



Original Research Article

Characterisation of undigested components throughout the gastrointestinal tract of broiler chickens fed either a wheat- or maize-based diet

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ABSTRACT

This study was to characterise the undigested nutrients present along the gastrointestinal tract of birds offered common wheat- or maize-based diets, with the goal of optimising utilisation of enzymes to enhance digestive efficiency. Wheat- and maize-based diets were offered to 240 mixed-sex broilers (10 birds/pen; $n = 12$) from 1 to 35 d post-hatch. Digestibility of dry matter, starch, crude protein and non-starch polysaccharides (NSP) were measured in the crop, gizzard, duodenum, jejunum, ileum, caeca and excreta at d 12 and 35 post-hatch. Analysis of nutrient levels in the excreta presented that more than 30% of nutrients provided in the feed was wasted, irrespective of wheat or maize diet type. On average, 92 g/kg crude protein, 92 g/kg insoluble NSP and 14 g/kg oligosaccharides were not utilised by birds at d 12 post-hatch. The quantity of water-insoluble NSP in the small intestine at d 12 was lower in birds offered the wheat-based diet compared to those fed the maize-based diet ($P < 0.05$), with the reverse being true for water-soluble NSP ($P < 0.001$). On average, 84 g/kg crude protein, 79 g/kg insoluble NSP and 9 g/kg oligosaccharides remained in the excreta at 35 d of age. At this time period, accumulation of feed in the gizzard was noted for birds offered both diets, but was more pronounced in those offered the maize-based diet ($P < 0.001$). Birds offered the maize-based diet demonstrated improved utilisation of oligosaccharides compared to those fed the wheat-based diet at both d 12 and 35 ($P = 0.087$ and $P = 0.047$, respectively). Protein utilisation in the jejunum and ileum was greater in birds offered the wheat-based diet compared to those fed the maize-based diet ($P = 0.004$ and $P < 0.001$, respectively). Thus, while both diets supported standard growth performance of birds, the degree and flow of nutrient disappearance along the gastrointestinal tract was influenced by cereal type and bird age.

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1. Introduction

The digestive system of modern broilers is adapted to promote highly efficient digestion in order to sustain rapid growth.

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Nevertheless, incomplete digestion of nutrients at the ileal and total tract levels is still a notable concern for feed manufacturers and poultry producers. Dietary components that escape digestion within the small intestine become substrates for hindgut bacteria where microbial fermentation may occur (Qaisrani et al., 2015b). Putrefactive fermentation of protein in the lower gut can produce toxic compounds, increasing the risk of diarrhoea and dehydration, decreasing bird productivity (Qaisrani et al., 2015a, 2015b; Apajalahti and Vienola, 2016). This often leads to inaccuracies in the prediction of the nutritive value of diets, due to variations in nutrient digestibility between small intestinal digestion and large intestinal fermentation. For example, starch fermentation by hindgut microbiota generates volatile fatty acids as an energy

source that are not used as efficiently as glucose in the small intestine (Sharma et al., 2008). Fibre digestion is considered beneficial in that it maintains an anaerobic intestinal environment that subsequently stabilises the resident microflora throughout the gastrointestinal tract (Engberg et al., 2004; Hetland et al., 2004). However, high fibre contents in the diet often hinder nutrient digestion in the small intestine, due to anti-nutritive impacts such as heightened viscosity and nutrient encapsulation (Hetland et al., 2004; Kiarie et al., 2014).

This complexity of digestive mechanisms has necessitated the evaluation of factors that influence nutrient utilisation in the different regions of the digestive system of birds. Furthermore, many poultry studies do not fully characterise digestion of dietary fibre along the digestive tract and instead analyse crude fibre. However, as much as 10% of the diet may be unaccounted for or 'missing' due to inadequate chemical definitions derived using the crude fibre system (Choct, 2015). Various nutritional modulations have been extensively discussed to optimise feed utilisation; however, responses have been largely inconsistent in broilers, due to the wide variety of dietary components and physicochemical properties.

Once the undigested fractions of the feed are fully characterised, it becomes possible to precisely determine what influences the underutilised components present in the gut, and develop strategies to minimise the undigested dietary fractions and maximise feed efficiency. Therefore, the present study aimed to determine the amount and types of nutrients that escape digestion in broilers offered standard industry wheat- or maize-based diets, with a focus on insoluble and soluble non-starch polysaccharides (NSP).

2. Materials and methods

2.1. Animal ethics

The experimental procedures used in the present study were reviewed and approved by the Animal Ethics Committee of University of New England (AEC 18-089).

2.2. Experimental design and diets

Birds were allocated to 24 pens, with 2 dietary treatments and 12 replicate pens per treatment, and 10 birds per pen. The ingredients and chemical composition of the diets are presented in Tables 1 and 2, respectively. Wheat- and maize-based diets were formulated to meet or exceed Cobb 500 broiler performance and nutrition recommendations (Cobb-Vantress, 2018). Neither diet contained animal protein sources or antimicrobial agents. The dietary treatments were offered in 3 phases (starter; d 0 to 12, grower; d 13 to 24 and finisher; d 25 to 35). Titanium dioxide (TiO₂; 5 g/kg) was incorporated into all diets as an inert dietary marker. The nutrient profile of wheat, maize and soybean meal were estimated using near-infrared reflectance spectrometry (Foss NIR 6,500, Denmark) standardised using Evonik AMINONIR Advanced calibrations. The diets were cold-pelleted ($\phi = 3.5$ mm, 65 °C) and the starter diets were crumbled.

2.3. Animal and housing

A total of 240 one-day-old Cobb 500 mixed-sex broiler chicks were obtained from a commercial hatchery (Baiada, Australia). Birds were housed in 24 floor pens (1.2 m × 0.77 m) with 10 birds per pen. Upon arrival, birds were weighed and allocated per pen to ensure that there was no significant difference in initial body weight between the 2 treatments. All pens were equipped with a bell feeder

Table 1
The composition of experimental diets (% as fed basis).

Item	Starter, d 0 to 12		Grower, d 13 to 24		Finisher, d 25 to 35	
	Wheat	Maize	Wheat	Maize	Wheat	Maize
Ingredients						
Wheat	64.1	–	69.5	–	71.6	–
Maize	–	60.6	–	66.3	–	67.5
Soybean meal, 47%	29.0	33.4	23.4	27.5	19.9	25.0
Canola oil	2.17	1.15	2.87	1.72	4.31	3.19
Limestone	1.17	1.13	1.12	1.08	1.06	1.02
Dicalcium phosphate	1.63	1.74	1.53	1.65	1.40	1.52
Salt	0.20	0.21	0.22	0.23	0.20	0.17
Sodium bicarbonate	0.22	0.26	0.11	0.16	0.18	0.31
TiO ₂	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix ¹	0.18	0.18	0.18	0.18	0.18	0.18
Choline chloride, 70%	0.07	0.13	0.07	0.13	0.06	0.11
L-Lysine HCl	0.31	0.28	0.24	0.22	0.26	0.22
DL-Methionine	0.31	0.34	0.24	0.28	0.22	0.25
L-Threonine	0.13	0.10	0.10	0.08	0.08	0.06
Calculated value						
ME, kcal/kg	2,950	2,950	3,050	3,050	3,150	3,150
Crude protein	23.0	22.0	20.8	19.5	19.5	18.4
Calcium	0.90	0.90	0.85	0.85	0.79	0.79
Available P	0.45	0.45	0.43	0.43	0.40	0.40
Digestible Lys	1.24	1.24	1.05	1.05	0.98	0.98
Digestible Met	0.58	0.63	0.49	0.55	0.45	0.51
Digestible Met + Cys	0.90	0.90	0.80	0.80	0.75	0.75
Digestible Thr	0.80	0.80	0.70	0.70	0.63	0.64
Digestible Trp	0.24	0.21	0.22	0.18	0.20	0.17
dEB, mEq/kg	247.9	243.8	213.7	206.5	203.9	213.7

dEB = dietary electrolyte balance.

¹ Vitamin-mineral concentrate supplied per kilogram of diet: retinol 12,000 IU; cholecalciferol, 5,000 IU; tocopherol acetate, 75 mg; menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 µg; biotin, 200 µg; cereal-based carrier, 277 mg; mineral oil, 6.25 mg; Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg.

and 2 nipple drinkers. Wood shavings were used as litter (depth = 7 cm). Birds had ad libitum access to feed and fresh water throughout the study. Birds were reared following a photoperiod of 24 h of light for the first 3 d post-hatch, and then 20 h of light: 4 h of dark for the remaining duration of the study. Housing temperature was maintained at 32 °C from d 1 to 3 and then gradually reduced by 1 °C every 3 d until a final temperature of 22 °C was reached.

2.4. Data and sample collection

Body weights and feed intake were measured at the end of each growth phase (d 12, 24 and 35). Mortality was recorded daily. Feed conversion ratio was calculated by dividing total feed intake by weight gain, corrected for mortality. Excreta samples were collected per pen on d 12 and 35; a plastic tub was placed in each pen to collect the excreta and avoid wood shaving inclusion. Four birds per pen on d 12 and three birds per pen on d 35 were randomly selected and euthanised by cervical dislocation. Gut contents from the crop, gizzard, duodenum, jejunum, ileum and caeca were then collected and pooled on a pen basis, and frozen at –20 °C for laboratory analysis. The duodenum was defined as the pyloric junction to the distal point of insertion of the duodenal mesentery, the jejunum was defined as the end of the duodenum to the Meckel's diverticulum, and the ileum was determined as from the Meckel's diverticulum to the ileocecal junction.

2.5. Laboratory analysis

All digesta and excreta samples were freeze-dried and, along with the experimental diets, were finely ground using a centrifuge mill to pass through a 0.5-mm screen (Model ZM 200, Retsch, Haan, Germany). Diets, digesta and excreta samples were analysed for dry matter, gross energy, nitrogen, NSP, uronic acids, oligosaccharides, TiO₂, starch and Klason lignin. The determination of dry matter was carried out according to the standard methods of AOAC (2012) (method 930.15). Nitrogen was determined by the combustion method (LECO Corp., St. Joseph, MI) using standard procedures as outlined in Siriwan et al. (1993), and the assayed nitrogen values were multiplied by a factor of 6.25. Gross energy was determined using an adiabatic bomb calorimeter (Model 6,400, Parr Instruments, Moline, IL), standardised with benzoic acid. The constituent sugar components of NSP were determined by gas–liquid chromatography (Model CP3800, Varian Inc., Palo Alto, CA) as alditol acetates, following the procedure of Englyst et al. (1994) with some modifications as described by Theander et al. (1995) and Morgan et al. (2019). Uronic acids were measured using a spectrophotometer at 450 and 400 nm (UV–1,600PC, VWR, Darmstadt, Germany), according to the method of Scott (1979). Klason lignin was determined gravimetrically after acid hydrolysis of insoluble NSP residues according to the standard method of AOAC (2012) (method 973.18). The total starch content was analysed using Megazyme Total Starch Assay kit (Megazyme, Wicklow, Ireland, UK) as described in McCleary et al. (1997). TiO₂ was quantified by UV–spectroscopy at 410 nm (Cary 50 Bio UV–Visible spectrophotometer equipped with a Cary 50 MPR microplate reader, Varian Inc., Palo Alto, CA), as illustrated by Short et al. (1996).

2.6. Calculations

Calculation of the disappearance of nutrients in each gut section was conducted by considering the concentration of the respective nutrients in the undigested dry matter fractions of the digesta or excreta. Firstly, dry matter digestibility was calculated by the indirect method using TiO₂ as an inert marker to determine the quantity of undigested dry matter. Then, the undigested nutrients were expressed in grams per kilogram undigested dry matter. The equations are as below:

$$\text{Dry matter digestibility (\%)} = \left\{ 1 - \left[\frac{\text{TiO}_2 \text{ diet (g/kg)}}{\text{TiO}_2 \text{ digesta/excreta (g/kg)}} \right] \times \left(\frac{\text{Nutrient digesta/excreta (g/kg)}}{\text{Nutrient diet (g/kg)}} \right) \right\} \times 100$$

$$\text{Undigested dry matter (g/kg)} = (100 - \text{Dry matter digestibility\%}) \times 10$$

$$\text{Undigested nutrients digesta/excreta (g/kg)} = \text{Undigested dry matter (g/kg)} \times \left(\frac{\text{Nutrient digesta/excreta (g/kg)}}{1,000} \right).$$

The greater utilisation of the nutrient reduces the amount of the nutrient that is detected in the sample, meaning presented lower values are an indication of greater nutrient digestion.

2.7. Statistical analysis

All data were analysed as a completely randomised design using the software IBM SPSS Statistics 25, with pen means as the experimental unit. Data were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test). The sex ratio of each pen did not vary significantly and all data were normally distributed. One-factor analysis of covariance (ANCOVA), with the male/female ratio as a covariate, was performed for the growth performance data, and an independent *t*-test was applied for the

undigested nutrients data. The differences were considered significant at $P < 0.05$.

3. Results

3.1. Chemical composition of experimental diets

The analysed composition of the experimental diets is shown in Table 2. Based on the laboratory analysis, the expected nutrient contents of the experimental diets were met. The 2 diets were iso-caloric and iso-nitrogenous. The starter and grower wheat-based diets had higher total NSP concentration compared to the maize-based diets. However, the maize-based finisher diet had higher total NSP content than the wheat-based finisher diet, by 0.59%. For the maize-based diet, soluble NSP concentration was approximately half of that observed in the wheat-based diet.

3.2. Growth performance

Mortality was <3% during the experiment and was not related to dietary treatment. The effect of cereal type on growth performance from d 0 to 35 is illustrated in Table 3. Within the starter phase (d 0 to 12), birds offered the wheat-based diet presented 4.3% higher weight gain ($P = 0.049$), and feed conversion ratios were 3.3% lower (0.038 points; $P = 0.036$) relative to those fed the maize-based diet.

Diet type had no significant impact on body weight, weight gain or feed intake over the grower (d 13 to 24) and finisher (d 25 to 35) phases.

Table 2
The analysed chemical composition of experimental diets (% DM)¹.

Item	Starter, d 0 to 12		Grower, d 13 to 24		Finisher, d 25 to 35	
	Wheat	Maize	Wheat	Maize	Wheat	Maize
Dry matter	88.4	88.0	88.8	89.6	89.3	88.7
Gross energy, kcal/kg	3,893.4	3,907.7	4,348.7	4,404.2	4,524.9	4,543.5
Crude protein ²	24.8	25.0	21.6	22.3	21.2	21.3
Ash	6.8	7.7	6.4	5.9	5.7	6.0
Crude fat	4.2	3.5	4.9	4.7	5.6	5.1
Total starch	47.2	44.4	49.6	48.1	48.9	49.2
Klason lignin	1.6	1.2	1.5	1.3	1.6	1.4
Oligosaccharides	4.6	4.8	4.5	4.3	4.1	4.2
Total NSP	13.75	12.00	11.33	10.90	9.81	10.40
Soluble						
Rhamnose	0.004	0.003	0.005	0.003	0.004	0.005
Fucose	0.006	0.006	0.005	0.005	0.003	0.005
Ribose	0.04	0.03	0.04	0.03	0.04	0.03
Arabinose	0.38	0.13	0.34	0.10	0.51	0.11
Xylose	0.46	0.04	0.41	0.04	0.64	0.06
Mannose	0.14	0.13	0.23	0.16	0.19	0.27
Galactose	0.22	0.17	0.23	0.15	0.22	0.16
Glucose	0.10	0.06	0.16	0.06	0.18	0.13
Uronic acid	0.25	0.31	0.15	0.22	0.15	0.18
Total soluble	1.42	0.79	1.39	0.68	1.72	0.86
Insoluble						
Rhamnose	0.06	0.08	0.05	0.09	0.06	0.06
Fucose	0.11	0.13	0.10	0.12	0.08	0.10
Ribose	0.04	0.03	0.04	0.03	0.04	0.03
Arabinose	2.89	2.19	2.18	2.00	1.84	1.96
Xylose	3.06	2.09	2.54	2.03	1.91	1.90
Mannose	0.25	0.24	0.22	0.17	0.17	0.20
Galactose	1.78	2.09	1.42	1.80	1.17	1.75
Glucose	2.68	2.54	2.48	2.45	2.48	2.32
Uronic acid	2.38	3.13	2.11	2.74	1.53	2.34
Total insoluble	12.33	11.21	9.94	10.22	8.11	9.53

NSP = non-starch polysaccharides.

¹ Samples were analysed in duplicate.

² Crude protein = N × 6.25.

Table 3
Growth performance of broiler chickens fed a wheat- or maize-based diet from d 0 to 35¹.

Item	Wheat	Maize	SEM	P-value
Body weight, g				
Initial	46.3	46.5	0.20	0.632
d 12	389.3	375.1	3.61	0.053
d 24	1319.5	1323.5	13.55	0.842
d 35	2437.6	2447.3	26.35	0.836
Weight gain, g				
Starter (d 0 to 12)	342.8 ^a	328.7 ^b	3.59	0.049
Grower (d 13 to 24)	929.6	949.1	11.66	0.419
Finisher (d 25 to 35)	1118.0	1123.9	18.90	0.883
Overall (d 0 to 35)	2390.4	2401.7	26.33	0.839
Feed intake, g				
Starter (d 0 to 12)	390.5	386.5	3.73	0.636
Grower (d 13 to 24)	1234.8	1207.5	15.39	0.688
Finisher (d 25 to 35)	1826.0	1844.5	25.55	0.748
Overall (d 0 to 35)	3451.3	3453.4	34.41	0.968
Feed conversion ratio ² , g/g				
Starter (d 0 to 12)	1.139 ^b	1.177 ^a	0.009	0.036
Grower (d 13 to 24)	1.328	1.276	0.015	0.055
Finisher (d 25 to 35)	1.636	1.647	0.019	0.801
Overall (d 0 to 35)	1.444	1.440	0.009	0.799

SEM = standard error of the mean.

^{a, b} Means within rows with no common superscript differ significantly ($P < 0.05$).

¹ Each mean represents values from 12 replicates (starter from d 0 to 12, 10 birds/replicate; grower and finisher from d 13 to 35, 6 birds/replicate). Male percentage of each pen was used as a covariate for the performance analysis.

² Corrected for mortality.

3.3. Disappearance of nutrients

The disappearance of nutrients along the gastrointestinal tract of broiler chickens fed a wheat- or maize-based diet at 12 and 35 d of age is shown in Table 4.

Table 4

The disappearance of dry matter, starch and crude protein throughout the gastrointestinal tract of broiler chickens fed a wheat- or maize-based diet on d 12 and 35 (g/kg DM digesta)¹.

Item	d 12				d 35			
	Wheat	Maize	SEM	P-value	Wheat	Maize	SEM	P-value
Dry matter								
Diet	1,000.0	1,000.0			1,000.0	1,000.0		
Crop	—	—	—	—	1,219.1	1,259.2	17.04	0.272
Gizzard	1031.5 ^b	1921.5 ^a	100.93	<0.001	1,548.3 ^b	2,996.9 ^a	194.42	<0.001
Duodenum	—	—	—	—	1,829.8	1,665.6	93.89	0.416
Jejunum	487.0	439.2	14.79	0.108	547.7	515.8	9.84	0.106
Ileum	348.5	355.8	4.38	0.433	324.5	324.8	5.70	0.980
Caeca	953.5 ^a	526.2 ^b	73.42	<0.001	659.6	699.1	96.52	0.846
Excreta	343.1	352.1	4.02	0.273	320.4	314.9	4.09	0.518
Starch								
Diet	472.3	444.1			488.7	491.9		
Crop	—	—	—	—	545.9	595.4	12.35	0.060
Gizzard	148.0 ^b	633.3 ^a	54.92	<0.001	331.2 ^b	1,440.7 ^a	132.14	<0.001
Duodenum	—	—	—	—	453.1 ^a	284.9 ^b	29.78	0.001
Jejunum	46.8	44.6	8.04	0.897	103.4 ^a	72.1 ^b	1.54	0.013
Ileum	11.2	10.3	0.66	0.530	31.3	17.7	4.31	0.115
Caeca	51.9 ^a	26.1 ^b	5.10	0.004	32.8	17.6	5.07	0.141
Excreta	9.6 ^a	4.4 ^b	0.80	<0.001	18.7	14.7	2.29	0.401
Crude protein								
Diet	248.2	250.2			212.2	213.4		
Crop	—	—	—	—	149.8	140.5	2.90	0.097
Gizzard	92.7 ^b	180.8 ^a	10.72	<0.001	188.8 ^b	326.5 ^a	20.11	<0.001
Duodenum	—	—	—	—	679.8	704.5	58.50	0.845
Jejunum	110.4	114.2	7.50	0.813	87.5 ^b	105.2 ^a	3.25	0.004
Ileum	67.4	74.3	3.34	0.326	39.7 ^b	48.6 ^a	1.35	<0.001
Caeca	463.6 ^a	280.2 ^b	32.49	<0.001	338.6	391.8	55.13	0.648
Excreta	91.7	92.1	1.54	0.907	87.4	80.1	2.26	0.109

SEM = standard error of the mean.

^{a, b} Means within rows with no common superscript differ significantly ($P < 0.05$).

¹ Each mean represents values from 12 replicates and samples were analysed in duplicate.

The quantity of undigested dry matter was determined to be higher ($P < 0.001$) in the gizzard of birds offered the maize-based diet compared to those fed the wheat-based diet, at both 12 and 35 d of age. The dietary treatments had no impact on the quantity of undigested dry matter determined in the jejunum, ileum and excreta. A greater amount of undigested dry matter was seen in the caeca of birds offered the wheat-based diet ($P < 0.001$) compared to those offered the maize-based diet at bird age d 12, but not at d 35.

The amount of undigested starch was greater ($P < 0.001$) in the gizzard of birds offered the maize-based diet compared to birds fed the wheat-based diet at 12 d of age. No dietary effects were observed on the quantities of undigested starch in the jejunum and ileum. However, birds offered the wheat-based diet presented a greater amount of undigested starch in the caeca ($P = 0.004$) and excreta ($P < 0.001$) compared to those fed the maize-based diet. At 35 d of age, there tended to be comparatively more undigested starch in the crop of birds offered the maize-based diet compared to the wheat-based diet ($P = 0.060$). The gizzard of birds fed the maize-based diet also contained a greater ($P < 0.001$) amount of undigested starch relative to that of birds fed the wheat-based diet. Conversely, undigested starch determined in the duodenum ($P = 0.001$) and jejunum ($P = 0.013$) were comparatively greater for birds offered the wheat-based diet. No dietary effects on the disappearance of starch were observed from the ileum to total tract at 35 d of age.

A higher quantity of undigested protein was observed in the gizzard of birds offered the maize-based diet compared with birds offered the wheat-based diet at both bird age d 12 and 35 ($P < 0.001$). The crop of birds offered the wheat-based diet tended ($P = 0.097$) to contain a greater amount of undigested protein relative to that of birds fed the maize-based diet at 35 d of age. However, birds fed the maize-based diet presented a greater quantity of undigested protein in the gizzard ($P < 0.001$), jejunum ($P = 0.004$) and ileum ($P < 0.001$) compared to those offered the wheat-based diets.

Table 5
The disappearance of NSP along the gastrointestinal tract of broiler chickens fed a wheat- or maize-based diet on d 12 and 35 (g/kg DM digesta)¹.

Item	d 12				d 35			
	Wheat	Maize	SEM ²	P-value	Wheat	Maize	SEM	P-value
Soluble NSP								
Diet	14.2	7.9			17.2	8.6		
Crop	–	–	–	–	15.4 ^a	5.5 ^b	1.21	<0.001
Gizzard	17.4	14.4	0.94	0.114	30.1 ^a	16.9 ^b	2.24	0.002
Duodenum	–	–	–	–	25.8 ^a	13.7 ^b	1.89	<0.001
Jejunum	12.9 ^a	6.2 ^b	1.07	<0.001	12.8 ^a	5.6 ^b	0.81	<0.001
Ileum	12.7 ^a	4.9 ^b	1.21	<0.001	14.3 ^a	6.6 ^b	0.84	<0.001
Caeca	43.8 ^a	15.5 ^b	4.75	<0.001	13.8 ^a	9.9 ^b	2.04	0.356
Excreta	13.6 ^a	6.5 ^b	0.79	<0.001	10.9 ^a	7.3 ^b	0.50	<0.001
Insoluble NSP								
Diet	123.3	112.1			81.0	95.3		
Crop	–	–	–	–	115.5	118.0	1.41	0.390
Gizzard	405.0 ^b	598.5 ^a	26.3	<0.001	341.1 ^b	577.1 ^a	38.95	0.002
Duodenum	–	–	–	–	93.3	86.5	4.30	0.454
Jejunum	106.2	98.2	2.91	0.150	79.9	86.3	1.89	0.092
Ileum	86.3 ^b	95.7 ^a	2.03	0.012	73.2 ^a	80.1 ^b	1.68	0.035
Caeca	76.3 ^a	20.1 ^b	9.02	<0.001	35.0	23.4	4.53	0.209
Excreta	86.5 ^b	98.1 ^a	1.93	0.001	76.6	81.9	1.62	0.101
Oligosaccharides								
Diet	45.5	47.8			41.4	41.9		
Crop	–	–	–	–	17.8 ^b	23.8 ^a	1.26	0.026
Gizzard	15.6 ^b	27.2 ^a	1.43	<0.001	33.9	42.1	2.43	0.092
Duodenum	–	–	–	–	116.1	126.6	13.55	0.721
Jejunum	65.6 ^a	43.1 ^b	4.46	0.005	94.4 ^a	78.0 ^b	3.21	0.008
Ileum	24.3	24.4	1.54	0.997	33.7 ^a	24.5 ^b	1.40	<0.001
Caeca	13.8 ^b	7.5 ^a	1.15	0.001	8.1	5.2	1.05	0.172
Excreta	15.1	12.5	0.77	0.087	10.6 ^a	7.9 ^b	0.69	0.047

NSP = non-starch polysaccharides; SEM = standard error of the mean.

^{a, b} Means within rows with no common superscript differ significantly ($P < 0.05$).

¹ Each mean represents values from 12 replicates and each sample was analysed in duplicate.

3.4. Disappearance of non-starch polysaccharides

The disappearance of NSP along the gastrointestinal tract in broiler chickens fed wheat- or maize-based diets is shown in Table 5.

On 12 d of age, greater quantities of undigested soluble NSP were observed in the jejunum, ileum, caeca and excreta ($P < 0.001$) in birds offered the wheat-based diet compared with those offered the maize-based diet. Similarly, at 35 d of age, birds offered the wheat-based diet displayed higher ($P < 0.05$) amounts of soluble NSP in all gut segments, except the caeca, compared to those fed the maize-based diet.

The amount of undigested insoluble NSP were comparatively higher ($P < 0.05$) in the gizzard and ileum of birds offered the maize-based diet on both 12 and 35 d of age. Birds offered the wheat-based diet presented a greater ($P < 0.001$) amount of insoluble NSP in the caeca relative to those fed the maize-based diet at 12 d of age, whilst a greater ($P = 0.001$) amount of insoluble NSP was observed in total tract of birds fed the maize-based diet than those offered the wheat-based diet. No significant difference in the amounts of undigested insoluble NSP between the 2 diets was observed based on analysis of the excreta of birds at 35 d of age.

Birds offered the wheat-based diet had lower amounts of oligosaccharides in the gizzard ($P < 0.001$) compared to those on the maize-based diet at 12 d of age. In contrast, birds offered the maize-based diet presented a lower amount of oligosaccharides in the jejunum ($P = 0.005$) and caeca ($P = 0.001$), and tended to excrete less oligosaccharides ($P = 0.087$) compared to birds offered the wheat-based diet. With results following a similar pattern to d 12, at d 35 birds offered the maize-based diet presented a greater amount of oligosaccharides in the crop ($P = 0.026$), and lower amounts of oligosaccharides in the jejunum ($P = 0.008$) and ileum ($P < 0.001$), compared to those offered the wheat-based diet.

4. Discussion

The present study characterised the quantities of major nutrients that were not utilised along the gastrointestinal tract of broiler chickens offered common wheat- or maize-based diets. By characterising the undigested nutrients, it is possible to understand what influences the digestion of nutrients in the bird, how nutrient wastage may be minimised and how digestive efficiency can be improved. Across both diets, appreciable amounts of nutrients provided were excreted at 12 and 35 d of age of birds. Although the difference in the quantity of undigested nutrients between the 2 diets was not significant on a total tract level, the extent of the disappearance of nutrients in each gut segment was dictated by diet type and bird age, suggesting that a targeted nutritional approach is required over each diet phase to maximise the utilisation of the undigested dietary components.

Crude protein was determined to be the most wasted nutrient on a total tract level. However, it should be noted that excreta contains substantial amounts of endogenous losses and proteins synthesised by the hindgut bacteria, alongside non-dietary bacterial protein, which may confound total nitrogen content measurements in the excreta (Moughan et al., 2014). Thus, analysis of ileal digesta likely provides a more accurate representation of protein utilisation in the bird. At the ileal level, older birds (35 d of age) utilised more protein than young birds (12 d of age) by 41% and 34% for the wheat- and maize-based diets, respectively. This age-related increase in the capacity of the small intestine to digest and absorb nitrogen (i.e., amino acids) is consistent with previous reports (Hughes, 2001; Batal and Parsons, 2002). Regardless of improved protein utilisation in older birds, 39.7 and 48.6 g/kg crude protein still remained undigested in the ileum of birds fed the wheat- and maize-based diet, respectively. This undigested protein that escapes the small intestine provides a substrate for microbial

digestion in the hindgut, which is energetically expensive, increases metabolic heat production, and results in production of ammonia, amines and toxic compounds, producing a poor shed environment (Gilbert et al., 2018).

Starch and protein digestion rates in the small intestine differ between wheat- and maize-based diets. At 35 d of age, the majority of maize starch digestion was complete by the jejunum, and essentially no further digestion was observed within the ileum. However, a tangible amount of wheat starch escaped the small intestine and was digested via hindgut fermentation. Conversely, birds offered the maize-based diet generated a greater proportion of protein digestion in the latter portions of the small intestine compared to those fed the wheat-based diet. It has previously been suggested that starch digestion rate may influence intestinal uptakes of amino acids and glucose, thereby affecting protein deposition and feed conversion efficiency (Liu et al., 2013; Truong et al., 2015). In the present study, wheat starch was more slowly digested than maize starch, resulting in greater protein digestion in the jejunum and ileum in birds fed the wheat-based diet compared to those fed the maize-based diet. The slow digestion rate of wheat starch is likely due to heightened viscosity caused by soluble NSP, which may further promote hindgut fermentation of starch (Choct et al., 1999). More pronounced impact seen in older birds than young birds may indicate that this asynchrony of digestive flows is age-related, and improved protein digestibility with advancing age may accelerate competition for intestinal uptake of nutrients.

Insoluble NSP appeared to be the least utilised component of the feed, regardless of diet type or bird age. This observation was expected as insoluble NSP are mostly inert and therefore dilute poultry feed in the absence of exogenous enzymes (Hetland et al., 2004). The disappearance of insoluble NSP in both diets began within the jejunum, indicating that insoluble NSP fractions are partially degraded in jejunal environmental conditions. However, the quantities of undigested insoluble NSP determined in the ileum and excreta did not differ, suggesting it is unlikely that insoluble fibre is fermented in the hindgut, regardless of diet type or bird age. It is no coincidence that the majority of insoluble NSPs could not collect in the caeca, as only fine soluble materials with low molecular weight can enter (Svihus et al., 2013). It has been demonstrated that the utilisation of insoluble NSP is possible if polysaccharides are rendered soluble by enzymes or bacteria (Khadem et al., 2016), although the response of birds to supplemental enzymes have been largely inconsistent (Choct, 2006). A better understanding of the characteristics of NSP remaining along the gastrointestinal tract will allow for more successful NSP-degrading enzyme selection.

An improvement in soluble NSP utilisation with bird age was noted in this study, particularly with the wheat-based diet. At 12 d of age, soluble NSPs were not utilised by birds, irrespective of the feed grain, indicating that young birds were incapable of depolymerising complex soluble NSP. Lee et al. (2017) observed that a lack of digestion, and thus accumulation, of soluble NSP in the caeca of young birds (11 d of age). Caecal accumulation of soluble NSP also occurred in the present study, and this was comparatively more pronounced in birds fed the wheat-based diet. Consequently, at 35 d of age, birds offered the wheat-based diet utilised 37% of the ingested soluble NSP (6.3 g/kg) following caecal fermentation, compared to only 15% NSP (1.3 g/kg) in birds fed the maize-based diets. This may suggest that intestinal microflora are better established in birds fed the wheat-based diet, due to the caecal accumulation of soluble NSP, which probably stimulates a fibre-fermenting microbiome (Bautil et al., 2020). In addition, Kiarie et al. (2014) noted that birds offered wheat-based diets exhibited greater caecal fermentation relative to those fed maize-based diets,

by producing more caecal volatile fatty acids. Free oligosaccharides from the maize-based diet appeared to be more digestible than those from the wheat-based diet throughout the digestive tract. It is speculated that low levels of soluble NSP present in the maize-based diet allow intestinal microbes to primarily utilise the oligosaccharides as substrates.

Both wheat- and maize-based diets accumulated dry matter within the gizzard to levels greater than that within the diet. Additionally, there was a greater accumulation of undigested material in the gizzard of birds offered the maize-based diet. Poultry diets based on maize have been shown to improve gizzard functionality, primarily by increasing gizzard size (Abdollahi et al., 2010; Masey-O'Neill et al., 2014). The gizzard is described as the 'pace-maker' of gut motility (Moss et al., 2017) and a more developed gizzard may promote gastric-duodenal reflux, as well as increasing grinding capacity. In this sense, the numerical and statistical differences in the quantity of undigested materials seen in the gizzard may suggest that birds offered the maize-based diet exhibited increased gastric reflux. There is the tendency for lower levels of solid marker to be present in the gizzard with enhanced motility (Svihus et al., 2002). However, it is possible that the separation of TiO₂ and digesta, due to the very fine particle size of TiO₂, was a contributing factor that confounds nutrient digestibility determination within the proximal end of the digestive tract (Li et al., 2002; Svihus et al., 2002; Sacranie et al., 2012).

Similar to the results seen in the gizzard, accumulation of undigested material was observed in the caeca of birds fed both diets, with birds fed the wheat-based diet exhibiting more pronounced value at 12 d of age. Caeca functionality has been largely influenced by the dietary level of fibre (Borin et al., 2006; Walugembe et al., 2015). Thus, it is assumed that highly fermentable components present in the wheat-based diet allowed the caeca to accommodate more materials, diluting TiO₂ (De Vries et al., 2014).

Although diet type resulted in differences in the degree of nutrient disappearance along the gastrointestinal tract, the overall growth performance did not differ between wheat- and maize-based diets. An explanation for greater performance in birds offered the wheat-based diet compared to those offered the maize-based diet during the starter period is not clear; however, it is assumed that it may be attributed to the starter wheat-based diet containing more starch (47.2% vs. 44.4%) and fat (4.2% vs. 3.5%) compared to the maize-based diet. This may have increased the available energy to the chicks, thereby contributing to the observed higher body weight during the starter period.

In summary, the present study indicates that dietary components present in wheat- or maize-based diet behave differently along the digestive tract of birds. Although either wheat- or maize-based diets without exogenous enzymes supported a standard performance of birds, it is noteworthy that more than 30% of the feed provided was wasted, irrespective of diet type. In both diets, insoluble NSPs are the least utilised components, suggesting that novel targeted approaches to reduce undigested components are required, mainly focusing on NSP.

Author contributions

Eunjoo Kim: Data curation, Formal analysis, Methodology, Investigation, Writing-Original draft preparation; **Natalie K. Morgan:** Conceptualisation, Investigation, Methodology, Writing-Review and Editing; **Amy F. Moss:** Writing-Review and Editing; **Lily Li:** Resources, Writing-Review and Editing; **Peter Ader:** Resources, Writing-Review and Editing; **Mingan Choct:** Conceptualisation, Data curation, Project administration, Writing-Reviewing and Editing, Supervision, funding acquisition.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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