2.66	2.55
Lysine	11.50	11.50	11.50	11.50	11.50	11.50	11.50	11.50
Methionine	6.01	5.82	6.49	6.33	5.78	5.55	6.43	6.29
Methionine + cysteine	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70
Threonine	7.70	7.70	7.70	7.70	7.70	7.70	7.70	7.70
Arginine	13.08	12.30	12.30	12.30	12.30	12.30	12.30	12.30
Histidine	4.96	4.83	3.86	3.80	4.73	4.72	3.80	3.80
Isoleucine	7.83	8.10	7.80	7.80	7.86	7.86	7.80	7.80
Leucine	16.13	16.97	13.44	14.13	13.45	13.45	12.70	12.70
Tryptophan	2.14	2.23	1.80	1.80	2.35	2.35	1.80	1.80
Valine	8.70	8.76	8.70	8.70	8.70	8.70	8.70	8.70
Phenylalanine	9.26	8.99	7.13	6.75	9.07	8.88	5.84	5.57
Phenylalanine + tyrosine	15.05	14.81	11.58	11.16	13.68	13.55	8.10	7.86
Glycine	7.31	7.29	8.75	8.79	7.25	7.16	9.37	9.36
Serine	9.05	9.08	7.03	6.97	9.13	9.27	6.16	6.17
Glycine equivalents	13.77	13.77	13.77	13.77	13.77	13.77	13.77	13.77
Calcium	8.70	8.70	8.70	8.70	8.70	8.70	8.70	8.70
Total phosphorus	4.97	7.85	4.74	4.61	4.57	4.44	4.09	3.93
Available phosphorus	4.35	4.35	4.35	4.35	4.35	4.35	4.35	4.35
Dietary electrolyte balance (mEq/kg)	250	250	250	250	250	250	250	250
Sodium	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Potassium	9.51	8.71	8.16	8.71	8.17	7.80	8.00	8.46
Chloride	2.76	2.28	1.51	2.34	1.87	1.48	1.39	2.13
Crude fiber	20.7	19.6	18.6	17.0	21.7	20.7	18.3	16.7
Crude fat	70.5	64.6	52.4	46.6	70.4	65.5	43.7	55.2

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:

$$\frac{\mathrm{AME}_{\mathrm{diet}} = (\mathrm{feedintake} \times \mathrm{GE}_{\mathrm{diet}}) - (\mathrm{excretaoutput} \times \mathrm{GE}_{\mathrm{excreta}})}{(\mathrm{feedintake})}$$

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

Table 4. Analyzed protein (N), starch, and amino acid concentrations in experimental diets.

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	13.25	12.11	12.32
Histidine	5.42	5.31	4.21	4.05	5.16	5.00	3.94	3.93
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	15.58	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	3.25	3.44	2.46	2.62
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:

$$\frac{\mathrm{Nretention}_{(\%)} = (\mathrm{feedintake} \times \mathrm{N}_{\mathrm{diet}}) - (\mathrm{excretaoutput} \times \mathrm{N}_{\mathrm{excreta}}) \times 100}{(\mathrm{feedintake} \times \mathrm{N}_{\mathrm{diet}})}$$

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, α-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 then 16 amino acids were analyzed using the Waters AccQTag Ultra chemistry (Waters) on a Waters Acquity UPLC. Protein (N) and acid insoluble ash concentrations were determined as outlined in Siriwan et al. (1993). Apparent digestibility coefficients (**ADC**) of starch, protein (N), and amino acids were calculated by the following equation:

$$\frac{\mathrm{ADC} = (\mathrm{nutrient/AIA})_{\mathrm{diet}} - (\mathrm{nutrient/AIA})_{\mathrm{digesta}}}{(\mathrm{Nutrient/AIA})_{\mathrm{diet}}}$$

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:

$$\begin{aligned}&\mathrm{Disappearancerate} = \mathrm{dailyfeedintake(g/bird)}\\&\times \mathrm{dietarynutrient(g/kg)} \times \mathrm{ADC}\end{aligned}$$

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:

$$Yi/\surd\, Y_i = b_1 X1_i/\surd\, Y_i + b_2 X2i/\surd\, Y_i + b_3 X3_i/\surd\, Y_i$$

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

$$\begin{aligned}\Sigma Y_{i..k}/\surd\, Y_{i..k} &= b_1 \Sigma X1_{i..k}/\surd\, Y_{i..k} + b_2 \Sigma X2_{i..k}/\surd\, Y_{i..k}\\&\quad + b_3 \Sigma X3_{i..k}/\surd\, Y_{i..k}\end{aligned}$$

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.

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) or wheat (2,705 g/bird); however, there was an advantage of 6.74% (2628 vs. 2462 g/bird; $P = 0.016$) in favour of maize when birds were offered 180 g/kg CP diets. A treatment interaction ($P = 0.022$) 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% (1.415 vs. 1.374). 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

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				
Crude protein (g/kg)	Feed grain	Whey protein (g/kg)	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Mortality rates (%)	Relative fat-pad weights (g/kg)
220	Maize	0	2,690	3,583	1.338	2.38	8.02
		25	2,736	3,474	1.268	2.38	8.41
	Wheat	0	2,720	3,574	1.314	2.38	6.19
		25	2,690	3,630	1.350	0.00	6.53
180	Maize	0	2,598	3,586	1.385	0.00	10.16
		25	2,658	3,609	1.360	2.38	12.17
	Wheat	0	2,510	3,595	1.433	0.00	7.78
		25	2,414	3,570	1.479	0.00	7.79
SEM			44.91	56.84	0.0265	1.684	0.5043
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	0.543	< 0.001	0.322	< 0.001
Feed grain (FG)			0.008	0.475	0.004	0.322	< 0.001
Whey protein concentrate (WP)			0.870	0.732	0.864	1.000	0.061
CP × FG interaction			0.016	0.279	0.151	1.000	0.038
CP × WP interaction			0.689	0.755	0.468	0.322	0.369
FG × WP interaction			0.074	0.475	0.022	0.322	0.157
CP × FG × WP interaction			0.536	0.190	0.655	1.000	0.178

[ab]Means within columns not sharing a common suffix are significantly different at the 5% level of probability.
Average weight at d 7 was 177 g/bird.

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

Treatment			Nutrient utilization				
Crude protein (g/kg)	Feed grain	Whey protein (g/kg)	AME (MJ/kg DM)	ME:GE ratio (MJ/MJ)	N retention (%)	AMEn (MJ/kg DM)	Excreta N (mg/g)
220	Maize	0	13.62	0.820	82.88	12.19	4.62
		25	13.74	0.822	82.85	12.31	4.73
	Wheat	0	12.58	0.741	78.62	11.20	4.15
		25	12.42	0.731	77.98	10.98	4.22
180	Maize	0	13.71	0.847	87.01	12.34	3.52
		25	13.74	0.859	87.15	12.46	3.56
	Wheat	0	12.56	0.770	83.40	11.28	3.07
		25	12.70	0.785	84.01	11.44	2.82
SEM			0.1219	0.0075	0.5134	0.1276	0.0986
Main effects: Crude protein							
220			13.09	0.779^{a}	80.49^{a}	11.67^{a}	4.43^{b}
180			13.18	0.815^{b}	85.40^{b}	11.88^{b}	3.24^{a}
Feed grain							
Maize			13.70^{b}	0.837^{a}	84.88^{b}	12.32^{b}	4.11^{b}
Wheat			12.56^{a}	0.757^{b}	81.00^{a}	11.23^{a}	3.57^{a}
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	0.024	< 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 × FG interaction			0.610	0.341	0.178	0.526	0.454
CP × WP interaction			0.522	0.108	0.217	0.291	0.170
FG × WP interaction			0.633	0.657	0.850	0.402	0.258
CP × FG x WP interaction			0.249	0.477	0.637	0.313	0.384

[ab]Means 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 ($P = 0.122$).

Table 7. Effects of dietary treatments on apparent starch digestibility coefficients and disappearance rates (g/bird/day) in distal jejunum and distal ileum at 35 d post-hatch.

Treatment			Jejunum		Ileum	
Crude protein (g/kg)	Feed grain	Whey protein (g/kg)	Digestibility coefficient	Disappearance rate	Digestibility coefficient	Disappearance rate
220	Maize	0	0.948^{b}	44.85^{ab}	0.998^{b}	47.39^{b}
		25	0.938^{b}	42.57^{a}	0.994^{ab}	45.02^{a}
	Wheat	0	0.942^{b}	43.38^{ab}	0.997^{b}	45.94^{ab}
		25	0.958^{b}	45.12^{b}	0.998^{b}	47.04^{ab}
180	Maize	0	0.948^{b}	51.18^{c}	0.996^{ab}	53.83^{c}
		25	0.958^{b}	67.11^{e}	0.999^{b}	69.94^{e}
	Wheat	0	0.946^{b}	61.74^{e}	0.999^{b}	65.19^{d}
		25	0.905^{a}	51.01^{c}	0.991^{a}	55.90^{c}
SEM			0.0071	0.8351	0.0018	0.7882
Main effects: Crude protein						
220			0.946	44.00	0.997	46.36
180			0.939	57.76	0.996	61.23
Feed grain						
Maize			0.947	51.43	0.997	54.04
Wheat			0.938	50.32	0.996	53.52
Whey protein						
0			0.945	50.29	0.998	53.09
25			0.940	51.47	0.995	54.48
Significance (P =)						
Crude protein (CP)			0.165	< 0.001	0.693	< 0.001
Feed grain (FG)			0.057	0.069	0.693	0.350
Whey protein concentrate (WP)			0.352	0.052	0.085	0.016
CP x FG interaction			< 0.001	0.007	0.119	0.150
CP x WP interaction			0.040	0.020	0.910	< 0.001
FG x WP interaction			0.145	< 0.001	0.313	< 0.001
CP x FG x WP interaction			< 0.001	< 0.001	0.008	< 0.001

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

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, 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. 0.699), 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)	Feed grain	Whey protein (g/kg)	Digestibility coefficient	Disappearance rate	Digestibility coefficient	Disappearance rate	Jejunum	Ileum
220	Maize	0	0.707	19.35	0.849	23.23	2.33^{a}	2.04^{a}
		25	0.768	20.20	0.863	22.72	2.13^{a}	2.00^{a}
	Wheat	0	0.690	18.77	0.838	22.78	2.32^{a}	2.02^{a}
		25	0.747	20.91	0.860	24.19	2.17^{a}	1.94^{a}
180	Maize	0	0.784	18.16	0.869	20.14	2.83^{b}	2.68^{b}
		25	0.742	16.46	0.856	18.98	4.09^{e}	3.69^{e}
	Wheat	0	0.791	17.66	0.847	18.92	3.51^{d}	3.45^{d}
		25	0.734	16.32	0.828	18.36	3.16^{c}	3.07^{a}
SEM			0.0214	0.5931	0.0111	0.4403	0.0918	0.0665
Main effects: Crude protein								
220			0.727	19.81	0.852	23.23	2.34	2.00
180			0.763	17.15	0.850	19.10	3.40	3.22
Feed grain								
Maize			0.750	18.54	0.859^{b}	21.27	2.84	2.60
Wheat			0.740	18.42	0.843^{a}	21.06	2.80	2.62
Whey protein								
0			0.743	18.48	0.851	21.27	2.75	2.55
25			0.747	18.48	0.852	21.06	2.89	2.67
Significance (P =)								
Crude protein (CP)			0.022	< 0.001	0.772	< 0.001	< 0.001	< 0.001
Feed grain (FG)			0.149	0.762	0.042	0.508	0.394	0.674
Whey protein concentrate (WP)			0.796	0.985	0.892	0.513	0.038	0.009
CP × FG interaction			0.509	0.645	0.256	0.027	0.276	0.238
CP × WP interaction			< 0.001	< 0.001	0.031	0.041	< 0.001	< 0.001
FG × WP interaction			0.714	0.330	0.978	0.018	< 0.001	< 0.001
CP × FG × WP interaction			0.907	0.580	0.663	0.292	< 0.001	< 0.001

^{abcde}Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

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 jejunum, 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

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

Treatment										
CP (g/kg)	Feed grain	WP (g/kg)	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl-alanine	Threonine
220	Maize	0	0.845	0.793	0.771	0.796	0.814	0.889	0.798	0.740
		25	0.866	0.831	0.823	0.842	0.854	0.912	0.828	0.798
	Wheat	0	0.803	0.748	0.734	0.716	0.772	0.858	0.762	0.690
		25	0.797	0.754	0.743	0.745	0.776	0.860	0.763	0.710
180	Maize	0	0.867	0.800	0.810	0.813	0.851	0.919	0.797	0.789
		25	0.856	0.772	0.805	0.798	0.839	0.912	0.765	0.770
	Wheat	0	0.818	0.725	0.755	0.731	0.800	0.896	0.687	0.707
		25	0.799	0.690	0739	0.712	0.780	0.886	0.644	0.693
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^{a}	0.788^{b}	0.734
180			0.835	0.747	0.777	0.764	0.817	0.903^{b}	0.723^{a}	0.740
Feed grain										
Maize			0.859^{b}	0.799^{b}	0.802^{b}	0.812^{b}	0.839^{b}	0.908^{b}	0.797^{b}	0.774^{b}
Wheat			0.804^{a}	0.729^{b}	0.743^{a}	0.726^{a}	0.782^{a}	0.875^{a}	0.714^{a}	0.700^{a}
Whey protein										
0			0.833	0.767	0.767	0.764	0.809	0.891	0.761	0.732
25			0.830	0.761	0.778	0.774	0.812	0.893	0.750	0.742
Significance (P =)										
Crude protein (CP)			0.636	0.110	0.677	0.648	0.484	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	0.881	0.845	0.658	0.649
CP × FG interaction			0.941	0.680	0.950	0.922	0.879	0.419	0.192	0.818
CP × WP interaction			0.463	0.216	0.363	0.257	0.326	0.331	0.284	0.261
FG × WP interaction			0.560	0.653	0.548	0.830	0.569	0.577	0.681	0.740
CP × FG × WP interaction			0.765	0.761	0.722	0.894	0.726	0.689	0.850	0.661

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

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

Treatment										
CP (g/kg)	Feed grain	WP (g/kg)	Valine	Alanine	Aspartic acid	Cysteine	Glutamic acid	Glycine	Proline	Serine
220	Maize	0	0.752	0.772	0.727	0.691	0.831	0.743	0.788	0.765
		25	0.805	0.823	0.794	0.759	0.856	0.790	0.834	0.810
	Wheat	0	0.712	0.670	0.687	0.668	0.833	0.700	0.783	0.718
		25	0.718	0.704	0.741	0.694	0.837	0.701	0.800	0.739
180	Maize	0	0.799	0.797	0.795	0.713	0.832	0.824	0.809	0.767
		25	0.788	0.776	0.749	0.700	0.812	0.805	0.798	0.728
	Wheat	0	0.746	0.576	0.719	0.616	0.810	0.770	0.763	0.619
		25	0.724	0.587	0.679	0.591	0.787	0.744	0.745	0.609
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^{a}	0.801	0.758^{b}
180			0.764	0.684	0.736	0.655	0.810	0.786^{b}	0.777	0.681^{a}
Feed grain										
Maize			0.786^{b}	0.792^{b}	0.766^{b}	0.716^{b}	0.832	0.790^{b}	0.805	0.767^{b}
Wheat			0.725^{a}	0.634^{a}	0.706^{a}	0.642^{a}	0.817	0.729^{a}	0.773	0.672^{a}
Whey protein										
0			0.752	0.704	0.732	0.672	0.826	0.759	0.876	0.718
25			0.759	0.723	0.741	0.686	0.823	0.760	0.792	0.721
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	0.623	0.599	0.824	0.979	0.733	0.895
CP × FG interaction			0.918	0.115	0.449	0.274	0.621	0.841	0477	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	0.750	0.640	0.687	0.600

[ab]Means 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 11. Effects of dietary treatments on apparent amino acid digestibility coefficients in distal ileum.

Treatment										
CP (g/kg)	Feed grain	WP (g/kg)	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl-alanine	Threonine
220	Maize	0	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	0.905	0.866	0.866	0.862	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	0.940	0.898	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	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
SEM			0.0071	0.0136	0.0141	0.0149	0.0114	0.0060	0.0157	0.0191
Main effects: Crude protein										
220			0.917	0.884	0.883	0.891^{b}	0.898	0.938	0.900	0.833
180			0.913	0.847	0.872	0.868^{a}	0.888	0.942	0.849	0.817
Feed grain										
Maize			0.932	0.895	0.901	0.911	0.914	0.925	0.907	0.860
Wheat			0.898	0.836	0.854	0.849	0.871	0.928	0.843	0.790
Whey protein										
0			0.918	0.873	0.880	0.884	0.896	0.941	0.886	0.828
25			0.912	0.858	0.875	0.876	0.890	0.939	0.864	0.822
Significance (P =)										
Crude protein (CP)			0.418	< 0.001	0.245	0.034	0.218	0.281	< 0.001	0.230
Feed grain (FG)			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Whey protein concentrate (WP)			0.214	0.114	0.555	0.486	0.425	0.731	0.060	0.704
CP × FG interaction			0.013	0.001	0.010	0.016	0.007	0.061	< 0.001	0.026
CP × WP interaction			0.022	0.005	0.012	0.013	0.004	0.003	0.008	0.013
FG × WP interaction			0.126	0.074	0.090	0.162	0.085	0.170	0.058	0.224
CP × FG × WP interaction			0.418	0.124	0.201	0.117	0.199	0.165	0.097	0.240

^{ab}Means within columns not sharing a common suffix are significantly different at the 5% level of probability.

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

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

Treatment										
CP (g/kg)	Feed grain	WP (g/kg)	Valine	Alanine	Aspartic acid	Cysteine	Glutamic acid	Glycine	Proline	Serine
220	Maize	0	0.864	0.869	0.875	0.807	0.913	0.848	0.875	0.876
		25	0.888	0.894	0.898	0.848	0.924	0.871	0.890	0.895
	Wheat	0	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	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	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
SEM			0.0145	0.0189	0.0201	0.0210	0.0085	0.0135	0.0082	0.0161
Main effects: Crude protein										
220			0.864	0.857	0.871	0.814	0.918	0.845	0.883	0.872
180			0.855	0.793	0.798	0.771	0.893	0.862	0.873	0.808
Feed grain										
Maize			0.885	0.884	0.879	0.827	0.917	0.877^{b}	0.883	0.878
Wheat			0.834	0.766	0.790	0.758	0.895	0.829^{a}	0.873	0.802
Whey protein										
0			0.863	0.827	0.836	0.793	0.911	0.856	0.882	0.847
25			0.856	0.822	0.832	0.792	0.900	0.850	0.874	0.834
Significance (P =)										
Crude protein (CP)			0.409	< 0.001	< 0.001	0.005	< 0.001	0.073	0.092	< 0.001
Feed grain (FG)			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.070	< 0.001
Whey protein concentrate (WP)			0.474	0.706	0.768	0.990	0.083	0.526	0.184	0.240
CP × FG interaction			0.013	< 0.001	< 0.001	0.005	< 0.001	0.052	0.043	< 0.001
CP × WP interaction			0.011	0.017	0.139	0.014	0.002	0.013	< 0.001	0.005
FG × WP interaction			0.082	0.288	0.614	0.139	0.085	0.194	0.090	0.208
CP × FG × WP interaction			0.193	0.198	0.624	0.219	0.160	0.393	0.138	0.197

^{ab}Means 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 13. Effects of dietary treatment on estimated proportions of dietary, endogenous, and microbial amino acids in distal ileal digesta.

Treatment			Amino acid proportions in ileal digesta		
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	23.3 ± 1.67	27.9 ± 8.69
Coefficient of variation			19.8%	7.2%	31.2%

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	$r = -0.503$	$P < 0.001$	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	$r = -0.795$	$P < 0.001$

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)

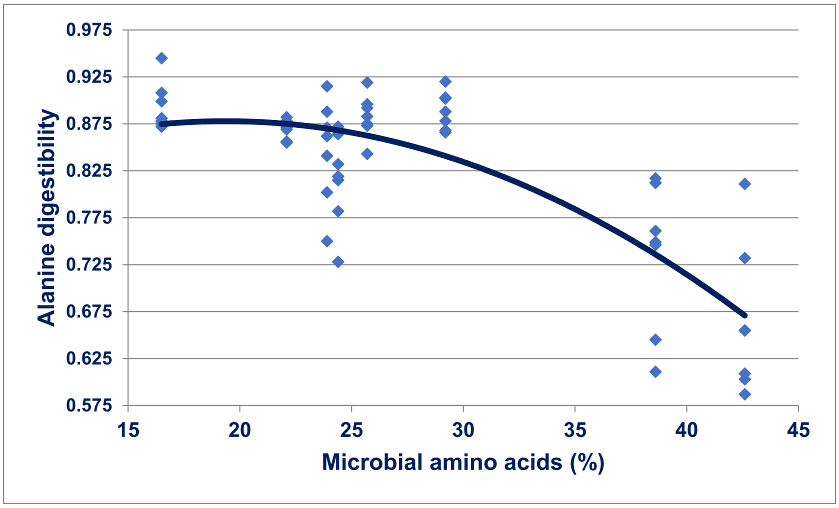

Figure 1. Quadratic relationship ($r = 0.851$; $P < 0.001$) between proportions of microbial amino acids (MAA) in distal ileal digesta and apparent ileal alanine digestibility coefficients where $y = 0.8596 + 0.0066*\mathrm{MAA} - 0.000261*\mathrm{MAA}^2$.

comparison, although the differences are of a lesser magnitude. The superiority of maize in terms of growth performance may be related to lower non-bound amino acid inclusions in maize-based reduced-CP diets; in 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 (NH_3), which is inherently toxic (Stern and Mozdziak, 2019); thus, high levels of deamination coupled with inadequate NH_3 detoxification could result in 'NH_3 overload'. Indeed, several studies (Namroud et al., 2008; Ospina−Rojas et al., 2013, 2014) have associated elevated NH_3 plasma concentrations with depressed growth performance. It is then noteworthy that Wilson et al. (1968) found that the intravenous LD_{50} of ammonium acetate in broiler chickens is half that of mice (2.72 vs. 5.64 mmol/kg). This illustrates the potential of 'NH_3 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 paradox, 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 Robin, 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 (Giuberti 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 attracts 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 (Apajalahti 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, Choct et al. (1996) found that soluble NSP increased small intestinal fermentation and this is partly responsible for the anti−nutritive effects soluble NSP. Accordingly, Hübener 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.

ACKNOWLEDGMENTS

The authors wish to acknowledge Ms Preethi Ramesh (Evonik Methionine SEA Pte Ltd) for the determinations of amino acid concentrations in diets and digesta and Dr David Cantor and Dr Mahmud Morshed (Australian Proteome Analysis Facility, Macquarie University) for size-exclusion chromatography of whey powder. The authors also wish to thank Ms Joy Gill, Ms Kylie Warr, Mr Duwei Chen and Mr Peter Bird (Poultry Research Foundation) and Mr Adam Crawford (Birling Avian Laboratories) for their invaluable technical support.

The feeding study was financially supported by Evonik Operations GmbH as part of a three-year project.

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.

REFERENCES

Acheson, K. J., Y. Schutz, T. Bessard, K. Anantharaman, J. P. Flat, and E. Jequier. 1988. Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man. Amer. J. Clin. Nutr. 48:240–247.

Ameer, F., L. Scandiuzzi, S. Hasnain, K. Kalbacher, and H. Zaidi. 2014. De novo lipogenesis in health and disease. Metab 63:895–902.

Apajalahti, J., and K. Vienola. 2016. Interaction between chicken intestinal microbiota and protein digestion. Anim. Feed Sci. Technol. 221:323–330.

Choct, M., R. J. Hughes, J. Wang, M. R. Bedford, A. J. Morgan, and G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poult. Sci. 37:609–621.

Chrystal, P. V., A. F. Moss, A. Khoddami, V. D. Naranjo, P. H. Selle, and S. Y. Liu. 2020. Impacts of reduced-crude protein diets on key parameters in male broiler chickens offered maize-based diets. Poult. Sci. 99:505–516.

Chrystal, P. V., A. F. Moss, A. Khoddami, V. D. Naranjo, P. H. Selle, and S. Y. Liu. 2020. Effects of reduced crude protein levels, dietary electrolyte balance and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein and amino acid metabolism. Poult. Sci. 99:1421–1431.

Chrystal, P. V., S. Greenhalgh, B. V. McInerney, L. R. McQuade, P. H. Selle, and S. Y. Liu. 2021. Maize-based diets are more conducive to crude protein reductions than wheat-based diets for broiler chickens. Anim. Feed Sci. Technol. 275:114867.

Dangin, M., C. Guillet, C. Garcia-Rodenas, P. Gachon, C. Bouteloup-Demange, K. Reiffers-Magnani, J. Fauquant, O. Ballèvre, and B. Beaufrère. 2003. The rate of protein digestion affects protein gain differently during aging in humans. J. Physiol. 549:635–644.

Daniel, H. 2004. Molecular and integrative physiology of intestinal peptide transport. Ann. Rev. Physiol. 66:361–384.

Duvaux, C. P., R. Guilloteau, R. Toullec, and J. W. Sissons. 1990. A new method of estimating the proportions of different proteins in a mixture using amino acid profiles: application to undigested proteins in the preruminant calf. Ann. Zootech 39:9–18.

Fang, R., M. Mantle, and H. Ceri. 1993. Characterization of quail intestinal mucin as a ligand for endogenous quail lectin. Biochem. J. 293:867–872.

Giuberti, G., A. Gallo, C. Cerioli, and F. Masoero. 2012. In vitro starch digestion and predicted glycemic index of cereal grains commonly utilized in pig nutrition. Anim. Feed Sci. Technol. 174:163–173.

Greenhalgh, S., B. V. McInerney, L. R. McQuade, P. V. Chrystal, A. Khoddami, M. A. M. Zhuang, S. Y. Liu SY, and P. H. Selle. 2020. 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 from 7 to 35 days post-hatch. Anim. Nutr 6:168–178.

Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587–603.

Hübener, K., W. Vahjen, and O. Simon. 2002. Bacterial responses to different dietary cereal types and xylanase supplementation in the intestine of broiler chicken. Arch. Tierern 56:167–187.

Jéquier, E. 1994. Carbohydrates as a source of energy. Amer. J. Clin. Nutr. 59:682S–685S.

Lehmann, U., and F. Robin. 2007. Slowly digestible starch – its structure and health implications: a review. Trends Food Sci. Technol. 18:346–355.

Lemme, A., H. S. Rostagno, A. Petri, and L. F. Albino. 2005. Standardised ileal digestibility of crystalline amino acids. Pages 462–464 in Proc. 15th Euro. Symp. Poult. Nutr. WPSA.

Li, Z., J. Li, X. L. Liu, D. D. Liu, H. Li, J. L. Li, R. L. Han, Y. B. Wang, X. J. Liu, X. T. Kang, F. B. Yan, and Y. D. Tian. 2019. Effects of different starch sources on glucose and fat metabolism in broiler chickens. Br. Poult. Sci. 60:449–456.

Lien, K. A., W. C. Sauer, and M. Fenton. 1997. Mucin output in ileal digesta of pigs fed a protein-free diet. Zeitschrift Ernährungs 36:182–190.

Liu, S. Y., P. H. Selle, S. G. Court, and A. J. Cowieson. 2013. Protease supplementation of sorghum-based broiler diets enhances amino acid digestibility coefficients in four small intestinal sites and accelerates their rates of digestion. Anim. Feed Sci. Technol. 183:175–183.

Liu, S. Y., and P. H Selle. 2015. A consideration of starch and protein digestive dynamics in chicken-meat production. Worlds Poult. Sci. J. 71:297–310.

Liu, S. Y., and P. H Selle. 2017. Starch and protein digestive dynamics in low-protein diets supplemented with crystalline amino acids. Anim. Prod. Sci. 57:2250–2256.

Liu, S. Y., S. P. Macelline, P. V. Chrystal, and P. H Selle. 2021. Progress towards reduced-crude protein diets for broiler chickens and sustainable chicken-meat production. J. Anim. Sci. Biotechnol. 12:20.

Liu, J., Q. Liu, Y. Yang, S. Zhao, Z. Jin, K. Zhu, L. Xu, and A. Jiao. 2022. Effects of whey protein on the in vitro digestibility and physicochemical properties of potato starch. Int. J. Biol. Macromol 193 Part B:1744–1751.

Mahasukhonthachat, K., P. A. Sopade, and M. J. Gidley. 2010. Kinetics of starch digestion and functional properties of twin-screw extruded sorghum. J. Cereal Sci 51:392–401.

Munyaka, P. M., N. K. Nandha, E. Kiarie, C. M. Nyachoti, and E. Khafipour. 2016. Impact of combined β-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. Poult. Sci. 95:528–540.

Musharaf, N. A., and J. D. Latshaw. 1999. Heat increment as affected by protein and amino acid nutrition. Worlds Poult. Sci. J. 55:233–240.

Namroud, N. F., M. Shivazad, and M. Zaghari. 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poult. Sci. 87:2250–2258.

Nolles, J. A., A. M. Verreijen, R. E. Koopmanschap, M. W. A. Verstegen, and V. V. A. M. Schreurs. 2009. Postprandial oxidative losses of free and protein-bound amino acids in the diet: interactions and adaptation. J. Anim. Physiol. Anim. Nutr. 93:431–438.

Ospina-Rojas, I. C., A. E. Murakami, I. Moreira, K. P. Picoli, R. J. B. Rodrigueiro, and A. C Furlan. 2013. Dietary glycine+serine responses of male broilers given low-protein diets with different concentrations of threonine. Br. Poult. Sci. 54:486–493.

Ospina-Rojas, I. C., A. E. Murakami, C. R. A. Duarte, C. Eyng, C. A. L. Oliveira, and V. Janeiro. 2014. Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. Br. Poult. Sci. 55:766–773.

Pineda-Quiroga, C., A. Camarinha-Silva, D. Borda-Molina, A. R. Ruiz, and A. García-Rodríguez. 2017. Feeding broilers with dry whey powder and whey protein concentrate affected productive performance, ileal digestibility of nutrients and cecal microbiota community. Anim 12:692–700.

Ravindran, V., P. C. H. Morel, S. M. Rutherfurd, and D. V. Thomas. 2009. Endogenous flow of amino acids in the avian ileum as influenced by increasing dietary peptide concentrations. Br. Poult. Sci. 101:822–828.

Ravindran, V. 2021. Progress in ileal endogenous amino acid flow research in poultry. J. Anim. Sci. Biotech. 12:5.

Selle, P. H., A. F. Moss, A. Khoddami, P. V. Chrystal, and S. Y. Liu. 2021. Starch digestion rates in multiple samples of commonly used feed grains in diets for broiler chickens. Anim. Nutr. 7:450–459.

Siriwan, P., W. L. Bryden, Y. Mollah, and E. F. Annison. 1993. Measurement of endogenous amino-acid losses in poultry. Br. Poult. Sci. 34:939–949.

Stern, R. A., and P. E. Mozdziak. 2019. Differential ammonia metabolism and toxicity between avian and mammalian species, and effect of ammonia on skeletal muscle: a comparative review. J. Anim. Physiol. Anim. Nutr. 103:774–785.

Szczurek, W., B. Szymczyk, A. Arczewska-Włosek, D. Józefiak, and M. N. Alloui. 2013. The effects of dietary whey protein concentrate level on performance, selected intestinal tract and blood parameters, and thiobarbituric acid reactive substances in the liver and breast meat of broiler chickens. J. Anim. Feed Sci. 22:342–353.

Tang, J. E., D. R. Moore, G. W. Kujbida, T. A. Tarnopolsky, and S. M. Phillips. 2009. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J. Appl. Physiol. 107:982–992.

Van Milgen, J. 2021. The role of energy, serine, glycine, and 1-carbon units in the cost of nitrogen excretion in mammals and birds. Anim 15:100213.

Wagner, D. D., and P. Thomas. 1977. Influence of diets containing rye or pectin on the intestinal flora of chicks. Poult. Sci. 57:971–975.

Wang, G., W. K. Kim, M. A. Cline, and E. R. Gilbert. 2017. Factors affecting adipose tissue development in chickens: A review. Poult. Sci. 96:3687–3699.

Wilson, R., M. Muhrer, and R. Bloomfield. 1968. Comparative ammonia toxicity. Comp. Biochem. Physiol. 25:295–301.

Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids 37:1–17.

Xia, S. K., L. Chen, and J. Q. Liang. 2007. Enriched selenium and its effects on growth and biochemical composition in Lactobacillus bulgaricus. J. Agric. Food Chem. 55:2413–2417.

Yang, N., Y. Liu, J. Ashton, E. Gorzyca, and S. Kasapis. 2013. Phase behaviour and *in vitro* hydrolysis of wheat starch in mixture with whey protein. Food Chem 137:76–82.

Yin, D., P. V. Chrystal, A. F. Moss, S. Y. Liu, and P. H. Selle. 2020. Effects of reduced crude protein and whole grain feeding on performance and amino acid metabolism in broiler chickens offered wheat-based diets. Anim. Feed Sci. Technol. 260:114386.

Zhang, S., C. Yang, S. Zhu, F. Zhong, D. Huang, and Y. Li. 2022. Understanding the mechanisms of whey protein isolate mitigating the digestibility of corn starch by *in vitro* simulated digestion. Food Hydrocoll 124 Part A:107211.