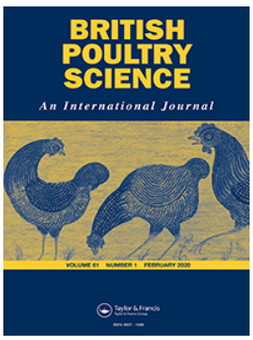

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Effects of three major protein sources on performance, gut morphology and fermentation characteristics in broilers

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ABSTRACT

1. This study determined the effects of three protein sources (PS), each at two digestibility crude protein (DCP) levels, on performance, gut morphology and fermentation characteristics in the hindgut of broilers.
2. It was hypothesised that broilers fed ingredients high in indigestible CP, i.e. rapeseed meal (RSM) or maize gluten (MG), could potentially cause reduced growth, impaired gut health, and more protein fermentation products in caecal digesta. Increasing the DCP level in each of the indigestible CP diets may compensate for these detrimental effects.
3. In total, 288 one-d-old male Ross 308 broilers were used in a completely randomised 3 × 2 factorial design, with six replicate pens per treatment. Three PS: soybean meal (SBM), rapeseed meal (RSM) or maize gluten (MG), and two DCP levels: 15.8 and 17.2% were used.
4. Broilers fed SBM had increased feed intake and BWG and improved FCR compared with those fed RSM and MG diets. Broilers fed high DCP had better performance compared with those on low DCP. No significant effects of PS or DCP level were found on gastrointestinal tract development, caecal ammonia or volatile fatty acid concentrations.
5. Broilers fed SBM had longer villi, smaller crypts and increased villus height to crypt depth ratio compared with those fed RSM and MG diets. Broilers fed RSM diet had a lower caecal pH, and had 16.5% and 14.9% more branched chain fatty acid contents in caecal digesta compared with those fed SBM and MG diets, respectively, indicating more proteolytic fermentation.
6. Replacing SBM by RSM and MG negatively affected growth performance and gut morphology. Hindgut protein fermentation was substantially increased in RSM fed birds.
7. To a certain extent, retarded growth performance in RSM and MG fed birds could be counterbalanced by increasing the dietary level of digestible CP.

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(in-)digestible protein; broilers; caecal fermentation; gut morphology; performance

Introduction

Broilers can attain a BW of 2 kg by consuming 3 kg of feed within 35 d (Choct 2009). Such modern broilers have been selected to achieve a low FCR and a high growth rate. This rapid growth rate requires a high concentration of digestible protein (e.g. digestible essential amino acids). Due to the growing world human population and increasing demand for proteins for food (Alexandratos and Bruinsma 2012), in the coming decades increased competition for high-quality proteins between feed and food is expected. A scarcity of commonly-used protein sources for feed, e.g. soybean meal, might occur. As a consequence, the feed sector has to cope with low-quality protein sources, with increased levels of indigestible protein.

Some dietary proteins are not well digested in the small intestine, and consequently undigested dietary protein enters, together with undigested endogenous proteins, into the caeca and colon. Proteins, peptides and amino acids that bypass the ileum, are a potential substrate for fermentation by microbiota in the caeca. These undigested protein substances may stimulate the growth of N-utilising microbiota (Reid and Hillman 1999), leading to increased levels of toxic compounds, such as biogenic amines, phenols, and cresols (Apajalahti and Vienola 2016). These toxic compounds may be detrimental for bird performance and gut health (Thomke and Elwinger 1998). Moreover, as a result of protein fermentation, higher levels of branched chain fatty acids and elevated levels of ammonia per gram of non-starch polysaccharides (NSP) may be produced (Bikker et al. 2006).

Gut morphology is an important indicator of intestinal health (Zang et al. 2009). There is a scarcity of published data regarding the effects of protein source on gut morphology in broilers. In addition, the available data show contradictory results. Buwjoom et al. (2010) found no significant effect for three different dietary CP levels (10, 16 and 22%) on villus height and crypt depth in broilers. Contrary to this, Laudadio et al. (2012) observed higher villus heights in broilers fed soybean meal-based medium and high CP diets compared with those fed a low CP diet, and recommended a 20.5% dietary CP level to optimise growth performance. It is thought that feeding protein sources with similar amounts of essential amino acids, but with varying amounts of indigestible protein, may have a different impact on broilers.

It was hypothesised that broilers fed ingredients high in indigestible CP, i.e. rapeseed meal (RSM) or maize gluten (MG), would potentially show reduced growth, impaired gut health, and more protein fermentation products in the caecal digesta. Moreover, it was hypothesised that increasing DCP level to a certain extent might compensate for these negative

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effects, e.g. by modifying the ratio between digestible and indigestible CP, and by providing more digestible amino acids from other protein ingredients to stimulate bird performance. The current study investigated the effects of three dietary protein sources, varying in indigestible protein content, supplied at two dietary digestible CP levels on growth performance, gut morphology and hindgut protein fermentation characteristics in broiler chickens.

Materials and methods

Animal ethics

Experimental procedures were in accordance with the Wageningen University and the Netherlands Animal Experimental Committee guidelines and code of practice. Ethical approval was granted before the conduct of the study.

Bird management

In total, 288 male (Ross 308), one-d-old broilers were purchased from a commercial hatchery (Morren Breeds B.V., Lunteren, The Netherlands). Upon arrival, broilers were individually weighed and steel wing tagged before being allotted to one of 36 floor pens (eight birds per pen), equally distributed over three identical climate-controlled rooms, so that each pen had a similar initial total BW. Each pen (1.15 × 1.75 × 0.80 m; L × W × H) had three drinking nipples with a cup underneath connected to a water tank of 10 litres capacity. Pens were separated by solid walls to prevent contact between broilers from different treatment groups. A feeding tray was placed on the floor during week 1 and replaced by a feeding trough thereafter. Feed and water were available *ad libitum* throughout the experiment. Wood shavings were used as litter material. The lighting schedule was maintained at 23l:1d for the first three days and, thereafter, maintained at 16l:8d, with an intensity of approximately 20 lux at bird level, throughout the experimental period. During the first three days, room temperature was set at 32°C, and was gradually decreased to a constant value of 22°C in week 4, which was maintained until the end of the experiment.

Experimental design and treatments

The study was conducted as a completely randomised design (six replicate pens per treatment with eight birds per pen), and treatments were equally distributed over rooms. All birds received the same standard starter diet for the first 7 d of the experiment (CP: 21%; ME: 2895 kcal/kg). After week 1, six dietary treatments were provided which contained three different protein sources: soybean meal (SBM), rapeseed meal (RSM) or maize gluten (MG) each at 15.8% or 17.2% digestible crude protein (DCP) levels. The diets were formulated to meet or exceed the nutrient recommendations for boilers (CVB (Central Bureau for Livestock Feeding) 2007). These dietary treatments were randomly assigned to pens in three rooms (3 × 2 × 6 = 36). As shown in Table 1, the diets were formulated to contain different concentrations of indigestible CP, based on calculated faecal digestible protein contents. Each diet was formulated to have similar concentrations of digestible essential amino acids and to be iso-energetic, on an AMEn basis. Differences in DCP levels within PS were achieved by varying the digestible non-essential amino acid (NEAA) content, through upgrading other protein ingredients. All six dietary treatments were offered as pellets with a size of 2.5 mm for the starter and 4.0 mm for the grower diet. The diets did not contain antimicrobial growth promoters.

Traits measured

Feed intake (FI), water intake (WI) and body weight (BW) gain per pen in each room were recorded at d 8, 15, 22, 29 and 33. Feed intake, BW gain and WI were expressed per bird per d. Weights of dead birds were determined and their BW gain and FI included in the calculation of feed conversion ratio (FCR) per pen.

At the end of the experiment (d 34), six of the eight birds per pen, selected to be closest to the average weight of the group, were euthanised by intravenous T-61 injection and the abdominal cavity opened. On the day of dissection, all birds had free access to feed and water up until the moment of euthanasia. Birds in a pen were euthanised in order of replicate number. The different parts of the gastrointestinal tract (GIT), i.e. the crop, proventriculus, gizzard, duodenum (from the pyloric junction to the pancreo-billiary duct), jejunum (from the pancreo-billiary duct to Meckle's diverticulum), ileum (from Meckle's diverticulum to the ileo-caecal junction), caecum (from the ostium) and colon were segmented. The digesta contents from each segment were immediately removed by gentle squeezing and the empty segments weighed. To obtain sufficient amount of material for analysis, the caecal content of six birds in a pen were quantitatively pooled, thoroughly mixed and the pH determined using a calibrated pH meter before the samples were freeze dried at −20°C pending volatile fatty acid (VFA) and ammonia analyses.

Tissue collection and histological measurements

A gut segment (approximately 2 cm) in the middle of the duodenum was excised, rinsed with cold physiological saline (0.9%) and immediately placed in Bouin's fluid. Thereafter, the samples were transferred into 70% ethanol within 24 h. The samples were embedded in paraffin and sliced at 5 µm thickness for histological examination. Six cross-sections per bird were processed using standard haematoxylin and eosin methods, as described by Owusu-Asiedu et al. (2002). Villus heights and crypt depths were measured on 10 intact, well-oriented villi (from 0.5 cm in the middle of the duodenum) per bird using a compound light microscope equipped with a video camera. Villus height was measured from the tip of the villous to the crypt-villous junction, whereas crypt depth was measured from the crypt-villous junction to the base.

Chemical analysis

Dry matter, organic matter and N contents in the experimental diets were analysed according to standard methods (AOAC International 2006). Ammonia-N in caecal digesta was analysed by the indole phenol-blue method (Novozamsky et al. 1974). The samples were deprotonated by adding 10% (w/v) trichloroacetic acid solution followed by centrifugation. The ammonium was transformed by phenol and hypochlorite in an alkaline solution into a blue

Table 1. Dietary ingredients, and calculated nutrient composition of the experimental diets (g/kg, as-fed basis).

Protein source	Soybean meal		Rapeseed meal		Maize gluten	
Digestible CP level (%)	15.8	17.2	15.8	17.2	15.8	17.2
Ingredients						
Maize	312.8	241.2	300.0	459.6	300.0	305.7
Rapeseed	0.0	0.0	280.7	209.6	0.0	0.0
Maize gluten feed	0.0	0.0	0.0	0.0	185.6	165.5
Soybean meal (>48% CP)	212.5	247.9	20.0	20.0	130.6	118.2
Wheat	149.3	288.6	20.0	20.0	20.0	20.0
Peas	125.0	20.0	10.0	20.0	10.0	20.0
Soy oil	77.9	75.5	81.7	76.8	82.8	82.6
Lucerne	49.6	41.7	40.1	61.7	60.0	75.0
Maize starch	20.0	20.0	155.5	10.0	109.0	85.9
Potato protein (ash<10)	17.0	20.0	63.7	37.3	70.9	73.9
Fish meal (63-68% CP)	0.0	15.0	0.0	70.8	0.0	31.9
Limestone	11.3	10.7	8.6	4.7	11.1	8.7
Monocalcium phosphate	6.8	5.3	6.0	0.1	6.0	3.4
Premix[1]	5.0	5.0	5.0	5.0	5.0	5.0
DL-Methionine	2.7	2.0	1.0	0.6	2.0	1.4
Sodium-bicarbonate	2.7	0.0	0.0	0.0	0.0	0.0
Phytase	2.0	2.0	2.0	2.0	2.0	2.0
L-Threonine	1.9	1.3	0.4	0.3	0.8	0.1
L-Lysine HCL	1.8	0.9	1.2	0.0	1.8	0.0
Salt	1.4	2.9	3.4	1.4	1.3	0.6
L-Tryptophan	0.3	0.0	0.1	0.1	0.2	0.1
L-Arginine	0.0	0.0	0.6	0.0	0.9	0.0
Calculated contents						
ME (MJ/kg)	12.6	12.6	12.6	12.6	12.6	12.6
CP (analysed between brackets)	200.0 (202)	214.0 (214)	210.0 (201)	224.0 (218)	210.0 (200)	224.0 (215)
Digestible CP[2]	158.0	172.0	158.0	172.0	158.0	172.0
Indigestible CP	42.0	42.0	52.0	52.0	52.0	52.0
CP from studied ingredient	103.4	120.6	108.9	81.3	44.5	39.7
Crude fat	102.7	101.1	105.6	112.7	109.8	112.3
Starch	342.9	336.6	333.6	311.9	311.7	297.7
Sugars	37.2	39.0	33.9	30.4	25.9	25.2
NSP[3]	154.0	148.0	167.2	171.5	185.8	185.2
Crude fibre	38.3	33.6	43.0	53.6	43.0	45.8
Digestible lysine	10.6	10.6	10.6	10.6	10.6	10.6
Digestible M + C	7.7	7.7	7.7	7.7	7.7	7.7
Digestible threonine	8.0	8.0	8.0	8.0	8.0	8.0
Digestible tryptophan	2.2	2.2	2.2	2.2	2.1	2.2
Digestible EAA[4]	55.0	56.8	56.1	57.0	56.2	57.7
Digestible NEAA	103.0	115.2	101.9	115.0	101.9	114.3

[1]Premix composition: 12,000 IE retinol, 2,400 IE cholecalciferol, 50 mg dl-a-tocopherol, 1.5 mg menadione, 2.0 mg thiamine, 7.5 mg riboflavin, 3.5 mg pyridoxine, 20 mcg cyanocobalamins, 35 mg niacin, 12 mg D-pantothenic acid, 460 mg choline chloride, 1.0 mg folic acid, 0.2 mg biotin; 80 mg iron, 12 mg copper, 85 mg manganese, 60 mg zinc, 0.40 mg cobalt, 0.8 mg iodine, 0.1 mg selenium, 125 mg anti-oxidant mixture.
[2]Based on data from CVB (Central Bureau for Livestock Feeding) (2007).
[3]NSP = Non-starch polysaccharides, calculated by subtracting the crude protein, fat, starch, sugar, and ash content from the dry matter content.
[4]Som of digestible lysine, methionine+cysteine, threonine, tryptophan, isoleucine, valine and arginine.

coloured, indole phenol-blue by the Berthelot reaction and measured spectroscopically at 623 nm.

For the determination of VFAs, 5 g of caecal samples and 5 ml 0.1 M phosphoric acid were shaken at 100 rpm before being centrifuged (7000 x *g*) for 10 min. Residues were collected and the supernatants again centrifuged (20.817 x *g*) for 10 min. Thereafter 600 µl of the supernatant was taken in a crimp vial and mixed with 600 µl of phosphoric acid containing iso-capronic acid (2.29 g/l concentration) as an internal standard. Volatile fatty acids were separated by gas chromatography using an EM-1000 (30 m × 0.53 mm) column from Alltech (Deerfield, IL, USA) with helium as the mobile phase and detection by a fluorescent infrared detector. Quantification of VFAs was based on a chemical standard solution (Merck) after internal standard correction.

Statistical analysis

The data were analysed with the use of PROC MIXED in SAS (version 9.2; SAS Inst. Inc., Cary, NC) by using the following statistical model:

$$Y_{ijk} = \mu + P_i + L_j + P_i \times L_j + e_{ijk}$$

where Y_{ijk} was the measured response, µ overall mean effect, P_i the i^{th} fixed protein source effect (i = SBM, RSM or MG), and L_j the j^{th} fixed digestible CP level effect (j = 15.8 or 17.2%). $P_i \times L_j$ was the interaction between protein source and digestible CP level, and e_{ijk} the residual error. There were no significant room effects, and so this factor was omitted from the model. Differences were considered significant at a probability level of 5% ($P < 0.05$).

Results

Bird performance

Overall, mortality was low (1.7%) during the study. All performance data of broilers were corrected for mortality by day. PS influenced ($P < 0.001$) FI, whereby broilers fed the RSM (116.5 g/d) diet had a lower FI compared with those fed the SBM diet (120.5 g/d), and FI of birds fed the MG diet (118.9 g/d) was in between (Table 2). FI of broilers fed the high DCP diet had a higher ($P < 0.001$) FI compared with those fed the low DCP diet (120.1 *vs.* 117.2 g/d). PS affected ($P < 0.001$) BWG, with broilers fed the RSM (69.7 g/d) had a lower BWG compared with those fed the SBM diet (77.4 g/d), while BWG of birds fed the MG diet was in between (71.9 g/d). Broilers fed the

Table 2. Least squares means[1] of performance parameters in broilers from 9 to 33 days of age as affected by protein source and digestible crude protein level.

Main effects	Feed intake (g/d)	BWG (g/d)	FCR (g/g)
Protein source			
Soybean meal	120.5[a]	77.4[a]	1.51[a]
Rapeseed meal	116.5[c]	69.7[c]	1.63[b]
Maize gluten	118.9[b]	71.9[b]	1.61[b]
Pooled SE	0.47	0.40	0.013
Digestible CP level			
15.8	117.2[y]	70.7[y]	1.61[y]
17.2	120.1[x]	75.4[x]	1.56[x]
Pooled SE	0.55	0.33	0.011
P-value			
PS	< 0.001	< 0.001	< 0.001
DCP	< 0.001	< 0.001	< 0.001
PS × DCP	0.229	0.198	0.292

[a-c, x-y]Means without a common superscript within a column and a parameter significantly ($P < 0.05$) differ.
[1]Each value represents the mean of 6 replicates (8 birds per replicate) determined over 4 periods (d9-15, d16-22, d23-29, d30-d33).

high DCP diets had a higher (P < 0.001) BWG compared with those fed the low DCP diets (75.4 *vs.* 70.7 g/d), regardless of the protein source. FCR was affected (P < 0.001) by PS, indicating that broilers fed the SBM diet had an improved FCR (1.51) compared with those fed the RSM (1.63) and MG (1.61) diets. Broilers fed the high DCP diet showed an improved FCR (P = 0.001) compared with those fed the low DCP diet (1.56 *vs.* 1.61), regardless of dietary protein source.

Results of WI and water to feed (WF) ratio were affected by a PSxDCP interaction, as shown in Table 3. In low DCP diet, the RSM fed birds had a lower WI compared to the SBM and MG fed birds, whereas in high DCP diet the RSM and MG fed birds had higher WI compared to the SBM fed birds. The difference in WI between broilers fed the low and high DCP level was 16.5% for the RSM diet, whereas this difference was 12.8% and 11.8% for broilers fed the MG and SBM diets, respectively. In the low DCP diet, WF ration was not affected by PS, whereas in the high DCP diet, WF ratio was increased in birds fed the MG and RSM diet compared to birds fed the SBM diet.

Digestive tract measurements

Protein source, as well as DCP level, did not influence the relative empty weights of the gut components and there were no interactions between these treatments (Table 4).

Table 3. Interaction effects of protein source and digestible crude protein level (%) on water intake and water to feed ratio in broilers from 9 to 33 d of age.

Main effects	Water intake (ml/d)[1]	Water to feed ratio (ml/g)[1]
15.8% Digestible protein		
Protein source		
Soybean meal	225.9[c]	1.89[c]
Rapeseed meal	219.8[d]	1.89[c]
Maize gluten	227.1[c]	1.90[c]
17.2% Digestible protein		
Protein source		
Soybean meal	252.5[b]	2.05[b]
Rapeseed meal	256.1[a]	2.16[a]
Maize gluten	256.4[a]	2.12[a]
SE	1.08	0.015
P-value		
PS	0.005	0.001
DCP	< 0.001	< 0.001
PS × DCP	< 0.001	0.003

[a-d] Means without a common superscript within a column and a parameter significantly ($P < 0.05$) differ.
[1]Each value represents the mean of 6 replicates (8 birds per replicate) determined over 4 periods (d9-15, d16-22, d23-29, d30-d33).

Protein source influenced (P < 0.001) duodenal morphology. Villus height was increased by 18.2% and 17.7%, whereas crypt depth was reduced by 15.5% and 18.1%, and villus height to crypt depth ratio was increased by 29.0% and 30.9% in broilers fed the SBM compared with those fed the RSM and MG diets, respectively (Table 5). Duodenal morphology was not significantly affected by DCP level, nor by the interaction between protein source and DCP level.

Caecal digesta characteristics

Protein source influenced (P = 0.005) caecal pH (Table 6). Broilers fed the RSM diet resulted in lower pH in the caecal contents compared to birds fed the MG and SBM diets. Digestible CP level did not alter caecal pH. There were no interactions between DCP level and protein source for caecal pH. Total VFA contents and ammonia concentration in the caecal digesta were not influenced by either dietary protein source or DCP level.

Caecal branched chain fatty acids (BCFA) were altered by dietary protein source (P = 0.030; Table 7) and were increased by 16.5% and 14.9% in broilers fed RSM compared with those fed SBM and MG diets, respectively. The increase in BCFA in samples from birds fed the RSM diet was caused by a significant increase in iso-butyric acid (P = 0.023) and a tendency to increased iso-valeric acid (P = 0.072). Caecal propionic acid content was reduced by 11.8% in broilers fed RSM compared with those fed MG diets, where propionic acid content of the SBM fed birds was in between (P = 0.046). Caecal valeric acid content was highest in birds fed the RSM diet (2.03 mmol/kg DM), but was reduced in birds fed the MG diet (1.74 mmol/kg DM) and further reduced in birds fed the SBM diet (1.49 mmol/kg DM) (P < 0.001).

Discussion

The present study was designed to investigate the impact of three PS (SBM, RSM and MG), which are known to differ in their level of indigestible protein, on performance, gut morphology and caecal digesta characteristics in broilers. It was hypothesised that increasing the level of indigestible protein of a certain PS would result in more protein fermentation in the hindgut, which coincides with poor performance and reduced gut health. Therefore, gut morphology and fermentation characteristics were studied as explanatory variables. Additionally, it was questioned whether increasing the level of digestible CP, by either the PS or by the other digestible protein ingredients, could alleviate the negative effects caused by protein fermentation. Therefore, each PS was fed at two DCP levels.

Bird performance as related to gut health

Feed intake of MG fed birds did not differ from FI of SBM fed birds. The reduced FI for broilers fed the RSM diets was in agreement with several studies reporting adverse effects of RSM on FI if its inclusion level was more than 10% (Ahmad et al. 2007; Aftab 2009), potentially due to its poor palatability because of the presence of glucosinolates (Zeb 1998), and high fibre content (Naseem et al. 2006). RSM, additionally, contains a high amount of sulphur (1.14 *vs.* 0.44%) compared with SBM (Summers 1995), which may interact with calcium due to an alteration in anion-cation balance, resulting in

Table 4. Effects of protein source (PS) and digestible crude protein level (DCP, %) on mean relative weights[1] (g/100 g BW) of empty gastrointestinal segments in broilers.

Main effects	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Ceca	Colon
Protein source								
Soybean meal	0.34	0.38	0.93	0.62	1.09	0.85	0.38	0.19
Rapeseed meal	0.36	0.40	1.04	0.68	1.22	0.95	0.41	0.18
Maize gluten	0.35	0.40	1.03	0.67	1.20	0.94	0.41	0.18
Pooled SE	0.01	0.01	0.04	0.21	0.05	0.03	0.01	0.01
Digestible CP level								
15.8	0.35	0.40	0.99	0.65	1.16	0.91	0.40	0.19
17.2	0.34	0.39	1.01	0.66	1.18	0.92	0.40	0.17
Pooled SE	0.01	0.01	0.03	0.02	0.04	0.03	0.01	0.01
P-value								
PS	0.198	0.126	0.112	0.098	0.117	0.109	0.120	0.860
DCP	0.512	0.693	0.680	0.815	0.653	0.699	0.720	0.240
PS × DCP	0.812	0.655	0.361	0.397	0.356	0.365	0.635	0.570

[1]Each value represents the mean of 6 replicates (6 birds per replicate).

Table 5. Effects of protein source (PS) and digestible crude protein level (DCP, %) on villus height (µm), crypt depth (µm) and villus height to crypt depth ratio (VCR) in the duodenum of broilers[1].

Main effects	Villus height	Crypt depth	VCR
Protein source			
Soybean meal	1499[a]	277[b]	5.4[a]
Rapeseed meal	1226[b]	320[a]	3.8[b]
Maize gluten	1233[b]	327[a]	3.8[b]
Pooled SE	82.60	3.70	0.50
Digestible CP level			
15.8	1312	306	4.3
17.2	1327	309	4.3
Pooled SE	78.40	3.00	0.30
P-value			
PS	0.001	0.001	0.001
DCP	0.101	0.468	0.734
PS × DCP	0.490	0.853	0.344

[a-b]Means without a common superscript within a column significantly ($P < 0.05$) differ.
[1]Each value represents the mean of 6 replicates (6 birds per replicate).

Table 6. Effects of protein source (PS) and digestible crude protein (DCP, %) level on cecal digesta characteristics in broilers[1].

Main effects	Cecal pH	NH_3 (g/kg DM)
Protein source		
Soybean meal	6.54[a]	4.95
Rapeseed meal	6.20[b]	4.55
Maize gluten	6.37[ab]	4.85
Pooled SE	0.07	0.18
Digestible CP level		
15.8	6.43	4.72
17.2	6.32	4.86
Pooled SE	0.05	0.14
P-value		
PS	0.005	0.252
DCP	0.187	0.495
PS × DCP	0.415	0.558

[a-b]Means without a common superscript within a column significantly ($P < 0.05$) differ.
[1]Each value represents the mean of 6 replicates (6 birds per replicate).

poor FI (Summers and Bedford 1994). Increasing the DCP level for all PS diets resulted in an increase of FI by 2.4% (2.7%, 1.4% and 3.2% for RSM, MG and SBM, respectively). Ferguson et al. (1998) reported an overall lower FI level in broilers fed low dietary DCP levels.

BWG was reduced in birds fed the MG or RSM supplemented diets compared to SBM fed birds, potentially due to increased hindgut fermentation for the RSM fed birds. This was indicated by lower levels of caecal pH and higher BCFA, as well as poorer gut morphology in both MG and RSM-fed birds, indicated by shorter villi and higher crypt depths. Differences in caecal pH and BCFA between MG and RSM fed birds were not expected, because the calculated amounts of indigestible protein were similar in both diets. Whether or not protein is able to enter the caeca depends on its solubility and particle size. The caecal opening is controlled by an interdigitating meshwork of villi and musculature that acts as a filter, only allowing entry of fluid and fine particles (Ferrando et al. 1987; Clench and Mathias 1995). Most likely, the RSM particles that passed through the ileum met those criteria better than the MG particles.

The reduced growth performance recorded for the RSM diets might have been related to the presence of certain toxic compounds in RSM such as glucosinolates, tannins, phytase, erucic acid and sinapine (Khajali and Slominski 2012). The enzymatic degradation of these glucosinolates may lead to the production of goitrin, which inhibits proper functioning of the thyroid glands and suppresses the secretion of thyroxin (Tripathi and Mishra 2007). This may lead to a reduced BWG in broilers fed RSM. The isothiocyanates in RSM, likewise, may result in reduced FI and, consequently, impaired growth (McNeill et al. 2004).

Increasing the levels of non-essential amino acids in the high DCP diets increased overall BWG by 4.7 g/d compared to the BWG of birds fed the low DCP diets, which was in line with earlier findings (Corzo et al. 2005). Increasing DCP in all PS diets resulted in an increase in BWG by 6.6% (8.4%, 5.1% and 6.5% for RSM, MG and SBM, respectively). The alleviating effect of high DCP on BWG in RSM-fed birds from 66.9 to 72.5 g/d was not sufficient to reach the BWG levels seen in the low DCP-SBM fed birds (75.0 g/d). This indicated that additional supplementation of digestible NEAA could not fully compensate for the negative effects of increased indigestible protein levels and/or antinutritional factors in the diet. The alleviating effect of high DCP levels on BWG in MG-fed birds (an increase from 70.1 to 73.7 g/d) seemed to be sufficient to reach the same BWG levels in the low DCP-SBM fed birds (75.0 g/d).

Low protein digestibility in a diet means less amino acids were available for growth and potentially larger amounts of indigestible CP could enter the hindgut, leading to proteolytic fermentation. The latter findings were supported by the results of De Lange et al. (2003). Processing and excretion of nitrogenous compounds require more energy (Birkett and De Lange 2001), resulting in less energy availability for growth.

High DCP, as a treatment factor, was accompanied by high dietary CP levels. High DCP levels induced high WI as well as high WF ratios. Such findings have been previously reported by Alleman and Leclercq (1997), and Ziaei et al.

Table 7. Effects of protein source (PS) and digestible crude protein (DCP, %) level on cecal volatile fatty acids (VFA (mmol/kg DM)) concentrations[1] in broilers.

Main effects	VFA	Acetic acid[2]	Propionic acid[2]	Butyric acid[2]	Valeric acid[2]	Total BCFA[2,3]	Iso-butyric acid[2]	Iso-valeric acid[2]
Protein source								
Soybean meal	130.4	73.80	6.52^{ab}	16.01	1.49^{c}	2.18^{b}	1.00^{b}	1.18
Rapeseed meal	126.1	74.12	5.90^{b}	15.35	2.03^{a}	2.61^{a}	1.24^{a}	1.37
Maize gluten	125.3	74.40	6.69^{a}	14.92	1.74^{b}	2.22^{b}	1.06^{b}	1.15
Pooled SE	3.30	0.58	0.24	0.44	0.06	0.12	0.06	0.07
Digestible CP level								
15.8	127.6	73.90	6.46	15.62	1.73	2.29	1.09	1.20
17.2	126.9	74.30	6.25	15.30	1.76	2.39	1.12	1.27
Pooled SE	2.70	0.47	0.20	0.36	0.05	0.10	0.05	0.06
P-value								
PS	0.510	0.620	0.046	0.207	<0.001	0.030	0.023	0.072
DCP	0.862	0.533	0.440	0.572	0.508	0.459	0.667	0.366
PS × DCP	0.374	0.029	0.011	0.269	0.492	0.901	0.839	0.843

[a-c]Means without a common superscript within a column significantly ($P < 0.05$) differ.
[1]Each value represents the mean of 6 replicates (6 birds per replicate).
[2]Percentage of total VFA.
[3]BCFA = branched chain fatty acid (sum of iso-butyric acid and iso-valeric acids).

(2008). It was reported that a 1% increase in protein content resulted in 3% more water consumption (Larbier and Leclercq 1992). Difference in WI and WF ratio between the three proteins sources were, however, small.

Gut morphology

Gut morphology is a marker for gut health and can be assessed by villus height and crypt depth (Awad et al. 2009). There is a scarcity of published data regarding the effect of CP sources and their digestible levels on these parameters in broilers. The duodenum is the major site for digestion and absorption of nutrients in the small intestine. Duodenal histology, therefore, was measured to monitor the expected negative effects of nitrogenous substances on villus height (Nousiainen 1991). Shorter villi indicate a decrease in surface area for absorption of nutrients from the gut, as these structures are the functional areas for nutrient absorption (Zang et al. 2009). In rabbits, an increase in height enhanced nutrient transport across the villus surface (Tufarelli et al. 2010). The shorter villi with greater crypt depth in broilers fed RSM and MG diets may be an indication of more damage to the gut by harmful compounds produced by microbial fermentation. A deeper crypt indicated increased turnover of enterocytes and, thus, more protein and energy demand for this purpose. Crypt depth is an indicator of the number of crypt cells produced (Hampson 1986). It has been reported that broilers spend approximately 12% of their synthesised protein on GIT turnover (Choct 2009). The absence of significant effects for DCP levels on villus height and crypt depth were confirmed by the findings of Buwjoom et al. (2010) in broilers.

Caecal digesta characteristics

Feeding a diet with a low-digestible protein, such as one containing RSM, have approximately 7% of the seed nitrogen (N) in a tightly bound form, which will increase the amount of indigestible protein entering the caeca (Finlayson et al. 1973). Low ileal digestibility of protein in the RSM and MG diets compared to the SBM diet, likely resulted in more undigested protein entering the hindgut. This can stimulate protein fermentation (Hobbs et al. 1996), particularly if fermentable (insoluble) carbohydrates are present at low levels in the diet, as seen in the RSM diet (Table 1). Fermentable (insoluble) carbohydrates provide additional energy to gut microbes, and decrease the concentration of harmful compounds that are the result of protein fermentation (Swanson et al. 2002). The MG and RSM diets had similar high amounts of indigestible CP. The higher amounts of NSP in MG diets (186 and 185 g/kg, for low and high digestible CP levels, respectively) compared to RSM diets (167 and 172 g/kg), may be related to certain fermentation characteristics between MG and RSM diets. This was confirmed by the data in the current trial. For example, greater caecal BCFA and lower propionic acid concentrations in broilers fed the RSM diet compared to those fed the MG diet indicated more protein and less carbohydrate fermentation in broilers fed RSM. Propionic acid has been suggested to result from carbohydrate fermentation (Rodriguez et al. 2013), although propionic acid can be synthesised by bacterial fermentation of alanine and threonine (Macfarlane and Gibson 1995). BCFA are supposed to be a marker for protein fermentation (Macfarlane et al. 1992). The BCFA, i.e. isobutyric acid, 2-methylbutyric acid and isovaleric acid, are exclusively formed upon fermentation of the branched amino acids valine, isoleucine and leucine, respectively (Blachier et al. 2007; Gilbert et al. 2018). In the current study, caecal levels of isobutyric acid and total BCFA were higher in birds fed the RSM diets compared to the other diets, which indicated higher fermentation levels, especially for valine, and leucine. Moreover, caecal pH in broilers fed RSM diets was lower than in those fed the MG diets, and the highest pH was observed in broilers fed the SBM diets. Low caecal pH potentially indicated more proteolytic fermentation. RSM contains more sulphur amino acids (methionine, cysteine and taurine) compared to SBM (Okrouhlá et al. 2012). Fermentation of these by sulphate reducing bacteria results in the production of hydrogen sulphide (H_2S) which in turn lowers the pH (Lewis and Cochrane 2007).

Conclusions

In conclusion, feeding diets containing RSM and MG as the major protein source compared to SBM to broilers, resulted in poorer performance, reduced villus height and deeper crypts. Increased caecal BCFA concentrations, as observed in the RSM-fed birds, were indicative of proteolytic fermentation in the hindgut, which may cause poor gut morphology and impair FCR. The results of this study showed that rapeseed meal could be used as a model for hindgut protein

fermentation in broilers. To a certain extent, retarded growth performance for RSM- and MG-fed birds could be counterbalanced by increasing the dietary level of digestible CP. The compensation in growth performance, however, was limited.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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