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A.J.W. Mens[1], A.J.M. Jansman[1], E. Willems[2] and M.M. van Krimpen[1†]

1 Animal Nutrition, Wageningen Livestock Research, Wageningen University & Research, P.O. Box 338, 6700 AH, Wageningen, the Netherlands

2 Agrifirm Feed Innovation Centre

† The author Dr. van Krimpen sadly passed away in December 2019. Please direct any correspondence to the other authors.

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Foreword

The study “Improvement of protein digestibility by changing digesta passage kinetics in broilers” was conducted by Wageningen Livestock Research and the private partner Agrifirm as part of the Public Private Partnership “Feed4Foodure”. The work was funded by Vereniging Diervoederonderzoek Nederland (VDN) and the Ministry of Agriculture, Nature and Food Quality (LNV). The authors thank VDN and LNV for their support, and the members of the Cluster “Poultry” of VDN for their valuable and inspiring contribution to the research. The skilful and devoted contribution of staff of the experimental facility of Agrifirm at which the research was conducted, and of colleagues involved at Agrifirm is highly acknowledged.

Annemarie Mens and Alfons Jansman

Summary

Due to the increasing world population, the human consumption and thus the demand for animal products increases as well. As a result, the feed and food sector compete for nutrient resources. This competition can be reduced by increasing the nutritional value of non-human edible feed ingredients. Such ingredients often contain high levels of fibre in which protein is present that is less accessible for poultry to digest. The non-digestible fraction of protein ends up as protein and nitrogen (N) in the excreta and contributes to the N-excretion and reduces the efficiency of N-utilization. Several strategies to unlock protein in fibre rich materials have been considered, e.g. mechanical or chemical processing of feed ingredients, the use of fibre or NSP degrading enzymes as well as feeding strategies to increase the mean retention time (MRT) of digesta in the crop and foregut (proventriculus and gizzard) of broilers.
Although these methods seem promising, none have evaluated the effect of fibre addition, in the form of insoluble NSP, on unlocking the N and protein fraction present in the fibre fraction of ingredients. Therefore, the aim of the present study was to evaluate the effects of dietary supplementation of coarse oat hulls as source of insoluble NSP on the kinetics of protein (N) digestion in broilers using diets with protein sources containing a variable fraction of protein entrapped in the fibre (NDF) fraction.

The experiment was set up as a digestibility study over the period of d 17 – 24 of age in a completely randomized block design with eight dietary treatments and six replicates per treatment. The treatments followed a 4x2 factorial design, with the factors (1) dietary protein source with different concentrations of N linked to the fibre (NDF) fraction (a mix of purified ingredients, wheat, rapeseed expeller and soybean meal, respectively) and (2) supplementation of either a fine or coarse fibre source to the diet. In total, 624 female day-old broilers (Ross 308) allotted in two rounds to 12 pens (26 birds per pen). On day 16 the birds were graded and reduced per round to a total of 288 experimental birds in 24 pens (12 birds per pen). From d 0 to d 16, all birds were fed with the same starter diet. The starter diet included 5% of oat hulls to stimulate proper gizzard development. From d17 to d24, the experimental diets were provided. The basal diet consisted of maize starch, gelatinized maize starch, wheat, potato protein, crystal sugar, egg white powder and soy oil as main ingredients. In the other experimental diets wheat (41%), rapeseed expeller (25%) or soybean meal (25%) were included respectively by replacing in proportion the main ingredients in the basal diet for these ingredients. In addition, the diets contained either 5% of Arbocel as source of fine fibre or 5% of oat hulls as source of coarse fibre. Titanium dioxide (TiO_2) was added on top of the formulation as an inert marker (0.5%) to determine mean retention time of the solid phase of digesta and partial nutrient digestibility in different compartments of the intestinal tract. Cobalt-EDTA (0.1%) was added manually to the diets at the experimental farm to measure mean retention time of the liquid phase of digesta in different compartments of the digestive tract.
The performance of the birds was measured by BW, feed intake, growth performance (weight gain, ADFI and FCR). All birds were stunned and slaughtered at d24 to weigh, collect and pool per pen the digesta of the crop, gizzard, duodenum, jejunum, proximal ileum and distal ileum. Digesta of the GIT was used to determine the pH, MRT and digestibility coefficients at ileal and excreta level.
Due to the differences in nutritional value of the diets related to the design of the study, the performance parameters were affected by dietary treatment, mostly regardless of the fibre source supplementation. Inclusion of oat hulls to the diet resulted in a lower FCR compared to the inclusion of Arbocel. Furthermore, BW at day 24, FI between day 17-24 and weight gain between day 17-24 were all highest in the diets including soybean meal (BW: 1320 g, FI: 791 g and weight gain: 604 g) , followed by the basal diet (resp. 1225 g, 752 g and 504 g) and rapeseed expeller diet (1245 g, 738 g and 524 g), and lowest values were found in the wheat diet (1165 g, 778 g and 449 g) ($P < 0.001$). Including coarse fibres to the diets resulted in a prolonged MRT of both the solid and liquid phase in the gizzard, but shortened the MRT in the duodenum and jejunum ($P < 0.05$). Within the gizzard, both the inclusion of protein sources with different levels of N-NDF, as well as the addition of oat hulls affected the MRT. Highest effects of coarse fibre supplementation were found on the basal diet, where

the MRT of digesta till the end of the terminal ileum was shortened by 73 min by supplementing oat hulls. Furthermore, supplementation with oat hulls resulted in higher gizzard weights (empty, full and digesta), an improved development of the gizzard and lower digesta pH ($P < 0.001$). As a result, the partial DM and CP digestibility of the diets in the crop and the gizzard were affected by both ingredient composition and source of fibre supplementation (interaction $P < 0.001$). In all segments, the DM digestibility coefficients were highest for the basal diets, lowest for the rapeseed expeller diets and intermediate for the soybean meal and wheat based diets. The CP digestibility of the diets in the gizzard was highest for the wheat based diets, followed by the basal diets and lowest for the rapeseed expeller and soybean meal based diets. This effect was enlarged by including oat hulls in the diets. Furthermore, ingredient composition affected the CP digestibility in the jejunum, proximal ileum and distal ileum. In all segments the wheat based diets had the highest protein digestibility, and the basal diets showed the lowest values. The partial protein digestibility in the jejunum and proximal ileum were 5 till 12% and 2 till 6% higher when oat hulls were supplemented to the diets. The distal ileal protein digestibility was increased by 1 to 3 % by supplementing the diets with oat hulls as a source of coarse fibre, the effect varying between diets containing ingredients with a different level of N linked to the NDF fraction. The estimated digestibility of the N in NDF fraction was increased by oat hull supplementation from 83 to 84% in the basal diets, from 16 to 21% in the RSE diet, from 76 to 80 in the SBM diet and from 54 to 63% in the wheat based diet. The latter values should be considered with care, however, related to a relatively high inaccuracy of the determination of the N-NDF concentration in diet and digesta samples, in particular when the concentrations are low as was the case in the diets and excreta samples in the present study.

It can be concluded from the present study that inclusion of 5% coarse oat hulls in broiler diets increases digesta retention time in particular in the proximal part of the digestive tract, reduces pH of digesta in the gizzard and increases ileal protein digestibility by at least 1 till 3%. Part of the improvement in ileal protein digestibility might be related to the physical release of a potentially digestible protein fraction linked to the fibre fraction present in wheat, soybean meal and rapeseed expeller, containing different concentrations of N and protein in the fibre (NDF) fraction. The ileal digestibility of the N-NDF fraction in the diet, although difficult to measure experimentally, seems to increase to a limited extent by oat hull inclusion in the diet.

1 Introduction

According to FAO (2012) in the coming decades the demand for animal products for human consumption such as meat, milk, and eggs will tremendously increase related to the growth of the world population. The former will also increase the demand for protein in diets for livestock. As a result, the competition for nutrient resources for animal feed and human food needs to be reduced. In these conditions, maximization of the nutritional value of feed ingredients not fit for human consumption may contribute to an efficient nutrient utilization.

Protein present in the fibre fraction of raw materials might be not or less accessible for digestive enzymes (proteases) in the intestinal tract of poultry and as a result be less digestible compared to other protein fractions in feed ingredients. The non-digestible fraction of protein ends up as protein and nitrogen (N) in the excreta, contributes to the N-excretion and reduces the efficiency of N-utilization. In common feed ingredients for broilers, a substantial fraction of N (crude protein; CP) of the total N-fraction can be found in the fibre (neutral detergent fibre; NDF) fraction e.g. 7.5% in soybean meal, 17% in wheat, and 26% in rapeseed meal (van Krimpen, 2019). Further analysis showed that the majority of the N (CP) in the fibre fraction is present as true protein, and thus serves as a potential source of amino acids. Strategies that could help to unlock the fibre fraction in raw materials could thereby contribute to improving the protein and amino acid digestibility in broilers. Several strategies to unlock protein in fibre rich materials have been considered, e.g. mechanical or chemical processing of feed ingredients, the use of fibre or NSP degrading enzymes as well as feeding strategies to increase the mean retention time (MRT) of digesta in the crop and foregut (proventriculus and gizzard) of broilers. Several studies showed that dietary supplementation of coarse insoluble non-starch polysaccharides (NSP) sources prolonged the MRT of digesta in the foregut of poultry (e.g. Ferrando et al., 1987; Van Krimpen et al., 2011; Nisshii et al., 2016). Moreover, it was demonstrated that the N (CP) digestibility of broiler diets increased up to 10% after inclusion of fibrous materials such as oat hulls (e.g. Hetland et al., 2001; Jiménez-Moreno et al., 2010, 2013) or pea hulls (Mateos et al., 2012).

Although these results seemed promising for improving protein digestibility in broilers, neither of these studies evaluated the effect of fibre (NSP) addition on unlocking the N and protein fraction present in the fibre fraction of raw materials as such. Therefore, the exact mode of action of fibre (NSP) addition on N-digestibility of feed ingredients in broilers remains largely unclear. It also can be hypothesised that inclusion of fibrous insoluble NSP sources in the diet affects the kinetics of protein digestion throughout the entire digestive tract. An increased mean retention time (MRT) of digesta in the foregut prolongs the exposure time of digesta to host digestive enzymes and low pH conditions in the foregut could open and loosen the fibre structure thereby making the fibre linked protein fraction more prone to digestion in the intestinal tract. Measurement of parameters related to digestive physiology and digestion kinetics (e.g. MRT of the solid and liquid digesta in different compartments of the gastro-intestinal tract, pH and viscosity of digesta, and kinetics of protein digestibility along the digestive tract), might contribute to a better understanding of the impact of addition of coarse fibre-rich ingredients on unlocking and improving digestibility of protein linked to the fibre (NDF) fraction of dietary ingredients.

The aim of the present digestibility study was to evaluate the effects of dietary supplementation of coarse oat hulls as source of insoluble NSP on the kinetics of protein (N) digestion in broilers using dietary protein sources containing a variable fraction of protein trapped in the fibre (NDF) fraction. It is hypothesized that adding a coarse insoluble NSP source to a broiler diet would prolong the MRT of digesta in the foregut and reduce the pH of digesta in the proximal digestive tract and would loosen and open the structure of the fibre fraction of protein sources thereby increasing the exposure of digesta to e.g. pepsin and bile acids and increasing N and protein digestibility.

2 Materials and Methods

2.1 Experimental design and treatments

The experiment was set up as a digestibility study over the period of d 17 – 24 of age in a completely randomized block design with eight dietary treatments and six replicates per treatment. The treatments followed a 4x2 factorial design, with the factors (1) dietary protein source with different concentrations of N linked to the fibre (NDF) fraction (a mix of purified ingredients, wheat, rapeseed expeller and soybean meal, respectively) and (2) supplementation of either a fine or coarse fibre source to the diet. The dietary protein sources were chosen based on a literature assessment and chemical analysis (van Krimpen, 2019) on the amount of NDF and the N present in the NDF fraction in these ingredients and their applicability in commercial diets. In addition, the choice was based on the origin of the ingredients and differences in pre-processing imposed to the ingredients (a cereal grain, a heat treated (toasted) solvent defatted meal and a mechanically processed expeller meal). To test whether the nature of the supplemented fibre source could influence the extent of release of protein (N) linked to the NDF fraction in the protein sources a fine or a coarse source of fibre was included in the diets (Arbocel and oat hulls, respectively). The study was performed in two rounds with three replicates per treatment per round.

The dietary treatments are presented below (Table 1).

Table 1 *Overview of experimental (dietary) treatments in the digestibility study.*

	Incl. Arbocel	Incl. oat hulls
Basal diet (BD)	**T1**	**T5**
BD + rapeseed expeller	**T2**	**T6**
BD + soybean meal	**T3**	**T7**
BD + wheat	**T4**	**T8**

2.2 Experimental animals

In total, 624 female day-old broilers (Ross 308) were allotted in two rounds to 12 pens (26 birds per pen). Day old chicks were supplied by hatchery Probroed en Sloot, The Netherlands. Birds were originating from the same breeder flock for both rounds. Birds were vaccinated against IB in the hatchery and against Newcastle Disease on 14 d of age. On day 16 the birds were graded and reduced per round to a total of 288 experimental birds in 24 pens (12 birds per pen). Birds with locomotive disorders, visual abnormalities and deviations in weight were removed.

2.3 Experimental facility

The experiment was conducted in the metabolic broiler pens located at the Research Farm Laverdonk (Heeswijk Dinther, The Netherlands) in two consecutive rounds. The room was automatically controlled by a Fancom climate computer. The incoming air is heated before entry into the room. The ventilation system was adjusted to provide a minimum ventilation improving the air circulation into the room. The sensors of the thermometers were connected with the climate control system and data were registered automatically.
Birds received 20 h of light and 4 h of darkness during first 6 days. From d 6 till the end of the study, 18 h of light and 6 h of darkness (from 17:00 to 21:00 and from 03:00 to 05:00) was provided. Light intensity was 90 lux at chicken height during the light period. The room temperature was 33-35°C on the day of arrival, and gradually decreased till 20-22°C at the end of the experiment. The pens were equipped with a 2-bulb light per unit of pens.

Starter period (d0 to d16)
During the starter period, 12 pens divided over 2 levels and 3 units were used. All pens had 2.13 m^2 effective floor surface (2.35 x 0.905 m). During the first week of life, pens were equipped with a grit mat and rubber mat until d 8 in order to accommodate the small birds. Wood shavings were placed on top of the rubber mats. From d 8 onwards, the mats were removed, and birds were housed on trays with wood shavings on top. Feed and water were *ad libitum* available; For the first few days, birds were fed from small feeders (egg trays) and water was available from round drinkers. Afterwards, feed was available in a feeder over the total length of the pen and water was distributed via drinking nipples with cups. Impressions of the housing of the chicks are depicted in Figure 1.

Adaptation and experimental period (d17 to d24)
The 12 cages were divided into two cages by putting a metal plate between adjacent pens. As a result, during the experimental period 24 cages with 1,05 m^2 effective floor surface (1.16 x 0,905 m) were used. The wood shavings were removed from the trays and the grid floor was placed into the cages for excreta collection. Feed and water were provided *ad libitum*, feed was available in one feeder over the total length of the pen (1.16 m) and water was distributed via five drinking nipples with cups per pen.

Figure 1 *Impression of housing of the chicks.*

2.4 Experimental diets

From d 0 to d 16, all birds were fed with the same starter diet. The starter diet included 5% of oat hulls to stimulate proper gizzard development. From d17 to d24, the experimental diets were provided.
The basal diet consisted of maize starch, gelatinized maize starch, wheat, potato protein, crystal sugar, egg white powder and soy oil as main ingredients. In the other experimental diets wheat (41%), rapeseed expeller (25%) or soybean meal (25%) were included respectively by replacing in proportion the main ingredients in the basal diet for these ingredients. In addition, the diets contained either 5% of Arbocel as source of fine fibre or 5% of oat hulls as source of coarse fibre. As a result, the experimental diets were not iso-energetic with $ME_{broilers}$ values ranging from 13.7 for the basal diet till 11.9 MJ per kg for the diets with rapeseed expeller and soybean meal, respectively. The diets were neither supplemented nor balanced for essential amino acids. Only L-arginine was supplemented to all diets. The mineral composition of the diets for Ca, P and sodium was identical for all diets and assumed to fulfil the requirements of the birds for these minerals.

The starter diet and experimental diets were produced by Research Diet Service (Wijk bij Duurstede, The Netherlands). The starter diet was pelleted at a 2.5 mm diameter and the experimental diets were pelleted at a 4 mm. The diets included phytase but did not contain NSP degrading enzymes. Titanium dioxide (TiO_2) was added on top of the formulation as an inert marker (0.5%) to determine mean retention time of the solid phase of digesta and partial nutrient digestibility in different compartments of the intestinal tract.

Cobalt-EDTA (0.1%) was added manually to the diets at the experimental farm to measure mean retention time of the liquid phase of digesta in different compartments of the digestive tract. Comprehensive pre-tests were executed to determine the homogeneity of the marker distribution on the pellets. The Cobalt-EDTA marker was dissolved in water and sprayed on each of the diets (per diet 100 kg of feed) within a Nauta mixer. Dosage of the marker took 5 min, after which mixing was continued for another 2 min.

The diets were analysed chemically by Nutricontrol BV (Veghel, The Netherlands) and the laboratory of the Animal Nutrition Group of Wageningen University and Research. Dietary ingredient and calculated nutrient composition of the experimental diets are given in Table 2 and of the starter diet can be found in Appendix 1.

Table 2 *Dietary ingredients, and calculated nutrients of the experimental diets (g/kg, as-fed basis)*

Diet[1]	**Basal**		**RSE**		**SBM**		**Wheat**	
Fibre source	*Fine*	*Coarse*	*Fine*	*Coarse*	*Fine*	*Coarse*	*Fine*	*Coarse*
Ingredient								
Maize starch	25.00	25.00	18.06	18.06	18.06	18.06	13.64	13.64
Maize starch (heat treated)	17.65	17.65	12.74	12.74	12.74	12.74	9.63	9.63
Wheat	13.74	13.74	9.92	9.92	9.92	9.92	7.50	7.50
Potato protein	10.10	10.10	7.30	7.30	7.30	7.30	5.51	5.51
Sugar	10.00	10.00	7.22	7.22	7.22	7.22	5.46	5.46
Egg albumin powder	9.28	9.28	6.70	6.70	6.70	6.70	5.06	5.06
Soy oil	4.00	4.00	2.89	2.89	2.89	2.89	2.18	2.18
Arbocel	5.00		5.00		5.00		5.00	
Oat hulls (coarse)		5.00		5.00		5.00		5.00
Mono-Calcium phosphate	1.58	1.58	1.15	1.14	1.27	1.27	1.42	1.41
Calcium carbonate	1.08	1.08	0.88	0.88	1.05	1.05	1.12	1.13
Potassium carbonate	0.74	0.74	0.21	0.17			0.46	0.42
Premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Diamol (insoluble ash)	0.33	0.33	1.38	1.42	1.29	1.30	0.54	0.58
L-Arginine	0.23	0.23	0.17	0.17	0.17	0.17	0.13	0.13
Sodium bicarbonate	0.18	0.17	0.28	0.28	0.28	0.28	0.36	0.36
Magnesium oxide	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase (Axtra PHY)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Rapeseed expeller			25.00	25.00				
Soybean meal					25.00	25.00		
Wheat							40.89	40.89
Calculated content[3]								
AME_n (MJ/kg)	13.6	13.7	11.9	12.0	11.9	11.9	12.3	12.4
Dry Matter	907.1	906.3	905.2	904.4	903.5	902.7	893.3	892.5
Crude ash	53.8	55.9	68.9	71.0	67.4	69.5	55.8	58.0
Crude protein	180.0	181.3	211.1	212.5	249.1	250.5	143.9	145.2
Crude fat	46.9	46.8	52.7	52.6	37.7	37.6	31.0	30.9
Crude fibre	38.7	20.1	66.4	47.8	46.6	28.0	46.3	27.7
Starch	443.9	443.6	334.9	334.6	320.9	320.6	482.7	482.4
Sugar	110.0	110.5	105.0	105.5	105.5	106.0	71.3	71.6
NDF	65	65	115	115	77	77	103	103
N in NDF	0.4	0.6	3.6	3.8	1.7	1.9	1.5	1.6
N in NDF, relative to total N (%)	1.5	2.0	10.7	11.1	4.3	4.7	6.6	7.2
ADF	31.0	30.7	72.7	72.4	39.9	39.6	40.7	40.4
ADL	11.3	1.9	28.4	19.0	11.8	2.4	13.6	4.2

Diet[1]	Basal		RSE		SBM		Wheat	
Ca	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
P	4.4	4.5	5.7	5.7	5.1	5.1	4.9	4.9
IP	0.5	0.5	2.4	2.4	1.4	1.4	1.1	1.1
K	5.8	6.0	5.3	5.4	6.8	7.1	5.2	5.2
Na	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Cl	1.8	1.8	1.4	1.4	1.4	1.5	1.2	1.3
Dig. Lys	10.4	10.4	11.0	11.0	13.9	14.0	6.7	6.8
Dig. met	4.5	4.5	4.6	4.6	4.7	4.8	3.1	3.1
Dig. Cys	3.0	3.1	3.6	3.6	3.7	3.7	2.5	2.5
Dig. Met+cys	7.6	7.6	8.2	8.2	8.4	8.4	5.6	5.6
Dig. Thr	7.6	7.7	8.3	8.3	9.5	9.5	5.2	5.2
Dig. Trp	2.3	2.3	2.5	2.5	3.1	3.1	1.7	1.7
Dig. Ile	8.3	8.3	8.5	8.5	10.8	10.8	5.8	5.9
Dig. Arg	10.9	10.9	12.2	12.3	15.8	15.8	7.7	7.8
Dig. Phe	6.8	6.8	7.6	7.6	10.4	10.4	5.5	5.5
Dig. His	3.0	3.0	4.0	4.0	5.0	5.1	2.5	2.5
Dig. Leu	9.8	9.9	12.0	12.0	15.2	15.2	7.9	7.9
Dig. Tyr	4.6	4.6	5.2	5.3	7.2	7.3	3.5	3.6
Dig. Val	9.6	9.6	10.2	10.2	11.9	11.9	6.8	6.9
Dig. Ala	6.3	6.4	7.4	7.4	8.9	9.0	4.7	4.8
Dig. Asp	12.4	12.5	14.0	14.1	21.3	21.4	8.6	8.7
Dig. Glu	20.7	20.8	26.6	26.7	34.2	34.3	23.4	23.5
Dig. Gly	4.4	4.5	6.5	6.6	7.4	7.4	3.8	3.9
Dig. Pro	6.1	6.1	8.3	8.4	9.8	9.8	7.2	7.3
Dig. Ser	7.5	7.5	8.3	8.4	10.7	10.8	5.9	6.0
Phytase (FTU)	500	500	500	500	500	500	500	500

[1] Diets: Basal = basal diet, RSE = basal diet + rapeseed expeller, SBM = basal diet + soybean meal, wheat = basal diet + wheat

[2] Provided per kilogram of complete diet: vitamin A 10.000 IE; vitamin D3 2.500 IE; vitamin E 50 mg; vitamin K_3 1.5 mg; vitamin B_1 2.0 mg; vitamin B_2 7.5mg; vitamin B_6 1.0 mg; vitamin B_{12} 20 µg; niacin 35 mg; D-pantothenic acid 12 mg; choline chloride 460 mg; folic acid 1.0 mg; biotin 0.2 mg; iron 80 mg; copper 12 mg; manganese 85 mg; zinc 60 mg; iodate 0.8 mg; selenium 0.15 mg.

[3] CVB matrix values (CVB, 2011) were used for diet formulations.

2.5 Measurements

2.5.1 Performance measurements

The following measurements were performed:

- The body weight was recorded per pen on d 0 and individually at d 17 and d 24.
- Feed intake was recorded per pen at d 17 and d 23, and d 24.
- On d 17, birds were redistributed over pens to get groups with a similar mean body weight and standard deviation per pen.
- Growth performance: average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated over the experimental period (d 17-24)
- Mortality and irregularities were checked daily with registration of the probable cause of death. In case of dead birds, feed consumption of the birds in the pen till that time point was determined.

2.5.2 Apparent ileal and faecal digestibility of nutrients

For the faecal digestibility study, the excreta output was collected per pen for 3 consecutive days (d 22 to d 24). Excreta was collected twice per day, frozen and stored at -20°C until further analysis. A representative sample of excreta was collected which was not contaminated by feed or feathers.

For ileal digestibility determination and intestinal digesta collection, all birds were stunned and slaughtered on d24 by electrocution. Individual bird weight was recorded. The digesta of the following parts of the gastrointestinal tract was collected, weighed and pooled per pen:

- Crop
- Gizzard
- Duodenum
- Jejunum
- Proximal ileum
- Distal ileum

The crop was defined as the out-pocketing of the oesophagus, which is located in the neck region, just above the body cavity. The gizzard was separated from the proventriculus and defined as the muscular part with thick lining. The small intestine was separated from the gizzard and divided into the duodenum, jejunum and ileum. The duodenum was defined as the part of the GIT that starts from the end of the gizzard and ends at the hepatopancreatic duct. The jejunum and ileum have no clear demarcation. The jejunum was defined as the part of the small intestine that starts at the hepatopancreatic duct and ends at the Meckel's diverticulum, after which it continues as the ileum until 2 cm before the split between ileum and caeca. The digesta was collected by gently flushing the gut segment with distilled water into a labelled box container. The pH was defined of all pooled digesta samples. The digesta was frozen in dry ice after collection and stored at -20°C until further analysis. The digesta was collected in the order of pen numbering.

2.5.3 Mean retention time

Retention time of the solid (RT_{solid}) and liquid fraction (RT_{liquid}) of digesta in the GIT segments was calculated as follows:

$$\text{Retention time (min)} = \frac{1440 \times \text{Marker}_{digesta} \times W_{digesta}}{FI_{24h} \times \text{Marker}_{diet}} \quad \text{(I)}$$

where $\text{Marker}_{digesta}$ (%) is the marker (Ti or Co) content in the freeze-dried digesta samples, $W_{digesta}$ (g) is the weight of freeze-dried digesta samples from the four segments of the SI, FI_{24h} (g) is the feed intake over 24 h prior to digesta sampling, Marker_{diet} (%) is the marker (Ti or Co) content in the experimental diets (as-fed basis), and the factor 1440 is used to convert time from days to minutes.

2.5.4 Digestibility calculations

Based on the analysed composition of the experimental diets and excreta the faecal digestibility of DM, CP, NDF and N in NDF digestibility of the diet was calculated using the equation (II).
Based on the analysed content in the experimental diets and digesta the pre-caecal (ileal) NDF and N in NDF digestibility of the diets was calculated (equation I).

$$\text{Digestibility (\%)} = 100 - [100 \times (M_{Diet} \times \text{Nutrient}_{Digesta/excreta}) / (M_{Digesta/excreta} \times \text{Nutrient}_{Diet})] \quad \text{(II)}$$

With

- M_{Diet} and $M_{Digesta}$ are the analysed concentration of the marker (TiO_2) in diet and digesta or excreta (g/kg)
- Nutrient_{Diet} and $\text{Nutrient}_{Digesta}$ are the analysed concentration of the nutrient in the diet and digesta or excreta (g/kg)

2.5.5 Score of gizzard development

To assess the gizzard development the following measurements were performed:

- Assessment of the passage of the proventriculus to gizzard (scale 1-5; Figure 2)
- Weight gizzard full and empty (fat must be removed as much as possible)
- Weight gizzard content is determined by difference between full and empty gizzard

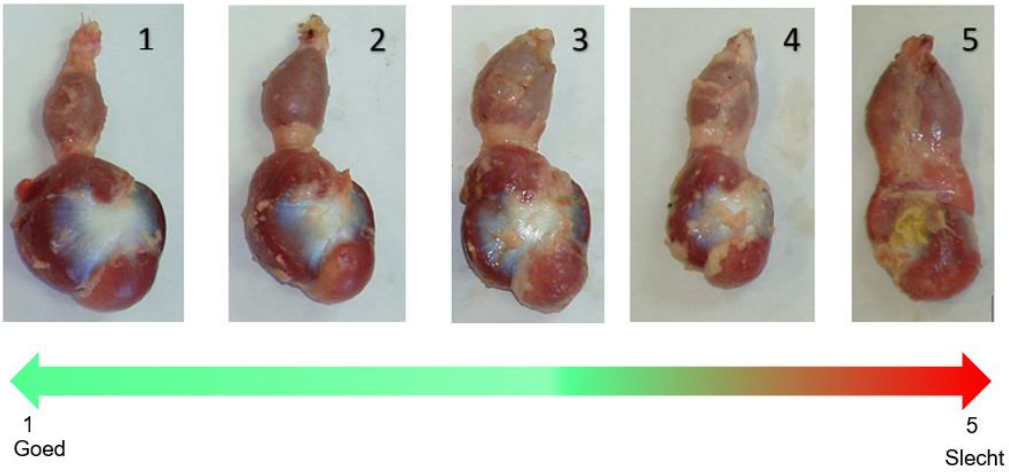

Figure 2 *Gizzard development score from 1 ("well developed") to 5 ("poorly developed").*

2.5.6 Analysis of feed, digesta and excreta samples

Feed samples were taken during production (2x 500 g) and at the experimental facilities during feeding (1x 300 g). Table 3 represents the analysis of the feed samples and the responsible lab, Nutricontrol (Veghel, The Netherlands) or the lab of the Animal Nutrition Group at Wageningen University.

Digesta samples were analysed by Nutricontrol and excreta samples were analysed by the lab of the Animal Nutrition Group at Wageningen university. After defrosting, digesta samples were homogenized. All samples were analysed in duplicate. For determination of the DM content in digesta, samples were freeze-dried according to International Organization for Standardization (ISO) method number 6496 (1998). Following freeze-drying, samples were ground to pass a 1 mm screen and kept for analysis. Air-dry samples were dried in a forced air oven at 103 °C to a constant weight according to ISO 6496 (1998). Kjeldahl nitrogen content in feed was measured according to ISO 5983 (1997) in fresh samples. CP content was calculated as nitrogen * 6.25. For determining crude ash content, feed samples were incinerated at 550°C in a muffle furnace according to ISO 5984 (2002). Titanium oxide was determined according to the method developed by Short et al. (1996) and further refined by Myers et al. (2004). This method is based on digestion of the sample in sulphuric acid and addition of hydrogen peroxide to produce an intense orange/yellow colour that is read colorimetrically at 408 nm by use of an UV- visible spectrophotometer (Varian, CARY 50 probe).

For the determination of the NDF fraction in the excreta NDF was gravimetrically determined as the remaining insoluble organic fraction after 60 minutes of hydrolysis with ND-reagens, containing termamyl, and 30 minutes of enzymatic incubation with amylase and alcalase at pH 7. This procedure is derived from Forage Fibre Analyses, Agricultural Handbook no. 379 by H.K. Goering and P.J. van Soest. N in NDF was determined by measuring the N fraction within the NDF fraction by using the Kjeldahl method with $CuSO_4$ as catalyst (ISO 5983).

Table 3 *Overview of analyses in feed samples.*

Experimental diets	Lab
Moisture 103°C (4h)	NutriControl
Ash (550°C)	NutriControl
Crude protein	NutriControl
Crude fat (after acid hydrolysis)	NutriControl
Starch (Ewers)	NutriControl
Crude fibre	NutriControl
Ca	NutriControl
P	NutriControl
Na	NutriControl
NDF	WUR
N-NDF	Nutricontrol & WUR
TiO_2	NutriControl
Cobalt	NutriControl
Wet sieve analysis	NutriControl

2.6 Statistical analyses

Performance data (average daily gain, average daily feed intake and feed conversion ratio) and digestibility data were analysed according to the following model using the analysis of variance (ANOVA) of the statistical package GenStat. The general model was used to test the differences between the diets:

$$Y_{ijkl} = \mu + Round_i + Diet_l + Fibre_{k+} e_{ijkl}$$

in which:

Y_{ijkl} = dependent variable,
μ = overall mean
$Round_i$ = round effect (i=1,2)
$Diet_l$ = effect of dietary treatment (k= 1, 2, 3, 4)
$Fibre_k$ = effect of fibre source (k= 1, 2)
e_{ijkl} = residual error

If an overall statistical treatment effect was found (P value < 0.05), a Fisher protected t-test was used to analyse pairwise differences. Pairwise differences are marked with a letter in superscript. Differences among means with 0.05 < P < 0.10 were accepted as representing tendencies to differences.

3 Results

3.1 General

The experiment was performed in accordance with the proposed protocol, and no abnormalities were detected. Although the study was not meant to be a performance study and diets were not fully nutritionally balanced, the overall performance of the birds during the experimental period was qualified as good (Table 4) and met the standards of Ross 308 performance data (Ross 308, 2019).

The body weight (BW) on day 24 and feed intake (FI), body weight gain and feed conversion ratio (FCR) over days 17-24 all differed between dietary treatments ($P<0.05$ for BW and $P < 0.001$ for the other parameters) regardless of the fibre source included. There were no interactions observed between dietary ingredient composition and fibre source supplementation. BW at day 24, FI and body weight gain over d 17-24, were highest for the diets including soybean meal, resulting in the lowest FCR over the related period. Feeding the wheat-based diets resulted in the lowest body weight gain and highest FCR. Birds on the basal diet and the rapeseed expeller diet showed an intermediate performance. The differences in performance parameters were a direct effect of differences in nutritional value of the diets. Only the FCR of the birds was affected by dietary fibre source. Inclusion of oat hulls resulted in a lower FCR compared to inclusion of Arbocel.

Table 4 *Effect of supplementing fine or coarse fibre to four diets differing in ingredient composition on feed intake, body weight, growth and feed conversion ratio (FCR).*

		Diet[1]					P-value[3)]		
	Fibre source	Basal diet	RSE	SBM	Wheat	LSD[2]	Fibre source	Diet	Fibre source * Diet
Body weight,	Fine	721	720	717	715				
D17 (g)	Coarse	722	723	715	717				
Feed intake,	Fine	765	732	788	779	38.0	0.19	<0.001	0.50
d17-24 (g)	Coarse	739	744	794	777				
	Diet	752^{bc}	738^{c}	791^{a}	778^{ab}				
Body weight,	Fine	1236	1229	1313	1155	33.0	0.18	<0.001	0.13
d24 (g)	Coarse	1215	1262	1327	1174				
	Diet	1225^{b}	1245^{b}	1320^{a}	1165^{c}				
Weight gain,	Fine	515	509	596	440	31.9	0.20	<0.001	0.12
d17-24 (g)	Coarse	493	539	612	457				
	Diet	504^{b}	524^{b}	604^{a}	449^{c}				
Feed ratio	Fine	1.49	1.44	1.32	1.77	0.057	0.020	<0.001	0.13
conversion	Coarse	1.50	1.38	1.30	1.70				
d17-24	*Diet*	1.50^{b}	1.41^{c}	1.31^{d}	1.74^{a}				

[1] Diets: Basal = basal diet, RSE = basal diet + rapeseed expeller, SBM = basal diet + soybean meal, wheat = basal diet + wheat

[2] LSD for interaction between fibre source and diet are presented.

[abcd] Means with a different superscript within a parameter represent a significant difference ($P < 0.05$).

3.2 Calculated and analysed nutrients of the diets

The calculated and analysed nutrient composition of the experimental diets are presented in Table 5. The analysed values for crude protein, crude fat, starch, calcium and phosphorous were close to the calculated values of the diets. The crude fibre content was especially lower in all the diets with Arbocel included (T1, T2, T3 and T4) compared to the calculated values.

The analysed values for the concentration N in NDF were higher for both basal diets and lower for the other diets compared to the calculated values.
The wet sieve analyses of the diets showed only small differences in particle size distribution between the diets (Table 6). Major part of all diets (range 72 – 83%) consisted of particles smaller than 106 µm, in line with the semi-purified nature of the experimental diets. No differences were found in particle size distribution between the diets containing Arbocel as fine source of fibre or more coarse oat hulls.

Table 5 *Calculated and analysed nutrient content of the experimental diets (g/kg).*

Diet[1]		**Basal**		**RSE**		**SBM**		**Wheat**	
Fibre source[2]		*Fine*	*Coarse*	*Fine*	*Coarse*	*Fine*	*Coarse*	*Fine*	*Coarse*
Nutrient	*Method*								
Dry matter	*Calc.*	907	906	905	904	903	903	893	892
	Ana.	887	887	885	884	883	879	880	881
Ash	*Calc.*	54	56	69	71	67	70	56	58
	Ana.	45	46	56	58	57	59	49	50
Crude protein	*Calc.*	180	181	211	212	249	250	144	145
	Ana.	179	183	211	214	247	249	149	151
Crude fat	*Calc.*	47	47	53	53	38	38	31	31
	Ana.	41	42	50	55	32	35	27	28
Crude fibre	*Calc.*	39	20	66	48	47	28	46	28
	Ana.	23	16	43	42	25	25	27	26
Starch	*Calc.*	444	444	335	335	321	321	483	482
	Ana.	453	462	335	343	343	344	478	493
Calcium	*Calc.*	7.26	7.28	7.26	7.26	7.26	7.26	7.26	7.26
	Ana.	7.24	7.39	7.33	7.35	7.34	7.14	7.92	7.40
Phosphorus	*Calc.*	4.42	4.47	5.69	5.72	5.09	5.12	4.90	4.93
	Ana.	4.40	4.55	5.86	5.93	5.15	5.23	5.30	4.88
Sodium	*Calc.*	1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66
	Ana.	2.03	2.00	1.85	1.78	1.88	1.79	1.85	1.83
Titanium dioxide	*Calc.*	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	Ana.	4.6	4.6	4.4	4.60	4.6	4.5	4.5	4.8
NDF	*Calc.*	65	65	115	115	77	77	103	103
	Ana.	81	58	106	99	75	81	86	89
N in NDF	*Calc.*	0.42	0.59	3.62	3.79	1.73	1.90	1.51	1.67
	Ana.	1.64	1.11	1.15	1.22	0.78	0.79	0.92	0.95

[1] Diets: Basal = basal diet, RSE = basal diet + rapeseed expeller, SBM = basal diet + soybean meal, wheat = basal diet + wheat

[2] Fibre source consisted out of Arbocel (fine) or oat hulls (coarse)

Table 6 *Results of the wet sieve analyses of the eight diets and two fibre sources (% DM).*

Diet	Basal		RSE		SBM		Wheat	
Fibre source	*Fine*	*Coarse*	*Fine*	*Coarse*	*Fine*	*Coarse*	*Fine*	*Coarse*
< 106 µm	77.2	76.4	83.5	80.1	72.4	72.6	76.1	77.0
> 106 µm	11.0	11.9	8.5	8.5	9.9	8.8	10.2	9.7
> 250 µm	7.9	6.4	4.7	5.4	6.5	6.3	6.1	5.0
> 500 µm	2.6	3.1	2.4	3.8	7.0	8.1	4.0	4.9
> 1000 µm	1.1	1.5	0.5	1.3	3.3	3.0	2.0	2.2
> 1400 µm	0.3	0.6	0.4	0.8	1.0	1.1	1.3	1.2
> 2000 µm	0.0	0.2	0.1	0.3	0.2	0.2	0.4	0.2

3.3 Mean retention time per segment

The mean retention time (MRT) per segment (crop, gizzard, duodenum, jejunum, proximal ileum and distal ileum) are presented in Table 7 (solid fraction of digesta) and Table 8 (liquid fraction of digesta). No interaction was observed between ingredient composition and source of supplemented fibre for the MRT of the solid and liquid fraction of digesta, except for the MRT of the liquid phase of digesta in the duodenum.

Adding coarse fibres to the diets affected the kinetics of transit of digesta through the digestive tract. The coarse fibres resulted in a longer MRT of the solid and liquid phase in the gizzard, and shorter retention times in the duodenum (only solid part) and jejunum (both solid and liquid part) ($P < 0.05$). Furthermore, the MRT tended to be shorter in the proximal ileum when coarse fibres were added ($P = 0.072$). Supplementation of coarse fibres prolonged the MRT of digesta in the gizzard for the rapeseed expeller diet (+12 min), the soybean meal diet (+20 min) and the wheat diet (+17 min) compared to the basal diet (Figure 3). Highest effects of coarse fibre supplementation were found on the basal diet, where the MRT of digesta till the end of the terminal ileum was shortened by 73 min by supplementing oat hulls. In the rapeseed expeller diet the MRT was shortened with 26 min, in the soybean meal diet with 3 min and in the wheat diet the MRT was prolonged with 25 min compared to the diets without coarse fibre supplementation. Ingredient composition of the diet only affected the MRT of the solid fraction in the gizzard and tended to affect the MRT of the solid fraction in the jejunum. Inclusion of wheat in the diet resulted in the longest MRT in the gizzard, while inclusion of rapeseed expeller resulted in a shorter MRT compared to the MRT of the basal diet ($P < 0.05$). The diets with wheat and rapeseed expeller tended to have a longer MRT than the basal diet in the jejunum ($P < 0.10$).

Table 7 *Effect of ingredient composition and fibre supplementation (Arbocel or oat hulls as fine and coarse fibre sources, respectively) on the mean retention time of the solid fraction of digesta in different segments of the digestive tract (min).*

		Diet[1]					P-value[3)]		
	Fibre source	Basal diet	RSE	SBM	Wheat	LSD[2]	Fibre source	Diet	Fibre source * Diet
Crop	Fine	68	65	39	55	36.8	0.30	0.73	0.72
	Coarse	52	55	41	66				
	Diet	60	60	40	60				
Gizzard	Fine	16	13	11	20	5.4	<0.001	<0.001	0.19
	Coarse	31	25	31	37				
	Diet	23^{b}	19^{c}	21^{bc}	28^{a}				
Duodenum	Fine	0.7	1.1	0.8	0.6	0.49	0.003	0.35	0.22
	Coarse	0.3	0.5	0.3	0.7				
	Diet	0.5	0.8	0.5	0.7				
Jejunum	Fine	49	54	48	49	12.5	<0.001	0.09	0.19
	Coarse	26	39	31	44				
	Diet	37	47	40	46				
Proximal ileum	Fine	50	32	29	32	23.2	0.070	0.55	0.43
	Coarse	23	24	22	31				
	Diet	37	28	25	31				
Distal ileum	Fine	41	30	25	28	15.5	0.14	0.81	0.18
	Coarse	20	26	25	30				
	Diet	31	28	25	29				
Cumulative till	Fine	225	196	153	183	74.0	0.30	0.35	0.30
distal ileum[3]	Coarse	153	170	150	208				
	Diet	189	183	152	196				

[1] Diets Diets: Basal = basal diet, RSE = basal diet + rapeseed expeller, SBM = basal diet + soybean meal, wheat = basal diet + wheat

[2] LSD for interaction between fibre source and diet are presented.

[3] Represents the sum of the segments

[abcd] Means with a different superscript within a parameter represent a significant difference (P < 0.05).

Table 8 *Effect of ingredient composition and fibre supplementation (Arbocel or oat hulls as fine and coarse fibre sources, respectively) on the mean retention time of the liquid fraction of digesta in different segments of the digestive tract (min).*

		Diet[1]					P-value[3)]		
	Fibre source	Basal diet	RSE	SBM	Wheat	LSD[2]	Fibre source	Diet	Fibre source * Diet
Crop	Fine	69	65	39	52	37.3	0.81	0.32	0.713
	Coarse	55	55	41	55				
	Diet	62	60	40	58				
Gizzard	Fine	13	13	10	12	4.3	<0.001	0.23	0.18
	Coarse	13	19	16	16				
	Diet	13	16	13	14				
Duodenum	Fine	0.4	0.8	1.0	0.5	0.6	0.11	0.21	0.071
	Coarse	0.2	0.3	0.4	0.9				
	Diet	0.3	0.6	0.7	0.7				
Jejunum	Fine	44	47	41	38	12.7	<0.001	0.50	0.15
	Coarse	25	32	26	28				
	Diet	34	39	34	38				
Proximal ileum	Fine	44	29	27	26	16.8	0.056	0.45	0.25
	Coarse	22	21	22	28				
	Diet	33	25	24	27				
Distal ileum	Fine	33	27	27	25	12.3	0.22	0.97	0.19
	Coarse	19	25	23	29				
	Diet	26	26	25	27				
Cumulative till	Fine	204	182	145	153	69.1	0.19	0.49	0.28
distal ileum[3]	Coarse	134	152	128	178				
	Diet	169	167	136	165				

[1] Diets: Basal = basal diet, RSE = basal diet + rapeseed expeller, SBM = basal diet + soybean meal, wheat = basal diet + wheat

[2] LSD for interaction between fibre source and diet are presented.

[3] Represents the sum of the segments.

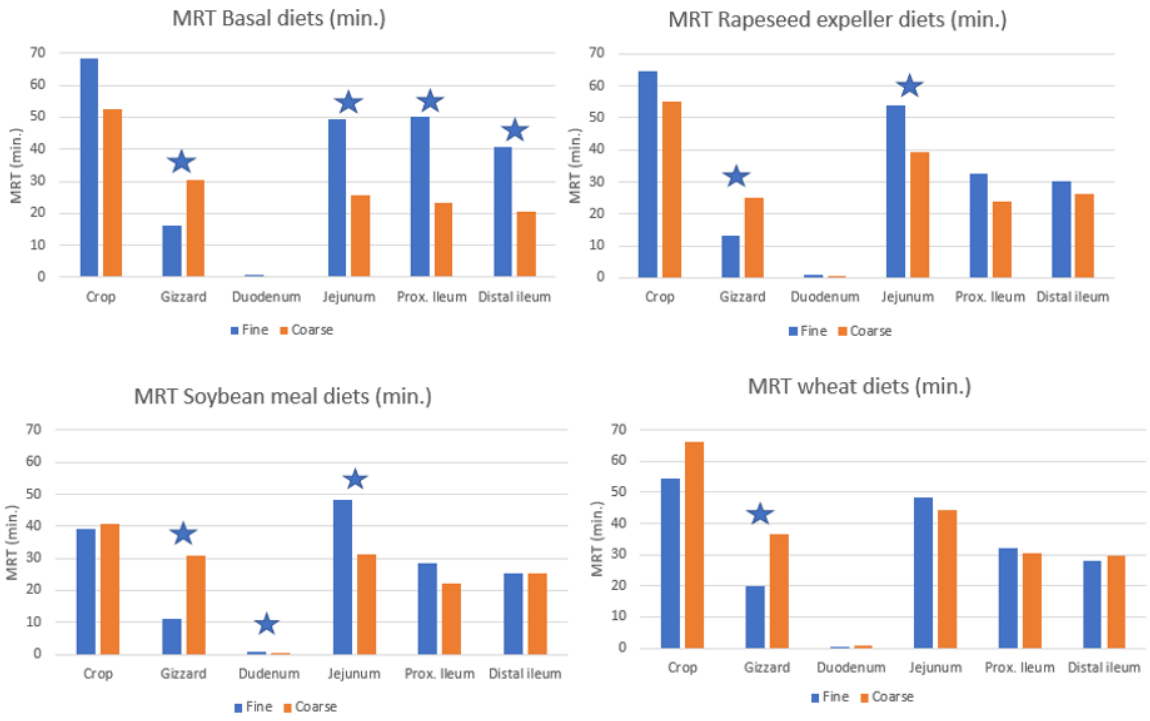

Figure 3 *The mean retention time (MRT) of the solid phase of digesta per segment for a basal diet, a basal diet with rapeseed expeller, a basal diet with soybean meal and a basal diet with wheat, all supplemented with a fine (Arbocel; blue bars) or coarse (oat hulls; orange bars) fibre source. Stars indicate a significant difference (P < 0.05).*

3.4 Digestibility of the diets

The partial digestibility of dry matter and crude protein in different segments of the digestive tract are presented in Table 9. The dry matter (DM) digestibility of the diets in the crop and the gizzard was affected by both ingredient composition and source of fibre supplementation (interaction $P < 0.001$). Diet ingredient composition affected DM digestibility in all segments except for the duodenum. In all segments, the digestibility coefficients were highest for the basal diets, lowest for the rapeseed expeller diets and intermediate for the soybean meal and wheat based diets. Furthermore, supplementing the diets with coarse fibre (oat hulls) reduced the dry matter (DM) digestibility in the duodenum and jejunum (Figure 4). Negative digestibility values were found for the DM digestibility in the crop, gizzard and duodenum likely reflecting a-synchronic transit of digesta and marker through these compartments resulting in less realistic digestibility values for the diets in these compartments.

The crude protein (CP) digestibility of the diets in the crop and gizzard were affected by the interaction between ingredient composition and supplementation of fibre source ($P < 0.001$; Table 9). The CP digestibility of the diets in the gizzard was highest for the wheat based diets, followed by the basal diets and lowest for the rapeseed expeller and soybean meal based diets (Figure 5). This effect was enlarged by including oat hulls in the diets. Furthermore, ingredient composition affected the CP digestibility in the jejunum, proximal ileum and distal ileum. In all segments, the wheat based diets had the highest protein digestibility, and the basal diets showed the lowest values. Furthermore, the protein digestibility of rapeseed expeller and soybean meal diets were intermediate in the jejunum and proximal ileum. In the distal ileum, however, differences in protein digestibility between diets were smaller, but for the wheat based diet still significantly higher than for the basal diet (81 vs. 78%, respectively).

The supplementation of oat hulls as coarse fibre source increased the CP digestibility in the jejunum, proximal and distal ileum. The partial protein digestibility in the jejunum and proximal ileum were 5 till 12% and 2 till 6% higher when oat hulls were supplemented to the diets. The distal ileal protein digestibility was increased by 1 to 3 % by supplementing the diets with oat hulls as a source of coarse fibre, the effect varying between diets containing ingredients with a different level of N linked to the NDF fraction. The ileal digestibility of the NDF fraction was affected by diet ingredient composition and oat hulls instead of cellulose (Arbocell) supplementation. Digestibility values for the dietary treatments ranged from 9 till 31% for the diets with cellulose inclusion, and from -13 till 17% for the diets supplemented with oat hulls. The estimated digestibility of the N in NDF fraction was increased by oat hull supplementation from 83 to 84% in the basal diets, from 16 to 21% in the RSE diet, from 76 to 80% in the SBM diet and from 54 to 63% in the wheat based diet. However, the latter values should be considered with care, related to a relatively high inaccuracy of the determination of the N-NDF concentration in diet and digesta samples, in particular when the concentrations are low as was the case in the diets and excreta samples in the present study.

By the difference method, the ileal dry matter and protein digestibility of rapeseed expeller, soybean meal and wheat were calculated (Table 10). The digestibility values of the ingredients were numerically slightly higher for the estimates based on the oat hull supplemented diets compared to the cellulose (Arbocell) diets. Due to the relatively high analytical error and related inaccuracy of the N-NDF analysis in the diets and digesta samples no reliable estimates for the digestibility values of the N-NDF fraction in the ingredients could be derived.

The faecal digestibility for dry matter of the diets are presented in Table 11. The average DM digestibility was 81.7%. Numerically, the DM digestibility was highest for the basal diet, followed by the wheat based diet, the soybean meal and the rapeseed expeller based diets. Faecal dry matter digestibility was numerically slightly higher in the oat hull supplemented diets compared to the diets supplemented with cellulose.

Table 9 *Effect of supplementing fibre sources to a basal diet, a basal diet with rapeseed expeller, a basal diet with soybean meal and basal diet with wheat on nutrient digestibility (%) in different segments of the digestive tract.*

			Diet[1]				P-value[3)]		
	Fibre source	Basal diet	RSE	SBM	Wheat	LSD	Fibre source	Diet	Fibre source * Diet
Dry matter									
Crop	Fine	2.1	-0.1	0.1	-0.4	2.21	0.073	0.007	<0.001
	Coarse	0.5	-1.0	1.3	4.9				
	Diet	1.3	-0.6	0.7	2.2				
Gizzard	Fine	20.4	-7.2	7.0	14.8	12.42	<0.001	<0.001	<0.001
	Coarse	-30.1	-73.6	-38.6	3.4				
	Diet	-4.9	-40.4	-15.8	9.1				
Duodenum	Fine	-17.4	-21.4	-40.2	-50.0	47.80	0.019	0.58	0.082
	Coarse	-84.0	-55.2	-76.8	-28.7				
	Diet	-50.7	-38.3	-58.5	-39.4				
Jejunum	Fine	68.2	60.4	62.2	62.5	3.02	0.002	<0.001	0.064
	Coarse	67.2	64.4	64.6	66.8				
	Diet	67.7^{a}	62.4^{c}	63.4^{b}	64.7^{b}				
Proximal ileum	Fine	71.6	66.3	68.3	69.3	3.68	0.20	<0.001	0.95
	Coarse	72.7	66.7	69.7	71.2				
	Diet	72.1^{a}	66.5^{c}	69.0^{b}	70.3^{a}				
Distal ileum	Fine	73.3	67.1	68.8	69.6	3.04	0.19	<0.001	0.87
	Coarse	73.8	67.7	69.7	71.7				
	Diet	73.5^{a}	67.4^{c}	69.3^{b}	70.6^{b}				
Crude protein									
Crop	Fine	0.5^{ab}	-0.2^{b}	2.5^{ab}	-0.6^{bc}	3.97	0.921	0.020	0.036
	Coarse	0.8^{ab}	-4.2^{c}	1.4^{ab}	3.8^{a}				
	Diet	0.7	-2.2	1.9	1.6				
Gizzard	Fine	14.7^{d}	3.9^{ef}	3.3^{f}	26.8^{c}	7.95	<0.001	<0.001	<0.001
	Coarse	30.7^{bc}	11.3^{de}	36.3^{ab}	43.2^{a}				
	Diet	22.7	7.6	19.8	35.0				
Jejunum	Fine	51.3	58.4	55.9	65.2	3.86	<0.001	<0.001	0.092
	Coarse	58.4	64.7	67.8	70.6				
	Diet	54.8^{c}	61.5^{b}	61.9^{b}	67.9^{a}				
Proximal ileum	Fine	67.8	74.0	72.8	77.4	3.91	<0.001	<0.001	0.261
	Coarse	71.6	75.5	79.4	79.5				
	Diet	69.7^{c}	74.8^{b}	76.1^{ab}	78.4^{a}				
Distal ileum	Fine	77.6	79.2	79.0	80.1	2.77	0.008	0.025	0.57
	Coarse	79.0	80.0	82.3	82.2				
	Diet	78.3^{b}	79.6^{ab}	80.6^{a}	81.2^{a}				
NDF[3]									
Distal ileum	Fine	8.8	26.5	31.0	21.0	19.1	0.007	<0.001	0.66
	Coarse	-13.4	21.3	17.3	8.0				
	Diet	-2.3^{b}	23.9^{a}	24.2^{a}	14.5^{a}				
N-NDF[4]									
Distal ileum	Fine	82.8	16.4	75.5	54.0	7.0	0.007	<0.001	0.48
	Coarse	84.1	21.4	80.1	63.0				
	Diet	83.5^{a}	18.9^{d}	77.8^{b}	58.5^{c}				

[1] Diets: Basal = basal diet, RSE = basal diet + rapeseed expeller, SBM = basal diet + soybean meal, wheat = basal diet + wheat

[2] LSD for interaction between fibre source and diet are presented.

[abcd] Means with a different superscript within a parameter represent a significant difference (P < 0.05).

[3]Calculated based on the calculated NDF concentrations of the diet upon diet formulation.

[4]Based on analysed concentrations of N-NDF in the diets, which deviated substantially from the calculated values upon diet formulation.

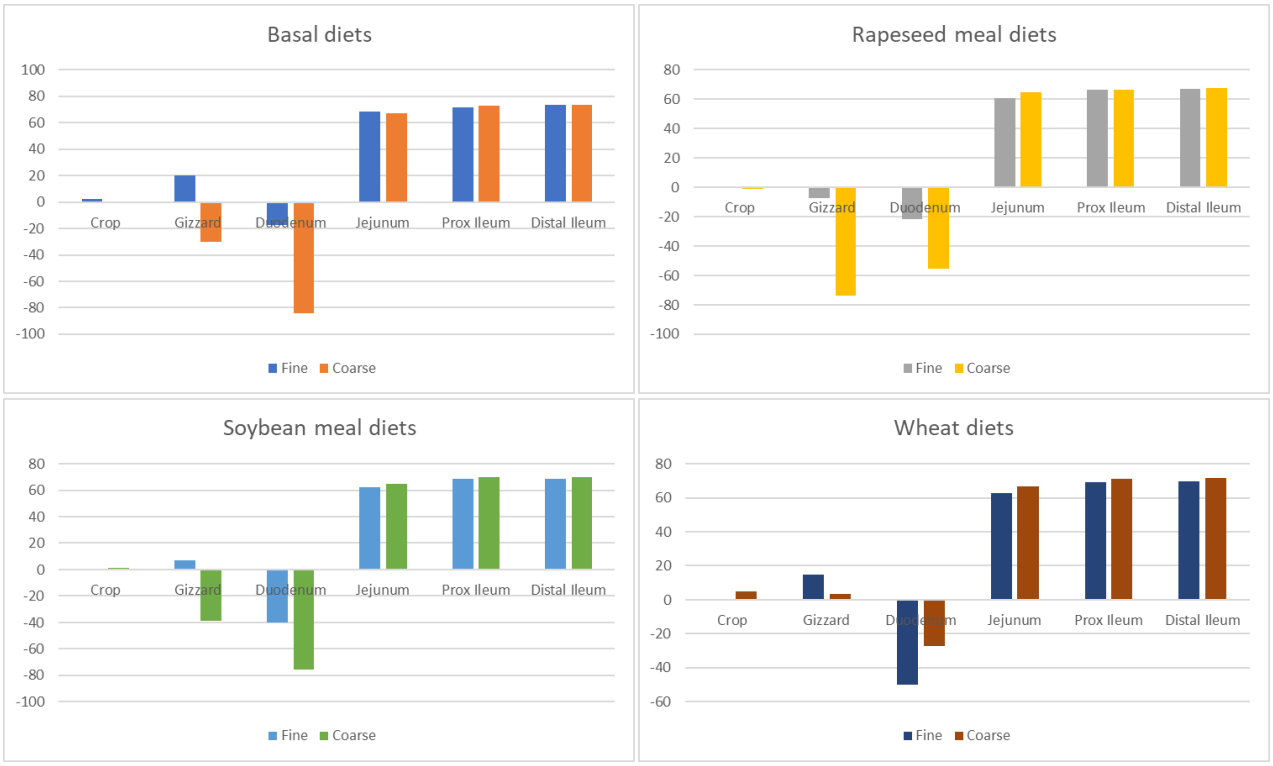

Figure 4 *The digestibility of dry matter (DM %) per segment for a basal diet, a basal diet with rapeseed expeller, a basal diet with soybean meal and a basal diet with wheat, all supplemented with a fine or coarse fibre source (Arbocel or oat hulls, respectively).*

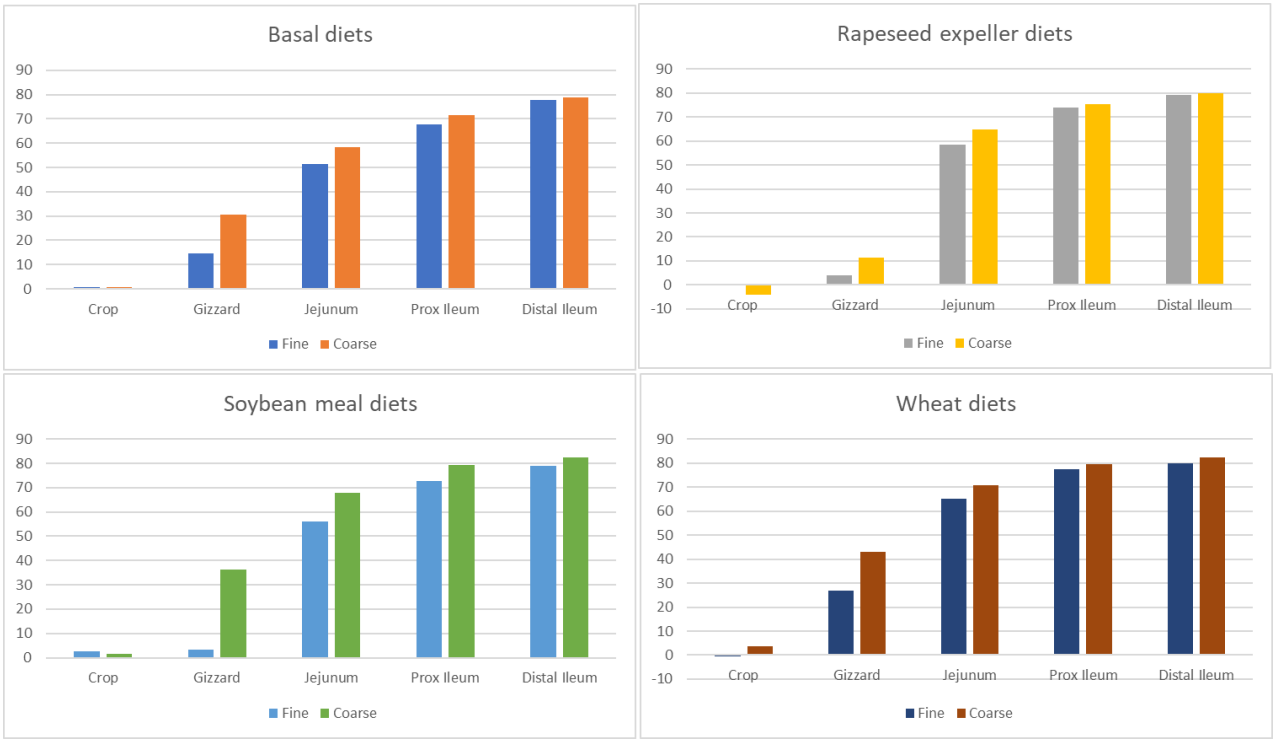

Figure 5. *The crude protein digestibility (CP %) per segment in a basal diet, basal diet with rapeseed expeller, basal diet with soybean meal and basal diet with wheat, all supplemented with a fine or coarse fibre source (Arbocel or oat hulls, respectively).*

Table 10 *Ileal digestibility (%) of dry matter and protein of rapeseed expeller, soybean meal and wheat calculated by difference from the experimental and basal diets supplemented with Arbocel or oat hulls.*

		Ingredient		
	Fibre source	RSE	SBM	Wheat
Dry matter	Fine	55.7	60.5	66.0
	Coarse	56.4	62.0	69.6
Crude protein	Fine	81.1	80.1	83.6
	Coarse	81.3	85.4	87.0

[1] Diets: Basal = basal diet, RSE = basal diet + rapeseed expeller, SBM = basal diet + soybean meal, wheat = basal diet + wheat

[2] LSD for interaction between fibre source and diet are presented.

[abcd] Means with a different superscript within a parameter represent a significant difference (P < 0.05).

Table 11 *Faecal digestibility of dry matter of the basal diet, the basal diet with rapeseed expeller, the basal diet with soybean meal and the basal diet with wheat supplemented with a fine or coarse fibre source.*

Diet	Basal		RSE		SBM		Wheat	
Fibre source	*Fine*	*Coarse*	*Fine*	*Coarse*	*Fine*	*Coarse*	*Fine*	*Coarse*
Dry matter	85.6	86.8	78.1	79.3	78.0	80.3	81.9	83.8

3.5 Gizzard parameters

Data for the weight of digesta and the full gizzard weight showed an interaction between diet ingredient composition and supplementation of fibre source (Table 12). For both parameters, all diets dietary treatments containing coarse fibres did not differ among each other, and were higher than the four in which Arbocel was included. The full gizzard weight was highest for the wheat diet with fine fibres, followed by the other diets containing Arbocel. Furthermore, the digesta weight was also highest in birds fed the wheat-based diet with fine fibres and the rapeseed expeller -based diet with fine fibres, followed by the basal and soybean meal diet containing fine fibres. Adding coarse fibres to the diets resulted in higher empty gizzard weight (24.6 vs 22.7 g per kg BW; P < 0.001) and a lower gizzard score (1.8 vs 2.4; P < 0.001) compared to the diets containing Arbocel. Dietary ingredient composition did not affect any of the gizzard related parameters.

Table 13 *Effects of diet composition and fibre source on empty gizzard weight, full gizzard weight, gizzard content (g per kg BW), and gizzard score.*

		Diet[1]					P-value[3)]		
	Fibre source	Basal diet	RSE	SBM	Wheat	LSD[2]	Fibre source	Diet	Fibre source * Diet
Weight, empty	Fine	22.5	22.4	22.1	23.9	1.43	<0.001	0.112	0.414
	Coarse	24.9	24.7	24.1	24.8				
	Diet	23.7	23.6	23.1	24.4				
Weight, full	Fine	27.1^{c}	27.7^{c}	27.0^{c}	30.7^{b}	2.39	<0.001	0.203	0.042
	Coarse	33.7^{a}	34.6^{a}	34.2^{a}	33.5^{a}				
	Diet	30.4	31.2	30.6	32.1				
Digesta weight	Fine	4.5^{c}	5.3^{bc}	4.9^{c}	6.7^{b}	1.45	<0.001	0.172	0.019
	Coarse	8.8^{a}	9.9^{a}	10.1^{a}	8.7^{a}				
	Diet	6.7	7.6	7.5	7.7				
Score	Fine	2.3	2.3	2.6	2.3	0.5	<0.001	0.838	0.715
	Coarse	1.8	1.7	1.8	1.9				
	Diet	2.0	2.0	2.2	2.1				

[1] Diets : Basal = basal diet, RSE = basal diet + rapeseed expeller, SBM = basal diet + soybean meal, wheat = basal diet + wheat

[2] LSD for interaction between fibre source and diet are presented.

[abcd] Means with a different superscript within a parameter represent a significant difference (P < 0.05).

3.6 pH of digesta in segments of the digestive tract

Both diet composition and supplemented fibre source affected the pH of digesta in the gizzard and the duodenum and tended to affect the pH of digesta in the jejunum and distal ileum (Table 13). However, the results differed between segments.

The pH of digesta in the jejunum, proximal ileum and distal ileum was affected by the supplementation of fibre (Table 14). Digesta in all the segments had a higher pH due to coarse fibre supplementation, except for the gizzard in which the pH was lower (Figure 8). The ingredient composition also affected the pH of the crop, jejunum, proximal ileum and distal ileum. These results also differed between segments. In the crop, the pH of digesta of the basal diet was highest, followed by the soybean meal and wheat-based diet and was lowest for the rapeseed expeller diet. The wheat diet and the basal diet had a comparable pH in the jejunum, which was higher than the soybean meal diet while the lowest pH was observed for the rapeseed expeller diet. In the proximal ileum the pH in the wheat diet was lower compared to the other three diets, and in the distal ileum the wheat and soybean meal diets were lower compared to the basal and rapeseed diets.

Table 14 *Effects of diet composition and fibre source on the pH of digesta in different segments of the digestive tract.*

		Diet[1]					P-value[3)]		
	Fibre source	Basal diet	RSE	SBM	Wheat	LSD[2]	Fibre source	Diet	Fibre source * Diet
Crop	Fine	6.16	5.03	5.55	5.58	0.332	0.801	<0.001	0.641
	Coarse	6.09	5.01	5.41	5.72				
	Diet	6.12^{a}	5.02^{c}	5.48^{b}	5.65^{b}				
Gizzard	Fine	4.56^{a}	3.97^{b}	4.45^{a}	3.85^{bc}	0.255	<0.001	<0.001	0.009
	Coarse	3.61^{c}	3.05^{e}	3.47^{d}	3.42^{d}				
	Diet	4.09	3.51	3.96	3.64				
Duodenum	Fine	6.25^{b}	6.16^{bc}	6.07^{c}	6.02^{c}	0.173	0.001	0.251	0.004
	Coarse	6.23^{b}	6.24^{b}	6.19^{bc}	6.45^{a}				
	Diet	6.24	6.20	6.13	6.23				
Jejunum	Fine	6.22	6.08	6.19	6.19	0.084	<0.001	<0.001	0.099
	Coarse	6.33	6.21	6.23	6.38				
	Diet	6.28^{a}	6.15^{c}	6.21^{b}	6.28^{a}				
Proximal ileum	Fine	7.08	7.19	7.14	6.66	0.234	<0.001	<0.001	0.166
	Coarse	7.62	7.41	7.51	7.20				
	Diet	7.35^{a}	7.30^{a}	7.32^{a}	6.93^{b}				
Distal ileum	Fine	7.68	7.60	7.04	7.13	0.225	<0.001	<0.001	0.072
	Coarse	8.06	7.82	7.67	7.66				
	Diet	7.87^{a}	7.71^{a}	7.35^{b}	7.39^{b}				

[1] Diets: Basal = basal diet, RSE = basal diet + rapeseed expeller, SBM = basal diet + soybean meal, wheat = basal diet + wheat

[2] LSD for interaction between fibre source and diet are presented.

[abcd] Means with a different superscript within a parameter represent a significant difference (P < 0.05).

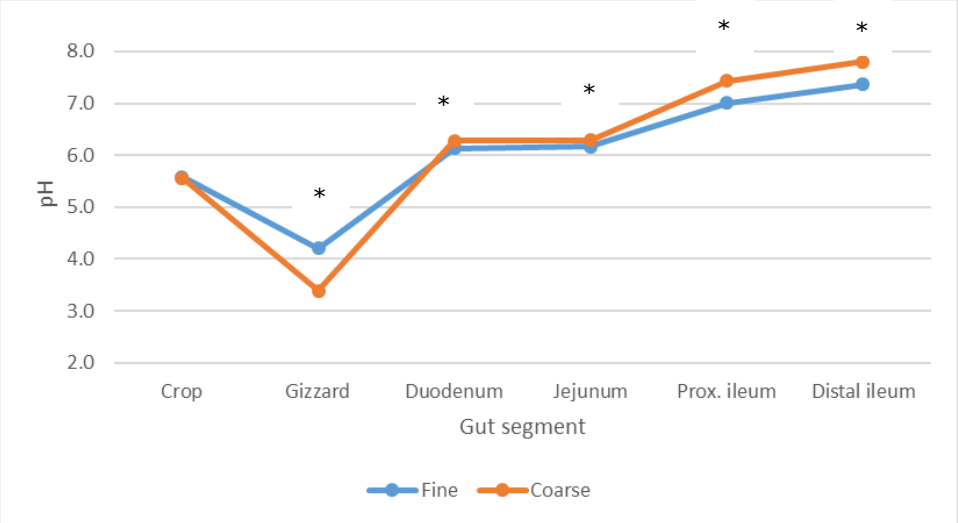

Figure 8 *Effect of fibre supplementation (fine cellulose or coarse oat hulls) on digesta pH per gut segment. Star indicates a significant difference (P < 0.05).*

4 Discussion

This study was conducted to study the extent and kinetics of protein digestion in broilers as affected by the inclusion of fine or coarse sources of dietary fibre in the form of either fine cellulose powder (Arbocel) as reference or coarse oat hulls. It was hypothesized that inclusion of coarse oat hulls would prolong the MRT of digesta in the foregut and reduce digesta pH which could increase protein digestibility, especially in conditions when a relatively high proportion of dietary protein is entrapped in or linked to the fibre (NDF) fraction. Ingredients differ in the concentration and proportion of N linked to the NDF fraction. In the present study wheat, soybean meal and rapeseed expeller were included in the experimental diets, these ingredients having a N-NDF concentration of 3, 6 and 13 g N-NDF per kg, respectively. In the study largely purified basal diets were used in which for the other experimental diets wheat (41%), rapeseed expeller (25%) or soybean meal (25%) were included by replacing in proportion the main ingredients (maize starch, wheat, potato protein, sucrose, egg albumin, and soy oil) in the basal diet. Preliminary in vitro work showed that in these ingredients at least 71, 65 and 68% of the N in the NDF fraction consists of N present in amino acids, excluding the contribution of Met, Cys and Trp in these estimations (van Krimpen, unpublished; Appendix 2). In the experimental diets the calculated proportion of nitrogen linked to or entrapped in the NDF fraction was about 11% in the RSE diet, 7% in the wheat based diet and 4-5% in the SBM based diet.

In contrast to expectation, wet sieve analysis did not reveal differences in particle size distribution between the diets containing either purified cellulose or oat hulls, where the latter fraction was supposed to be more coarse of nature. The purified nature of ingredients as major part of the composition of the experimental diets and the physical/mechanical conditions during pelleting of the diets might have reduced the contrast in particle size distribution between the diets containing the different sources of fibre. Visual inspection of the oat hull diets, however, clearly showed the presence of oat hull particles in the diets.

The results of the study show that kinetics of transit of digesta in the GIT of broilers is affected by inclusion of coarse oat hulls in the diet, while also ileal protein digestibility was improved when including oat hulls in the diet. The former is in line with the hypothesis of the study.

Kinetics of digesta passage and nutrient digestibility

The digesta passage rate and the mean retention time of the solid and liquid part of digesta was clearly affected in various segments of the digestive tract by the source of fibre supplementation (cellulose or oat hulls) and by ingredient composition only for the solid part of digesta in the gizzard. The overall retention time of the solid phase of digesta till the distal ileum ranged from 153 – 225 min in birds fed diets supplemented with cellulose and from 150 - 208 min in birds fed the diets supplemented with oat hulls. The overall retention time of the liquid phase of digesta till the distal ileum ranged from 145 - 204 min in birds fed the diets supplemented with cellulose and from 128 – 178 min in birds fed the diets supplemented with oat hulls. Within dietary treatment, the mean retention time of the liquid fraction of digesta was in most segments slightly shorter compared retention times of the solid phase, in line with results of e.g. Chen (2017).

The results of this study show an overall improved (distal) ileal protein digestibility by 1–3 % by supplementing the diets with oat hulls as a source of coarse fibre, the effect varying between diets containing ingredients with a different level of N linked to the NDF fraction. One should consider that the magnitude of the effect might be somewhat reduced by the fact that supplementation of oat hulls to the diet also increased the amount of protein linked to the NDF fraction as oat hulls (CP, 35 g/kg) were assumed to contain almost 3 g N-NDF per kg, equivalent to 18 g CP per kg diet. If this fraction would have an assumed low N-digestibility, as also supposed to be mainly entrapped in the fibre matrix, this could have reduced the ileal CP digestibility of the experimental diets by up to 1%. The former is under the assumption that (Arbocel) cellulose is very low in N and N-NDF content.

The largest effect of oat hull supplementation on ileal protein digestibility was observed for the diet with 25% rapeseed expeller. Similar, but generally larger effects of oat hull inclusion on intestinal protein digestibility were also noticed in more proximal segments of the intestinal tract (gizzard, jejunum, and proximal ileum). As the protein digestibility values of the diets reflect the digestibility of the sum of all protein present in ingredients composing the diet, it cannot be derived to which extent the additional "digestive extraction" of protein is related to the improvement of the digestibility of the N and protein fraction linked to the NDF fraction in the test ingredients as the digestibility of the N-NDF fraction in the ingredients could not be estimated properly in the present study (see below).

As hypothesized, the MRT of both the solid and the liquid phase of digesta in the gizzard was prolonged from 11 - 20 to 25 - 37 min, depending on the ingredient composition of the diet, when the diets were supplemented with oat hulls instead a purified cellulose as source of fibre. As a consequence of the prolonged retention time, the pH of the digesta in the gizzard was lower when supplementing oat hulls. The longer retention time and the lower pH in the gizzard likely have resulted in a longer exposure of dietary protein, including protein linked to the fibre fraction, towards pepsin, which could explain the larger and more rapid digestion of the protein fraction in the remainder of the digestive tract. In addition, ingredient composition interacted with the supplementation of oat hulls with respect to the pH in the gizzard. The rapeseed expeller and wheat based diet lowered the pH in the gizzard compared to the basal and SBM diet, the effect being enforced by the supplementation of oat hulls. In other segments of the GI-tract, the pH was also affected by diet ingredient composition and supplementation of fibre source, however, no statistical interaction between both factors was observed. Furthermore, supplementation of oat hulls also increased the empty weight of the gizzard, indicating a stronger development of this organ and a potentially higher capacity for physical grinding and mixing of stomach digesta and related reduction of digesta particle size, which also could contribute to increasing nutrient digestion rate. The results of the present study did not show effects of ingredient composition using mostly purified ingredients or additional inclusion of RSE, SBM or wheat, as sources of protein and energy, on the gizzard development, indicating a rather specific effect of the inclusion of 5% oat hulls. Similar effects of oat hulls in broilers were found by Jiménez-Moreno et al. (2009), Hetland et al. (2003) and Sacranie et al. (2012). Sacranie et al. (2012) diluted a diet with 15% coarse hulls (an equal mixture of hulls from oats and barley) in broilers, which resulted in an increased weight and digesta content in the gizzard and a lower gizzard pH, while growth performance was not reduced.

The improved ileal protein digestibility when supplementing coarse oat hulls indicates that the effect on the digestibility of the protein in the diet per se is higher than the possible negative effect of including more fibre in the diet, related to the potential increased loss of endogenous protein when the dietary fibre level is increased. The presence of non-fermentable fibre in coarse form in the digestive tract could exert abrasive effects on the intestinal mucosa, thereby stimulating mucosal cell turnover and loss of mucus proteins and other mucosal proteins (endogenous proteins), potentially lowering the apparent digestibility of protein and amino acids as measured in the present study. It was concluded by Kluth and Rodehutscord (2009) that the dietary fibre level using a purified α-cellulose can affect the amount of AA inevitably lost at the terminal ileum. The results in that study suggested that there was no effect of an enhanced fibre level in the diet on AA composition of ileal endogenous CP loss in broilers. Results of the present study did not allow to discriminate between N, as a marker for protein, from dietary and endogenous origin in the digesta samples obtained. Moreover, it should be mentioned that in our study results of the effects of oat hull supplementation were compared with the inclusion of Arbocel (cellulose) having a fine, powder like structure, meaning that effects observed in the present study relative to the treatments with cellulose in the diets are related to the use of oat hulls with a coarse structure.

Originally it was the intention to also determine the ileal digestibility of the N-NDF fraction in the diets and of this fraction in the test ingredients wheat, SBM and RSE. The latter could principally be estimated applying the so called difference method using also the digestibility values of this fraction for the purified basal diets and the experimental diets in the study. For this purpose, the concentration of N in the NDF fraction in both the experimental diets and in ileal digesta and excreta of the birds was analysed. The analysed concentration of N in the NDF fraction of the diets, however, differed rather largely and inconsistently from the calculated concentration in the diets upon diet formulation.

Some additional analysis on this fraction in the same samples in a different laboratory confirmed the difficulty to quantify this fraction in samples with a relatively low concentration of N linked to the NDF fraction (<1.7 g N-NDF/kg diets and 0.04-0.26 g N-NDF/kg dried ileal digesta). Therefore, the analytical data did not allow us to calculate accurate and reliable ileal digestibility values for the N-NDF fraction in the experimental diets and in wheat, SBM and RSE, analysed to contain 3, 6 and 13 g N-NDF per kg, equivalent to about 81, 38 and 19 g CP-NDF per kg, respectively. An additional complicating factor for the calculation of the N-NDF fraction in the test feed ingredients (RSE, SBM and wheat) was the presence of a substantial level of N-NDF in oat hulls (3 g N-NDF per kg) used as coarse fibre fraction in half of the experimental diets. Analysis of the N in NDF fraction is based on a N-analysis in the NDF residue of a sample. The NDF residue is obtained as a residue after an intensive solubilization procedure involving boiling of a sample in a neutral detergent. The procedure seems to be subject to considerably high between sample run and lab variation. The accuracy of analysis of the N-NDF fraction in feed and digesta samples is therefore relatively low, in particular when concentrations are relatively low.

The results of the present study suggest that protein and amino acid digestibility of diets can be improved by inclusion of high fibre ingredients which increases retention time of digesta in the proximal part of the digestive tract and improves grinding and incubation function of the gizzard. To further evaluate such concept using more practical diets more knowledge needs to be acquired on whether or not other fibre sources than oat hulls induce similar effects. In addition, optimal dose levels of fibre sources need to be established which maximize the positive effect on protein digestion. The latter is also important in relation to the consequences of using fibre rich ingredients on the energy density of the diet and related effects on voluntary feed intake and growth performance, when using nutrient balanced diets. Also effects on gut health and functionality need to be evaluated when including higher levels of fibre sources to the diet. In addition, effects of technological or enzymatic pre-processing of fibre rich ingredients on the effects of digesta transit and improvement of N and amino acid digestibility of the diet are worthwhile to study in order to maximize nutritional and functional value of the future diets for poultry, likely containing more fibre rich ingredients.

5 Conclusions

It can be concluded from the present study that inclusion of 5% coarse oat hulls in broiler diets increases digesta retention time in particular in the proximal part of the digestive tract, reduces pH of digesta in the gizzard and increases ileal protein digestibility by at least by 1-3%. The actual effect on protein digestibility might be somewhat higher as a result of the negative effect of the presence of a low digestible N-NDF fraction in oat hulls which were used in the present study to provide a source of coarse crude fibre in the diet, which interfered with the estimation of the quantitative improvement in ileal protein digestibility of the diets. Part of the improvement in ileal protein digestibility might be related to the physical release of a potentially digestible protein fraction linked to the fibre fraction present in wheat, soybean meal and rapeseed expeller, containing different concentrations of N and protein in the fibre (NDF) fraction. The ileal digestibility of the N-NDF fraction in the diet, although difficult to measure experimentally, seems to increase to a small extent by oat hull inclusion in the diet.

Further research is required to optimize the effects of coarse fibre ingredients on the digestive function of the gut in broilers and to evaluate the consequences for the improvement of protein digestibility of diets containing relative high levels of protein entrapped in the fibre matrix. Such diets could be more common in future poultry production in which fibre rich co-products are used as part of more circular production systems for human food.

Appendix 1 Ingredient and nutrient composition of the starter diet

Dietary ingredients (%)	
Ingredient	
Maize	40.00
Soyabean meal	23.94
Wheat	16.77
Oat hulls	5.00
Potato protein	4.00
Soy oil	3.50
Palm oil	2.00
Monocalcium phosphate	1.63
Chalk (fine)	1.50
Premix[1]	0.50
Sodium bicarbonate	0.41
DL-Methionine	0.30
L-Lysine HCl	0.28
L-Threonine	0.09
Salt	0.05
Clinacox	0.02
Axtra PHY (phytase)	0.01
Rovabio Excel AP[3]	0.01
Calculated content[3]	g/kg
AME_n (MJ/kg)	11.90
Dry Matter	886.63
Crude ash	62.73
Crude protein	201.47
Crude fat	76.77
Crude fibre	37.13
Starch	348.69
Sugar	36.02
NDF	115.94
ADF	48.51
ADL	3.85
Ca	9.20
P	6.96
K	7.70
Na	1.40
Cl	1.50
Dig. Lys	11.50
Dig. met	5.91
Dig. Cys	2.62
Dig. Met+cys	8.51
Dig. Thr	7.50
Dig. Trp	2.07
Dig. Ile	7.62
Dig. Arg	11.12
Dig. Phe	9.17
Dig. His	4.53
Dig. Leu	15.17
Dig. Tyr	6.78
Dig. Val	8.50
Dig. Ala	8.12
Dig. Asp	17.61
Dig. Glu	31.38
Dig. Gly	6.82
Dig. Pro	10.55
Dig. Ser	8.62
Phytase (FTU)	500

[1] Provided per kilogram of complete diet: vitamin A 10.000 IE; vitamin D3 2.500 IE; vitamin E 50 mg; vitamin K3 1.5 mg; vitamin B1 2.0 mg; vitamin B2 7.5mg; vitamin B6 1.0 mg; vitamin B12 20 µg; niacin 35 mg; D-pantothenic acid 12 mg; choline chloride 460 mg; folic acid 1.0 mg; biotin 0.2 mg; iron 80 mg; copper 12 mg; manganese 85 mg; zinc 60 mg; iodate 0.8 mg; selenium 0.15 mg.

[2] CVB matrix values (CVB, 2011) were used for diet formulations.

[3] Rovabio Excel, NSPase containing xylanase, β-glucanase, cellulase, and pectinase activities.

Appendix 2. Contents of nitrogen and amino acids in NDF fraction in various feed ingredients

Ingredient	CP	Crude fat	Crude fibre	Starch	NDF	N in NDF	N in NDF (%)	N as AA in NDF (%)
Sunflower meal (CP high)	339	8	188	10	292	3.1	5.7	75.4
Soybean meal	484	5	37	10	94	5.8	7.5	65.1
Sunflower meal (CP low)	246	10	265	24	396	3.5	9.0	61.6
Maize	76	33	20	588	73	1.5	12.4	68.2
Wheat	117	13	22	542	100	3.1	16.8	70.7
Lucerne	145	16	298	12	434	5.9	25.5	80.0
Rapeseed meal	305	26	132	22	287	12.5	25.6	68.3
Maize DDGS	279	128	62	10	303	12.6	28.4	74.1
Peas (conventional)	191	19	47	428	114	2.0	6.5	
Peas (organic)	196	20	48	435	94	2.5	7.8	
Field beans	260	18	64	322	148	3.8	9.2	
Lupins	331	50	140	6	232	2.5	4.7	
Sunflower meal	341	27	169	4	297	7.1	12.9	
Sorghum	94	32	21	606	80	4.4	29.2	

Data: M. van Krimpen (2018)

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To explore the potential of nature to improve the quality of life

Wageningen Livestock Research
P.O. Box 338
6700 AH Wageningen
The Netherlands
T +31 (0)317 48 39 53
E info.livestockresearch@wur.nl
www.wur.nl/livestock-research

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