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Original Research Article

# Reduced-particle size wheat bran and endoxylanase supplementation in broiler feed affect arabinoxylan hydrolysis and fermentation with broiler age differently

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ABSTRACT

Since the caecal microbiota of young broilers are not yet able to ferment the dietary fibre (DF) fraction of the feed to a large extent, increasing the accessibility of DF substrates along the gastrointestinal tract is crucial to benefit from the health stimulating metabolic end-products (e.g. butyric acid) generated upon microbial DF fermentation. Therefore, the present study aimed to evaluate the potential of reduced-particle size wheat bran (RPS-WB) and endoxylanases as feed additives to stimulate arabinoxylan (AX) hydrolysis and fermentation along the hindgut of young broilers. To this end, RPS-WB and endoxylanase supplementation were evaluated in a 2 × 2 factorial design using a total of 256 male 1-d-old chicks (Ross 308). Broilers were assigned to 4 dietary treatments: a basal wheat-based diet with (1) no feed additives (control, CTRL), (2) an endoxylanase (XYL; Econase XT 25 at 0.10 g/kg diet), (3) 1% wheat bran with an average reduced particle size of 297 μm (RPS-WB) and (4) an endoxylanase and 1% RPS-WB (RPS-WB + XYL). Each dietary treatment was replicated 8 times and on d 10 and 28, respectively, 24 and 16 broilers per treatment group were euthanised to analyse AX degradation, short-chain fatty acid production and digesta viscosity in the ileum and caecum. Broilers receiving XYL in their diet showed increased AX solubilisation and fermentation at both d 10 and 28 compared to the CTRL group ($P < 0.05$). Adding RPS-WB to the diet stimulated wheat AX utilisation by the primary AX degraders in the caecum at 10 d of age compared to the CTRL group, as observed by the high AX digestibility coefficient for the RPS-WB supplemented group at this young age ($P < 0.05$). At 28 d, RPS-WB supplementation lowered body-weight gains but increased butyric acid concentrations compared to the XYL and CTRL group ($P < 0.05$). Although no synergistic effect for RPS-WB + XYL broilers was observed for AX hydrolysis and fermentation, these findings suggest that both additives can raise a dual benefit to the broiler as a butyrogenic effect and improved AX fermentation along the ileum and caecum were observed throughout the broiler's life.


## 1. Introduction

Due to the potential transmission of antibiotic-resistant bacteria and infections to humans, the non-therapeutic use of in-feed antibiotics in poultry feed is banned or tightly regulated worldwide (Dibner and Richards, 2005; Marshall and Levy, 2011). This ban on in-feed antibiotics in 2006 in Europe has pushed scientists to search for alternative and effective strategies to control poultry health status and performance (Crisol-Martínez et al., 2017; Huyghebaert et al., 2011; Melo-Durán et al., 2019). Nourishing the beneficial gut microbiota has become an emerging strategy in this

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research area. Research has revealed that oligosaccharides derived from dietary fibre (DF) are the most important source of nutrients for the gut microbiome and can sustain a healthy gut environment and the zootechnical performance of broilers (De Maesschalck et al., 2015; Yacoubi et al., 2018). Feed additives such as non-starch carbohydrate (NSC) degrading enzymes and prebiotics can contribute to this. Indeed, prebiotics, defined as "a substrate that is selectively utilised by host microorganisms conferring a health benefit" (Gibson et al., 2017), can largely contribute to the stimulation of butyrate-producing bacteria (Onrust et al., 2015). It is well-documented that butyrate generates different positive gastrointestinal responses in broilers, such as reducing inflammation and pathogenic colonisation by *Salmonella enteritidis*, and improving intestinal villus structure, thereby optimising gut health (González-Ortiz et al., 2019; Guilloteau et al., 2010; Van Immerseel et al., 2005; Vermeulen et al., 2017). This has led to an increased interest in the use of the prebiotic xylo-oligosaccharides (XOS) and arabinoxylan-oligosaccharides (AXOS), produced from the main dietary fibre arabinoxylan (AX), as additives in poultry diets in the last ten years (Bautil et al., 2020; Ding et al., 2018; Onrust et al., 2015; Pourabedin et al., 2015; Ribeiro et al., 2018). Next to the direct inclusion of these prebiotic substrates, the addition of endoxylanases which can solubilise and hydrolyse the dietary AX into low molecular weight AX substrates, i.e. AXOS, is one of the major strategies used nowadays to improve feed digestibility and broiler health and performance (Courtin et al., 2008; Craig et al., 2020; Dale et al., 2020; Ribeiro et al., 2018; Singh et al., 2021; Yacoubi et al., 2018). Despite the wide use of these feed additives, the majority of them fail to increase the proportion of dietary fibre that enters the primary fermentation segment of the broiler's gastrointestinal (GI) tract, i.e. the caecum, amongst others, due to dietary fibre inaccessibility. It can be hypothesised that the majority of both soluble, but mainly insoluble fibre is probably too large to enter the caecum (Bautil, 2020; Svihus et al., 2013). The current hypothesis states that only soluble and small particles will enter the caeca (Svihus et al., 2013). Although there is no direct proof of the range of average sizes of DF particles that can enter the caeca, the current literature clearly indicates that the addition of rather small percentages (e.g. 1%) of DF-rich fractions with average particle sizes ranging from 20 to 280 μm to the feed can alter the composition and fermentation dynamics of the caecal microbiota (De Maesschalck et al., 2019; Svihus et al., 2013; Vermeulen et al., 2017, 2018). Therefore, to allow better exploitation of DF in the caeca of broilers, it is of interest to increase the susceptibility and fermentability of the AX structures from the cereal grains or the flow of DF-rich fractions into the caeca. This is particularly of interest for young broilers during the starter period (d 1 − 10), as their caecal microbiota are rather incompetent to hydrolyse and ferment the majority of the DF of the cereal cell walls (Bautil et al., 2019). A further improvement of DF fermentability, especially in the young broiler, and hence feed digestibility and broiler's performance and health might be obtained in this way.

Compared to the common strategy of endoxylanase supplementation, one way to improve and allow a more efficient microbial fermentation of the dietary AX and its multifactorial beneficial effects in broilers might be to increase the accessible surface of the fermentable substrates in the feed to the intestinal bacterial or exogenous supplemented enzymes. Wheat bran with a reduced particle size (below 300 μm, further referred to as RPS-WB) compared to regular wheat bran (±2,000 μm) is, in this sense, a potentially interesting and rich source of easily accessible AX (De Paepe et al., 2019; Vermeulen et al., 2017). Indeed, previous research by Vermeulen et al. (2017) found that the addition of 1% wheat bran with an average particle size reduced from approximately 2,000 to 280 μm can have a butyrogenic effect and consequently may help to control Salmonella infections in broilers. It was also observed by De Paepe et al. (2018), Vermeulen et al. (2018) and De Paepe et al. (2019) that, in particular, inclusion of 1% RPS-WB (150 − 280 μm) can form a crucial dietary platform for colonisation of beneficial microbiota such as Bifidobacteriaceae and butyric acid-producing species. The increase in specific surface area of this RPS-WB compared to regular wheat bran provides a larger contact for both the bacteria and their enzymes to more easily and efficiently access the fermentable substrates of bran (De Paepe et al., 2019; Jacobs et al., 2015; Vermeulen et al., 2017). In this way, the fermentation rate and production of both propionic and butyric acid can be stimulated. Although beneficial microbial communities and microbial metabolites are positively stimulated with this approach, it remains to be investigated if the allocation of AX and subsequent fermentation of AX can also be improved by adding this small amount, i.e. 1%, of an RPS-WB fraction to the feed.

Furthermore, compared to the recalcitrant character of the NSC that make up the cell wall of regular wheat bran, in essence AX, β-glucan and cellulose (Hemdane et al., 2016; Roye et al., 2020), the different NSC components constituting the cell wall of RPS-WB are made more accessible to enzymes (Vermeulen et al., 2018). It can therefore be hypothesised that the simultaneous addition of an endoxylanase on top of RPS-WB could even accelerate and increase the production of AX hydrolysis products that can be efficiently fermented by the broiler's gut microbiome. Considering this approach, a synergistic effect with both feed supplements might be elicited in the gut, thereby allowing a better allocation of the DF towards the gut microbiome and exploitation of the energy from the AX in the broiler's feed.

Therefore, the objective of this study was to examine whether the addition of 1% RPS-WB, a commercial dose of endoxylanase (0.10 g/kg of complete feed) or the combination of both can heighten and stimulate AX hydrolysis and fermentation in the hindgut of young broilers. Hence, exploring the additive value of 1% RPS-WB and endoxylanase addition to the feed of broilers could unlock new feed additive strategies, which can further improve the functional value of DF and its benefits on the health status of broilers.

## 2. Materials and methods

### 2.1. Animal ethics

This broiler trial was approved by the Ethical Committee for experimental use of animals of the KU Leuven (P213/2015). The research and animal care protocols were in strict accordance with the European Directives (Directive, 2010/63/EU, 2010).

### 2.2. Wheat bran particle size reduction

Commercial coarse wheat bran (*Triticum aestivum* L.) was obtained from Dossche Mills (Deinze, Belgium). The particle size of this wheat bran was reduced using a Cyclotec 1093 Sample mill (FOSS, Höganäs, Sweden) installed with a grinding ring and a final mesh sieve of 1,000 μm according to the settings described by Jacobs et al. (2015). The average particle size ($d_{av}$) of the milled wheat bran sample was determined by sieving 20.0 g of this sample on a set of sieves having different mesh sizes, i.e. 1,000, 710, 500, 400, 250, 200, 150, 75, 50, and 38 μm using a vibratory sieve shaker (Retch, Aartselaar, Belgium). The sample was shaken for 15 min at a frequency of 1.5 $s^{-1}$, and after every 15 min, each sieve was gently brushed to avoid clogging the sieve pores. Afterwards, the remaining material on each sieve was weighed and the $d_{av}$ was calculated using the following formula:

$$d_{av} = \sum d_i \times m_i$$

with $d_i$ the average particle size of particles on sieve $i$ and $m_i$ the mass percentage of the fraction retrieved from sieve $i$ (Jacobs et al., 2015). This procedure was repeated twice. Wheat bran with an average reduced particle size of 297 ± 6 μm was obtained and used in 2 out of 4 experimental diets during the broiler trial, as described below.

This particle size below 300 μm was chosen for this study since Vermeulen et al. (2018) have shown that adding 1% of RPS-WB on top of the broiler's feed can positively stimulate the xylan degraders and the production of butyric acid in the caeca. However, it remains to be investigated how the AX hydrolysis and fermentation processes are affected with 1% addition of RPS-WB.

### 2.3. Experimental diets

A total of 4 experimental diets were used in this broiler trial. The diets were wheat-soy based and formulated in accordance with the nutrition guidelines of Aviagen (2014a) to meet Ross 308 broiler nutrient requirements. The nutrient specifications of the basal diet can be found in Table 1. These basal diets were non-supplemented (control diet, CTRL) or supplemented with a commercial endoxylanase preparation Econase XT 25 (an endoxylanase derived from *Nonomurea flexuosa*, AB Vista, Marlborough, UK; 160,000 U/kg) at a commercial dosage rate of 0.10 g/kg of complete feed (XYL diet) or with 1.0% of wheat bran with reduced particle size (RPS-WB diet) added on top or with both additives, the endoxylanase preparation at 0.10 g/kg of complete feed and 1.0% of RPS-WB (RPS-WB + XYL diet) added on top. In particular an addition level of 1% was chosen as the goal was to study if the microbial modulating effect of 1% RPS-WB could stimulate AX hydrolysis and fermentation in young broilers as was previously observed by our research group with the addition of 0.5% of prebiotic oligosaccharides (AXOS) to wheat-based broiler diets (Bautil et al., 2020). In addition, diets contained the indigestible marker titanium dioxide ($TiO_2$) (5.0 g/kg of complete feed) and a coccidiostat (Sacox, Huvepharma, Antwerp, Belgium). To accurately assess the effect of RPS-WB and endoxylanase addition on broiler performance and the gastrointestinal parameters under study, phytase was not used in any experimental diet. All experimental diets were manufactured at Research Diet Services (Wijk bij Duurstede, The Netherlands). Broilers were fed in 2 phases: a starter (d 1 − 10) and a grower phase (d 11 − 28). Diets were provided as pellets during the grower phase. The pellets of the starter diet were crushed into a crumble for the young chicks.

**Table 1**
Ingredients and nutrient composition of the basal wheat-based starter (crumble) and grower (pellet) feed used during the broiler balance trial (as-fed, g/kg).

| Item | Starter | Grower |
|---|---|---|
| **Ingredients** | | |
| Wheat | 621 | 653 |
| Soybean meal (48.5% CP) | 292 | 259 |
| Soybean oil | 35.0 | 44.0 |
| Premix [1] | 5.00 | 5.00 |
| Limestone fine | 15.7 | 14.0 |
| Monocalciumphosphate | 15.5 | 11.0 |
| Salt | 1.50 | 1.70 |
| Sodium carbonate | 4.40 | 4.20 |
| L-Lysine HCl | 3.30 | 2.70 |
| DL-Methionine | 3.35 | 2.90 |
| L-Threonine | 1.85 | 1.50 |
| L-Arginine | 0.45 | 0.20 |
| L-Valine | 0.65 | 0.40 |
| L-Isoleucine | 0.35 | 0.20 |
| Magnesiumoxide | 0.00 | 0.00 |
| **Calculated nutrient content** | | |
| Energy, kcal/kg | 2,907 | 3,005 |
| DM | 873 | 873 |
| Crude ash | 63.1 | 55.3 |
| Crude protein | 220 | 205 |
| Crude fat | 48.8 | 58.1 |
| Crude fibre | 24.3 | 23.9 |
| Carbohydrates | 517 | 530 |
| Starch | 378 | 397 |
| Sugars | 46.9 | 44.4 |
| Calcium | 9.60 | 8.10 |
| Total phosphorus | 7.10 | 6.00 |
| Available phosphorus | 4.80 | 3.70 |
| Digestible lysine, % | 1.16 | 1.04 |
| Digestible leucine, % | 1.30 | 1.21 |
| Digestible methionine + cysteine, % | 0.86 | 0.79 |
| Digestible arginine, % | 1.24 | 1.12 |
| Digestible threonine, % | 0.78 | 0.70 |
| Digestible tryptophan, % | 0.23 | 0.22 |

CP = crude protein; DM = dry matter.
[1] Mineral-vitamin premix provided per kilogram diet: vitamin A 10,000 IU; vitamin $D_3$ 2,500 IU; vitamin E 50 mg; vitamin $K_3$ 1.5 mg; vitamin $B_1$ 2.0 mg; vitamin $B_2$ 7.5 mg; vitamin $B_6$ 3.5 mg; vitamin $B_{12}$ 20 μg; niacinamide 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; iodine 0.8 mg; selenium 0.15 mg.

### 2.4. Experimental design and broiler husbandry & management

A total of 256 male 1-d-old chicks (Ross 308) (Belgabroed, Merksplas, Belgium) were randomly divided upon arrival over the 4 dietary treatments in 4 floor pens covered with wood shavings litter. All broilers received water and feed ad libitum and were kept under conventional conditions of lighting, heating and ventilation as provided in the broiler management guide of Aviagen (2014b). More specifically, a lighting schedule of 23 h of light and 1 h of darkness was maintained during the first 7 d and changed to 18 h of light and 6 h of darkness from d 8 onwards until the end of the experiment (28 d). The housing temperature was set at 34 °C and gradually reduced to 20 °C throughout the experiment. Housing conditions, water and feed supply, and chicken behaviour, condition and mortality were checked daily.

To accurately collect digesta samples and avoid the interference of heteroxylan in wood shavings litter upon quantification of wheat AX in these samples afterwards, 5 and 3 broilers from each dietary treatment were placed in one digestibility cage at d 4 (no acclimatisation due to ethical and welfare considerations) and d 13 (with a 48 h of acclimatisation period) of the experiment, respectively. At the age of 10 and 28 d, 3 out of 5 and 2 out of 3 broilers residing in the cages were sampled. A total of 8 replicate digestibility cages was used in this study. In addition, by using digestibility cages in the experimental set-up, broiler performance parameters during the periods broilers resided in these cages could be estimated. The cages had a wire floor, a feed trough at the front, a drinking cup at the backside and a plastic tray underneath the cage. The digesta contents of 3 and 2 broilers residing in one cage were pooled together in one sampling tube during sampling at d 10 and 28, respectively, to have sufficient digesta material for the chemical measurements afterwards.

### 2.5. Performance parameters

Although the main objective of the study was to evaluate AX hydrolysis and fermentation in the broiler, the performance parameters of broilers were also evaluated during this experiment. Average body weights (BW) were recorded individually at d 1, 4, 10, 15, and 28. Feed intake (FI) was recorded and body-weight gains (BWG) were calculated during the periods that the chicks resided in digestibility cages, i.e. d 4 to 10 and d 15 to 28. The corresponding

feed conversion ratio (FCR) for these periods was calculated and corrected for chick mortality.

### 2.6. Sampling

At the end of the starter period (d 10) and the end of the trial (d 28), 3 and 2 broilers in one digestibility cage, respectively, were randomly selected, weighed and euthanised by electronarcosis followed by decapitation. Following decapitation, the pancreas and the full caecum were removed and weighed. Contents of the whole ileum and both caecal pouches were collected separately by gently finger-stripping these gastrointestinal segments and subsequently pooled per cage in one tube. In total, 8 replicate pools of digesta contents for each gastrointestinal part were collected on each sampling day. After collection, weights of the empty gizzard and small intestine (jejunum + ileum) were measured. The pH of the gizzard content, collected in a tube before measurement, was determined as well (Hamilton Liq-Glass lab pH electrode, Hamilton Company, Reno, NV, USA). Ileal digesta viscosity was immediately measured on freshly collected ileal content (2.0 g) for each replicate pool. The remaining digesta samples were divided over different sampling tubes and stored at − 20 °C until further analysis of the AX and $TiO_2$ content. The tubes containing ileal and caecal content (2.0 g) for short-chain fatty acid (SCFA) analysis were stored at − 80 °C.

### 2.7. Chemical analyses of feed and digesta samples

#### 2.7.1. Dry matter

The dry matter (DM) content of feed and digesta samples was calculated based on the analysed moisture content. The feed moisture content was determined by drying 2.0 g of feed at 130 °C for 15 h in a hot air oven. Moisture content in digesta samples (2.0 g) was determined using a specific temperature scheme in which the temperature gradually increased from 60 to 103 °C over 48 h, as previously described by Bautil et al. (2019).

#### 2.7.2. Feed extract and digesta viscosity

A sample of 500 μL of aqueous extracts of milled feed samples (1.0 g passed through a 1 mm screen) was analysed for its extract viscosity using a Brookfield DV-II + viscometer (Brookfield Engineering, Laboratories Inc, Stoughton, MA, USA) with a CP40 cone and a constant shear rate of 750 $s^{-1}$ at 37 °C, as described by Bautil et al. (2019).

Immediately after sampling, 2.0 g of ileal content was weighed into a centrifuge tube and centrifuged for 15 min at 21,000 × *g* (Himac CT15RE centrifuge, Hitachi, Japan). A sample of 500 μL of the resulting supernatant was immediately measured for viscosity using a Brookfield DV-II + viscometer with a CP 40 cone and a constant shear rate of 90 $s^{-1}$ at 37 °C.

#### 2.7.3. In-feed endoxylanase activity

In-feed endoxylanase activity levels in each experimental diet were measured according to the Xylazyme AX method (Megazyme, Bray, Ireland) as previously described by Bautil et al. (2019). In short, aqueous extracts of feed samples (milled to pass through a 1-mm screen) containing the enzymes were obtained by suspending 1.0 g in 10 mL of a sodium acetate buffer (25 mmol/L, pH 5.0), followed by shaking (30 min, 7 °C), centrifugation (10 min, 4,000 × *g*, 7 °C) and filtration of these suspensions. Subsequently, one azurine-crossed-linked AX tablet was added to 1.0 mL of (diluted) pre-equilibrated (10 min, 40 °C) aqueous feed extract. After appropriate incubation times, reactions were stopped by adding 10 mL Tris-(hydroxymethyl)aminomethane aqueous solution (10.0 g/L), followed by vortex stirring and filtration. Absorbance values of the filtered extracts were measured at 590 nm (Ultraspec II UV/vis spectrophotometer) against a control, prepared by incubating the feed extracts without a tablet. In addition, correction for the non-enzymatic release of the blue-coloured fragments from the AX tablet was made. In-feed endoxylanase activities were expressed as endoxylanase units (EU) per gram DM of complete feed. One unit is defined as the amount of endoxylanase activity needed to yield a corrected extinction value of 1.0 per hour of incubation under the conditions of the assay.

#### 2.7.4. AX content

Total AX (TOT-AX) and water-extractable arabinoxylan (WE-AX) contents were determined on feed samples and freeze-dried, homogenised digesta samples from the caecum and ileum using an adapted Englyst method (Englyst and Cummings, 1984; Gebruers et al., 2009). TOT-AX and WE-AX contents were determined by analysing the arabinose and xylose content in freeze-dried digesta as such and aqueous extracts, respectively by gas chromatographic analysis after acid hydrolysis of carbohydrates and reduction and acetylation of resulting monosaccharides as described by Bautil et al. (2019). To determine the total amount of WE-AX in feed and digesta samples, the hydrolysis step was preceded by an extraction procedure including two enzyme inactivation steps to yield the aqueous extracts containing the WE-AX. In the first step, the enzymes present were inactivated by evaporating the samples in water–ethanol solution (800 mL/L) at 95 °C. Secondly, the extraction procedure was carried out using a potassium chloride/hydrogen chloride (KCl–HCl) buffer (20 mmol/L, pH 3.0) instead of distilled water to prevent the modification of the native AX in the samples by endoxylanases during extraction (30 min, 7 °C), in case enzymes present were not fully inactivated by the evaporation step with ethanol. TOT-AX and WE-AX content were calculated by summing up 0.88 times the arabinose and xylose content present in the feed and freeze-dried samples themselves and their aqueous extracts, respectively. TOT-AX and WE-AX content were expressed on dry matter base of the analysed sample (g/kg DM).

#### 2.7.5. Quantification of titanium dioxide

The content of indigestible marker $TiO_2$ was measured in feed and digesta samples according to a downscaled method of Short et al. (1996) with modifications proposed by Myers et al. (2004) as previously described by Bautil et al. (2019). In short, dried samples (0.1 g) underwent acidic digestion (12 mL of 18.4 mol/L sulfuric acid) in macro-Kjeldahl tubes containing a copper catalyst (3.5 g $K_2SO_4$ and 0.4 g $CuSO_4$) at 420 °C for 60 min. Tubes were allowed to cool for 30 min after which 10 mL of a hydrogen peroxide solution (300 g/L) was added. After this precipitation step, cooling and filtration steps were subsequently performed. Extinction values were measured at 410 nm against a deionised water control. $TiO_2$ content was calculated using a calibration curve prepared with working standards (0.5 mg/mL $TiO_2$ dissolved in 18.4 mol/L sulfuric acid).

#### 2.7.6. Calculation of AX digestibilities

Digestibility coefficients for TOT-AX and WE-AX in the ileum and caecum were calculated according to the following formula:

$$\text{AX digestibility} = 1 - \frac{\left([TiO_2]_{feed} \times [AX]_{digesta}\right)}{\left([TiO_2]_{digesta} \times [AX]_{feed}\right)}$$

with $[TiO_2]_{feed}$ and $[TiO_2]_{digesta}$ the measured $TiO_2$ contents (g/kg DM) in the feed and digesta, respectively, and with $[AX]_{feed}$ and $[AX]_{digesta}$ the AX content (g/kg DM) (TOT-AX content for TOT-AX

digestibility calculation and WE-AX content for WE-AX digestibility calculation) in the feed and digesta, respectively. A more detailed description of the biological meaning of these AX digestibility coefficients was described earlier by Bautil et al. (2019). In short, the native AX population present in the feed can be solubilised through hydrolysis by added or microbe-derived endoxylanases present in the ileum and caecum. During this hydrolytic event, WE-AX is created. As a result, the pool of WE-AX will be enlarged along the gut compared to the pool of native WE-AX present in the feed. This solubilisation process will be noticed by negative WE-AX digestibility coefficients in our data. Simultaneously, these endoxylanases will further hydrolyse and depolymerise the native and created AX substrates into AX oligomers, which can be finally fermented by the microbiota. This latter fermentation process will be noticed by the occurrence of positive AX digestibility coefficients. It is of note that the term AX digestibility is used in the broad sense, i.e. as a collective term to describe the combination of hydrolytic events by endoxylanases on the AX polymers and microbial fermentation of AX oligomers resulting in the disappearance of AX in the broiler's gut.

#### 2.7.7. SCFA analysis

The amount of the non-branched SCFA acetic, propionic, butyric and valeric acid and the branched short-chain fatty acids (BCFA) isobutyric and isovaleric acid present in fresh samples of the caecal pouches when broilers were 28 d of age was analysed by gas chromatography according to the procedure previously described by Van Craeyveld et al. (2008) with slight modifications. In short, 0.1 mL of 2-ethyl butyric acid (internal standard), 0.4 mL NaCl solution (250 g/L), 0.2 mL 9.2 mol/L sulfuric acid and 0.8 mL diethyl ether were added to 0.5 g of fresh sample. After shaking (2 min, 60 rpm, 7 °C) and centrifugation (5 min, 1,000 × *g*, 7 °C), the diethyl ether phases containing the organic acids (1.0 μL) were analysed on the Agilent 6890 Series gas chromatograph (Wilmington, DE, USA) equipped with a capillary FFA packed column [J&W DB-FFAP GC column (Bellefonte, PA, USA), 30 m × 0.53 mm, film thickness 1.0 μm], and with helium as the carrier gas. The column temperature gradually increased from 100 to 235 °C, the injector and the flame ionisation detector temperatures were 200 and 245 °C, respectively. The content of each SCFA and BCFA was calculated using the internal standard and calibration standard solution (volatile free acid mix #46975-U, Sigma–Aldrich, Bornem, Belgium) containing 9.8 mmol/L, acetic acid, 9.9 mmol/L of formic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic and hexanoic acid, and 10.0 mmol/L n-heptane acid. SCFA and BCFA content were expressed as μmol/g DM of caecal sample.

### 2.8. Statistical analysis

This broiler trial was designed as a 2 × 2 factorial experiment with RPS-WB and endoxylanase supplementation (XYL) as factors. Due to the importance of broiler age for the parameter AX digestibility, the effect of age (d 10 and 28) was also included as a factor in the statistical model. The intestinal and performance parameters ($n = 8$) were analysed statistically using analysis of variance (ANOVA) with the fit model platform of the JMP Pro 15.0.0 software (SAS Institute Inc., Cary, NC, USA). Data for the intestinal parameters were tested using three-way ANOVA with RPS-WB, XYL, broiler age and their second-order interactions as model effects. A two-way ANOVA with XYL and RPS-WB as model effects was used for testing the measured compounds of the feed, the performance and the SCFA data. Significantly different means were further identified by performing a posthoc Tukey's HSD test. In addition to this test, data were also analysed using the posthoc Dunnett's test, which specifically identifies treatment means that significantly differ from the control group (CTRL). Mean differences were considered statistically significant at $P \leq 0.05$ and were interpreted as a tendency at $0.05 < P \leq 0.10$. All data are represented as mean ± standard error of the mean (SEM).

## 3. Results

### 3.1. Feed and broiler performance

The added RPS-WB had a TOT-AX and WE-AX content of 281.2 ± 11 g/kg DM and 6.60 ± 2 g/kg DM, respectively. Its TOT-AX and WE-AX arabinose to xylose (A:X) ratios were respectively 0.59 ± 0.02 and 0.85 ± 0.01. Endoxylanase activity and extract viscosity of this wheat bran preparation were 0.54 ± 0.00 EU/g DM and 1.00 ± 0.02 cP, respectively. The measured TOT-AX and WE-AX content, in-feed endoxylanase activity and extract viscosity are shown in Table 2. As expected, endoxylanase activity was present in the XYL-supplemented diets and the addition of 1.0% RPS-WB increased the TOT-AX and water-unextractable arabinoxylan (WU-AX) content.

As shown in Table 3, adding an endoxylanase significantly reduced the FI ($P = 0.004$) and lowered FCR at young broiler ages ($P = 0.03$). This positive effect of endoxylanase addition on FCR was not present anymore during the growing phase. The addition of RPS-WB reduced the average BWG at d 28 ($P = 0.04$).

No difference in the proportional weights (g/100 g of BW) of the pancreas, full caecum, empty gizzard and small intestine was observed when either XYL or RPS-WB was added to the basal diets. No effect of these additives was detected for the pH of the gizzard either (data not shown).

### 3.2. Viscosity

As shown in Fig. 1 and Supplementary Table S1, the addition of a XYL preparation in the broiler's feed significantly reduced ileal viscosity at all ages. Broilers receiving no endoxylanase in their feed showed a significant decrease in ileal viscosity with age ($P < 0.001$). The addition of RPS-WB did not affect the ileal viscosity. No specific interaction between XYL and RPS-WB addition was observed.

### 3.3. Wheat arabinoxylan content and digestibility in ileal and caecal digesta of young and old broilers

#### 3.3.1. Effect of XYL addition

As shown in Table 4, the AX contents in digesta decreased from the ileum to the caecum for all diets. An increase in the amount of WE-AX formed was observed with age in the ileal digesta ($P = 0.001$), whereas an overall decrease in the amount of AX was found in the caecal digesta ($P < 0.001$ and $P = 0.044$ for TOT-AX and WE-AX, respectively). The addition of a XYL preparation in the feed increased the solubilisation of WU-AX into WE-AX, as noted by the significantly higher WE-AX contents in both the ileum and caecum in XYL supplemented birds compared to non-XYL supplemented birds ($P < 0.001$ for the WE-AX content both at the ileum and the caecum). This increase in the WE-AX content led to highly negative values (ranging between −95% and −54%) for WE-AX digestibility in the hindgut at all broiler ages (Fig. 2). However, this increased solubilisation with XYL addition was only significantly present in the ileum (Fig. 2 and Supplementary Table S1, $P = 0.001$). For TOT-AX, an increase in the digestibility coefficient at all broiler ages was observed both in the ileum ($P = 0.016$) and caecum ($P < 0.001$) with XYL addition (Fig. 2 and Supplementary Table S1), suggesting better overall AX hydrolysis and fermentation in the hindgut of both young and old broilers when XYL is present as an additive (Econase XT 25 at 0.10 g/kg complete feed) in the feed.

**Table 2**
Total arabinoxylan (TOT-AX), water-extractable arabinoxylan (WE-AX) and water-unextractable arabinoxylan (WU-AX) content, arabinose to xylose (A:X) ratio, titanium dioxide ($TiO_2$) content, extract viscosity and endoxylanase activity of the control (CTRL), and XYL, 1.0% RPS-WB, and RPS-WB + XYL supplemented diets.[1]

| Item | TOT-AX, g/kg DM | WE-AX, g/kg DM | WU-AX, g/kg DM | TOT-AX A:X ratio | WE-AX A:X ratio | $TiO_2$, g/kg DM | Extract viscosity, cP | Endoxylanase activity, EU/g DM |
|---|---|---|---|---|---|---|---|---|
| **Starter** | | | | | | | | |
| CTRL | 53.7 $^{b}$ | 6.38 $^{z}$ | 47.3 $^{b}$ | 0.89 $^{a}$ | 1.10 | 6.86 | 0.87 | 0.31 $^{z}$ |
| XYL | 55.8 $^{b}$ | 7.39 $^{y}$ | 49.0 $^{b}$ | 0.87 $^{a}$ | 1.05 | 7.00 | 0.88 | 8.16 $^{y}$ |
| RPS-WB | 57.8 $^{a}$ | 6.32 $^{z}$ | 52.5 $^{a}$ | 0.81 $^{b}$ | 1.10 | 6.98 | 0.87 | 0.36 $^{z}$ |
| RPS-WB + XYL | 57.2 $^{a}$ | 6.72 $^{y}$ | 50.9 $^{a}$ | 0.86 $^{b}$ | 1.08 | 6.85 | 0.92 | 7.51 $^{y}$ |
| **Pooled SEM** | 0.8 | 0.23 | 0.83 | 0.01 | 0.03 | 0.33 | 0.01 | 1.08 |
| **Grower** | | | | | | | | |
| CTRL | 55.1 $^{b}$ | 6.42 $^{z}$ | 49.9 $^{b}$ | 0.89 $^{a}$ | 1.07 | 6.93 | 0.90 | 0.36 $^{z}$ |
| XYL | 54.1 $^{b}$ | 7.13 $^{y}$ | 48.5 $^{b}$ | 0.87 $^{a}$ | 1.05 | 6.78 | 0.86 | 7.99 $^{y}$ |
| RPS-WB | 57.0 $^{a}$ | 6.59 $^{z}$ | 51.6 $^{a}$ | 0.82 $^{b}$ | 1.06 | 7.09 | 0.92 | 0.36 $^{z}$ |
| RPS-WB + XYL | 58.3 $^{a}$ | 6.96 $^{y}$ | 51.9 $^{a}$ | 0.83 $^{b}$ | 1.00 | 7.09 | 0.91 | 10.24 $^{y}$ |
| **Pooled SEM** | 1.0 | 0.15 | 0.95 | 0.01 | 0.03 | 0.32 | 0.02 | 1.28 |
| ***P* ANOVA** | | | | | | | | |
| XYL | 0.68 | 0.018 | 0.82 | 0.74 | 0.35 | 0.95 | 0.90 | <0.001 |
| RPS-WB | 0.015 | 0.49 | 0.009 | <0.001 | 0.98 | 0.83 | 0.08 | 0.37 |
| RPS-WB × XYL | 0.89 | 0.37 | 0.67 | 0.12 | 0.88 | 0.96 | 0.82 | 0.40 |

DM = dry matter; RPS-WB = reduced-particle size wheat bran (297 μm); SEM = standard error of the mean; XYL = endoxylanase (Econase XT 25 at 0.10 g/kg diet).
[a,b] Means within a column having different superscripts indicate a significant difference for the main effect of RPS-WB addition ($P < 0.05$).
[y,z] Means within a column having different superscripts indicate a significant difference for the main effect of XYL addition ($P < 0.05$).
[1] Reported values are dietary treatment means analysed at least in triplicate ($n = 3$).

**Table 3**
Body-weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) of broilers in digestibility cages during the test period d 4 − 10, d 15 − 28 fed the control wheat-soy based diet (CTRL), and Econase XT 25 at 0.10 g/kg diet (XYL), 1.0% RPS-WB and RPS-WB + XYL supplemented wheat-soy based diets.

| Item | BWG, g/broiler | | FI, g/broiler | | FCR, g/g | |
|---|---|---|---|---|---|---|
| | 4 − 10 d | 15 − 28 d | 4 − 10 d | 15 − 28 d | 4 − 10 d | 15 − 28 d |
| **Dietary treatment means**[1] | | | | | | |
| CTRL | 166.0 | 1,221.2 | 212.4 | 1,660.6 | 1.29 | 1.38 |
| XYL | 173.3 | 1,224.6 | 192.3 | 1,648.9 | 1.11 * | 1.37 |
| RPS-WB | 175.5 | 1,152.7 | 201.5 | 1,611.4 | 1.15 | 1.38 |
| RPS-WB + XYL | 164.1 | 1,192.9 | 178.4 * | 1,667.5 | 1.10 * | 1.40 |
| Pooled SEM | 2.2 | 12.1 | 4.8 | 22.6 | 0.03 | 0.01 |
| **Main effects**[2] | | | | | | |
| **XYL addition** | | | | | | |
| Yes | 168.7 | 1,207.7 | 179.9 $^{b}$ | 1,658.2 | 1.10 $^{b}$ | 1.38 |
| No | 170.7 | 1,191.8 | 207.0 $^{a}$ | 1,634.4 | 1.22 $^{a}$ | 1.38 |
| **RPS-WB addition** | | | | | | |
| Yes | 169.8 | 1,175.7 $^{b}$ | 189.9 | 1,639.5 | 1.12 | 1.39 |
| No | 169.4 | 1,222.8 $^{a}$ | 203.0 | 1,654.3 | 1.21 | 1.37 |
| ***P* ANOVA** | | | | | | |
| XYL | 0.64 | 0.36 | 0.004 | 0.47 | 0.03 | 0.22 |
| RPS-WB | 0.96 | 0.04 | 0.42 | 0.62 | 0.42 | 0.29 |
| RPS-WB × XYL | 0.04 | 0.44 | 0.65 | 0.28 | 0.11 | 0.27 |

[1] Treatment means from 8 replicates of 5 and 3 broilers per digestibility cage, respectively, during the test periods d 4 − 10 and d 15 − 28 ($n = 8$). * Dietary treatment means are significantly different from the control, analysed according to the Dunnett's test ($P < 0.05$).
[2] [a,b] Means within a column having different superscripts indicate a significant difference for the main effect of RPS-WB or XYL supplementation ($P < 0.05$).NS = not significant; RPS-WB = reduced-particle size wheat bran (297 μm); XYL = endoxylanase (Econase XT 25 at 0.10 g/kg diet).

In the ileum, it was observed that the number of arabinose residues present on the water-extractable AX chains reduced upon endoxylanase addition ($P < 0.001$, Table 5). The opposite was true for the AX substrates in the caecal pouches: highly substituted AX polymers, i.e. A:X ratios ranging between 0.8 and 0.9 for both the water-extractable and the TOT-AX were observed with XYL addition ($P < 0.001$, Table 5). Very low TOT-AX A:X ratios of 0.2 to 0.3 were observed in the caecum when no XYL preparation was added to the feed (Table 5).

### 3.3.2. Effect of RPS-WB addition

As shown in Table 4, the addition of 1% RPS-WB in 2 out of 4 experimental diets did not considerably affect the concentration of AX in the hindgut. Only a significantly higher TOT-AX content was observed in the ileum of RPS-WB supplemented birds compared to non-RPS-WB supplemented birds (Table 4, $P = 0.049$), with no change in the ileal TOT-AX digestibility coefficient (Fig. 3 and Supplementary Table S1). However, the ileal digestibility coefficient of the dietary WE-AX fraction showed a negative trend (Fig. 3 and Supplementary Table S1, $P = 0.088$). When going further down in the gut, in the caecum, TOT-AX digestion was increased with the addition of RPS-WB, but this was only at 10 d of age (Fig. 3 and Supplementary Table S1, $P = 0.019$ for the interaction effect age × RPS-WB addition). No difference in WE-AX digestion and overall degree of arabinose substitution in the caecum was observed with adding 1% RPS-WB to the diet (Fig. 3, Supplementary Table S1 and Table 5).

### 3.3.3. The combined effect of XYL and RPS-WB addition

An interaction effect between the addition of the endoxylanase (XYL) and RPS-WB was not present in the data describing the AX content in the ileum and caecum. However, for the TOT-AX

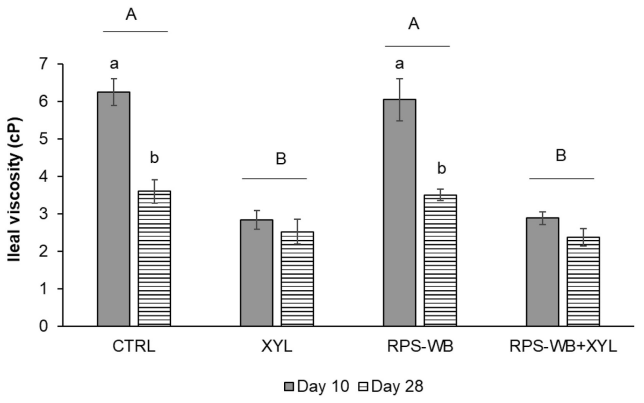


**Fig. 1.** Ileal viscosity at d 10 and 28 for broilers fed the control (CTRL), XYL, RPS-WB and RPS-WB + XYL diet. Capital letters A, B indicate significant dietary treatment differences (main effect of XYL addition, $P < 0.05$). Small letters a, b indicate significant age differences for broilers receiving a particular dietary treatment (interaction effect age × XYL addition, $P < 0.05$) ($n = 8$). Error bars denote the standard error of the mean (SEM). XYL = endoxylanase (Econase XT 25 at 0.10 g/kg diet); RPS-WB = reduced-particle size wheat bran (297 μm).

**Table 4**
Total arabinoxylan (TOT-AX) and water-extractable arabinoxylan (WE-AX) content in the ileum and caecum for young (d 10) and older (d 28) broilers fed the control diet (CTRL), and XYL, RPS-WB and RPS-WB + XYL supplemented wheat-soy based diets (g/kg DM).

| Item | Ileum | | | | Caecum | | | |
|---|---|---|---|---|---|---|---|---|
| | TOT-AX | | WE-AX | | TOT-AX | | WE-AX | |
| | d 10 | d 28 | d 10 | d 28 | d 10 | d 28 | d 10 | d 28 |
| **Dietary treatment means**[1] | | | | | | | | |
| CTRL | 182.7 | 169.0 | 25.1 | 29.2 | 78.0 | 23.1 | 8.5 | 3.2 |
| XYL | 171.5 | 179.0 | 36.3* | 40.0* | 27.0* | 6.3* | 9.5 | 3.1 |
| RPS-WB | 178.1 | 191.0 | 25.5 | 30.2 | 71.3 | 27.7 | 7.1 | 3.4 |
| RPS-WB + XYL | 182.9 | 189.5 | 33.2* | 44.8* | 28.0* | 9.6* | 11.6 | 4.7 |
| **Pooled SEM** | 3.1 | 3.9 | 1.2 | 1.8 | 6.5 | 2.6 | 0.8 | 0.3 |
| **Main effects**[2] | | | | | | | | |
| **Age** | | | | | | | | |
| Day 10 | 178.8 | | 29.8 [b] | | 52.0 [a] | | 8.9 [a] | |
| Day 28 | 181.9 | | 36.0 [a] | | 17.0 [b] | | 3.6 [b] | |
| **XYL addition** | | | | | | | | |
| No | 180.2 | | 27.4 [b] | | 47.8 [a] | | 5.4 [b] | |
| Yes | 180.5 | | 38.5 [a] | | 13.6 [b] | | 6.4 [a] | |
| **RPS-WB addition** | | | | | | | | |
| No | 175.6 [b] | | 32.6 | | 31.7 | | 5.7 | |
| Yes | 185.2 [a] | | 33.1 | | 32.8 | | 5.9 | |
| ***P* ANOVA** | | | | | | | | |
| Age | 0.49 | | 0.001 | | <0.001 | | <0.001 | |
| XYL | 0.89 | | <0.001 | | <0.001 | | 0.039 | |
| RPS-WB | 0.049 | | 0.63 | | 0.91 | | 0.80 | |
| Age × XYL | 0.44 | | 0.34 | | <0.001 | | 0.09 | |
| Age × RPS-WB | 0.19 | | 0.22 | | 0.41 | | 0.76 | |
| RPS-WB × XYL | 0.81 | | 0.97 | | 0.73 | | 0.33 | |

[1] Reported values are means from 8 replicates from each dietary treatment group ($n = 8$). * Treatment means are significantly different from the control group, analysed according to the Dunnett's test ($P < 0.05$).

[2] Means within a column having different superscripts indicate a significant difference for the main effect of age, XYL or RPS-WB supplementation, analysed according to the posthoc Tukey's HSD test ($P < 0.05$).DM = dry matter; NS = not significant; RPS-WB = reduced-particle size wheat bran (297 μm); XYL = endoxylanase (Econase XT 25 at 0.10 g/kg diet).

digestibility coefficients in the caecum, an interaction effect for the addition of XYL and RPS-WB was observed (Fig. 4 and Supplementary Table S1, $P = 0.022$). When no XYL preparation was provided in the feed, the addition of the RPS-WB resulted in a considerably higher TOT-AX digestibility, especially at 10 d of age. In the presence of XYL, the addition of RPS-WB did not influence

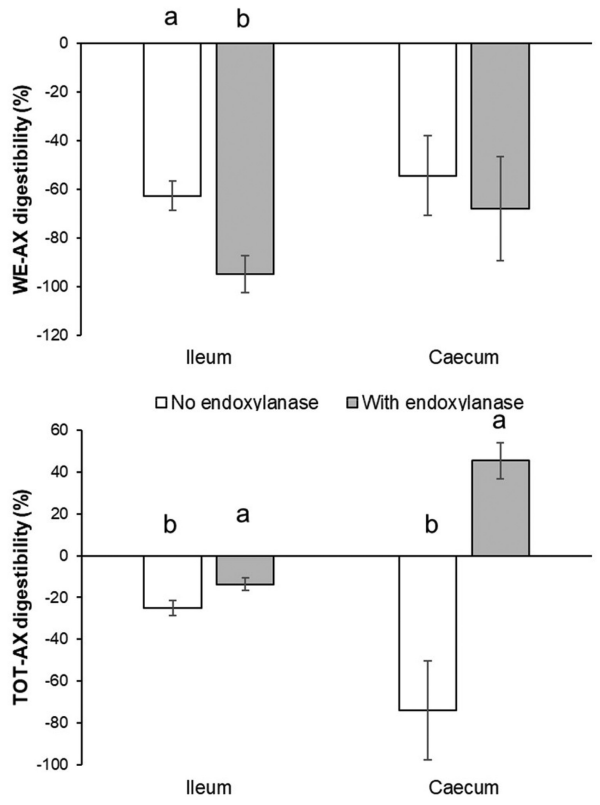


**Fig. 2.** Effect of endoxylanase addition (Econase XT 25 at 0.10 g/kg diet) on ileal and caecal water-extractable arabinoxylan (WE-AX) and total arabinoxylan (TOT-AX) digestibility (%) over all broiler ages. Small letters a,b denote a significant difference for the main effect of endoxylanase addition ($P < 0.05$). Error bars denote the standard error of the mean (SEM).

further the digestibility of the wheat AX along the hindgut. Hence, no synergistic effect of supplementing both additives on AX degradation along the gut was observed in this study.

### *3.3.4. SCFA content in caecal material with RPS-WB and XYL addition*

Total SCFA content, quantified as the sum of the acetic, propionic, butyric and valeric acid contents, at d 28 was not affected by the addition of XYL or RPS-WB (Fig. 5 − I and Supplementary Table S2). However, looking at the contents of the acids separately, significantly different acetic and butyric acid contents were observed upon supplementation of the CTRL diet with RPS-WB or XYL. XYL supplementation showed a tendency to lower the acetic acid content with 152.9 μmol/g DM (Fig. 5 − III and Supplementary Table S2, $P = 0.06$), while RPS-WB addition significantly increased the amount of butyric acid with 50 μmol/g DM (Fig. 5 − V and Supplementary Table S2, $P = 0.03$). Propionic and valeric acid content was not affected when these feed additives were supplemented to the broiler's diet (Fig. 5 − IV and VI). Total BCFA content was not significantly changed upon the addition of 1% RPS-WB or XYL (Fig. 5 − II).

## 4. Discussion

The objective of the present study was to investigate whether the addition of XYL (0.10 g/kg of complete diet), 1% RPS-WB, or the combination of both can trigger and increase AX hydrolysis and fermentation in the hindgut of both young and old broilers. In this section, we will integrate and discuss the results obtained to

**Table 5**
Total arabinoxylan (TOT-AX) and water-extractable arabinoxylan (WE-AX) arabinose to xylose (A:X) ratios in the ileum and caecum for young (d 10) and older (d 28) broilers fed the control diet (CTRL), and XYL, RPS-WB and RPS-WB + XYL supplemented wheat-soy based diets.

| Item | Ileum | | | | Caecum | | | |
|---|---|---|---|---|---|---|---|---|
| | TOT-AX A:X ratio | | WE-AX A:X ratio | | TOT-AX A:X ratio | | WE-AX A:X ratio | |
| | d 10 | d 28 | d 10 | d 28 | d 10 | d 28 | d 10 | d 28 |
| **Dietary treatment means**[1] | | | | | | | | |
| CTRL | 0.84 | 0.81 | 0.88 | 0.79 | 0.27 | 0.33 | 0.61 | 0.76 |
| XYL | 0.87 | 0.82 | 0.74* | 0.74* | 0.92* | 1.00* | 0.81 | 0.84 |
| RPS-WB | 0.86 | 0.79 | 0.87 | 0.79 | 0.26 | 0.21 | 0.75 | 0.66 |
| RPS-WB + XYL | 0.83 | 0.80 | 0.76* | 0.72* | 1.04* | 0.76* | 0.85 | 0.74 |
| **Pooled SEM** | 0.01 | 0.01 | 0.00 | 0.01 | 0.09 | 0.06 | 0.04 | 0.02 |
| **Main effects**[2] | | | | | | | | |
| **Age** | | | | | | | | |
| Day 10 | 0.85 [a] | | 0.81 [a] | | 0.59 | | 0.74 | |
| Day 28 | 0.80 [b] | | 0.76 [b] | | 0.56 | | 0.75 | |
| **XYL addition** | | | | | | | | |
| No | 0.82 | | 0.83 [a] | | 0.27 [b] | | 0.70 [b] | |
| Yes | 0.83 | | 0.74 [b] | | 0.92 [a] | | 0.80 [a] | |
| **RPS-WB addition** | | | | | | | | |
| No | 0.83 | | 0.79 | | 0.61 | | 0.75 | |
| Yes | 0.82 | | 0.79 | | 0.54 | | 0.74 | |
| ***P* ANOVA** | | | | | | | | |
| Age | <0.001 | | <0.001 | | 0.41 | | 0.37 | |
| XYL | 0.56 | | <0.001 | | <0.001 | | 0.003 | |
| RPS-WB | 0.17 | | 0.59 | | 0.28 | | 0.47 | |
| Age × XYL | 0.77 | | 0.001 | | 0.34 | | 0.13 | |
| Age × RPS-WB | 0.53 | | 0.37 | | 0.06 | | 0.006 | |
| RPS-WB × XYL | 0.13 | | 0.69 | | 0.97 | | 0.75 | |

[1] Reported values are means from 8 replicates from each dietary treatment group ($n = 8$). * Treatment means are significantly different from the control group, analysed according to the Dunnett's test ($P < 0.05$).
[2] Means within a column having different superscripts indicate a significant difference for the main effect of age, XYL or RPS-WB supplementation; analysed according to the posthoc Tukey's HSD test ($P < 0.05$).NS = not significant; RPS-WB = reduced-particle size wheat bran (297 μm); XYL = endoxylanase (Econase XT 25 at 0.10 g/kg diet).

provide additional insights into using a reduced-particle size fibre source and endoxylanase as feed additive strategies to improve further the functional value of AX in the broiler's feed and make use of their full fermentation potential.

### *4.1. Ileal viscosity is reduced upon XYL but not upon RPS-WB addition*

Given the previous reported microbial modulating effect of RPS-WB (De Paepe et al., 2019; Vermeulen et al., 2018), it was hypothesised that RPS-WB could stimulate AX solubilisation higher up in the gut, as previously observed with the addition of 0.5% AXOS (Bautil et al., 2020), thereby increasing the digesta viscosity compared to the CTRL birds. However, no effect on ileal viscosity with 1.0% RPS-WB addition was observed. In addition, the fact that wheat bran is rich in insoluble DF, especially WU-AX and cellulose (Hemdane et al., 2016) which generally has a lower potential to induce a high digesta viscosity in the gut of poultry compared to soluble DF such as high molecular weight WE-AX and β-glucans (Annison and Choct, 1991; Karunaratne et al., 2021) might also explain the lack of a viscosity effect.

In agreement with studies of Bautil et al. (2021), Dusel et al. (1997), Lee et al. (2017), Liu and Kim (2017) and Matthiesen et al. (2021), the addition of an endoxylanase preparation consistently reduced ileal viscosity over all broiler ages. This corresponds well with the increase in solubilisation and depolymerisation of the AX in the ileum in broilers receiving XYL-supplemented diets. The extra addition of 1.0% RPS-WB combined with an endoxylanase resulted in low viscosity values over time. When broilers received no endoxylanase, a decrease in ileal viscosity was observed as the broiler aged. The age-related microbial adaptation towards the high molecular weight WE-AX probably led to this decrease in viscosity with age (Bautil et al., 2019). As a result, at the end of the experiment (d 28), both broilers receiving XYL and non-XYL supplemented diets had comparable and low ileal viscosity values (±3 cP).

### *4.2. XYL addition induces a permanent effect on AX digestion in the broiler's hindgut*

In agreement with observations made by Bautil et al. (2021) and Lee et al. (2017), broilers receiving the endoxylanase hydrolysed the wheat AX to a greater extent compared to broilers receiving non-endoxylanase supplemented diets. The high ileal WE-AX contents and low viscosity values clearly demonstrated an effective dissolution of a portion of the TOT-AX coupled with depolymerisation of the wheat AX present in the aqueous extracts of the digesta by the XYL preparation. This effect of endoxylanase on AX hydrolysis and solubilisation resulted in an overall increased AX utilisation in the hindgut of both young and old broilers, as observed by the high TOT-AX digestibility coefficients for broilers receiving the XYL supplemented diets. A gradual increase in AX digestibility and decrease in viscosity profiles with age was noted for non-XYL supplemented birds, as explained by the increased complexity in the composition and substrate specificity of the gut microbiota with age towards the dietary AX entering the hindgut (Bautil et al., 2019; Lee et al., 2017; Lu et al., 2003). Hence, endoxylanases induce a permanent effect on the AX digestion kinetics and viscosity profiles throughout the broiler's life, which corroborates the findings of our previous study (Bautil et al., 2021).

As expected and observed previously by Bautil et al. (2019) and Lee et al. (2017), a high AX digestibility was observed especially in the caecum. This site has the highest bacterial density (Apajalahti et al., 2004) and therefore is the principal place for NSC fermentation in the broiler's gut (Svihus et al., 2013). The increased dissolution and depolymerisation of the AX chain with XYL

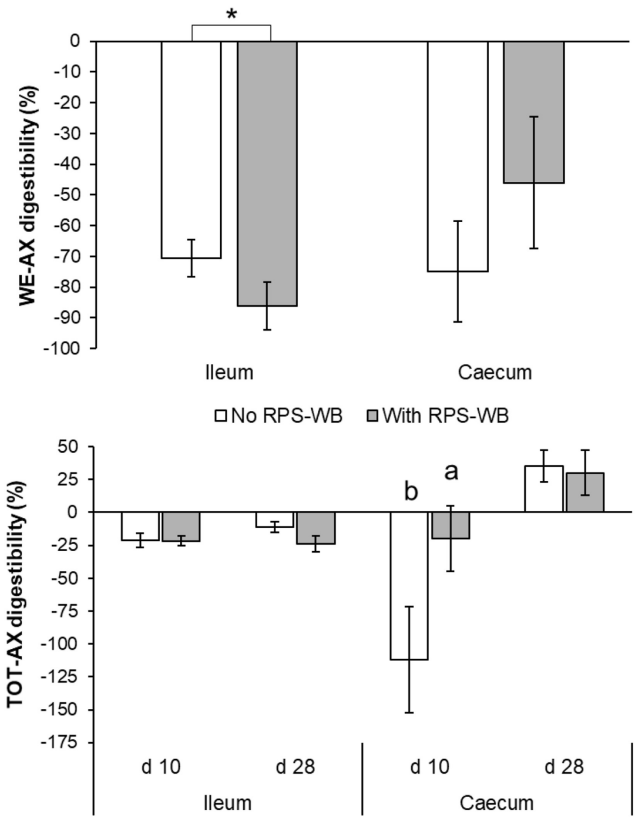


**Fig. 3.** Effect of 1.0% reduced-particle size wheat bran addition (297 μm, RPS-WB) on ileal and caecal water-extractable arabinoxylan (WE-AX) and total arabinoxylan (TOT-AX) digestibility (%) over all broiler ages. Small letters a,b indicate a significant difference for the effect of RPS-WB addition at a particular broiler age ($P < 0.05$). An asterisk denotes a trend for the main effect of RPS-WB addition ($P < 0.10$). Error bars denote the standard error of the mean (SEM).

supplementation probably led to an increased pool of soluble AX oligomers entering the caecum (Bautil et al., 2021; Lee et al., 2017). As a result, the endoxylanase facilitated a more rapid and complete fermentation of AX substrates and thus an increased AX

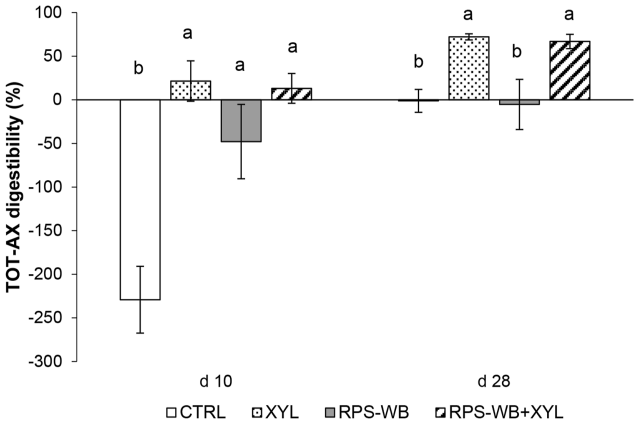


**Fig. 4.** Total arabinoxylan (TOT-AX) digestibility (%) in the caecum for broilers fed the control wheat-soy based diet (CTRL), and XYL, RPS-WB and RPS-WB + XYL supplemented wheat-soy based diets at 10 and 28 d of age. Small letters a, b indicate a significant age difference for broilers receiving a particular dietary treatment ($P < 0.05$). Error bars denote the standard error of the mean (SEM). XYL = endoxylanase (Econase XT 25 at 0.10 g/kg diet); RPS-WB = reduced-particle size wheat bran (297 μm).

digestibility from very early ages onwards compared to the CTRL, suggesting a higher rate of AX utilisation by the microbiome in XYL supplemented birds. In addition, this high AX utilisation with XYL is also corroborated by the very high A:X ratios of the residual AX substrates found in the caecum. As XYL-supplemented birds had an A:X ratio of 0.92 on average, while non-XYL supplemented birds had an A:X ratio of 0.27, a more robust AX fermenting gut microbiome seems to be colonising the caecum upon endoxylanase addition, as previously suggested by Bedford and Apajalahti (2018). Interestingly, this observation also seems to suggest that in the absence of an endoxylanase, there might be an active removal of arabinose residues as a result of bacterial arabinofuranosidase action and subsequent microbial fermentation of arabinose in the caecum, as previously suggested by research of Bautil et al. (2019). Furthermore, these high A:X ratios in XYL supplemented birds showed that the complex AX polymers present in a wheat kernel, i.e. AX of the pericarp having an A:X ratio of 1.1 (Saulnier et al., 2007), cannot be degraded upon endoxylanase addition.

A kick-starter effect on AX hydrolysis and fermentation, as seen for AXOS addition (Bautil et al., 2020), can also be induced by supplementing the correct dose and type of endoxylanase to the feed, as previously suggested by Bautil et al. (2021) and Ribeiro et al. (2018). Furthermore, this kick-starter effect on AX digestion at young ages transitions into a permanent effect, as improved AX digestibility values were observed in this study at both 10 and 28 d of age compared to the CTRL. This observation clearly indicates the multifactorial role of the endoxylanase in the gut. By reducing the intestinal viscosity in the foregut and supplying readily fermentable AX fragments in the hindgut, the host–diet interaction throughout the broiler's life is positively affected (Aftab and Bedford, 2018; Masey O'Neill et al., 2014).

To note, in contrast with other broiler digestibility experiments (Bautil et al., 2019, 2021), very negative TOT-AX digestibility coefficients were measured in the ileum in this trial, in some cases even with dietary endoxylanase addition. The reason for this is not clear. Probably, a different partitioning of digesta and marker over solid and liquid phases in vivo might explain these very negative AX digestibility (Choct et al., 1996; De Vries et al., 2014). In addition, a large accumulation of indigestible DF substrates, especially in the ileum, might have occurred as well. Furthermore, the AX digestibility coefficients are based on one point in time measurements and probably do not represent the entire dynamic processes of AX substrate modification and fermentation. Therefore, we want to stress that not the absolute values but the observed trends are of significant importance for understanding the pattern of AX digestion in the hindgut. The marker method is thus far the best approach to analyse and describe changes in DF digestibility in vivo, but it clearly needs development if we are to advance our understanding of DF fermentation.

Despite the improved TOT-AX digestibility with XYL supplementation, no increase in SCFA production was observed at d 28. This lack in SCFA response in vivo was also previously encountered in other studies (Bautil et al., 2021; Boets et al., 2015; González-Ortiz et al., 2019). Similarly, as for the AX digestibility coefficients, SCFA utilisation and production dynamics are not reflected in the measured values. Thus the full potential of the endoxylanase on in vivo SCFA production is possibly not captured in these data (González-Ortiz et al., 2019).

### *4.3. The addition of RPS-WB stimulates early wheat AX fermentation and butyric acid production in the caecum*

Our study did not reveal a strong effect of 1% RPS-WB on the digestion profiles of AX nor the viscosity profiles in the ileum. This was unexpected given the previously reported microbial

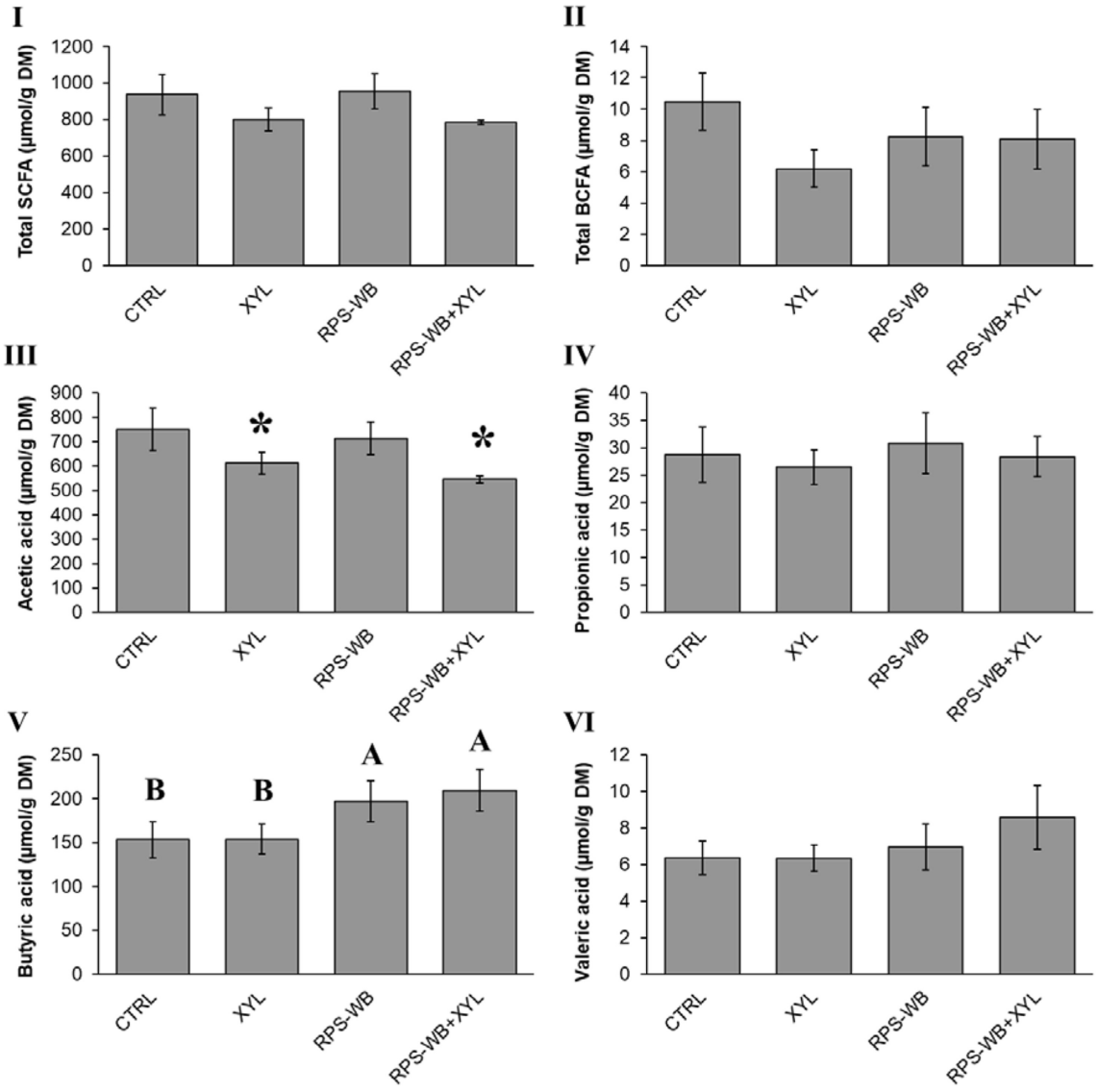


**Fig. 5.** Amount of total short-chain fatty acids (SCFA) (**I**), total branched-chain fatty acids (BCFA) (**II**), acetic acid (**III**), propionic acid (**IV**), butyric acid (**V**) and valeric acid (**VI**) produced in the caecum (µmol/g DM) for each dietary treatment. The amount of total SCFA was calculated as the sum of acetic, propionic, butyric and valeric acid. The amount of total BCFA was calculated as the sum of isobutyric and isovaleric acid. Capital letters A, B and an asterisk indicate significant differences for the main effect of 1.0% reduced-particle size wheat bran (297 µm, RPS-WB) and XYL addition (Econase XT 25 0.10 g/kg diet), respectively $P < 0.05$ and $P < 0.1$. Error bars denote the standard error of the mean (SEM). CTRL = broilers fed the control wheat-soy based diet; XYL = endoxylanase (Econase XT 25 at 0.10 g/kg diet); RPS-WB = reduced-particle size wheat bran (297 µm).

modulating effect of this mechanically modified wheat bran source (De Paepe et al., 2017, 2019; Vermeulen et al., 2018). The experiments of De Paepe et al. (2017) and Vermeulen et al. (2018) demonstrated that colonisation by primary AX degraders and butyrate producers could be improved with the use of RPS-WB (280 µm). The hypothesis that the addition of this RPS-WB as a rich source of easily accessible AX would initiate AX solubilisation and depolymerisation by the intestinal microbiota higher up in the gut (Vermeulen et al., 2017) was hence not confirmed here. Both the porosity and increased surface area of the added RPS-WB might have been detrimentally impacted during pelleting (De Paepe et al., 2019; Guillon et al., 1998) which might have muted the anticipated in vivo effects. Other microbial communities might have colonised the bran particles in this study, compared to the previous measurements in research of Vermeulen et al. (2018, 2017), in which mash feed conditions were applied. Besides, it can be questioned if the short transit time in the small intestine allows sufficient growth of particle-associated communities on RPS-WB. However, we cannot confirm this statement since the microbial composition was not studied in this trial.

As the indigestible dietary constituents are retained for several hours in the caecum and a high bacterial richness and diversity of fibre-degrading microbiota are generally found there (Sergeant et al., 2014; Svihus et al., 2013), it could be expected that the effects of RPS-WB addition on AX content and digestion profiles are strongly affected in this part of the gut. This was indeed the case for the TOT-AX digestibility coefficient measured in the caecum at d 10 (Fig. 3.). The addition of RPS-WB to the diet seems to provide an accessible substrate platform for the caecal microbiota, thereby stimulating wheat AX digestion at 10 d of age compared to the non-RPS-WB supplemented broilers. This might suggest that, in contrast to what was observed in the ileum, the relative proportion of primary AX degraders was enhanced in the caecum of the young broiler, which resulted in this improved AX degradation and the

notable decrease in FCR during the starter phase (d 4 to 10) compared to the CTRL. On the other hand, it could also be that the bran particles were more fermentable in the caecum due to their improved passage through the caecal sieves at 10 d of age. RPS-WB addition did not alter the A:X ratio of the remaining AX substrates in the caeca. Probably, a higher amount and/or diversity of AX-debranching enzymes which facilitate enhanced hydrolysis of the AX chain, were initially secreted by the first colonising caecal microbiota when only RPS-WB and no endoxylanase preparation was added to the feed. It was previously demonstrated that the production and activity of carbohydrate degrading enzymes and microbial metabolites rely on the dominance of microbial species colonising the gut (Reichardt et al., 2018; Van Den Broek et al., 2008). Although the microbial population was not measured in this study, it is likely that the relative proportion of AX fermenting and butyrate-producing microbiota were increased in the caecum of broilers receiving RPS-WB in their diet. We can speculate this based on our observations of high TOT-AX digestibility and very low TOT-AX A:X ratios in broilers at d 10 and the increase in butyric acid concentrations at d 28. In previous in vitro and in vivo research by Stewart and Slavin (2009) and Vermeulen et al. (2018, 2017), supplementation of RPS-WB significantly increased the concentration of butyric acid. This butyrogenic effect of RPS-WB was confirmed in this in vivo study.

### *4.4. Lack of synergy in AX fermentation with the combined addition of RPS-WB and XYL*

It was hypothesised that an endoxylanase, which can partly hydrolyse AX, and RPS-WB, a more accessible AX-rich substrate for microbial and exogenous supplemented enzymes than regular wheat bran (Vermeulen et al., 2017), can act in synergy. Adding both could improve the formation of AX hydrolysis products which in turn can be more efficiently fermented by the microbiota than AX hydrolysis products formed upon the addition of the single feed additive. However, no superior effect of adding the XYL and RPS-WB together on AX degradation in the hindgut and broiler performance was observed. The lack of a superior AX digestibility response was not expected, given that the added RPS-WB (297 μm) had an increase in specific surface area through particle size reduction (Jacobs et al., 2015), which is known to form a more favourable substrate platform for AX degrading microbiota and enzymes compared to native wheat bran AX of ground wheat (1,700–2,000 μm) in the CTRL diet (De Paepe et al., 2019; Vermeulen et al., 2018; Wang et al., 2001). It might be that there was an asynchrony between the site of action of the endoxylanase and the added RPS-WB in the gut or bias that is inherent to point-in-time measurements of AX digestibility and SCFA quantification. The change in porosity of the matrix for RPS-WB compared to regular wheat bran (Jacobs et al., 2015) might also have muted the anticipated in vivo response. Due to the increase of nanometer to micrometre size pores in RPS-WB, enzyme accessibility to the bran constituents might also have been hindered (De Paepe et al., 2019; Jacobs et al., 2015). As a result, no increased transfer of easily fermentable carbohydrates from these bran particles into the intestinal lumen was observed when both supplements were provided in the diet. In addition, RPS-WB might not have been the preferred substrate for the endoxylanase from *Nonomureae flexuosa* present in the Econase preparation (Bautil et al., 2021).

Dietary composition and the physical form of the feed are hence major contributing factors in evoking the desired fibre digestibility and performance response. Despite the lack of synergy between both additives, the main effect of each additive was still elicited in the broiler. Indeed, the provision of both XYL and RPS-WB raised a dual benefit throughout the broiler's life by stimulating early AX hydrolysis and fermentation on the one hand and by increasing the butyric acid production on the other hand.

### *4.5. Performance of young broilers is improved when XYL is added to a wheat-soy-based diet*

In spite of the marginally higher AX content (on average 2.75 g/kg) in RPS-WB supplemented diets compared to non-RPS-WB supplemented diets, BWG were considerably lower at d 28, but without negatively affecting the FCR. This observation is in contrast to the previously reported positive performance effects on FCR and BWG when 0.5% to 1.0% of dietary fibre with a reduced particle size (below 250 μm) was added to the diet of broilers (De Maesschalck et al., 2019; Rezaei et al., 2011). Indeed, Rezaei et al. (2011) observed improved FCR and BWG for 42 d old broilers when 0.5% of Vitacel, a commercial dietary fibre by-product of wheat with an average particle size below 250 μm, was added to the diet. Similar observations for FCR and BWG were observed during the starter period (13 d) with the addition of 0.5% of micronised cellulose (Arbocel, with an average fibre thickness of 20 μm) in the study by De Maesschalck et al. (2019). Still, a numerical improvement of 14 points in FCR with 1.0% RPS-WB addition compared to the CTRL group during the starter phase (d 4 to 10) is of considerable importance commercially but could not be detected as statistically significant, probably due to the high variability within this data as a consequence of the short periods broilers resided on cages during this feeding phase.

A commercial endoxylanase preparation significantly reduced the FCR at young ages (d 4 to 10). Hence, performance effects upon endoxylanase addition were more pronounced during the starter phase than the grower phase, similar to the observations of Gonzalez-Ortiz et al. (2017) and Lee et al. (2017). Since the functioning of the GI tract of young birds is prone to dietary anti-nutritive effects and wheat AX is known to exert a considerable anti-nutritive effect, the value of adding exogenous endoxylanases will be greater in younger birds compared to older ones, as observed here. In addition, the microbiome of young broilers lacks the ability to efficiently ferment wheat AX (Bautil et al., 2019; Lu et al., 2003; Uni et al., 1999). Therefore, a more pronounced decrease in FCR was observed with 1% RPS-WB addition compared to the CTRL due to its positive stimulation of the AX fermentation dynamics at 10 d. The lack of a synergistic effect of both 1% RPS-WB and XYL addition on AX hydrolysis and fermentation might explain why no effect on performance for broilers receiving the RPS-WB + XYL diet was observed. The simultaneous reduction in the anti-nutritive effects of AX, as demonstrated by the decrease in viscosity, and the improved AX degradation with XYL supplementation led to improved performance of young broilers and seemed to nullify the increase in AX fermentation and butyric acid production at respectively, d 10 and 28 with 1% RPS-WB addition. To note, in this study, the aim was to gain insight into the change of AX hydrolysis and fermentation processes with the addition of 1% RPS-WB to the broiler's feed. To anticipate on the possible intereference with heteroxylan of wood shavings in the AX data, digestibility cages formed an inherent part of the experimental design. Performance parameters were hence only analysed during the short periods that broilers resided on the cages. It is recommended to analyse the performance effects induced by the addition of 1% RPS-WB, and 1% RPS-WB and XYL more in-depth in a future performance trial (d 1 to 42).

## 5. Conclusion

The potential of endoxylanase and RPS-WB as feed additives to stimulate AX hydrolysis and fermentation in the hindgut of broilers

was investigated in this study. Supplementation of broiler diets with endoxylanase reduced digesta viscosity and improved AX utilisation in the ileum and caecum throughout the broiler's life, respectively, which led to a superior performance outcome, particularly in young broilers. Adding RPS-WB to broiler diets seems to be a promising strategy to enhance substrate accessibility to the first colonising microbiota, suggested by the stimulation of AX utilisation in the young broiler and butyric acid production at 28 d in the caecum. The combination of RPS-WB and an endoxylanase supplementation could not produce a synergistic effect on AX digestibility in the hindgut and broiler performance. In spite of this, such a combination might raise a dual benefit to the broiler by improving AX hydrolysis and fermentation and decreasing the anti-nutritional response during the starter period, and increasing butyric acid production towards slaughter age.

## Author contributions

**An Bautil**: Conceptualisation, Methodology, Formal analysis, Validation, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualisation. **Michael R. Bedford**: Conceptualisation, Writing – Review & Editing. **Johan Buyse**: Resources, Writing – Review & Editing. **Christophe M. Courtin**: Conceptualisation, Resources, Supervision, Writing – Review & Editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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