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A dynamic model to measure retention of solid and liquid digesta fractions in chickens fed diets with differing fibre sources

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ABSTRACT

Dietary fibres impact multiple digestive processes, and insights into the effects of various types of fibre on digesta retention time are required to optimise current feed formulation systems. Therefore, the objective of this study was to apply a dynamic modelling approach to generate estimates for the retention time of solid and liquid digesta in broilers fed different fibre sources. A maize-wheat-soybean meal control diet was compared against three diets in which wheat was partially substituted with oat hulls, rice husks, or sugar beet pulp (3% w/w). Non-starch polysaccharide (**NSP**) digestibility was evaluated in broilers between 23 and 25 days of age (n = 60 birds/treatment) using titanium dioxide (TiO_2, 0.5 g/kg) as a marker, after feeding the experimental diets for 21 days. Digesta mean retention time (**MRT**) was measured in another 108 birds at 30 days of age by the administration of an oral pulse dose of chromium sesquioxide (Cr_2O_3) as solid marker and Cobalt-EDTA as liquid marker, and subsequent measurement of marker recovery in compartments of the digestive tract (n = 2 or 3 replicate birds/time point/treatment). Marker recovery models to estimate fractional passage rates for solid and liquid digesta in crop, gizzard, small intestine, and caeca compartments of the gastrointestinal tract were developed to predict MRT of solid and liquid digesta for each dietary treatment. The models were composed of a series of first-order differential equations, representing the variation of marker concentration in a compartment over time. Estimated MRT of solid and liquid digesta in the gizzard varied from 20 min for oat hulls and 34 min for rice husks diets to 14 min for sugar beet pulp and 12 min for control diets. In the caeca, liquid MRT was decreased compared to the control diet (989 min) for the sugar beet pulp diet (516 min), while it was increased for both the oat hulls and rice husks diets (≈1 500 min). Overall, these estimates are greater than those previously reported, suggesting that liquid digesta retention in the caeca previously has been underestimated. Digestibility of total NSP was increased by dietary fibre inclusion, regardless of the fibre type, although degradation of constituent sugars of NSP varied among diets. In conclusion, the inclusion of fibre sources at a low level (3% w/w) in the diet of broiler modulated retention time mainly in the gizzard and caeca, and increased digestibility of NSP.

Implications

Fibre-rich agricultural by-products are increasingly used in poultry diets but their effects on digestive process are poorly understood, hampering accurate predictions of nutritional values of fibre-rich diets. The combination of *in vivo* measurements on digesta retention and digestibility with a mathematical model developed in the present work allows more insight in the modulation of digesta retention behaviour and fibre degradation in the digestive tract of birds. The developed model provides a basis for further studies on retention time in birds and can be used to evaluate the effects of fibres on digesta retention behaviour to fine-tune feed formulation models.

Introduction

Competition for the use of human-edible resources urges the poultry sector to use more by-products from agricultural and food industries that cannot be used for human consumption. These by-products typically have high fibre contents compared with

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traditional ingredients. However, dietary fibres may interfere with the digestion of other nutrients in the diet due to their pronounced effect on physicochemical properties of the digesta, thereby influencing digesta retention time and nutrient digestibility (Mateos et al., 2012). These effects must be considered to accurately predict the nutritional value of diets for broilers.

Dietary fibres encompass a diverse group of polymers with different physicochemical properties (Jones, 2014). These properties together with others such as water solubility, hardness and, post-processing particle size may differentially affect digesta retention time in the gastrointestinal tract (**GIT**). Past literature has highlighted the importance of insoluble dietary fibre on digesta flow in the upper digestive tract of broilers (Hetland et al., 2004). Insoluble fibres are known to prolong digesta retention time in the gizzard, and this is associated with improved particle size reduction (Hetland et al., 2005). Hence, the addition of insoluble fibre to the diet typically has a positive effect on nutrient digestibility (Hetland et al., 2004). However, the inclusion of fibres in the diet may affect digesta retention not only in the gizzard but also in other compartments of the avian GIT. For example, high fibre diets increase the separation of solid and liquid digesta contents in the GIT (de Vries et al., 2014a) and the amount of fibre directed to the caeca appears to also be influenced by dietary fibre level and fibre properties (de Vries et al., 2014b).

To study digesta mean retention time (**MRT**), van Krimpen et al. (2011) developed a multi-compartment model based on the recovery of an oral pulse dose with an inert marker (Luckey et al., 1975). The compartments of the GIT were represented as pools (g digesta) linked by equations representing the flows of digesta (g/min). However, this model only followed the flow of solids, using the marker titanium dioxide (TiO_2), and omitted the caeca. Digesta fractions such as solids and liquids, fine and coarse particles tend to separate as they pass through the GIT (de Vries et al., 2014a), leading to different retention times for the various fractions. For example, the gizzard selectively retains large (>1 mm) particles (Amerah et al., 2009), while the caeca are only accessible for liquids and small particles (Vergara et al., 1989). Data on retention of liquids in the caeca in chickens are scarce and MRT in the caeca has been estimated only once, giving a range between 33 and 117 min (Shires et al., 1987). However, other authors found a significant amount of digesta in the caeca up to 24 h after ingestion (Hinton et al., 2000), suggesting that retention in the caeca could be longer than previously estimated.

The aim of the present study was to use dynamic modelling to separately estimate MRT of solid and liquid digesta fractions in each compartment of the GIT, including the caeca. To this end, the effect of dietary inclusion of different fibre sources, varying in water solubility and physical structure, on retention time of digesta was assessed in broiler chickens. In addition, the effect of fibre type on total tract non-starch polysaccharide (**NSP**) digestibility was also investigated.

Material and methods

Experimental design

The experiment described herein was part of a larger study, testing the effects of adding fibre sources to a standard diet based on wheat, maize, and soybean meal (control diet) in broiler chickens between 0 and 36 days of age. In this manuscript, total tract nutrient digestibility was evaluated in a subset of birds between 22 and 24 days of age and MRT in a subset of birds at 30 days of age for the control diet and three fibre-supplemented diets, i.e. oat hulls (**OH**), rice husks (**RH**), and sugar beet pulp (**SBP**).

Animals, housing, and diets

A total of 4 160 one-day-old male broiler chicks were randomly assigned to 80 floor pens (52 birds/pen). The dimension of each pen was 2.5 m × 1.6 m, and the floor was covered with wood shavings. Birds had *ad libitum* access to water and pelleted feed over the duration of the experiment. Each pen was randomly assigned to one of eight dietary treatments, resulting in ten replicate pens per treatment; only four dietary treatments (control, OH, SBP, and RH) were used in the experiment described in this manuscript. Ambient temperature was set at 33 °C for day 1, and gradually decreased to 20 °C by day 36. Photoperiod was 24L:0D during the first 3 days and 18L:6D from day 3 onwards. At the hatchery, birds were vaccinated against infectious bronchitis, Marek's disease, and infectious bursal disease. No additional medication was administered during the experiment. Birds were fed a starter diet from 1 to 14 days and a grower diet from 14 day to the end of the measurement period. Diets were formulated to meet or exceed nutrient requirements for broiler chickens with wheat, maize, and soybean meal as the main ingredients (CVB, 2018). Wheat in the control diet was substituted for OH, RH, or SBP (3% w/w), to obtain experimental contrasts (Table 1). With the exception of OH, SBP, and RH that were used unground, the rest of the feed ingredients were ground by a hammer mill with 2.5 mm screen (Full Circle Screen Hammer Mills, ROSAL, Barcelona, Spain) and pelleted using a 2 mm (starter diet) or 3 mm (finisher diet) die. No additional grit was provided. Feed intake, BW, and mortality per pen were recorded on day 14 and day 28.

Total tract non-starch polysaccharide digestibility

On day 14, six birds per pen (240 in total) were randomly selected and transferred to metabolism cages. Birds of one replicate pen were housed in six adjacent individual cages and considered one experimental unit (with 10 experimental units/treatment). Birds received the same experimental diet, supplemented with TiO_2 (0.5 g/kg) as an inert marker for the evaluation of digestibility. After 7 days of acclimatisation, representative composite samples of clean excreta were collected for 3 d. Excreta were pooled per cage and stored (−20 °C) for further analysis.

Digesta retention time

At day 25, another 27 birds per treatment (108 in total) were randomly selected and transferred to metabolism cages. After being transferred, birds received the same grower diets according to the treatment they were assigned to. After 5 days of acclimatisation, birds received an oral pulse dose of marker and were subsequently euthanised after 5, 30, 60, 180, 300, 420, 1 440, or 2 880 min (2–3 replicate birds/time-point/diet) to obtain digesta. During the procedure, birds were fasted for 2 h, then access to feed was given again 30 min before marker administration. The marker pulse dose consisted of 0.1 g Cr_2O_3 and 0.4 g Co-EDTA in the form of a fine powder (<0.1 mm), which was loaded into a gelatin capsule which was gently inserted by hand into the oropharyngeal cavity. Immediately after euthanasia, tie-wraps were placed between GIT compartments to prevent mixing of digesta. Then, the GIT was removed and digesta was collected per compartment (crop, proventriculus and gizzard combined, small intestine, both caeca and, colon) and tissues were flushed with demineralised water to allow for quantitative recovery. Total excreta voided between the moment of marker pulse dose and dissection was also collected. Samples were stored at −20 °C for further analysis. A selection of samples (1–3 replicate birds/treatment/time-point) were analysed for marker contents, resulting in a total of 24 observations for each compartment for the control diet, 24 for the OH

Table 1
Ingredient composition and calculated nutrient contents of the experimental broiler diets (in g/kg as-fed, unless indicated otherwise for the grower phase (14–28 days)) fed during the digestibility and retention time measurements.

Items	Control	Oat hulls	Rice husks	Sugar beet pulp
Ingredient				
Wheat	450.0	420.0	420.0	420.0
Maize	182.6	182.6	182.6	182.6
Soybean meal (47%)[1]	251.2	251.2	251.2	251.2
Maize gluten meal (61%)[1]	32.7	32.7	32.7	32.7
Oat hulls		30.0		
Rice husks			30.0	
Sugar beet pulp				30.0
Soya oil	56.6	56.6	56.6	56.6
Salt (NaCl)	1.7	1.7	1.7	1.7
Monocalcium phosphate	4.9	4.9	4.9	4.9
Calcium carbonate, fine	5.9	5.9	5.9	5.9
Sodium bicarbonate	2.3	2.3	2.3	2.3
L-Lysine HCl 98%	2.5	2.5	2.5	2.5
DL-Methionine 99%	1.8	1.8	1.8	1.8
L-Threonine 98%	0.4	0.4	0.4	0.4
Premix[2]	7.5	7.5	7.5	7.5
Calculated nutrient composition[3]				
DM	849	851	851	851
CP	205	203	204	204
Ether extract	25.3	26.1	29.4	26.9
Crude fibre	23.6	31.0	30.9	28.0
Starch	386	374	373	369
aME[4] (MJ/kg)	12.9	12.5	12.5	12.5
Digestible lysine[5]	10.9	10.8	10.8	10.8
Digestible methione[5]	4.7	4.7	4.7	4.7
Digestible threonine[5]	6.5	6.4	6.4	6.4
Digestible tryptophan[5]	2.0	2.0	2.0	2.0
Digestible arginine[5]	11.3	11.2	11.2	11.2
Digestible isoleucine[5]	7.5	7.4	7.4	7.4
Digestible valine[5]	8.2	8.1	8.1	8.1
Calcium	5.1	5.1	5.1	5.1
Phosphorous	4.5	4.5	4.5	4.5
Analysed NSP content (g/kg)	100	109	107	115

Abbreviations: NSP = Non-starch polysaccharides.
[1] CP content of the feed ingredients.
[2] The premix supplied (per kg of feed): 10 000 IU, vitamin A (trans-retinyl acetate); 2 500 IU, vitamin D3 (cholecalciferol); 50 IU, vitamin E (all-rac-tocopheryl-acetate); 2.0 mg, vitamin B1 (thiamine mononitrate); 6 mg, vitamin B2 (riboflavin); 40 mg, vitamin B3 (niacin); 4.0 mg, vitamin B6 (pyridoxine HCl); 25 µg, vitamin B12 (cyanocobalamin); 2.0 mg, vitamin K3 (bisulfate menadione complex); 10 mg, pantothenic acid (d-Ca pantothenate); 1.0 mg, folic acid; 300 mg, choline (choline chloride); 150 µg, d-biotin; 0.25 mg, Se (Na2SeO3);1.0 mg, I (KI); 15 mg, Cu (CuSO4·5H2O); 65 mg, Fe (FeCO3); 90 mg, Mn (MnO2); 80 mg, Zn (ZnO); 2.25 mg/kg, butylated hydroxyanisole; 11.25 mg/kg, butylated hydroxytoluene.
[3] Calculated composition (g/kg, as-fed) based on data from CVB (2018), unless indicated otherwise.
[4] Apparent metabolisable energy for broiler chickens.
[5] Apparent total tract digestible amino acids for broiler chickens.

diet, 21 for the SBP diet, and 23 for the RH diet. In total, marker recovery for all GIT segments were available for 18 birds.

Sample preparation and analytical methods

For digestibility calculations, diets and excreta (d 21–23) were analysed for contents of DM according to Association of Official Analytical Chemists (AOAC, 2005) method 930.15, titanium (AOAC, 2005, method 540.91, using a SpectrAA 300 atomic absorption spectrophotometer, Varian B.V., Middelburg, the Netherlands), and total NSP measured as neutral sugars and uronic acids (Englyst et al., 1994). Before analyses of neutral sugar and uronic acid contents in diets, NSP was extracted from diet samples (Englyst et al., 1994). First, starch was gelatinised and enzymatically degraded, and subsequently, NSP was precipitated using acidified ethanol. Neutral sugar composition was analysed according to the method of Englyst et al. (1994). In short, after pretreatment with 72% (w/w) H_2SO_4 for 30 min at 35 °C, samples were hydrolysed with 2 M H_2SO_4 at 100 °C for 1 h. Constituent monosaccharides were derivatised into their corresponding alditol acetates and analysed using gas chromatography (Varian 450-GC, Agilent, Santa Clara, CA, US). Inositol was used as an internal standard. Uronic acid content was analysed according to the colorimetric m-hydroxydiphenyl assay (Williams et al., 1962), using a spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MAUS). Galacturonic acid was used for calibration.

For digesta retention time calculations, samples of digesta and excreta (d 30–32) were freeze-dried, incinerated (550 °C for 3 h; AOAC, 2005, method 942.05), and weighed. Finally, chromium and cobalt contents were analysed after acid hydrolysis (Williams et al., 1962) using an inductively coupled plasma atomic emission spectrometer (ICP-MS, Nexion 2000, Perkin Elmer, Waltham, USA).

Model description

Two multi-compartment models were developed, based on earlier work by van Krimpen et al. (2011), representing the liquid digesta fraction (based on Co-EDTA; Fig. 1, A; Eqs. (1)–(5)), and the solid digesta fraction (based on Cr_2O_3; Fig. 1, B; Eqs. (6)–(9)). The main difference in the current digesta flow models and the previous work of van Krimpen et al. (2011) is that the liquid digesta model developed here additionally represents the caeca. For the solid marker (Cr_2O_3), recovery was sufficiently low in the caeca (<0.1% w/w) to exclude it from the model. For both models, the colon was excluded due to too few measurements being

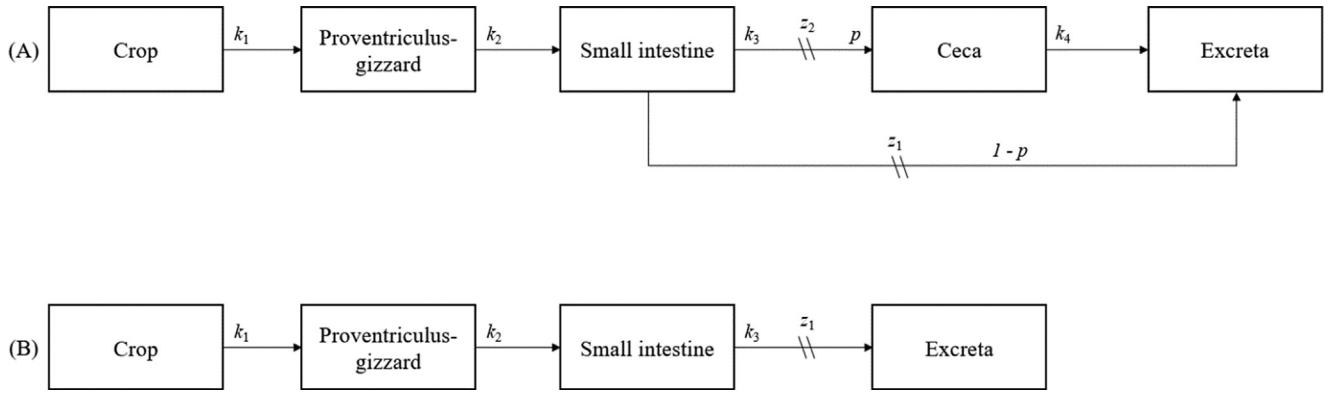

Fig. 1. Schematic overview of the multi-compartment models to estimate fractional passage rate of liquid (A) and solid (B) digesta, through various compartments of the gastrointestinal tract (GIT) of broilers. The parameters k_1, k_2, k_3 and k_4 (min^{-1}) are the rates of filling and emptying of the compartments; z_1 and z_2 (min) the delay between emptying of the small intestine and filling the excreta (z_1) or caeca (z_2) compartments, and p the fraction of liquid digesta entering the caeca.

available and the very short retention time typically measured for this compartment. Both liquid and solid digesta models consisted of a series of ordinary differential equations. The instantaneous rate of change of marker (dQ/dt, % marker min^{-1}) per compartment of the digestive tract at time (t, min) was modelled using the following series of dynamic differential equations for the liquid digesta:

$$dQ_{crop}/dt = -k_1 Q_{crop}(t) \quad (1)$$

$$dQ_{prov\_gizz}/dt = k_1 Q_{crop}(t) - k_2 Q_{prov\_gizz}(t) \quad (2)$$

$$dQ_{small\_intestine}/dt = k_2 Q_{prov\_gizz}(t) - k_3 Q_{small\_intestine}(t) \quad (3)$$

$$dQ_{caeca}/dt = p \times k_3 Q_{small\_intestine}(t - z_2) - k_4 Q_{caeca}(t) \quad (4)$$

$$dQ_{excreta}/dt = k_4 Q_{caeca}(t) + (1 - p) \times k_3 Q_{small\_intestine}(t - z_1) \quad (5)$$

and the following series of differential equations were used for the solid digesta fraction:

$$dQ_{crop}/dt = -k_1 Q_{crop}(t) \quad (6)$$

$$dQ_{prov\_gizz}/dt = k_1 Q_{crop}(t) - k_2 Q_{prov\_gizz}(t) \quad (7)$$

$$dQ_{small\_intestine}/dt = k_2 Q_{prov\_gizz}(t) - k_3 Q_{small\_intestine}(t) \quad (8)$$

$$dQ_{excreta}/dt = k_3 Q_{small\_intestine}(t - z_1) \quad (9)$$

where k_1 is the fractional rate of emptying of Q_{crop} into Q_{prov_giz}, k_2 is the fractional rate of emptying of Q_{prov_giz} into $Q_{small\_intestine}$, k_3 is the fractional rate of emptying of $Q_{small\_intestine}$ into the caeca/excreta and k_4 (min^{-1}) is the fractional rate of emptying of Q_{caeca} into the excreta (liquid). The parameters z_1 and z_2 (min) represent time delays in between the emptying and the filling of two compartments. The delay z_1 was used to model passage through the colon, in the absence of the colon being represented explicitly in either model, while the delay z_2 was used to represent the process of reverse peristalsis for the filling of the caeca. The fraction of liquid digesta entering the caeca is represented by p and ranges from 0 to 1.

Data manipulation

In total, there were five (liquid) or four (solid) curves to be fit (one for each of the GIT compartments: crop, proventriculus/gizzard, small intestine, caeca, excreta) across the time points 5, 30, 60, 180, 300, 420, 1 440, or 2 880 min after administration of the pulse marker dose for four dietary treatments (control, OH, BP, and RH) to parameterise Eqs. (1)–(9). Marker recovery was calculated as the amount of marker found in a compartment or the excreta, relative to the initial amount of marker provided as pulse dose (%). For model fitting, it was assumed that at t = 0, 100% of the marker was in the crop. Unfortunately, all observations on the marker recovery at t = 1 440 min had to be excluded from the datasets. Marker recovery in the excreta at t = 1 440 min was low and highly variable (64 ± 32% for Cr; 48 ± 35% for Co). This low level of marker recovery at t = 1 440 min in the excreta was not considered to be realistic based on previous literature (van der Klis et al., 1993), as well as based on marker recovery in the other GIT compartments at this time-point. Therefore, these observations were removed, and it was assumed that for the insoluble marker, 100% of the Cr was in the excreta by t = 3 000 min, and for the soluble marker, it was assumed that 100% of the Co marker was in the excreta by t = 3 600 min. These values were considered to exceed by far the longest estimates of retention time in broiler, for both solid and liquid digesta (Hinton et al., 2000). While an assumption, these changes were required to allow adequate model fitting.

Outliers were identified using a combination of three methods: boxplots, studentised residuals greater than 2.0, and values which were higher or lower than the mean minus two SDs. Only the values highlighted by at least two of these three procedures were identified as potential outliers. The values were then removed from the dataset if they also were considered unrealistic given the current state of knowledge on digesta retention in chickens. As a result, one observation at t = 420 min in the crop (control) and one observation at t = 5 min in the small intestine (control) were additionally excluded.

Model fitting

The models were fitted for each of the GIT compartments against marker recovery (% of dosed) over time. The fitting was done per dietary treatment, pooling all replicate observations of all time-points included. Estimates of the parameters were obtained by iterative fitting using the FME package (Soetaert and Petzoldt, 2010) in R (Version 4.0.3), in order to minimise the residual sum of squares of the overall model. The model as a whole was fitted (all pools fitted simultaneously to minimise their combined residual sum of squares), although alternate fitting approaches were investigated (e.g. fitting each pool sequentially). After the fitting procedure, an estimate of model parameters was obtained for the liquid (k_1, k_2, k_3, k_4, z_1, z_2, p) and the solids (k_1, k_2, k_3, z_1) models, for each dietary treatment.

Calculations

Apparent total tract digestibility (**ATTD**) was calculated from analysed NSP and Ti content in the diet and excreta using the following formula:

$$\text{ATTD} = (1 - \left(\frac{([TiO_2]_{diet} \times [NSP]_{excreta})}{([TiO_2]_{excreta} \times [NSP]_{diet})} \times 100\%\right)$$

where $[NSP]_{excreta}$, $[NSP]_{diet}$, $[TiO2]_{excreta}$, $[TiO2]_{diet}$ are concentrations of NSP and Ti (g/kg DM) in the excreta or diet samples. The MRT in each compartment was calculated as the inverse of fractional passage rate as follows:

$$\text{MRT (min)} = 1/k$$

where k is the fractional passage rate of the marker (soluble or insoluble) for the corresponding compartment.

Overall MRT was calculated as the sum of the MRT in each compartment (crop, gizzard, small intestine, caeca for liquid fraction; crop, gizzard, small intestine for solid fraction) plus the time delays (z_2 for liquid fraction entering the caeca; z_1 for solid fraction and liquid fraction bypassing the caeca). For the soluble marker, total tract retention was calculated both including and excluding the caeca, to account for the fact that part of the liquid digesta enters the caeca (p) while the rest does not ($1 - p$).

Statistical analysis

Digestibility values were analysed by a general linear model (Proc GLM, SAS, version 9.4, SAS Institute Inc., Cary, NC), using diet as fixed effect. Model assumptions for homogeneity and normality were verified by visual inspection of model residuals using histograms, QQ-plots, and studentised residuals. Least squares means were compared using Tukey adjustments for multiple comparisons. Data are presented as estimated least squares means and pooled SEM unless stated otherwise. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences.

In order to assess the adequacy of the models, two indicators were calculated. Mean square prediction error (**MSPE**) for each compartment was calculated as following:

$$\text{MSPE} = \sum_{i=1}^{n} (O_i - P_i)^2/n$$

where n is the number of observations, O_i is the observed value, and P_i is the predicted value. The square root of the MSPE, expressed as a proportion of the observed mean, gives an estimate of the overall prediction error ($\sqrt{}$MSPE, %).

Correspondence between predicted and observed values was also assessed by the concordance correlation coefficient (**CCC**), which was calculated as:

$$\text{CCC} = R \times C_b$$

where C_b is a bias correction factor (a measure of accuracy), and R is the Pearson correlation coefficient (a measure of precision). The C_b variable is calculated as:

$$C_b = \frac{2}{(v + \frac{1}{v} + \mu^2)}$$

where

$$v = \frac{s_o}{s_p}$$
$$\mu = \frac{m_o - m_p}{\sqrt{s_o \times s_p}}$$

where m_o is the mean of the observed values and m_p that of the predicted values, s_o is the SD of the observed values and s_p that of the predicted ones.

Results

Fibre composition and digestibility

Oat hulls and RH supplemented mainly insoluble NSP, whereas the NSPs of SBP were more soluble (~44% (w/w); Table 2). Total NSPs of OH and RH were mainly composed of xylosyl and glucosyl, whereas total NSPs of SBP were mostly composed of arabinosyl, glucosyl, and uronyl. At 30 days, the BW of broilers were 2 012 ± 244 g for the control diet, 2 036 ± 236 g for OH, 2 081 ± 144 g for RH and 1 985 ± 203 g for SBP. ATTD of total NSP was greater in birds fed diets supplemented with a fibre source compared with the control diet (Table 3) (P = 0.0002), mainly explained by arabinosyl-, xylosyl-, and glucosyl residues. There was no difference in ATTD of uronyl- (P = 0.701) and galactosyl residues (P = 0.398) among dietary treatments.

Digesta retention time

For the birds for which all segments were analysed (n = 18), the average marker recovery was 88 ± 30% (w/w) for Cr and 86 ± 26% (w/w) for Co. In the crop, estimated MRT ranged from 6 to 78 min for the liquid fraction and from 5 to 67 min for the solid fraction (Table 4). Estimated MRT in the crop was shorter for the RH diet compared with other diets, for both the liquid and solid fractions. In the gizzard, estimated MRT ranged from 10 to 27 min for the liquid fraction and from 13 to 40 min for the solid fraction. Differences in predicted MRT in the gizzard in between solid and liquid fractions were highest with the RH diet (27 min for liquids vs 40 min for solids). Both solid and liquid digesta were estimated to be retained longer in the gizzard in birds fed the OH and RH diet than in birds fed the control or SBP diets. In the small intestine, estimated MRT ranged from 87 to 129 min for the liquid fraction and from 110 to 158 min for the solid fraction. For the solid digesta, retention in the SI constituted the largest part (33–45%) of total tract retention time. In the caeca, estimated MRT ranged from 516 min for the SBP diet and 989 min for the control diet to 1 537 min for the OH and 1 547 min for the RH diet. The proportion of liquid digesta entering the caeca (p) was estimated to be similar among diets, ranging from 0.30 to 0.35. Total tract estimated MRT of solids was on average 337 min, the estimated total tract MRT of liquid digesta entering the caeca was on average 1 443 min, and the estimated total tract MRT of liquid digesta bypassing the caeca was 310 min on average. Liquid digesta that did not enter the caeca had a shorter estimated MRT than the solid digesta. The delay between the emptying of the small intestine and the filling of the caeca (z_2) was estimated to be on average 125 min. The delay in between the emptying of the small intestine and the excretion of the marker (z_1) was calculated to be 139 min for both the solid and for the liquid digesta.

Model evaluation

Overall, the assessment of model fit was good, with an average CCC of 0.81 and $\sqrt{}$MSPE of 98 % across all diets and compartments (Table 5). As an example, the curve fits for the control treatment are presented in Figs. 2 and 3. A complete report of curve fits for all treatments is available as online supplementary material S1. As all compartments (within a treatment) were fitted simultaneously and the overall residual sum of squares minimised, it was inevitable that some compromises in predictability between

Table 2
Contents of total and water-insoluble non-starch polysaccharides (NSP) of oat hulls, sugar beet pulp, and rice husk ingredients used in the experimental broiler diets (g/100 g as-fed, unless indicated otherwise).

Items	Oat hulls	Rice husks	Sugar beet pulp
Non-starch polysaccharides (NSP)	59.8	51.0	65.5
Molar composition of NSP[1]			
Arabinosyl	5	4	27
Xylosyl	32	27	2
Galactosyl	2	2	7
Glucosyl	59	64	30
Uronyl	2	3	32
Residual sugars	0	0	2
Insoluble NSP	57.3	47.7	36.6
Molar composition of insoluble NSP[1]			
Arabinosyl	5	4	28
Xylosyl	36	27	3
Galactosyl	2	1	7
Glucosyl	55	64	49
Uronyl	2	3	10
Residual sugars	0	0	3

[1] Presented as anhydrous sugar moieties.

compartments would be made. For the crop, the RH diet had the best fit for both markers, with a CCC of 0.85 and $\sqrt{}$MSPE of 90%. The quality of predictions was the lowest in the gizzard and the small intestine, for all treatments. It can be seen visually that there is a wide scatter of points around the curve for the gizzard and the small intestine (Figs. 2 and 3). For the caeca, the quality of prediction was relatively good, with a CCC of 0.82 and a $\sqrt{}$MSPE of 82%, on average. Finally, the excreta compartment gave the best prediction, the CCC was 0.92 and the $\sqrt{}$MSPE 48%, on average.

Discussion

The aim of the present study was to develop a dynamic modelling approach to estimate the retention time of solid and liquid digesta fractions in broiler chickens. To this end, diets containing fibres varying in physiochemical properties were used as a mean to increase the digesta dynamics in the gastrointestinal tract. Estimation of MRT of liquid digesta in the caeca, accounting for the fractional inflow of liquids, ranged between ~500 and 1 500 min, depending on diet. Although the experimental set-up did not allow statistical analyses of MRT among dietary treatments, descriptive estimates of MRT for the various diets are provided.

Ability of the modelling approach to estimate retention time

The model developed in this study enabled the estimation of MRT in each of the compartments of the GIT and in the total tract for both liquid and solid digesta fractions. Despite the preliminary nature of the study, the obtained predicted MRT in the crop, the gizzard, and the small intestine are in the range found in the literature (Shires et al., 1987; van der Klis et al., 1993; van Krimpen et al., 2011).

Data on digesta retention time in the avian caeca are scarce. To our knowledge, only one study previously estimated MRT in the caeca of chickens, with a reported range of between 33 and 117 min (Shires et al., 1987). These estimates were based on the pool size of a ruthenium chloride marker found in the caeca in a spot-sample taken at a single time-point after continuous supplementation of this marker in the feed. However, this method relies on the assumption of constant tracer pool size in each GIT compartment, as a result of continuous tracer ingestion and plug flow. It does not account for the fact that emptying of the caeca occurs infrequently and incompletely (Duke, 1989) and that only a certain fraction of the liquids enters the caeca (Vergara et al., 1989), thereby presumably resulting in underestimation of MRT. In our study, we estimated MRT in the caeca to range between ~516 and 1 547 min. These values are in agreement with previous studies in which feed material was still found in the caeca 24 h after feed withdrawal (Hinton et al., 2000). In our study, we also found up to 25% of the liquid marker present 24 h after pulse dose, under *ad libitum* feeding conditions. These results suggest that MRT of liquids in the caeca far exceeds retention in all other compartments of the broiler's GIT, and is considerably longer than previously assumed.

Previous studies have shown that only a certain fraction of liquid digesta enters the caeca (reviewed by Björnhag, 1989), and this fraction was previously estimated to be 55% in chickens (Vergara et al., 1989). In this study, the estimated fraction of liquids entering the caeca was lower, ranging between 30 and 35%. The processes of caeca filling and emptying are poorly understood and the scarce available data seem to indicate that the fraction of liquids entering the caeca is variable and may be influenced by dietary and animal factors (e.g. feed consumption). In addition, a fraction of the soluble material entering the caeca will be urine, coming from the cloaca as the result of reverse peristalsis (Björnhag, 1989). By using an assumed inert marker, the flux of urine going into the caeca was not accounted for in the model.

The parameter z_1, which represents the delay between emptying of the small intestine and excretion of the marker, was estimated to be on average 139 min for both the liquid and solid fraction. This could be an overestimate, as the colon is only around 10–15 cm long, and MRT was previously estimated to be in the range of 5–50 min (van Krimpen et al., 2011). The high estimated delay (z_1) could have arisen from the gizzard or the small intestine in which MRT might have been underestimated, because they are the compartments in which the quality of prediction was lowest.

Table 3
Apparent total tract digestibility (ATTD, in %) of non-starch polysaccharide (NSP) and constituent sugars in broilers fed a standard maize, wheat, soybean meal diet without (control) or with the addition of 3% (w/w) oat hulls, rice husks, or sugar beet pulp, measured between 23 and 25 days of age using a TiO_2 marker.[1]

	Control		Oat hulls		Rice husks		Sugar beet pulp		
Items	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*-value[2]
n[1]	7		7		8		5		
Total NSP	7.4^{a}	0.02	23.2^{b}	0.02	19.2^{b}	0.02	22.2^{b}	0.03	<0.001
Arabinosyl	21.3^{a}	0.02	33.5^{b}	0.02	28.0^{ab}	0.02	33.5^{b}	0.02	<0.001
Xylosyl	17.4^{a}	0.02	32.0^{b}	0.02	22.3^{a}	0.02	25.3^{ab}	0.02	<0.001
Glucosyl	−28.0^{a}	0.04	9.6^{b}	0.04	5.3^{b}	0.04	5.9^{b}	0.05	<0.001
Uronyl	19.7	0.02	17.5	0.02	16.2	0.02	18.9	0.03	0.701
Galactosyl	22.3	0.04	28.4	0.04	30.8	0.05	30.2	0.05	0.398
Residual sugars	37.5^{b}	0.02	23.7^{a}	0.02	47.4^{c}	0.02	44.5^{bc}	0.02	<0.001

[1] Number of replicate adjacent pens, of six individually housed broilers each.
[2] Model established *P*-values for the fixed effect of diet. Means within a line without a common superscript differ ($P < 0.05$).

Table 4
Estimated mean retention time (MRT, min) of solid and liquid digesta fractions in various compartments of the gastrointestinal tract as well as the fraction of liquid digesta that enters the caeca (p), and time delays of the models (z_1, z_2) in 30-days broilers fed a standard maize, wheat, soybean meal diet without (control) or with the addition of 3% (w/w) oat hulls, rice husks, or sugar beet pulp.[1,2]

	Control		Oat hulls		Rice husks		Sugar beet pulp	
Digesta fraction	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
MRT (min)								
Crop	63	44	78	67	6	5	44	36
Gizzard	10	13	17	23	27	40	13	14
Small intestine	129	158	87	110	107	155	102	126
Caeca	989	–	1 537	–	1 547	–	516	–
Total tract, w/o . caeca[3]	403	–	275	–	258	–	302	–
Total tract[4]	1 302	354	1 811	335	1 838	344	818	314
Parameters								
p[5]	0.35	–	0.35	–	0.30	–	0.31	–
z_1[6]	201	139	93	135	118	144	143	138
z_2[7]	111	–	92	–	151	–	144	–

[1] Estimated using multi-compartment models after an oral pulse dose of 0.1 g Cr (as Cr_2O_3) and 0.4 g (as Co-EDTA) and subsequent measurement of pool sizes in each gastrointestinal compartment at 5, 30, 60, 120, 180, 300, 420, 1 440, or 2 880 min after receiving the pulse dose. Where Cr_2O_3 is considered to represent the solid digesta fraction and Co-ETDA the liquid digesta fraction.
[2] Daily feed intake in g/day between 0 and 36 days of age were 118.7[a] for control-, 119.3[a] for oat hulls-, 120.3[a] for rice husks-, and 115.7[b] for sugar beet pulp diets, SEM = 0.62 (P-value < 0.0001 for the fixed effect of diet, means lacking a common superscript differ).
[3] Total tract MRT of liquid digesta that does not enter the caeca, calculated by summing MRT in the crop, gizzard, small intestine plus the delay z_1.
[4] Total tract MRT, calculated by summing MRT in each compartment plus the delay z_1 for the solid marker, or z_2 for the liquid marker entering the caeca.
[5] Fraction of liquid digesta entering the caeca.
[6] Delay between emptying of the small intestine and excretion of the marker (min).
[7] Delay in between emptying of the small intestine and filling of the caeca (min).

It is also possible that error accumulated in this delay parameter, due to the process of fitting the model to all compartments of the GIT at the same time. This could have led to an overestimate of retention in the colon, but our estimated total tract retention time is in the range of what is commonly found in literature (300–360 min) (Svihus and Itani, 2019). Fitting the model sequentially per compartment (results not presented) was also tried, but it gave prominence to the initial parameters, while their error accumulated in the latter compartments. By fitting sequentially, parameter estimation was optimal for the crop and gizzard, but less precise for the caeca and the excreta. Additionally, there was strong sensitivity to initial parameter values when fitting sequentially per compartment. As predicting MRT of liquids in the caeca was of primary interest, we chose to fit the model as a whole, acknowledging the possibility of compromises in fitting spread across the GIT.

The delay between emptying of the small intestine and filling of the caeca (z_2) was also relatively long (125 min), with high variation among treatments, ranging from 92 to 151 min. Again, it is possible that some fitting error accumulated in estimating this parameter. However, the range in delay values could be attributed to the complex biological process that control filling the caeca; filling the caeca involves a backflow of liquids, including urine, and fine particles from the cloaca through the colon to the caeca (Björnhag, 1989). However, this study did not find any significant amount of marker in the caeca before 300 min, which supports the hypothesis that even if the delay was overestimated, there is probably a certain amount of time required for the liquid fraction to reach the caeca after leaving the small intestine.

Difference in retention between the solid and liquid phases

In the crop, the estimated MRT was longer for the liquid phase than the solid phase for all treatments, although being equivalent for the RH diet. These results are in contrast with Vergara et al. (1989) who did not find a difference in crop emptying rate between markers varying in water solubility or particle size. In the gizzard, the average predicted MRT of the liquid phase was ~5 min shorter than average predicted MRT of the solid phase. Selective emptying of solids and liquids was expected to lead to a shorter MRT of liquids (Vergara et al., 1989). Particularly, coarse particles (>0.6 mm) are known to be selectively retained in the gizzard (Hetland et al., 2002). Therefore, while an insoluble fine particle marker such as Cr_2O_3 (<0.1 mm) is quickly emptied from the gizzard, mordanted coarse particles might be more suited to assess the selective retention mechanism in the gizzard.

The average total tract retention of the liquid digesta fraction that entered the caeca was ~1 100 min longer than the total tract MRT of the solid digesta, due to the relatively longer retention time in the caeca. The fraction of liquid digesta that does not enter the caeca is, however, excreted quicker than the solid fraction (310 vs 337 min). This might be explained by the process of digesta flow and intestinal contractile activity. The contractions in the intestine tend to extrude liquids from the bolus; then, extruded liquids are not entirely reabsorbed into the bolus because the latter reduces in size during transit, hence, liquids transit faster than solids in the intestine (Lentle and Janssen, 2008). This difference can be seen in the small intestine, in which estimated MRT for liquids was 30 min shorter than for solids (106 vs 137 min).

Effect of fibre type on mean retention time

In this study, predicted MRT in the crop was ~5 min for the RH diet, while it ranged between 36 and 78 min for the other diets. Regulation of retention in the crop is a complex mechanism in which the gizzard plays a central role, as reviewed by Classen et al. (2016). The shorter predicted MRT in the crop for the RH diet coincided with a longer retention in the gizzard without affecting daily feed intake, speculatively indicating that mechanical distention in the gizzard and subsequent satiety-related effects on feed intake behaviour – i.e. meal duration and frequency – (Savory, 1999) may explain the lower predicted MRT in the crop for the RH diet compared with the other diets.

Apart from the OH diet, also for the RH diet, predicted MRT in the gizzard was longer than for the control diet. Oat hulls and RH are rich in insoluble fibres and exert high grinding resistance (Jiménez-Moreno et al., 2019) compared with the control diet. This is in line with previous studies which highlighted the fact that the addition of a structural component in the diet prolongs the grinding process in the gizzard (Hetland et al., 2005; Mateos et al.,

Table 5
Evaluation of the quality of the curve fitting, for each dietary treatment, for the liquid and solid fraction and per compartment of the gastrointestinal tract in broilers.[1]

		Control		Oat hulls		Rice husks		Sugar beet pulp	
Compartment	Digesta fraction	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Indicator									
Crop	CCC[2]	0.72	0.73	0.75	0.74	0.85	0.85	0.70	0.72
	R	0.72	0.73	0.77	0.99	0.86	0.85	0.71	0.72
	C_b	1.00	1.00	0.98	0.74	0.99	0.99	0.99	1.00
	√MSPE[3] (%)	92	101	89	95	89	91	96	99
	ECT[4] (%)	0.4	0.0	9.1	5.5	4.3	4.8	1.4	0.8
	ER[4] (%)	17.1	12.6	14.6	12.0	8.7	6.6	18.7	15.9
	ED[4] (%)	82.6	87.4	76.4	82.5	14.0	88.7	80.0	83.3
Proventriculus-gizzard	CCC	0.56	0.63	0.40	0.54	0.46	0.62	0.67	0.61
	R	0.60	0.67	0.51	0.56	0.69	0.70	0.67	0.61
	C_b	0.94	0.93	0.78	0.96	0.67	0.88	0.99	1.00
	√MSPE[3] (%)	122	126	325	177	301	160	126	114
	ECT[4] (%)	2.2	2.3	27.9	7.3	13.5	6.6	2.1	0.1
	ER[4] (%)	2.5	0.2	38.7	14.2	72.5	55.7	11.3	13.9
	ED[4] (%)	95.2	97.5	33.4	78.5	14.0	37.7	86.7	86.0
Small intestine	CCC	0.54	0.59	0.54	0.60	0.62	0.65	0.65	0.67
	R	0.59	0.62	0.57	0.64	0.62	0.65	0.97	0.69
	C_b	0.92	0.95	0.94	0.93	0.99	0.99	0.98	0.97
	√MSPE[3] (%)	106	111	131	120	99	88	111	107
	ECT[4] (%)	0.6	0.6	0.8	0.2	1.4	0.7	0.3	0.7
	ER[4] (%)	0.9	1.9	3.0	0.3	25.5	6.8	3.3	1.8
	ED[4] (%)	98.5	97.5	96.2	99.6	73.1	92.5	96.4	97.5
Caeca	CCC	0.89	–	0.67	–	0.90	–	0.81	–
	R	0.89	–	0.71	–	0.90	–	0.81	–
	C_b	0.99	–	0.94	–	1.00	–	0.99	–
	√MSPE[3] (%)	78	–	115	–	51	–	106	–
	ECT[4] (%)	0.0	–	0.3	–	1.3	–	3.8	–
	ER[4] (%)	0.0	–	0.0	–	4.1	–	8.9	–
	ED[4] (%)	100.0	–	99.7	–	94.6	–	87.2	–
Excreta	CCC	0.95	0.92	0.92	0.92	0.94	0.91	0.94	0.88
	R	0.95	0.92	0.92	0.92	0.94	0.92	0.94	0.88
	C_b	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00
	√MSPE[3] (%)	44	54	46	49	40	50	42	61
	ECT[4] (%)	0.0	1.5	3.0	1.2	0.0	0.1	0.9	1.6
	ER[4] (%)	0.1	3.8	7.5	1.5	0.0	1.1	1.0	2.8
	ED[4] (%)	99.9	94.7	89.5	97.3	100.0	98.9	98.1	95.6
Overall	CCC	0.80	0.79	0.82	0.82	0.83	0.82	0.82	0.79
	R	0.81	0.79	0.83	0.82	0.83	0.82	0.82	0.80
	C_b	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00
	√MSPE (%)	102	101	101	97	95	87	103	98
	ECT[4] (%)	0.0	0.1	3.0	2.1	0.5	0.1	0.7	0.6
	ER[4] (%)	5.3	6.0	7.5	5.8	11.9	5.9	8.8	7.7
	ED[4] (%)	94.7	93.9	90.0	92.1	87.7	94.0	90.4	91.8

Abbreviations: C_b = Bias correction factor; CCC = Concordance correlation coefficient; ECT = Error due to mean bias; ED = Error due to disturbance; ER = Error due to regression; MSPE = Mean square prediction error; R = Pearson correlation coefficient.
[1] Two systems of differential equations, one for the liquid fraction (five compartments) and one for the solid fraction (four compartments), were fitted to the observations of percentage of marker recovery against time.
[2] CCC = $r \times C_b$.
[3] Square root MSPE, % of observed mean.
[4] Error as % of total MSPE.

2012). In contrast, the predicted MRT in the gizzard for the SBP diet was similar to the control diet. The proportions of the insoluble fibre fractions NDF, ADF, ADL (Van Soest et al., 1991) in the gizzard were found to be increased by the inclusion of OH in the diet compared with a control diet, while it was not the case for the inclusion of SBP (Mateos et al., 2012). Therefore, it seems that the presence of SBP in the diet does not lead to prolonged grinding in the gizzard.

Predicted MRT in the caeca was longer for the OH and RH diets (1 537 and 1 547 min), being rich in insoluble NSP, compared with the control diet (989 min). Addition of SBP in the diet reduced MRT in the caeca compared with the control diet, without increasing the fraction of digesta going in the caeca (*p*). This could be because the soluble fibre present in the SBP was easily fermented (Barry et al., 1995) and quickly excreted from the caeca. However, the emptying mechanism of the caeca is not well understood in poultry and would need to be further investigated to better understand what affects MRT in the caeca.

Influence of fibre sources on non-starch polysaccharide digestibility

Overall, total tract NSP digestibility was greater for all diets, including additional fibre sources (OH, RH, SBP), compared with the control diet, although total NSP content of these fibre diets was only increased from 10 to 10.7–11.5 g/100 g. The greater total NSP digestibility can be explained by (1) better degradation of NSP of the added fibre sources relative to the substituted wheat NSP from the control diet, or (2) better degradation of the NSP from maize, wheat, and soybean meal due to indirect effects on digestion processes, such as muscularity and grinding activity of the gizzard, and digesta retention. The observed increase in total NSP digestibility for the fibre diets was higher than what can be

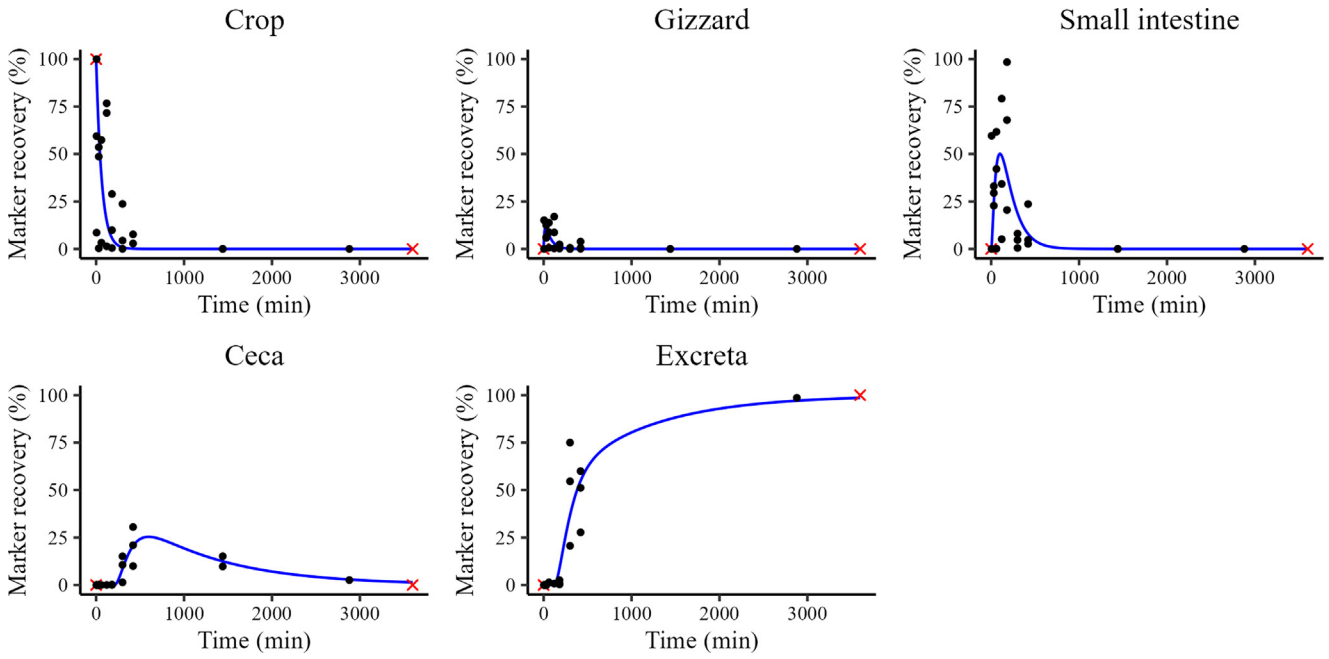

Fig. 2. Observed and predicted Co recovery (% (w/w) of dosed) per compartment of the gastrointestinal tract between 0 and 3 600 min after an oral pulse dose of Co-EDTA in 30-day-old broilers fed a standard maize, wheat, soybean meal diet (control). Black dots are individual observations; red crosses are assumed points; lines are fitted curves obtained by fitting a 5 compartments model of connected differential equations. Co-EDTA was used as a marker to represent liquid digesta.

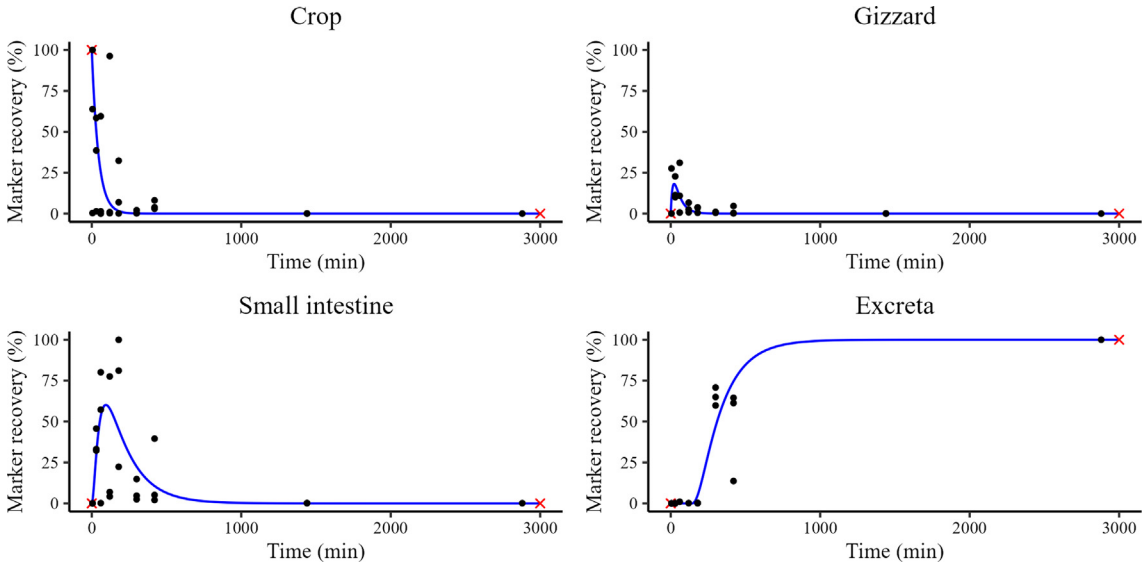

Fig. 3. Observed and predicted Cr recovery (% (w/w) of dosed) per compartment of the gastrointestinal tract between 0 and 3 000 min after an oral pulse dose of Cr_2O_3 in 30-day-old broilers fed a standard maize, wheat, soybean meal diet (control). Black dots are individual observations; red crosses are assumed points; lines are fitted curves obtained by fitting a four compartments model of connected differential equations. Cr_2O_3 was used as a marker to represent solid digesta.

explained by the added NSP coming from the different fibre sources, even if one would assume these sources would be fully degraded (e.g. the OH diet contained 0.86 g/100 g additional NSP compared with control diet, while an additional 1.78 g/100 g of NSP were degraded). It is therefore reasonable to assume that supplementation of fibre sources (OH, RH, SBP) also enhanced degradation of NSP originally present in the control diet. Although the NSP present in OH and RH can be expected to be poorly degradable based on their chemical and physical structure (Bach Knudsen, 2014; CVB, 2018), the observed greater NSP digestibility for OH and RH diets in our study corresponded with a longer predicted MRT in the gizzard for OH and RH diets compared with the control diet. This would suggest that gizzard grinding allows the release of easily solubilised fibres initially encapsulated in the cell matrix, and subsequent entrance into the caeca. For the SBP, diet and increased fermentability of NSP may be explained by the relatively easily degradable NSP from SBP compared with NSP originating from wheat in the control diet (Barry et al., 1995). Nevertheless, also for SBP, improved degradation of total NSP might be explained by the extent of gizzard development and activity (Jiménez-Moreno et al., 2013), although in our study, predicted MRT in the gizzard was similar to the control diet. The high water binding and swelling capacity of SBP (Serena and Bach Knudsen, 2007) could have been expected to hamper the entrance of liquids in

the caeca, thus reducing digestibility, but this is not what was observed. The increase in total NSP digestibility was not associated with prolonged digesta retention in the caeca nor an increased fraction of liquid digesta entering the caeca, although effects of fibre inclusion on caecal length, weight, and the microbiome (and thus fermentative capacity) cannot be excluded (Rougière and Carré, 2010).

For the OH diet, the increase in NSP digestibility compared with the control diet was mainly explained by the degradation of arabinosyl-, xylosyl-, and glucosyl residues, whereas for the RH diet, only increased degradation of glucosyl residues was observed. These results seem to correspond with the more complex, highly feruloylated, and therefore less degradable (glucurono)arabinoxylans found in RH vs OH (Watanabe et al., 1983; Bach Knudsen, 2014). For the SBP diet, the increased degradation of arabinosyl- and glucosyl residues may speculatively indicate the degradation of pectin (Bayashi et al., 1993).

In conclusion, caecal MRT of liquid digesta was estimated to range from ~500 to 1 500 min, which is longer than previously reported. The addition of fibre in the diet modulates retention time in the various GIT compartments differently, depending on the type of fibre used. Retention times of digesta in the gizzard and caeca were longer with the addition of insoluble fibre sources OH and RH, compared with the control diet, while the addition of SBP, which is rich in soluble fibre, did not increase digesta retention time in the gizzard and decreased retention time of liquids in the caeca compared with the control diet. Finally, NSP digestibility was greater with all diets including additional fibre sources regardless of the type of fibres used, although some variations in the degradation of constituent sugars of NSP were found among diets.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100867.

Ethics approval

All experimental procedures were approved by the Animal Ethics Committee of the Poultry Research Centre (Trouw Nutrition R&D) in compliance with the Spanish guidelines for the care and use of animals for research purposes (Boletín Oficial del Estado, 2013).

Data and model availability

None of the data were deposited in an official repository. The data that support the study findings are confidential.

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Declaration of interest

None.

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