UNIVERSIDAD POLITÉCNICA DE MADRID

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA, ALIMENTARIA Y DE BIOSISTEMAS

EFFECT OF FIBER CONCENTRATE ADDITION ON PERFORMANCE AND DIGESTIVE HEALTH IN YOUNG MONOGASTRICS

DOCTORAL THESIS

Submitted for the degree of Doctor by:

Agnieszka Rybicka
Veterinarian

Madrid, 2024

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Doctoral Degree in Agro-Environmental Technology for Sustainable Agriculture

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Disclaimer

The results of this PhD Thesis are already published:

Experiment 1:

A. Rybicka, R. del Pozo, M.D. Carro, J. García. 2024. Effect of type of fiber and its physicochemical properties on performance, digestive transit time, and cecal fermentation in broilers from 1 to 23d of age. Poultry Science, 103:103192 https://doi.org/10.1016/j.psj.2023.103192

Experiment 2:

A. Rybicka, P. Medel, M.D. Carro, J. García. 2024. Effect of dietary supplementation of two fiber sources differing on fermentability and hydration capacity on performance, nutrient digestibility and cecal fermentation in broilers from 1 to 42d of age. Poultry Science, 103:103957 https://doi.org/10.1016/j.psj.2024.103957

Experiment 3:

A. Rybicka, P. Medel, E. Gómez, M.D. Carro, J. García. 2024. Different physiochemical properties of novel fibre sources in the diet of weaned pigs influence animal performance, nutrient digestibility, and caecal fermentation. Animals, 14, 2612. https://doi.org/10.3390/ani14172612

ABSTRACT

The objective of this PhD was to evaluate the inclusion of novel fiber sources from agricultural residues which were finely ground, into the diets of broilers and weaned piglets, and explore their impact on digestive health of young animals.

Experiment 1 was a preliminary study that evaluated the effects of insoluble fiber (IF) sources differing on particle size and hydration capacity (HC) on performance, gastrointestinal tract (GIT) development, cecal fermentation, and digestive transit time in 550 broilers (Ross-308) from 1 to 23 d. There were five treatments in mash form: a Control diet (corn-soybean meal based diet) with no fiber addition, and four fiber-diluted diets with 1.5% of different IF sources: lignocellulose (LC), finely ground straw (FS), coarsely ground straw (CS), all of them with high HC, and almond shell (AS) with low HC. The birds were housed in 50 cages with 10 replicates per treatment. Fiber supplementation tended to affect negatively the feed conversion ratio (FCR; P = 0.053). Broilers supplemented with high-HC IF (LC, FS, and CS) had lower average daily feed intake (ADFI; P = 0.005) and average daily gain (ADG; P = 0.001) than those fed AS. Overall, the AS group showed higher ADFI and ADG and tended to improve FCR compared to the other fiber groups.

Experiment 2 analyzed the effects of IF sources differing in HC and fermentability on GIT development, apparent ileal digestibility (AID), and performance from 1 to 42 d. Three diets were prepared all in mash form: a wheat-soybean control (CON) diet, CON diet diluted with 1.5% of wood lignocellulose (LC) as a non-fermentable insoluble fiber with high hydration capacity; and CON diluted with 1.5% of a mixture of fibers (ISFC) containing IF and a prebiotic fraction from fructooligosaccharides. A total of 378 male Cobb-500 broilers were used, and there were 9 replicates per treatment (14 chickens/pen). Chickens fed LC and ISFC diets impaired FCR by 4% during the first 7 d (P = 0.003) compared with the CON group. From 21 to 42 d, the ISFC group showed the best ADG (P = 0.039) and at 42 d tended to show the highest body weight (P = 0.095), likely due to higher AID of dry matter (P = 0.033) and organic matter (P = 0.043) in the ISFC group, and the similar trend observed for protein digestibility (P = 0.099). These results suggest that combining IF sources with highly fermentable prebiotics like FOS can enhance nutrient digestibility and performance in broilers.

Experiment 3 evaluated the effects of fiber sources differing in fermentability and HC on performance, fecal digestibility, and cecal fermentation in piglets (Landrace x Duroc) weaned at 28 days. The experimental design consisted on a common barley-wheat-corn feed without additional fiber supplementation (CON); and three treatments differing in HC and fermentability of fiber sources added at 1.5% to the pre-starter (28-42 days) and starter (42-61 days) diets: LHC (low HC

with a prebiotic fraction (PF) from chicory root), MHC (medium HC with PF), and HHC (non-fermentable, high HC, wood-based fiber without PF). Piglets were housed in 32 pens (6 piglets/pen), with 8 replicates per treatment. Over the entire period (28-61 d), LHC and MHC piglets showed a 10% increase in ADG and ADFI ($P = 0.019$) and tended to have a reduced FCR ($P = 0.087$) compared to HHC piglets. At 42 days, fecal protein digestibility increased by 5% in the LHC and MHC groups compared with the HHC group ($P = 0.035$) and did not differ from the CON group. These results suggest that balanced soluble and insoluble fiber concentrates can improve piglet performance.

In conclusion, the findings of this PhD Thesis confirm that finely ground agricultural by-products can serve as effective fiber sources in monogastric animal diets. Combining IF with highly fermentable sources at 1.5% of the diet provides additional benefits for productivity and nutrient digestibility in piglets and poultry.

RESUMEN

El objetivo de esta Tesis Doctoral fue evaluar la inclusión de fuentes novedosas de fibra provenientes de residuos agrícolas finamente molidas en piensos de broilers y lechones destetados, y explorar su impacto en la salud digestiva de animales jóvenes.

El Experimento 1 evaluó los efectos de fibra insoluble (FI) con diferente tamaño de partícula y capacidad de hidratación (CH), sobre la productividad, el desarrollo del tracto gastrointestinal (TGI) y la fermentación cecal en 550 pollos de engorde (Ross-308) de 1 a 23 d. En total hubo 5 tratamientos basados en una dieta común en harina (maíz y harina de soja) sin fibra añadida (Control), y 4 basados en la dieta Control diluida con un 1,5% de diferentes fuentes de FI: lignocelulosa (LC), paja molida fina (FS) y paja molida gruesa (CS), caracterizadas por alta CH; y cáscara de almendra (AS) con baja CH. Los animales se alojaron en 50 jaulas con 10 réplicas por tratamiento. En comparación con las aves control, la FI tendió a afectar negativamente el índice de conversión (IC; P = 0,053). Los pollos suplementados con FI con alta CH (LC, FS y CS) mostraron menor consumo medio diario (CMD; P = 0,005) y ganancia media diaria (GMD; P = 0,001) que los AS. En resumen, los animales AS mostraron mayores CMD y GMD y tendieron a mejorar el IC en comparación con el resto de FI.

El Experimento 2 analizó los efectos de FI caracterizadas por diferente CH y fermentabilidad, sobre el desarrollo del TGI, digestibilidad íleal aparente (DIA) y productividad de 1 a 42 d. El diseño experimental se basó en 3 tratamientos: un pienso control sin fibra añadida (CON), pienso CON diluido con un 1,5% de lignocelulosa de madera (dieta LC) como FI no fermentable con alta CH; y CON diluido con un 1,5% de una mezcla de FI con una fracción soluble y prebiótica del fructooligosacaridos (FOS; dieta ISFC). Se utilizó un total de 378 pollos machos Cobb-500, y con 9 repeticiones por tratamiento. Los pollos LC y ISFC mostraron el IC un 4% peor solo durante los primeros 7 d (P = 0,003) que los CON. En el período de 21-42 d, el grupo ISFC mostró la mejor GMD (P = 0,039), y a los 42 d tendió a mejorar el peso vivo (P = 0,095), lo que pudo deberse a mayor DIA de materia seca (P = 0,033) y materia orgánica (P = 0,043) en el grupo ISFC, y una tendencia similar en DIA de la proteína (P = 0,099). Estos resultados muestran el potencial interés en combinar fuentes de FI con fuentes prebióticas muy fermentables, como los FOS, en broilers para mejorar la digestibilidad de los nutrientes y la productividad.

El Experimento 3 evaluó la adición de FI con diferente grado de fermentabilidad y CH sobre la productividad, digestibilidad fecal y la fermentación cecal en lechones (Landrace x Duroc) destetados a los 28 d. El diseño experimental consistió en un pienso común (cebada-trigo-maíz) sin fuentes adicionales de fibra (CON), y otros 3 con diferente CH y fermentabilidad de FI añadida en

un 1,5% al pienso prestarter (28-42 d) y starter (42-61 d): LHC (FI con baja CH y una fracción prebiótica (FP) de raíz de achicoria); MHC (FI con media CH y una FP); y HHC (FI a base de madera no fermentable con alta HC sin FP). Los animales se alojaron entre 32 boxes (6 lechones/box) con 8 repeticiones por tratamiento. Entre 28-61 d, los lechones LHC y MHC aumentaron un 10% el GMD y CMD (P 0,019) y tendieron a tener menor IC (P = 0,087) que los HHC. A los 42 d, la digestibilidad fecal de la proteína aumentó un 5% en los grupos LHC y MHC en comparación con HHC (P = 0,035) sin diferir del CON. Estos resultados sugieren que las fuentes de FI con una fracción altamente fermentable pueden mejorar el rendimiento productivo de los lechones en postdestete.

Los resultados Tesis Doctoral confirman que los subproductos en la dieta de los animales monogastricos. La combinación de FI con una fuente altamente fermentable administrados a un 1,5% del pienso proporciona una ventaja sobre la productividad y digestibilidad en los modelos de animales estudiados.

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List of Acronyms/ Lista de Acrónimos

ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
ADL	Acid detergent lignin
AID	Apparent ileal digestibility
AMEn	Nitrogen-corrected apparent metabolizable energy
AS	Almond shell
BCFA	Branched chain fatty acids
BW	Body weight
CD	Crypt depth
CH	Capacidad de hidratación
CLDN	Claudins (tight junction protein)
CMD	Consumo medio diario
CON	Control
CP	Crude protein
CS	Corse straw
DF	Dietary fiber
DIA	Digestibilidad ileal aparente
DM	Dry matter
EE	Ether extract
EU	European Union
FCR	Feed conversion ratio
FI	Fibra insoluble
FOS	Fructo-oligosaccharides/ fructooligosacáridos
FP	Fracción prebiótica
FS	Fine straw
GE	gross energy
GIT	Gastrointestinal tract
GMD	Geometric mean diameter
GMD	Ganancia media diartia

HC	Hydration capacity
IC	Índice de conversión
IDF	Insoluble dietary fiber
IL	Interleukins
ISFC	Dietary treatment based on insoluble fiber with low hydration capacity (Experiment 2)
LC	Lignocellulose- dietary treatment (Experiment 1, 2 and 3)
LHC	Dietary treatment with low hydration capacity and prebiotic activity (Experiment 3
MHC	Dietary treatment with medium hydration capacity and prebiotic activity (Experiment 3)
MOS	Mannan-oligosaccharides
NDF	Neutral detergent fiber
OCLN	Occluding (tight junction protein)
OM	Organic matter
PF	Prebiotic fraction
PS	Particle size
SBM	Soybean meal
SC	Swelling capacity
SCFA	Short chain fatty acids
SDF	Soluble dietary fiber
SEM	Standard error of the mean
SICR	Weight of full small intestine, colon and rectum
SID	Standardized ileal digestible
TDF	Total dietary fiber
TGI	Tracto gastrointestinal
TiO_2	Titanium dioxide
TTAD	Total tract apparent digestibility
VH	Villus high
VH:CD	Villus high to crypt depth ratio
WBC	Water binding capacity
WHC	Water holding capacity

INTRODUCTION

1.1. General Contextualization

Antimicrobial resistance is a global challenge, partially attributed to the excessive and inappropriate use of antimicrobials in both animal and human healthcare. This issue has been already addressed by the European Union (EU) through regulation that have prohibited the use of antibiotics as growth promoters since 2006, with the objective to reduce the antimicrobials usage in animal production by 50% by 2030 (European Commission, 2017) and banned the use of pharmacological levels of zinc oxide in post weaning diets for pigs (European Commission, 2016).

As a result, there is a significant sectoral demand for the development of effective tools to improve gut health, enhance performance, and control excessive mortality. These legislative changes have forced adaptations of the diet formulation in monogastric animals to maintain gut health. Several alternatives have been proposed to address this challenge, including phytogenic additives, probiotics, prebiotics, postbiotics, and fiber sources (Saettone et al., 2020; Silva Júnior et al., 2020; Arsène et al., 2021; Li et al., 2021; Shehata et al., 2022).

1.2. Fiber Sources

Traditionally, dietary fiber (DF) has been recognized as an 'anti-nutritive' factor, associated with poorer nutrient utilization, and reduced net energy value in pig and poultry (Lindberg, 2014). High DF is typically associated with poorer performance and nutrient digestibility (Freire et al., 2000; Khempaka et al., 2009). However, investigations performed over the lasts two decades have shown that young animals require a minimal amount of fiber in their diet to optimize intestinal physiology and performance (Mateos et al., 2007; Jiménz-Moreno et al., 2009). More recent studies suggest that moderate levels of DF may contribute to digestive health and has become one of the strategies used (Farré et al., 2020; Dai et al., 2022).

Different DF sources positively influence the gastrointestinal tract (GIT) development and gut health throughout the direct impact on the microbiome, intestinal mucus, epithelial cells, and gut-associated lymphoid tissue (Jha et al., 2019; Cronin et al., 2021). The proper gut microbiota balance may be crucial for the modulation of intestinal permeability and gut barrier integrity (Farré et al., 2020; Shehata et al., 2022). Also, DF acts as a substrate for beneficial microbiome proliferation, which metabolites contribute the maintenance of an anaerobic intestinal environment that prevent facultative pathogens from flourishing (Liu et al., 2018; Williams et al., 2019). Liu et al. (2018) attributed better gut health to the interaction between the microbiota and short chain fatty acids (SCFAs) production, induced by insoluble and soluble dietary fiber inclusion. Indeed, the correct integrity of the mucosal barrier promote the physiological function of the GIT, influences the nutrient digestibility and absorption of other nutrients, may decrease the incidence of post-weaning diarrhea, and improve the growth performance in piglets and poultry (Mahmood and Guo, 2020; Sun et al., 2021).

However, the profile and quantity of DF requiriments in young animals is mostly unknown. Since the source of DF can come from many raw materials, by-products or fiber concentrates, the knowledge about the effect of DF in young animals is limited.

1.3. Valorization of Agricultural Residues

The world's growing population and increasing demand for food are driving the expansion of the agricultural sector, leading to a rise in agricultural wastes production. According to 'Green Deal' it is essential to treat and, if possible, reuse these wastes to prevent potential environmental pollution (European Commission, 2019). The volume of residues generated by the food industry is substantial, highlighting the need to explore alternative recycling options. One promising approach for the revalorization of these by-products is to convert them into a high-fiber ingredients for animal nutrition, given that their primary components are cellulose, lignin and hemicellulose (Koul et al., 2022). Agricultural by-products are not only high in dietary fiber but may also be rich in functional metabolites such as polyphenol (including flavonoids), which can promote animal health, throughout the antioxidant capacities, or stabilize the microbiota (Chuang et al., 2021). This approach aligns with the global goal of "zero waste" in the environment.

Fiber sources such as sugar beet pulp, wheat bran, oat hulls, citrus pulp or soybean hulls have traditionally been used as feed ingredients for swine and poultry (Molist et al., 2009; González-Alvarado et al., 2010; Pascoal et al., 2012). However, many other residues have not yet been revalorized due to its hardness and size, which renders them unsuitable for use in animal feed, despite their potential as feed ingredients.

World dried fruit production had a positive trend over the last decade due to its high nutritional value and common application in food technology (Rybicka et al., 2021). The almonds (*Prunus amigdalis*) and walnuts (*Juglans regia*) are the most consumed nuts worldwide (INC, 2023). Besides, the edible seed of dry fruits also contain the brown skin (seed coat), shell (hardened endocarp), and hull (flesh but thin mesocarp, green shell cover), which are considered as a waste (Prgomet et al., 2017; Barral-Martinez et al., 2021; Gonçalves et al., 2023). Almond hulls or hazelnut skin have been already used in ruminants and poultry feeding with positive results due to its high antioxidant properties (Wang et al., 2021ab; Musati et al., 2024; Recalde et al., 2024).

However, the almond shells are a major waste from the almond processing industry, around 35–75% of the total fruit weight, depending on the year and variety (Ebringerová et al., 2008). Shells contains lower phenolic contents than hulls, but the main chemical constituents are cellulose, hemicellulose and lignin in around 38.5%, 28.8% and 29.5%, respectively (Esfahlan et al., 2010; Li et al., 2018a). In case of hazelnut by-products, the shells cover more than 50% of the total hazelnut weight, becoming an important by-product of the hazelnut processing industry. Shells contains lignocellulosic material with 24.6–30% hemicelluloses, 27–27.3% cellulose, and 40.7–41% lignin, become an excellent lignocellulosic source (Pérez-Armada et al., 2019).

Grape pomace is a solid residue after grape pressing during wine production. It contains grape skins, pulps, and seeds which constitutes about 20% of the total processed grape mass (Teixeira et al., 2014). The composition and characteristics of the pomace vary depending on the grape variety, winemaking techniques, or pressing efficiency (Ahmed et al., 2020). It contains 6, 19 and 31% of hemicellulose, cellulose and lignin, respectively (FEDNA, 2019). In addition to a source of fiber, it represents a natural source of bioactive compounds, such as flavonoids, stilbenes, lignans, phenolic acids, and anthocyanins, becoming a valuable by-product for potential valorization as an ingredient (Boff et al., 2022; Chedea et al., 2022). Therefore, grape by-products may provide health benefits, acting as free radical scavengers or antioxidants (Goñi et al., 2007; Fiesel et al., 2014; Kafantaris et al., 2017; Hosseini-Vashan et al., 2020).

The olive oil industry generates a considerable quantity of by-products (pulp, cake, olive kernels, skin, and water) that are potential environmental pollutants due to their high organic load (Chouchene et al., 2010). Olive leaves, cake or pulp have been effectively included into broilers and pigs' diets (Ferrer et al., 2018; Pečjak et al., 2020). Olive kernel is an important by-product, representing approximately 10% of olive fruit weight (Rodrigues et al., 2015). However, since the main chemical constituents of olive kernel are cellulose (28.1–40.4%), hemicelluloses (18.5–32.2%) and lignin (25.3–27.2%) there is a real possibly to utilization as a lignocellulosic source of fiber (Rodríguez et al., 2008; Matos et al., 2010).

The fiber composition of different agricultural by-products is shown in the Figure 1

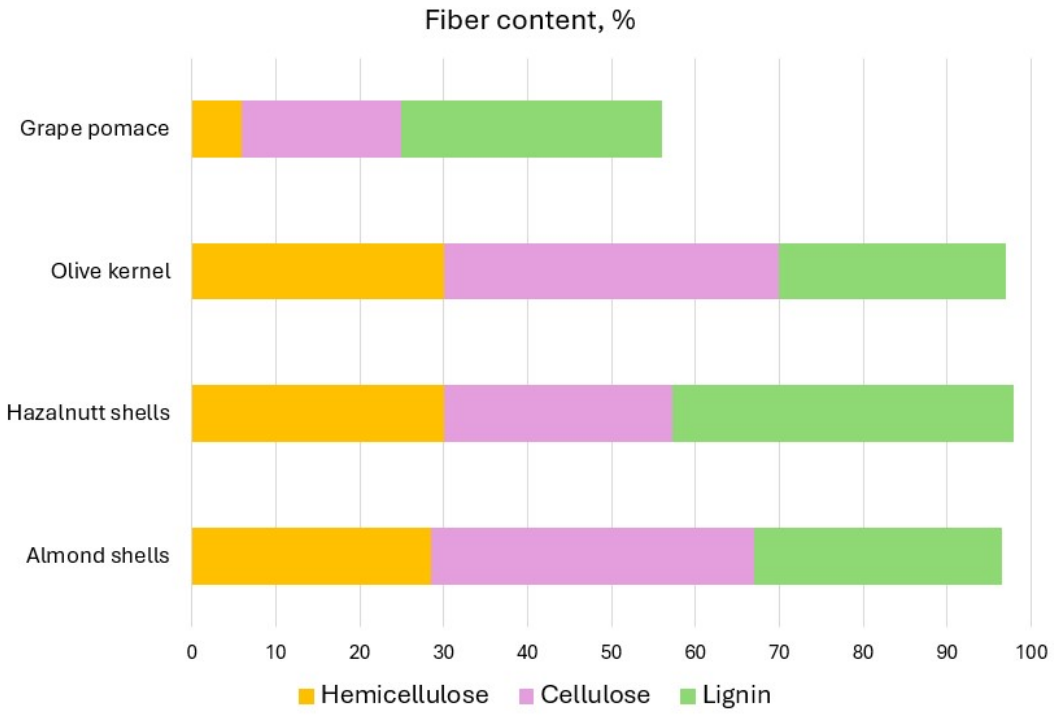


Figure 1. Concentration of different fiber fractions of four agriculture residues.

Hemicellulose: NDF-ADF, Cellulose: ADF-LAD, Lignin: LAD. Data from Esfahlan et al., 2010; Matos et al., 2010; FEDNA, 2019; Pérez-Armada et al., 2019

Overall, the agricultural wastes discussed above are characterized by a high calorific value and are often utilized in thermal processes or heating systems (Otero et al., 2021). However, since they are a suitable source of DF, their revalorization as feed ingredient for livestock resents an intriguing challenge (Chuang et al., 2021; Costa et al., 2022).

1.4. Micronization Process

Fiber sources traditionally used in animal feeding are frequently introduced into the feed mill with no specific pre-treatments. However, some of fiber rich by-products cannot be used directly due to their hardness and size, requiring technological processing to make them suitable for inclusion. In feed manufacturing, grinding is a standard process that applies force to break down the physical structures of ingredients, leading to particle size (PS) reduction (Lyu et al., 2020). Micronization is a potential technology that reduces a PS to micrometer dimensions, typically below 100 micrometers (Beutinger Bender et al., 2019a), thereby enabling inclusion of dried fruits by-products into farm animal diets.

Consequently, this process induces several modifications in the physical and chemical properties of fiber sources (Speroni et al., 2021). Generally, surface characteristics, porosity, hydration capacity, dissolution, and antioxidant activity, are frequently enhanced as a result of PS reduction, although depend deeply on the source (Gao et al., 2020). For instance, micronization of rye and barley increased porosity, and water retention capacity with PS reduction (Drakos, 2017). However, in other study superfine grinding decreased water holding capacity and swelling properties of pear pomace and grape pomace (Yan et al., 2019; Beutinger Bender et al., 2020). Ball-milling treatments of lotus root nodes and grape pomace produced a redistribution of fiber components from insoluble to soluble fractions due to destruction of the IDF constituents, such as cellulose and lignin, leading to increased SDF content (Hussain et al., 2017; Beutinger Bender et al., 2020). These modifications also may enhance the bioaccessibility of active compounds within the food matrix, thereby improving the antioxidant capacity of the products (Speroni et al., 2019).

Utilizing agro-wastes as a feed ingredient, supports the principles of a circular economy by converting waste into valuable resources. This closed-loop approach reduces environmental impact and promotes sustainability. Furthermore, incorporating these by-products into animal feed, enhances farm sustainability, adds value to these ingredients, and increases the circularity of the food chain, aligning with current societal demands and polices. To our best knowledge, no previous studies have investigated the use of mixtures of micronized, fiber-rich agricultural by-products in diets of young monogastrics.

STATE OF ART

2.1. Dietary Fiber

Generally, DF is a heterogeneous group of vegetal cell wall components that is not digested by the enzymatic secretion in the small intestine of monogastric animals (Trowell, 1972; Taghipoor et al., 2014). In consequence, it reaches the distal part of the GIT, where may act as a substrate for bacterial fermentation, modulating the microbial communities and their metabolic activities (Macfarlane and Macfarlane, 2012). However, the diversity of the chemical composition, physicochemical properties, and multiple physiological effects, make a development of accurate definition, very complex. Therefore, fiber can be defined following different criteria:

- Based on the chemical composition, fiber is constituted heterogeneous mixture of polysaccharides and associated substances mostly located in the plant cell wall. It is integrated by non-starch polysaccharides (NSP), including mainly celluloses, hemicelluloses (arabino-xylans, β-glucans, etc.), pectins, oligosaccharides, gums, and associated compounds like lignin (Selvendran, 1984; McDougall et al., 1996; Lindberg, 2014; Hald et al., 2016).
- From a nutritional point of view, it can be defined as cell-wall components of the plants that are resistant to hydrolysis by the monogastric's digestive enzymes, which reach the distal parts of the GIT, where are partially fermented by the microbiome (Trowell, 1972; Jha et al., 2019). Dietary fiber was defined by 1169/2011/EU, and updated in 2018, as carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: i) edible carbohydrate polymers naturally occurring in the food as consumed, ii) edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence, iii) edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence (European Commission, 2011).

The plant cell wall non-starch polysaccharides is a diverse group of molecules with varying degree of water solubility, complexity, and structure (Van Soest, 1994). **Cellulose** represents one of the most abundant polymers in nature. It is structurally homogenous, linear polymer of β-(1→4)-D-glucose units.

Hemicellulose is heterogeneous matrix of polysaccharides, which includes the cell wall components being neither cellulose nor pectin and possessing β-(1→4) linked backbones of glucose, mannose, or xylose. The term "hemicellulose" has been created when the structures and biosynthesis were not well understood. The high diversity of compounds suggest that it is inaccurate term, although it is still used as a single chemical entity. Therefore, arabinoxylans,

mixed linked ß-glucans, xyloglucans, mannans, galactomannans, galactans, arabinans are considered part of the hemicellulose (Xu et al., 2013; Choct, 2015; Agyekum and Nyachot, 2017). **Arabinoxylan** consists of major polysaccharide component of cell wall in cereals especially, wheat and rye. It is generally characterized by high viscosity and fermentability (Scheller and Ulvskov, 2010; Williams 2017, 2019). **β-Glucans** consist of D-glucose monomers linked by β-(1→4) and β-(1→3) glycosidic bonds. Its concentration varies among cereals with the highest content in oat and barley (2.8-4.1%). They provide considerable water solubility and gel-forming properties. **Xyloglucans** comprises a backbone of β-(1→4)-linked glucose residues, most of which are substituted with β-(1→6) linked xylose sidechains. **Xylans** are a diverse group of polysaccharides with a backbone of β-(1→4)-linked xylose residues. **Pectins** are highly heterogeneous soluble polysaccharides, characterized by large amount of galacturonic acid residues, linked by β-(1→4) bonds to the main backbone. **Gums** and **mucilages** are structurally similar, gel-forming soluble fiber contain galactose backbone linked by β-(1→3) and β-(1→6) bonds with side chains of arabinose, glucuronic acid, methyl-glucuronic acid or galactose (Soliman, 2019). **Resistant starch** is a homopolysaccharide of glucose forming a linear molecule of α-(1→4)-D-glucan. **Lignin** is extensively branched polymer composed of aromatic building blocks, associated with cell wall polysaccharides, interspersed between cellulose and hemicelluloses by ether and carbon-carbon bonds.

2.2. Physicochemical Properties of Fiber Sources

Fiber exerts different physiological functions through the gut, depending on its concentration and on the chemical composition and physicochemical properties, such as solubility, hydration capacity, fermentability, fermentation kinetics, and antioxidant capacity. According to solubility, fiber sources may be classified into soluble dietary fiber (SDF) and insoluble dietary fiber (IDF), which impact mostly the physiological effect of the DF (McCleary et al., 2010; Lindberg, 2014). Oligosaccharides, pectins, gums and mucilages, fructans, and part of hemicelluloses (e.g. xyloglucans, galactomannans mixed-linkage glucans), are included in SDF, whereas lignin, cellulose and most of hemicelluloses are considered as IDF (Navarro et al., 2019a; Williams et al., 2019). The PS seems to modulate the transit time and microbiota adherence into intestinal mucosa. The fermentability, and fermentation kinetics, are important factors affecting the intestinal microbiome. Finally, hydration properties (water holding, water binding and swelling properties) are also relevant to define the DF role on digestion (McConnell et al., 1974; Cadden, 1987; Zhou et al, 2018).

Thus, all these factors are relevant and affect the physiological effects of DF: i) transit time, ii) digestibility of nutrients in the small intestine, iii) fermentation, fermentation kinetics, and short chain fatty acids production, iv) water absorption in the large intestine, v) gut morphology and integrity of intestinal barrier. However, it is still difficult to find appropriate methods to

quantify the physical properties of feedstuffs that correlate well with their behavior in the digestive tract (Martens et al., 2019).

2.2.1. Solubility

Solubility is one of the most determinative properties of DF, which contributes to its physiological effect in the GIT. According to the grade of dispersibility of fibrous particles when mixed with water, SDF is highly dispersible, and commonly associated with greater potential to ferment, whereas IDF is not. Carbohydrate fermentation is generally a beneficial process in the large gut, protecting the host through the increase of the saccharolytic bacteria proliferation and SCFA production (Macfarlane and Macfarlane, 2012).

The fermentative capacity of IDF is limited, thus, its physiological effects depend mostly on the physical stimulation of the GIT, leading to increase the peristaltic movements, although it also depends on PS. In the stomach it modulates the empty speed (Lannuzel et al., 2024), and after it passes through the small intestine unaffected, increasing its proportion in the quimo progressively in relation to other nutrients (which are digested and absorbed). Its presence normally decreases the intestinal transit time, and increase fecal bulk, which promotes digestive regularity (Chesson, 1991).

In the large intestine, it may be partially used as substrate for bacteria fermentation, and modulate the proportion of the cellulolytic microbiota, which are able to produce SCFA, contributing to maintain the optimal environment of the GIT (Hald et al., 2016; Jha et al., 2019). Its medium to high capacity to bind water contribute to bulking properties of feces. Also, it may affect the pathogen bacteria adherence site in the mucosa, decreasing its proliferation (Molist et al., 2014).

The physiological effects of SDF are usually the opposite. Highly fermentable substrates have greater impact on the microbiota modification and their end products, which impair less the total tract digestibility than IDF (Zhao et al., 2020). However, an important factor to point out of SDF is the potential increase of intestinal viscosity especially associated to pectins and β-glucans (Pascoal et al., 2015). Consequently, it influences the rate and extent of digestion, interferes with nutrient absorption, as well as decreases the feed intake. Accumulation of digesta in the distal part of the GIT delays the gastric emptying, and increases the time of intestinal transit (Hedemann et al., 2006; Bach Knudsen et al., 2016).

In contrast, non-digestible oligosaccharides, another fraction of DF are soluble and highly fermentable sources with no negative effects on intestinal viscosity, which are considered as a prebiotics. Prebiotics are defined as a non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson and Roberfroid, 1995). The most commonly sources are fructooligosaccharides (FOS), galacto-oligosaccharides, manna-oligosaccharides,

oligofructose, trans-galacto-oligosaccharides, gluco-oligosaccharides, glico-oligosccharides, lactulose, lactitol, malto-oligosaccharides, xylo-oligosaccharides, stachyose, raffinose and inulin (Hajati and Rezaei, 2010; Markowiak and Śliżewska, 2018). These sources have been widely used in the livestock diets with generally beneficent effects on the microbial population with higher proportion of *Lactobacillus* or *Bifidobacterium* (Khoobani et al., 2019; Csernus and Czeglédi, 2020).

Therefore, the effects of SDF may be positive or negative depending on the balance between benefits associated with the high fermentability, and impairments by intestinal viscosity, or intestinal morphology. Likely low amounts of highly fermentable sources would provide the best respond. However, viscosity is less important in pigs than in poultry, due to higher water content in the GIT of the pig, and its negative effects normally decrease with aging (Saki et al., 2011a; Diao et al., 2020).

2.2.2. Particle Size

The mean PS and its distribution is of major importance, determining the role on the digestive tract: transit time, fermentation, fecal excretion (Hussain et al., 2017). Generally, finely grinding is associated with an increase of the particles number, and greater specific surface area, which contribute to a more extensive contact between digestive enzymes and substrates, making the enzymatic digestion of non-fibrous constituents more effective (Mavromichalis et al., 2000; Kiarie and Mills, 2019; Sun et al., 2019).

In pigs, PS reduction of the feed has been reported as one of the main reasons for ulcerations and gastric keratinization (Lancheros et al., 2020; Jo et al., 2021). However, low inclusion levels of finely ground fiber sources such as micronize lignocellulose did no promote gastric ulceration (Liesner et al., 2009).

In poultry, it is an important factor to determine the effect of DF on the GIT motility. Coarse particles increase the peristaltic and antiperistaltic movements, associated to the gizzard development (Amerah et al., 2007; Mateos et al., 2012). Large particles are selectively retained in the gizzard, allowing an increase of the musculature and digestive enzymes secretion, leading to better nutrient digestibility. On the other hand, finely ground particles escape easily and fasten the gizzard emptying (Amerah et al., 2009; Svihus, 2011, 2014). The effect of different fiber sources on gizzard and proventriculus development is shown in Table 1.

Also, PS reduction increases the soluble fraction and the antioxidant characteristics of IDF (Speroni et al., 2019; Yan et al., 2019), improving the microbial fermentation and SCFA production (Alam et al., 2014; Vermeulen et al., 2017).

In summary, it is not well known what part of the effect of the fiber is due to its chemical composition, its PS, or interaction between both.

Table 1. The effect of fiber sources inclusion on relative proventriculus and gizzard's development (relative to bird's body weight) in broilers, compared to non-supplemented animals (%)[1].

Fiber sources	Inclusion level, %	Age, d	CF feed[3], %	Proventriculus	Gizzard	Reference
Almond hulls	2.5	19	2		↓8	(Wang et al., 2021b)
Cellulose	0.3	42	nd		↓2	(Rezaei et al., 2011)
Cellulose	0.4	42	nd		↓2	(Rezaei et al., 2011)
Cellulose	0.5	42	nd		↓2	(Rezaei et al., 2011)
Lignocellulose	0.8	35	4.5-6.7		↓2	(Zeitz et al., 2019)
Lignocellulose partially fermentable	0.8	35	4.5-6.7		↓9	(Zeitz et al., 2019)
Lignocellulose	1.0	35	1.9-2.7		↑2	(Kheravii et al., 2017)
Lignocellulose	2.0	35	1.9-2.8		↑0.2	(Kheravii et al., 2017)
Lignocellulose	3.0	35	1.9-2.9	**↑17**	**↑10**	(Rezaei et al., 2014[2])
Cassava pulp fiber	0.5	21	nd		↑2	(Okrathok and Khempaka, 2020)
Cassava pulp fiber	0.5	42	nd	↑3	↑4	(Okrhatok and Khempaka, 2020)
Cassava pulp fiber	1.0	21	nd	↑2	**↑7**	(Okrhatok and Khempaka, 2020)
Cassava pulp fiber	1.0	42	nd	↓5	**↑11**	(Okrhatok and Khempaka, 2020)
Cassava pulp fiber	1.5	21	nd	↑2	**↑9**	(Okrhatok and Khempaka, 2020)
Cassava pulp fiber	1.5	42	nd	↓5	**↑11**	(Okrhatok and Khempaka, 2020)
Rice hulls	3.0	21	3.5-3.7	↑3	↑1	(Saadatmand et al., 2019
Wheat bran	3.0	21	nd	↑8	**↑25**	(Shang et al., 2020a)
Wheat bran	3.0	42	nd	↑13	**↑12**	(Shang et al., 2020a)
Sugar beet pulp	3.0	21	3.5-3.7	↑8	↓6	(Saadatmand et al., 2019)
Pectin and cellulose	2.0+1.0	21	0.8	↓14	↓12	(Saki et al., 2011b)
Pectin and cellulose	1.5+1.5	21	0.8	↓8	↓2	(Saki et al., 2011b)
Pectin and cellulose	1.0+2.0	21	0.8	↓15	↓5	(Saki et al., 2011b)
Pectin and cellulose	2.0+1.0	42	0.8	**↑20**	**↑20**	(Saki et al., 2011b)
Pectin and cellulose	1.5+1.5	42	0.8	↑1	**↓19**	(Saki et al., 2011b)
Pectin and cellulose	1.0+2.0	42	0.8	**↓18**	**↓34**	(Saki et al., 2011b)

nd: no data reported,

[1] Significant differences ($P<0.05$) are marked in bold; [2] Quails; [3] Crude fiber content of the control diet;

2.2.3. Hydration Capacity (HC)

Hydration properties of DF describe the way of interaction between DF and water. Raghavendra et al. (2006) defined the water holding capacity (WHC) as the ability to hold water within the feed matrix without the application of any external force. On the other hand, water binding capacity (WBC) refers to the quantity of water that bound to the hydrated fiber following the application of an external force, such as pressure or centrifugation. Swelling capacity (SC), measures the ratio of volume occupied when the sample is immersed in an excess of water and after equilibration to the actual weight.

The hydration or water retention properties of DF are related to the chemical structure of the polysaccharides, porosity, PS, ionic form, pH and temperature (Beutinger Bender et al., 2019ab). In general, HC is positively correlated with the amount of total dietary fiber (Ngoc et al., 2012; Brachet et al., 2015). Soluble sources such as sugar beet pulp, lime pulp and citrus pulp, are normally characterized by higher values (Jongaroontaprangsee et al., 2007; Jiménez-Moreno et al., 2009; Slama et al., 2019), due to high potential of pectin for taking water up into the amorfous phase (Kunzek et al., 1999). In contrast, HC of IDF sources is generally lower due to hydrophobicity and crystalline structure, except for lignocellulose, which is higher (Slama et al., 2019). However, surprisingly, IDF sources, specially lignocellulose, were observed to increase fecal bulking and decrease the faces humidity in pigs and poultry (Kim et al., 2008; Rezaei et al., 2011; Molist et al., 2012), although the mechanism involved in is not yet fully understood.

2.2.4. Fermentability of Fiber Sources

The indigestible components reach the distal part of the intestine where are partially fermented by intestinal microflora, providing a substrate to grow for resident bacteria. The fermentation characteristics of DF depends on the physicochemical properties, being the water solubility one of the most determining in monogastrics.

It is generally assumed that IDF is not fermented in the small intestine but in pigs it can disappear there up to a 50% depending on the source of fibre (Keys and DeBarthe, 1974; Graham et al., 1986; Schulze et al., 1994; Wilfart et al., 2007). In many cases, IDF fermentation is moderate and prolonged, taking place mostly in distal GIT (Urriola et al., 2010; Jaworski and Stein, 2017). In contrast, SDF seemed more easily fermented but its digestibility rendered even negative values in the small intestine (Wilfart et al., 2007). It was due to the interference of intestinal mucins in the determination of SDF of ileal digesta, and once corrected its ileal digestibility is greater than that for IDF (Abad-Guamán et al., 2015; Montoya et al., 2015). Anyway, SDF is also fermented in the cecum and proximal colon (Bach Knudsen et al., 2016).

The fermentation process results in a high number of bio-active end-products, which may be absorbed across the intestinal mucosa or have antimicrobial effect on specific bacteria species

(Hald et al., 2016; Williams et al., 2017). Fermentation of carbohydrates results predominantly in the production of SCFA: acetic, propionic, butyric and valeric acid, which are commonly associated with health benefits. The effects of different fiber sources on SCFA production are shown on the Table 2. Sources of IDF such as lignocellulose, wheat bran, corn bran, or soybean hulls increased butyrate (Molist et al., 2009; Zhao et al., 2018; Shang et al., 2019; Silva Guillen et al., 2022), whereas cellulose and wheat bran increased acetate levels in weaned piglets (Hanczakowska et al., 2008; Molist et al., 2012; Shang et al., 2019). Dietary FOS addition increased the acetic, propionic and butyric acids in weaned piglets, as well as total SCFA content in large intestine of suckling piglets (Correa Matos et al., 2003; Xu et al., 2005). On the other hand, effects of IDF sources on butyrate production in poultry are not so clear. Only wheat bran administration increased cecal butyrate levels *in vivo* (Shang et al., 2020b) and i*n vitro* (Vermeulen et al., 2017). Based on the results reported in the metanalysis performed by Worawang et al. (2022), the concentration of butyrate was not affected by prebiotic sources. However, inulin or FOS supplementation increased butyrate concentration in broilers (Cao et al., 2005) and turkeys (Juskiewicz et al., 2006).

On the other hand, protein fermentation leads to branched-chain fatty acids (BCFA), amines, phenols and sulphides production. Excessive levels of these metabolites may represents a potential risk factor for disrupting the intestinal ecosystem, and impairing animal health (Macfarlane and Macfarlane, 2012; Jha et al., 2019; Williams et al., 2019). Therefore, reducing protein fermentation is a crucial objective that can be achieved by lowering the protein content of the diet, or by administering fermentable carbohydrates, which would decrease both the protein fermentation and ammonia production (Kim et al., 2008).

2.2.5. Antioxidant Activity

Some DF sources, such as wine industry or olive by products, are rich in polyphenols (Speroni et al., 2019, 2021). These bioactive molecules have a beneficial action on the health status of the animals, preventing oxidative cells damage (Chedea et al., 2019). Grape by-products improved antioxidant status, and modulated the microbiota leading to better nutrient digestibility and growth performance in pigs and poultry (Goñi et al., 2007; Fiesel et al., 2014; Kafantaris et al., 2017; Hosseini-Vashan et al., 2020).

Table 2. The effect of dietary fiber inclusion on piglet's cecal SCFA production, compared to non-supplemented animals (%)[1].

Piglets									
Fiber source	Inclusion level, %	Age, d	NDF feed[2], %	Acetate	Propionate	Butyrate	Valeriate	Lactate	Reference
Lignocellulose	1.0	35	nd	↑7	↓12	↑46			(Silva-Guillen et al., 2022)
Lignocellulose	1.5	54	11-12	↑3	↓3	↑**8**			(Slama et al., 2020)[3]
Lignocellulose	2.0	35	nd	↑8		↑43			(Silva-Guillen et al., 2022)
Lignocellulose	3.0	35	nd	↑21	↑15	↑86			(Silva-Guillen et al., 2022)
Lignocellulose fermentable	1.5	54	11-12	↑4	↓16	↑**10**			(Slama et al., 2020)[3]
Corn bran	5.0	35	nd	↓6	↑16	↑93	↑**150**		(Zhao et al., 2018)[5]
Wheat bran, fine	4.0	33	14.5	↓13	↑2	↓15			(Molist et al., 2012)[4]
Wheat bran, coarse	4.0	33	14.5	↑25	↑31	↑6			(Molist et al., 2012)[4]
Wheat bran	5.0	35	nd		↑25	↑**77**	↑75		(Zhao et al., 2018)[2]
Wheat bran	6.0	56	9.5-10.5	↑**22**	↓0.9	↑**70**	↑14		(Shang et al., 2019)
Wheat bran	8.0	39	7.7	↑60	↑19	↑**207**		↑66	(Molist et al., 2009)
Wheat bran & sugar beet pulp	4+3	39	7.7	↑80	↑70	↑**167**		↓68	(Molist et al., 2009)
Soybean hulls	2.5	54	11-12	↑1	↓3				(Slama et al., 2020)[3]
Soybean hulls	5.0	35	nd	↑17	↑24	↑**76**	↑60		(Zhao et al., 2018)[2]
Sugar beet pulp	6.0	56	9.5-10.5	↑**27**	↑17	↑44			(Shang et al., 2019)
Sugar beet pulp	6.0	39	7.7	↑112	↑45	↑4		↑163	(Molist et al., 2009)
Fructooligosaccharides	0.4	54	nd	↑**20**	↑**56**	↑**44**	↓31		(Xu et al., 2005)
Inulin, short chain	4.0	27	nd	↓3	↑9	↓4	↑27		(Paßlack et al., 2012)
Inulin, long chain	4.0	27	nd	↓**15**	↑**32**	↑20	↑**82**		(Paßlack et al., 2012)
Inulin, solution	20.0	28	-	↑2	↑**12**	↓1	↑**20**		(Li et al., 2018)
Inulin, solution	30.0	28	-	↓1	↓6	↓16	↑3		(Li et al., 2018)

nd, no data reported; [1]Significant differences (P<0.05) are marked in bold; [2]Neutral detergent fiber content of the control diet; [3]Analyzed in ileal digesta samples, [4] Analyzed in colon digesta sample, [5]Analyzed with fresh fecal sample.

Table 2 (cont). The effect of dietary fiber inclusion on poultry cecal SCFA production compared to non-supplemented animals (%)[1].

Broilers									
Fiber source	Inclusion level, %	Age, d	CF feed[3], %	Acetate	Propionate	Butyrate	Valeriate	Lactate	Reference
Lignocellulose	0.25	42	nd	↑3	↑13	↓2		↑37	(Bogusławska-Tryk et al., 2015)
Lignocellulose	0.50	42	nd	↑12	↑18	↓5		**↑50**	(Bogusławska-Tryk et al., 2015)
Lignocellulose	0.80	35	4.5-6.7	↑23	↓18	↓4	↓27		(Zeitz et al., 2019)
Lignocellulose	1.0	42	nd	↓11	↑8	↓14		↑11	(Bogusławska-Tryk et al., 2015)
Lignocellulose fermentable	0.80	35	4.5-6.7	↓2	↓12	**↓26**	↓30		(Zeitz et al., 2019)
Sunflower hulls	3.0	21	nd	↑1	↓5	↑5	↑7		(Kimiaeitalab et al., 2018)
Wheat bran	3.0	21	nd	↑6	↑13	↑30	↑26		(Shang et al., 2020b)
Wheat bran	3.0	42	nd	↑16	↑40	**↑22**	↑24		(Shang et al., 2020b)
Fructooligosaccharides	0.40	42	nd	↑5	↓11	↓4	**↑54**		(Cao et al., 2005)
Fructooligosaccharides	0.50	21	nd	↓31			↓4		(Al-Khalaifa et al., 2019)
Fructooligosaccharides	0.50	56	3.4	↑11	↑12	↑32	↑15		(Juśkiewicz et al., 2006)[2]
Fructooligosaccharides	1.0	56	3.4	↑6	↑30	↑29	↑26		(Juśkiewicz et al., 2006)[2]
Fructooligosaccharides	2.0	56	3.4	↑30	↑3	**↑77**	↑42		(Juśkiewicz et al., 2006)[2]
Mannooligosaccharides	0.50	21	nd	↓19			↑41		(Al-Khalaifa et al., 2019)
Inulin	1.0	35	1.5	↓8	↓9	**↑103**	↑10		(Rehman et al., 2007)
Inulin	1.0	35	nd	↓6	↑7	**↑25**			(Rebolé et al., 2010)
Inulin	2.0	35	nd	↓2	↓1	**↑26**			(Rebolé et al., 2010)

nd, no data reported; [1]Significant differences (P<0.05) are marked in bold; [2]Turkeys; [3]Crude fiber content of the control diet

2.3. Physiological Implications of Dietary Fiber

2.3.1. The Effect ofDdietary Fiber on Gut Barrier Integrity and Intestinal Microbiota

A well-structured mucus layer is the first physical barrier against microbial invasion, particularly relevant in the post-weaning period and in young broilers (Shehata et al., 2022). The proper balance among the intestinal epithelial cell layer, tight junctions proteins (occludin - OCLN, claudins - CLDN, and zonula occludens), and the gut microbiota, along with their fermentation end products, is crucial for maintaining intestinal integrity and permeability (Farré et al., 2020; Mahmood and Guo, 2020). These components interact with each other, preventing the pathogens adhesion, toxins translocation and enteric disease (Gresse et al., 2017; Jha et al., 2019).

A wide variety of pathogenic bacteria species such as *Escherichia coli, Klebsiella spp., Campylobacter spp., Streptococcus spp., Clostridium perfringens, Clostridium difficile* or *Bacteroides fragilis* are protein fermenters, producing substantial amounts of ammonia and other potentially toxic compounds such as branched-chain fatty acids, indoles, thiols, phenols, and amines (Pieper et al., 2014; Jha and Berrocoso, 2015). The accumulation of these components may impair barrier function, causing colonic diseases and diarrhea problems (Macfarlane and Macfarlane, 2012; Williams et al., 2019).

Fiber supplementation is effective to maintain the barrier integrity, by enhancing the richness and diversity of the gut microbiota (Liu et al., 2018; Pu et al., 2020). However, the mechanism depends on the structure, composition and fermentability of the fibre sources (Hald et al., 2016; Bedford et al., 2024).

For instance, feeding 1% inulin resulted in greater microbial diversity and richness compared to the same level of lignocellulose (Chen et al., 2019b). Supplementation with 3% soybean hulls, or 9% citrus pulp reduced proliferation of potentially pathogenic *E. coli* proliferation in the small intestine and cecum of piglets (Pascoal et al., 2015). The increase of fiber-degradating bacteria enhances the hindgut fermentation and production of SCFA, decreasing the pH in the gut, which contribute to harmful conditions for pathogenic bacteria proliferation (Jha et al., 2019; Feng et al., 2024).

On the other hand, insoluble sources contribute to the exclusion of pathogenic bacteria from the epithelial surface (Dou et al., 2017; Mahmood and Guo, 2019), through abrasive action on the mucosal surface, thereby impairing the adherence of pathogenic bacteria (Molist et al., 2014). Purified cellulose and coarse ground wheat bran decreased the levels of *E. coli* and *C. perfringens,* blocking the adhesion sites, which reduced the ability of pathogens to remain in the gastrointestinal tract (Hanczakowska et al., 2008; Molist et al., 2012; Pascoal et al., 2012).

In broilers, lignocellulose inclusion (0.25-1.0%) reduced *Clostridium* and *E. coli* colonization, and increased population of *Lactobaciullus spp*. (Bogusławska-Tryk et al., 2015; Makivich et al., 2018; Sozcu, 2019).

Supplementation of FOS (0.25%) and mannooligosaccharides (0.025-0.05%) increased

Lactobacillus and decreased of *E. coli* compared to control broilers (Kim et al., 2011). Moreover, chicory root (0.1-0.2%) supplementation inhibited the growth of potentially pathogenic *E. coli,* and enhanced the growth of beneficial *Lactobacillus* gut bacteria (Khoobani et al., 2019).

The DF provides a fermentative substrate maintaining a viable and diverse microbiota and production of short-chain fatty acids in the GIT, which depends on the chemical composition and physicochemical properties of the dietary carbohydrates (Bach Knudsen et al., 2019; Yang et al., 2020).

Butyrate, in particular, is the most important fermentation end-product, which acts as the primary energy source for colonocytes proliferation (Jha et al., 2019; Lallès and Montoya, 2021). Additionally, it contributes to a wide range of process, resulting in enhancement of digestive tract functionality: i) improves the enterocyte morphology and tight junctions, ii) contributes to mucus secretion, iii) improves the biosynthesis of defense peptides and promote the immunity of host, and iv) prevents infectious diseases (Guilloteau et al., 2010). This stimulation of mucosal defense mechanisms inhibits the expression of inflammatory signal pathways, and proinflammatory cytokines (IL-1b, IL-6, and TNF-α; Sun et al., 2021).

Dietary fiber may improve the intestinal barrier functionality, through the physical effect or by the physiological effects produced by SCFA, especially butyrate. It may promote the expression of tight junction proteins, and alleviate the systemic and local inflammation, leading to an increase of the intestinal health status in monogastric animals (Shang et al., 2020b; Sun et al., 2021). Administration of inulin and lignocellulose were positively correlated with higher expression of CLDN-1, OCLN, Muc1 and IL-10 in piglets (Chen et al., 2019a). Also, high expression of tight junction protein was positively associated with the abundance of lactic acid-producing bacteria in the intestine of weanling pigs (Zhang et al., 2022). Wheat bran (3%) up-regulated ileal mRNA levels of occluding in piglets (Shang et al., 2021), and expression of zonula occluding and intracellular proteins in ileum in broilers, compared to control animals, suggesting better intestinal barrier function due to reduction of pro-inflammatory cytokines (Shang et al., 2020b). Thus, fiber alleviates local and systemic inflammation, providing the first line of defense against pathogens.

2.3.2. Intestinal Morphology

The intestinal morphology is relevant, since it forms part of the gut barrier integrity and interferes with nutrient digestibility. An optimal villus development, and high villus height to crypt depth ratio are indicators of the intestinal mucosa maturity and functionality (Jha and Mirsha, 2021). However, the positive effects of fiber supplementation would depend on different factors, including the level, chemical composition, PS, solubility and the animal's species and age. The effects of fiber supplementation on the intestinal morphology of monogastrics are summarized in Table 3.

In piglets, the greatest villus height in duodenum and jejunum, as well as the largest surface

area available for nutrients absorption, was observed in fiber supplementation at 12.2% neutral detergent fiber (NDF) (Nepomuceno et al., 2006). In poultry, moderate crude fiber levels (4%) of finely ground soybean hulls improved jejunal villus height to crypt depth ratio when compared to high crude fiber levels (8%), suggesting that too high fiber content may impair villus height due to its abrasive function (Tejeda and Kim, 2021a).

The DF solubility impacts the mucosa morphology, being IDF widely reported to improve intestinal morphology in pigs and poultry. Addition of 3% of wheat bran rich in hemicellulose and cellulose, or 1.27% of pine pollen rich in lignin, increased jejunum villus height, width and villus area (Schedle et al., 2008), whereas 2% of pure cellulose increased the jejunal villus high and deeper crypt depth (Hanczakowska et al., 2008).

In poultry, elongation of the villi in the ileum, and a gradual increase in villus height to crypt depth ratio in all sections of the small intestine, was observed in broilers receiving low levels (0.25, 0.5 and 1.0%) of lignocellulose (Bogusławska-Tryk et al., 2020), whereas cellulose (0.3-0.5%) improved ileal villus high to crypt depth ratio (Rezaei et al., 2011).

The contrary effect seems to be associated with SDF inclusion. Dietary inclusion of citrus pulp or sugar beet pulp impaired the gut mucosa morphology when compared with pigs fed wheat bran or purified cellulose (Pascoal et al., 2015; Shang et al., 2019). Also, those fed pectin-based diets had lower villus height and crypt depth, and lower mucin content, suggesting poorer protection against pathogenic bacteria when compared with pigs fed IDF diets (Hendemann et al., 2006).

In broilers, supplementation with 3% sugar beet pulp reduced the villus high at 12 and 21d, suggesting poorer nutrient absorptive capacity (Jiménez-Moreno et al., 2013; Sadeghi et al., 2015). Carboxymethyl cellulose addition at 2 and 4% to low crude fiber diet impaired the villus heigh, crypt depth and villus height to crypt depth ratio (Rajmatnejad and Saki, 2015). High digesta viscosity due to high SDF content is recognized as the main cause of intestinal mucosal atrophy, and lower enzyme diffusion into nutrients which impact negatively the digestive capacity (Tejeda and Kim, 2021b; Jha and Mishra, 2021).

However, the negative effects of high viscosity seem to decrease with ageing. The GIT maturation enhances its development and functionality by the adaptation of the morphological structures, affecting the intestinal absorption capacity (Molist et al., 2014). In growing pigs fed sugar beet pulp, higher villus height and better villus height to crypt depth ratio were found (Diao et al., 2020). In poultry, 3% inclusion level of sugar beet pulp produced no effects on mucosa morphology compared to control at 42d (Saadatmand et al., 2019; Sabour et al., 2019). Also, dietary supplementation with 2% pectin and 1% cellulose impaired the villi heigh, villi heigh to crypt depth ratio, and the villi surface area at 14 and 21d, with no negative effects at 42d (Saki et al., 2011a).

On the other hand, oligosaccharides do not increase GIT viscosity (Hartini et al., 2003) and may produce positive effects even in very young animals. Water solution of FOS supplementation in piglets during the suckling period, increased villi height and produced deeper crypt depth in the

jejunum at weaning (Schokker et al., 2018), whereas those receiving 0.2% dietary oligofructose, had higher crypt depth in the duodenum (Shim et al., 2005).

In poultry, birds fed the diets containing MOS had longer duodenal villi (Houshmand et al., 2012), whereas villus height and crypt depth were improved in the ileum of broiler chickens fed FOS (Xu et al., 2003; Shang et al., 2015).

In general, IDF exert more favorable effect on the intestinal morphology than SDF, mainly due to its high viscosity, with the exception of oligosaccharides, that may improve the absorption surface area, even in suckling piglets.

Table 3. The effect of fiber sources inclusion on the small intestine morphology: villus height (VH), crypt depth (CD) and villus height to crypt depth ratio (VH:CD) compared to non-supplemented piglets and broilers (%)[1].

Fiber source					Duodenum			Jejunum			Ileum			
Piglets	Inclusion level, %	Age, d	NDF feed[2], %	CF feed[3], %	VH	CD	VH:CD	VH	CD	VH:CD	VH	CD	VH:CD	Reference
Cellulose	0.5	35	nd	5.5				↓9	↓7	↓2				(Hanczakowska at al., 2008)
Cellulose	1.5	50	10	3.4	↓9	↓7	↓2	↓16	↓15					(Pascoal et al., 2015)
Cellulose	1.5	35	nd	5.5				↑**10**	↓**17**	↑33				(Hanczakowska at al., 2008)
Cellulose	2.0	35	nd	5.5				↑**13**	↓**15**	↑33				(Hanczakowska at al., 2008)
Lignocellulose	1.0	35	nd	nd	↑11	↑6	↑8	↑14	↑4	↑16				(Silva-Guillen et al., 2022)
Lignocellulose	2.0	35	nd	nd	↓6	↓1	↓2	↓8	↑**26**	↓30				(Silva-Guillen et al., 2022)
Lignocellulose	3.0	35	nd	nd	↓0.3	↑15	↓12	↑4	↑16	↓8				(Silva-Guillen et al., 2022)
Wheat bran	6.0	56	9.5-10.5	nd	↑3	↓1	↑3	↑3	↓1	↑3	↑11	↑4	↑**7**	(Shang et al., 2019)
Rice hulls and pectin	1.25+0.6	50	nd	4.16	↑3	↓3	↑7	↑21	↑12	↑8	↑30	↑19	↑8	(Taksinanan et al., 2020)
Rice hulls and pectin	2.25+0.7	50	nd	4.16	↑9	↑8	↑1	↑2	↑1	↑1	↑33	↑3	↑21	(Taksinanan et al., 2020)
Soyhulls	3.0	50	10	3.4	↑5	↑5	↑2	↓20	↓16	↓2				(Pascoal et al., 2015)
Sugar beet pulp	6.0	56	9.5-10.5	nd	↓1	↑5	↓6	↓1	↑7	↓8	↑0.5	↑1	↓1	(Shang et al., 2019)
Citrus pulp	9.0	50	10	3.4	↑2	↓6	↑8	↓16	↓16	↑2				(Pascoal et al., 2015)
Broilers														
Cellulose	0.3	42	nd	nd							↑10	↓14	↑**30**	(Rezaei et al., 2011)
Cellulose	0.4	42	nd	nd							↑11	↓14	↑**27**	(Rezaei et al., 2011)
Cellulose	0.5	42	nd	nd							↑21	↓21	↑**56**	(Rezaei et al., 2011)
Cellulose	2.0	21	nd	0.05							↑3	↓2	↑5	(Rajmatnejad and Saki, 2015)
Cellulose	4.0	21	nd	0.05							↑5	↑1	↑3	(Rajmatnejad and Saki, 2015)
Cellulose + pectin	1.0+2.0	21	nd	0.8				↓**19**	↓11	↓**9**				(Saki et al., 2011a)
Cellulose + pectin	1.5+1.5	21	nd	0.8				↓2	↓10	↑8				(Saki et al., 2011a)
Cellulose + pectin	2.0+1.0	21	nd	0.8				↑1	↓7	↑6				(Saki et al., 2011a)

Significant differences ($P<0.05$) are marked in bold; [2] Neutral detergent fiber content of the control diet; [3]Crude fiber content of the control diet.

nd no data reported

Table 3 (cont). The effect of fiber sources inclusion on the small intestine morphology: Villus height (VH), crypt depth (CD) and villus height to crypt depth ratio (VH:CD) compared to non-supplemented broilers (%)[1]

Broilers				Duodenum			Jejunum			Ileum			
	Inclusion level, %	Age, d	CF feed[2], %	VH	CD	VH:CD	VH	CD	VH:CD	VH	CD	VH:CD	Reference
Lignocellulose	0.25	21	2.8	↓11	↓31	**↑32**	↓10	**↓31**	**↑33**	↓2	**↓36**	**↑60**	(Bogusławska-Tryk et al., 2020)
Lignocellulose	0.4	28	3.4	↑4	↓1		↑0.2	↑4		**↑12**	↑5		(Makivich et al., 2018)
Lignocellulose	0.5	21	2.8	**↓15**	**↓41**	**↑48**	↓14	**↓35**	↑29	**↑11**	**↓38**	**↑82**	(Bogusławska-Tryk et al., 2020)
Lignocellulose	0.5	35	3.4-3.8				**↑22**	↓5	**↑28**				(Sozcu, 2019)
Lignocellulose	0.6	28	3.4	**↑18**	**↑25**		**↑13**	**↑18**		**↑18**	**↑19**		(Makivich et al., 2018)
Lignocellulose	1.0	21	2.8	↓2	↓28	**↑83**	↑15	**↓46**	**↑112**	**↑16**	**↓46**	**↑114**	(Bogusławska-Tryk et al., 2020)
Lignocellulose	1.0	35	3.4-3.8				**↑41**	**↓13**	**↑63**				(Sozcu, 2019)
Lignocellulose	2.0	35	3.4-3.8				**↑35**	↓6	**↑44**				(Sozcu, 2019)
Rice hulls	3.0	42	3.5-3.7				↓11	↓5	↓7	↑30	↑13	↓10	(Saadatmand et al., 2019)
Rice hulls	3.0	42	3.9-4.7	**↑13**	↓2	↑19	↓5	↑10	↓20	↓1	**↑28**	↓20	(Sabour et al., 2019)
Sunflower hulls	3.0	21	nd							↑6	↑2	↑3	(Kimiaeitalab et al., 2018)
Wheat bran	3.0	42	nd	↑8		↑6	**↑21**	↓5	**↑26**	**↑24**	↓0.6	**↑26**	(Shang et al., 2020a)
Carboxymethyl cellulose	2.0	21	0.05							**↓5**	**↑14**	**↑8**	(Rajmatnejad and Saki, 2015)
Carboxymethyl cellulose	4.0	21	0.05							**↓6**	**↑15**	**↓18**	(Rajmatnejad and Saki, 2015)
Sugar beet pulp	3.0	42	3.5-3.7				↑3	↓2	↑7	↑27	↑5	↓10	(Saadatmand et al., 2019)
Sugar beet pulp	3.0	42	3.9-4.7	↑5	↓4	↑14	↓7	↓5	↓16	↓14	↓4	↓12	(Sabour et al., 2019)
Fructooligosaccharides	0.5	21	nd	↑7	↑6	↓1	↓1	↓4	↑2	**↑16**	**↑10**	↑5	(Shang et al., 2015)
Fructooligosaccharides	0.2	49	nd	↓3	↑1	↓4	↑3	↓7	↑10	↑11	↓16	**↑31**	(Xu et al., 2003)
Fructooligosaccharides	0.4	49	nd	↑2	↑3	↓1	↑4	**↓17**	**↑26**	**↑16**	**↓26**	**↑55**	(Xu et al., 2003)
Fructooligosaccharides	0.8	49	nd	↓0.6	↑0.2	↓0.7	↑3	↓0.6	↑10	↑5	↓11	**↑18**	(Xu et al., 2003)
Xylo-oligosaccharides	0.02	35	2.3				**↑62**	↓26	↑40	**↑22**	↓21	↑51	(Yang et al., 2021)
Fructooligosaccharides	0.02	35	2.3				**↑11**	↓31	↑4	**↑32**	↓0.4	↑40	(Yang et al., 2021)
Inulin	1.0	35	nd				↑4	↓5	**↑14**				(Rebolé et al., 2010)
Inulin	2.0	35	nd				↑2	↑8	↓3				(Rebolé et al., 2010)

nd, no data reported; [1]Significant differences (P<0.05) are marked in bold; [2]Crude fiber content of the control diet.

2.3.3. Effect of DF on Nutrient Digestibility

The effects of DF on nutrient digestibility deeply depends on the physicochemical properties of fiber sources. The effect of main fiber sources on total tract apparent digestibility (TTAD) digestibility is summarized in the Table 4.

As a concept, DF is usually assumed to be indigestible in the small intestine, although positive results have been reported as it was previously indicated. Instead, it is well accepted that it can be partially fermented in the distal part of intestine providing energy (Jha et al., 2019). So, it is important to differentiate between apparent ileal digestibility (AID) and TTAD, which values changes with fermentable sources (Jiménez-Moreno et al., 2009; Berrocoso et al., 2015). Most of the bibliographic evidence reported below refers to TTAD values due to availability of data.

The digestibility of fiber is typically low, as it is classified as an indigestible component for monogastric animals, and largely dependents on its chemical composition. None of the components is digestible and evermore, the cellulose and lignin are in addition low or nonfermentable (Van Soest, 1994; Bachmann et al., 2021).

Presence of fiber fractions in the diet may affect also the digestibility of other nutrients. A negative effect of the fiber inclusion was reported on the energy digestibility (Freire et al., 2000). Le Sciellour et al. (2018) observed 0.8% reduction of energy TTAD for each 1% increase in NDF content in piglets. The main loss of energy due to DF may be associated to the gases of fermentation, the heat of fermentation and the heat due to metabolic utilization of SCFA (Bindelle et al., 2008).

Soluble sources are controversial, since its fermentability may increase the TTAD, although in practice it depends mostly on the physical-chemical properties, and age of the animals. In the study performed by Zhao et al. (2020), animals fed oat bran, sugar beet pulp and soybean hulls diets, had greater TTAD of fiber fractions than pigs fed corn bran, wheat bran and rice bran-based diets. Also, Supplementation of 2% sugar beet pulp, in comparison with the wheat bran, increased the TTAD of DM and energy by 2.4 and 1.7%, respectively (Freire et al 2000).

However, SDF may be also associated with digestibility reduction due to its effects on digesta viscosity. When SDF enter in contact with water, it creates an unstirred water layer in the intestinal surface, acting as a physical barrier between nutrients and digestive enzymes, reducing nutrient digestion (Jha and Berrocoso, 2015; Navarro et al., 2019b). As an example, sugar beet pulp, which inclusion at 6% decreased the TTAD of organic matter, gross energy and NDF in piglets at 42 and 56d (Shang et al., 2019).

The effect of IDF on TTAD is related with the balance between the positive effects on physiology, and its undegradability. High lignin amounts, which is not apparently degraded by pigs, decreases the digestibility of non-starch polysaccharides due to its covalent linkages with hemicelluloses and cellulose (Navarro et al., 2019a). Moreover, insoluble sources may increase the passage rate, allowing less time for digestive enzymes to interact with dietary components, which may decrease

digestion efficiency (Berrocoso et al., 2015; Agyekum and Nyachoti, 2017). For instance, high levels (5%) of corn bran or wheat bran reduced dry matter and organic matter TTAD in piglets compared to non-supplemented animal (Zhao et al., 2018).

In broilers, administration of 12 and 16% dried cassava pulp reduced dry matter and organic matter TTAD as compared to control animals (Khempaka et al., 2009). Administration of 10% cellulose inclusion decreased CP digestibility in comparison to 3.5% or non-supplemented animals (Cao et al., 2003). Also, broilers supplemented with 8% of microcellulose or soybean hulls reduced dry matter digestibility than those fed at 4% inclusion (Tejeda and Kim, 2021a).

However, in poultry, moderate DF inclusion may improve nutrient digestibility, throughout adaptation of the GIT. The effect of different fiber sources on nutrient digestibility is shown in Figure 2. The gizzard development and activity are normally improved by using lignified and coarse DF, which are retained more time in the gizzard (Jiménez-Moreno at al., 2009, 2010; Mateos et al., 2012). For example, IDF addition into low-fiber basal diet increased dry matter and organic matter digestibility (Jiménez-Moreno et al., 2009, 2010; Donadeli et al., 2019).

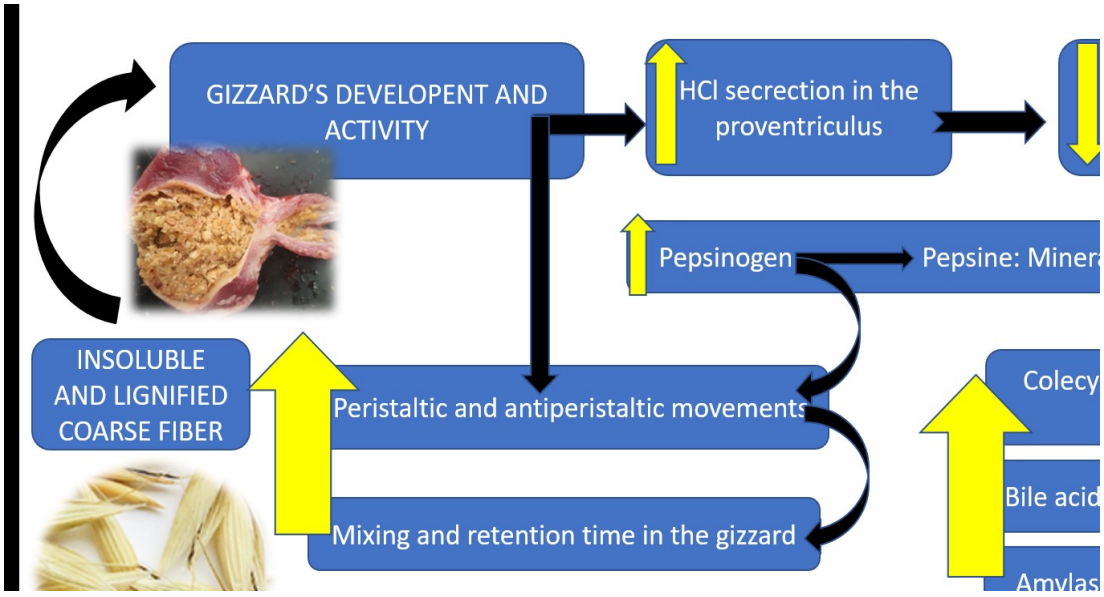


Figure 2. The effect of fiber sources on gizzard development and its influence on nutrient digestibility in broilers.

In broilers, the physical stimulation of the gizzard's musculature results in lower pH due to greater HCl secretion in the proventriculus and higher digestive enzyme (amylase, bile acids or cholecystokinin) production, allowing more efficacious nutrient utilization (Hetland et al., 2005; González-Alvarado et al., 2007). Inclusion of 3% wheat bran increased activities of amylase and trypsin in pancreas and jejunal mucosa at 21d (Shang et al., 2020a).

Table 4. The effect of fiber sources inclusion on total tract apparent digestibility (TTAD) of nutrients (GE: gross energy, DM: dry matter, OM: organic matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, TDF: total dietary fiber, IDF: insoluble dietary fiber) in weaned piglets and broilers compared to non-supplemented animals (%)[1].

	Inclusion level, %	Age, d	NDF feed[2], %	CF feed[3], %	GE	DM	OM	CP	ADF	NDF	TDF	IDF	Reference
Piglets													
Bamboo fiber	5.0	28	12	nd	↓**5**	↓**3**		↓1	↓1	↑3	↓**9**	↓**12**	(Yu et al., 2016)
Lignocellulose	1.0	38	nd	nd		↑**12**	↑**11**	↑**20**					(Chen et al. 2019a)
Lignocellulose	1.0	52	nd	nd		↑**4**	↑**4**	↑**5**					(Chen et al. 2019a)
Palm kernel expeller	6.0	28	12	nd	↓2			↓1	↑**20**	↑**13**	↓2	↑5	(Yu et al., 2016)
Wheat bran	10.0	28	12	nd	↓**3**	↓2		↓1	↓8	↓5	↓**9**	↓**12**	(Yu et al., 2016)
Wheat bran	6.0	14	9.5-10.5	nd	↓2	↓2	↓2	↓0.2	↓3	↓6			(Shang et al., 2019)
Soybean hulls	5.0	28	12	nd	↓2	↓1		↓1	↑2	↑4	↓**6**	↓8	(Yu et al., 2016)
Sugar beet pulp	6.0	42	9.5-10.5	nd	↓**4**	↓4	↓**4**	↓3	↓**27**	↓**29**			(Shang et al., 2019)
Sugar beet pulp	6.0	56	9.5-10.5	nd	↓**2**	↓2	↓**2**	↓0.4	↓6	↓**13**			(Shang et al., 2019)
Inulin	1.0	42	nd	nd		↑**9**	↑**9**	↑**13**					(Chen et al. 2019a)
Inulin	1.0	56	nd	nd		↑2	↑2	↓1					(Chen et al. 2019a)
Broilers													
Oat hulls	3.0	15	5.2	1.5		↑**3**	↑0.7						(Jiménez-Moreno et al., 2009)
Dried cassava pulp	4.0	25	nd	2.7		↓4	↑5						(Khempaka et al., 2009)
Dried cassava pulp	8.0	25	nd	2.7		↓6	↓4						(Khempaka et al., 2009)
Dried cassava pulp	12.0	25	nd	2.7		↓**14**	↓**12**						(Khempaka et al., 2009)
Dried cassava pulp	16.0	25	nd	2.7		↓**10**	↓**9**						(Khempaka et al., 2009)
Sunflower hulls	3.0	19	10	nd		↑0.4	↓0.1						(Kimiaeitalab et al., 2018)
Wheat bran	3.0	42	11-11.4	nd	↑**3**	↑**3**	↑**3**	↑**6**					(Shang et al., 2020a)
Sugar beet pulp	3.0	15	5.2	1.5		↑2	↓1		↓10	↑2			(Jiménez-Moreno et al., 2009)
Fructooligosaccharides	0.05	37	Nd	3		↑**8**		↑**7**					(Saleh et al., 2014)

nd, no data reported; [1]Significant differences (P<0.05) are marked in bold; [2]Neutral detergent fiber content of the control diet; [3]Crude fiber content of the control diet

However, inclusion of finely ground sources such as micronize cellulose produced no effects on gizzard development due to lack of structure (Jiménez-Moreno et al., 2010), but it improved the fat digestibility (Boguslawska-Tryk et al., 2015; Jiménez-Moreno et al., 2010). This inconclusive observation may indicate that the nutrient digestibility may be affected not only by the gizzard development, but also by the modification of the more distal compartments of the GIT and the enzymatic stimulation.

In piglets, DF supplementation is also a balance between the negative effects of DF on digestibility *per se*, since is not digestible, and the positive effects on physiology, morphology, stimulation of digestive enzymes activity, transit time, fermentation, and microbiota composition.

The activity of lactase, maltase, peptidases, and aminopeptidase were increased in pigs fed diets with high IDF content (Hedemann et al., 2006). The activity of pancreatic lipase, dipeptidyl peptidase IV, N-aminopeptidase and alkaline phosphatase in the ileum were increased with the inclusion of sugar beet pulp in the diet (Lizardo et al., 1997). However, compared with 5% inclusion of sugar beet pulp, the same level of wheat bran increased the sucrase activity in the duodenum (Shang et al., 2019).

Therefore, the final effect of fiber supplementation depends on the balance between the undigested fiber material, either at ileal or fecal level, and the positive or negative effects of the different types of DF on physiology and morphology.

2.3.4 Growth Performance

Addition of fibrous feedstuffs to monogastric animals' diets is recognized as a diluent of the energy and nutrients content. However, the effect would depend on DF source, physicochemical properties, inclusion level, and PS. In general, high DF levels impairs performance in piglets and poultry (Khempaka et al., 2009; Jiménez Moreno et al., 2011; Wang et al., 2021b). A summary of the effects of DF on piglet's and broiler's performance is shown in Tables 5 and 6, respectively.

Specially, SDF is considered problematic for growth performance due to high viscosity of intestinal contents. Post-weaning piglets, receiving diets containing citrus pulp had lower ADFI and ADG and worse FCR when compared to piglets fed cellulose, soybean hulls, or control diet (Pascoal et al., 2015). Also, pectin in the diets decreased ADFI and ADG compared with pigs fed no pectin (Hendenman et al., 2006), since highly fermentable DF may prolong the postprandial satiety, and decrease the feed motivation (De Leeuve et al., 2008). Inclusion of relevant quantities of soluble fermentable fiber sources immediately after weaning in piglets might be contraindicative due to the limited digestive capacity of young animals (Molist et al., 2014; Berrocoso et al., 2015).

In broilers, similar effects were observed. Sugar beet pulp supplemented at 3% reduced FCR in the starter period (Jiménez-Moreno et al., 2009). Its supplementation from 25 to 42 days of age, reduced feed intake with respect to the control diet, and feed intake and BWG with respect to the same level of oat hulls in the diet (González-Alvarado et al., 2010). Overall FCR from 1 to 42d was impaired

by 3% of sugar beet pulp supplementation (Sadeghi et al., 2015). Also, carboxymethyl cellulose, a source of soluble fiber administrated at 2 and 4%, decreased ADFI, ADG and increased FCR in starter phase (Rajmatnejad and Saki, 2015). Lower growth and impaired FCR were a consequence of the lower rate of feed passage through the gut and accumulation of the digesta, which decreased the feed intake in young chickens.

More positive effects were observed with inclusion of short non-digestible oligosaccharides. Feeding oligofructose to suckling piglets increased ADG during pre-weaning period (Shim et al., 2005). In post-weaned pigs, fructooligosaccharides addition at 0.4% diet improved ADG and FCR similarly to auromycin, in comparison to control diet (Xu et al., 2005). Also, 0.3% of mannooligosaccharides supplementation increased ADG from 1-42d, which was presumably associated with a direct effect on the immune cells in the gastrointestinal tract via its uptake into M-cells located in the Peyer's patches (Rozeboom et al., 2005). In poultry, inulin (5g/kg) or fructooligosaccharides (200 mg/kg) administration increased feed intake and growth from 1 to 21d in broilers (Huang et al., 2015; Yang et al., 2021).

On the other hand, low to moderate IDF levels are considered beneficial for performance, especially in young animals, due to an increase of intestinal development (Zhao et al., 2018ab; Chen et al., 2019a). Lignocellulose inclusion during the first 14 d after weaning, increased ADG and ADFI by 6.4 and 8.1 g/day for each 10 g/kg of NDF, resulting in higher body weight after 21 d (Pluske et al., 2014). Wheat bran supplementation at 8% increased ADFI and tended to increase ADG, compared to the control diet from 1 to 10d (Molist et al., 2009). Diet with 15% of wheat straw and oat hulls supplementation improved ADFI and FCR during 14d after weaning (Gerristen et al., 2012).

In poultry, wheat bran or oat hulls supplemented at 3% improved growth and FCR in starter period (Jiménez Moreno et al., 2009; Shang et al. 2020b). The inclusion of 2.5 and 5% of pea hulls improved FCR from 1-18d compared to the control diet (Jiménez Moreno et al., 2011). Gonzaléz-Alvarado et al. (2010) reported higher body weight gain and better feed to gain ratio in broilers fed 3 % oat hulls compared to non-fiber-supplemented animals from 1 to 42 days. Also, different levels of lignocellulose supplementation (0.25-0.75) improved final body weight and overall FCR (Sarikhan et al., 2010; Makivich et al., 2018).

However, animals do not need exclusively IDF or SDF, but an optimal combination of both, trying to obtain the benefits of IDF on physiology and morphology, and the benefit of a fermentable source for microbiota. Therefore, supplementation of mixtures of insoluble with soluble fiber sources has become a new interesting strategy to improve performance. Piglets fed cellulose with inulin had better ADG and FCR then those fed only inulin or the control diet (Chen et al., 2019a). Also, combination of wheat bran (4%) and sugar beet pulp (2%) increased overall ADG and final body weight of piglets, as compared to low fiber diet (Hermes et al., 2009). However, supplementation with the same levels of wheat bran (4%) and slightly higher sugar beet pulp (3%) produced no effects on performance (Molist et al., 2009). The discrepancy may be due to higher fiber levels of the control diet, or too high viscosity produced by the sugar beet pulp.

In broilers, the research performed by Sadeghi et al. (2015) reported that birds supplemented with 3% of sugar beet pulp had poorer feed intake from 14-28d, and worst FCR from 14-28 and 1-42 than birds supplemented with 1.5% sugar beet pulp and 1.5% rice hulls. Broilers supplemented with a diet containing a 2:1 ratio of pectin to cellulose, had lower feed intake in the starter and the whole rearing period than those supplemented with a diet containing a 1.5:1.5 or 1:2 ratio of pectin to cellulose, although no negative effects on growth were observed (Saki et al., 2011a). These finding may suggest that supplementation with both SDF and IDF, may alleviate the negative impact of SDF.

In summary, IDF inclusion in young animals provide better performance due to a synergetic effect throughout the GIT on the gut barrier, immunological status, and intestinal morphology resulting finally in enhancement of the digestive development. The use of SDF is more polemical due to high viscosity, which may impair the nutrient digestibility and reduce feeding motivation and feed intake. The effect of addition of SDF may vary depending on the health status, nature of SDF, its inclusion level, and the interaction with other dietary components. However, the negative effect decreases with ageing. Moreover, oligosaccharides may be considered as SDF which produce no negative effects on intestinal viscosity, and act as fermentable material in the hindgut, producing SCFA and modify the microbiota. Therefore, it makes sense to design combination with IDF and SDF, trying to obtain advantages and synergetic between both types of fiber.

Table 5. The effect of fiber sources inclusion on performance: average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) during prestarter (1-14d) and overall post-weaning period (1-28/35d) in piglets, compared to non-supplement animals (%)[1].

				Prestarter, 14d postweaning			Whole weaning period (1-28/35)			Reference
Source of fiber	Inclusion level, %	NDF feed[2], %	TDF feed[3], %	ADG	ADFI	FCR	ADG	ADFI	FCR	
Purified cellulose	1.5	9-11	19-23	↑14	↑2	↓12	↑11	↑8	↓4	(Pascoal et al., 2012)
Lignocellulose	1.0	nd	nd	↑11	↑2	↓**7**	↑3	↑2	↓3	(Chen et al., 2019a)
Lignocellulose	1.0	nd	16-20	↑10	↑8	↑1	↑10	↑14	↓5	(Silva-Guillen et al., 2022)
Lignocellulose	1.5	11-12	16-17	↑13	↓1	↓1				(Slama et al., 2020)
Lignocellulose	2.0	nd	16-20	↑34	↑25	↑8	↑8	↑16	↓8	(Silva-Guillen et al., 2022)
Lignocellulose	3.0	nd	16-20	↑31	↑18	↑12	↑11	↑16	↓5	(Silva-Guillen et al., 2022)
Lignocellulose fermentable	1.5	11-12	16-17	↑3	↑7	↑2				(Slama et al., 2020)
Corn bran	5.0	nd	nd	↑**10**		↓**10**	↑**6**	↓3	↓**8**	(Zhao et al., 2018)
Wheat bran	5.0	nd	nd	↑**7**		↓**8**	↑7		↓6	(Zhao et al., 2018)
Wheat bran	6.0	9.5-10.5	13.8-15	↑4	↑3	↓1	↑3	↑2	↓1	(Shang et al., 2019)
Wheat bran	10.0	12	nd				↑1	↑4	↑2	(Yu et al., 2016)
Out hulls	7.0	12	nd				↓9	↓5	↑4	(Yu et al., 2016)
Palm kernel expeller	6.0	12	nd				↓**10**	↓2	↑9	(Yu et al., 2016)
DDGS	7.5	7.3-8	nd	↑3	↓1	↑4	↑4	↑2	↑3	(Weber et al., 2008)

nd, no data reported; [1]Significant differences (P<0.05) are marked in bold; [2] Neutral detergent fiber content of the control diet; [3] Total dietary fiber content of the control diet.

Table 5 (cont). The effect of fiber sources inclusion on performance: average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) during prestarter (1-14d) and overall post-weaning period (1-28/35d) in piglets, compared to non-supplemented animals (%)[1]

				Prestarter, 14d postweaning			Whole weaning period (1-28/35)			Reference
Source of fiber	Inclusion level, %	NDF feed[2], %	TDF feed[3], %	ADG	ADFI	FCR	ADG	ADFI	FCR	
Lignocellulose and inulin	0.75+0.25	nd	nd	↑10	↑1	**↓8**	↑11	↑4	↓6	(Chen et al., 2019a)
Lignocellulose and inulin	0.5+0.5	nd	nd	↑11	↓1	↓11	↑7	↓1	**↓7**	(Chen et al., 2019a)
Soybean hulls	2.5	11-12	16-17	↓9	↑0.5	↓3				(Slama et al., 2020)
Soybean hulls	3.0	9-11	19-23	↑11	↓0.2	↓12	↑2	↑5	↑2	(Pascoal et al., 2012)
Soybean hulls	5.0	nd	nd	**↓5**	**↓6**	↓1	**↓7**	**↓8**	↓2	(Zhao et al., 2018)
Soybean hulls	7.5	7.3-8	nd	↑16	↑13	↑4	↑10	↑7	↑3	(Weber et al., 2008)
Soybean hulls	5.0	12	nd				↓7	↓2	↑6	(Yu et al., 2016)
Citrus pulp	7.5	7.3-8	nd	↓8	↓8	0	↓5	↓7	↑1	(Weber et al., 2008)
Citrus pulp	9.0	9-11	19-23	↑6	↓1	↓10	↓2	↑3	↑4	(Pascoal et al., 2012)
Sugar beet pulp	3.0	13.4	nd	↓16	↓13	↓6	↓7	↓9		(Yan et al., 2017)
Sugar beet pulp	6.0	13.4	nd	↓9	↓2	↓7		↑3	↑10	(Yan et al., 2017)
Sugar beet pulp	6.0	9.5-10.5	13.8-15	↓5	↓4	↑1	↓4	↓3	↑0.6	(Shang et al., 2019)
Sugar beet pulp	9.0	13.4	nd	↓5	↓6	↑3	↑16	↑2	↑19	(Yan et al., 2017)
Sugar beet pulp	12.0	13.4	nd	↓14	↓17	↑4	↑4	↓11	↑6	(Yan et al., 2017)
Inulin	1.0	nd	nd	↓3	↓7	↓2	↓2	↓4	↓1	(Chen et al., 2019a)
Fructooligosaccharides	0.4	nd	nd				↑31	↓0.2	↓24	(Xu et al., 2005)
Fructooligosaccharides	1.5	9	nd				↓4	↑0.1	↓7	(Pierce et al., 2005)

nd, no data reported; [1]Significant differences (P<0.05) are marked in bold; [2] Neutral detergent fiber content of the control diet; [3] Total dietary fiber content of the control diet.

Table 6. The effect of fiber sources inclusion on body weight (BW) and performance: average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) during initiation (1-19/21d of age) and grower (1-35/42d od age) periods in broilers, compared to non-supplemented animals (%)[1].

			1-19/21d				1-35/42d				
	Inclusion level, %	CF feed[2], %	BW, 21d	ADG	ADFI	FCR	BW, 42	ADG	ADFI	FCR	Reference
Almond hulls	2.5	2		↑1	↑1	↑0.6					(Wang et al., 2021b)
Almond hulls	5.0	2		↑6	↑11	**↑10**					(Wang et al., 2021b)
Almond hulls	7.5	2		↓1	↑1	**↑6**					(Wang et al., 2021b)
Almond hulls	10.0	2		**↓9**	↑2	**↑17**					(Wang et al., 2021b)
Fiber from cassava pulp	1.0	nd	↑0.5	↑0.3	↑0.2		↑0.1	↑0.2	↓2	↓2	(Okrhatok and Khempaka, 2020)
Fiber from cassava pulp	1.5	nd	↓1	↓1	↓1		↑0.4	↑1	↓0.4	↓1	(Okrhatok and Khempaka, 2020)
Dried cassava pulp	4.0	2.7	↓1		↑6	↑6	↓0.4		↑3	↑4	(Khempaka et al., 2009)
Dried cassava pulp	8.0	2.7	↑1		↓3	↓4	↓3		↑5	↑10	(Khempaka et al., 2009)
Dried cassava pulp	12.0	2.7	**↓26**		**↓17**	**↑22**	**↓11**		↓13	↓2	(Khempaka et al., 2009)
Dried cassava pulp	16.0	2.7	**↓13**		↓8	**↑14**	**↓15**		↓18	↓2	(Khempaka et al., 2009)
Cellulose	0.3	nd						↑1	↑1	↓2	(Rezaei et al., 2011)
Cellulose	0.4	nd						↑1	↑2	↓0.2	(Rezaei et al., 2011)
Cellulose	0.5	nd						**↑3**	↓0.2	**↓5**	(Rezaei et al., 2011)
Cellulose	2.0	0.05		↑1	↑2	↑1					(Rajmatnejad and Saki, 2015)
Cellulose	4.0	0.05		↓8	↓9	↓3					(Rajmatnejad and Saki, 2015)
Cellulose	4.0	2		↓4	↑4	↑8					(Tejeda and Kim, 2020)
Cellulose	6.0	2		↓2	↑5	↑8					(Tejeda and Kim, 2020)
Cellulose	8.0	2		↓17	↑4	**↑26**					(Tejeda and Kim, 2020)

nd, no data reported; [1]Significant differences (P<0.05) are marked in bold; [2] Crude fiber content of the control diet.

Table 6 (cont). The effect of fiber sources inclusion on body weight (BW) and performance: average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) during initiation (1-19/21d of age) and grower (1-35/42d od age) periods in broilers, compared to non-supplemented animals (%)[1]

					1-19/21d				1-35/42d				
	Inclusion level, %	NDF feed[2], %	TDF feed[3], %	CF feed[4], %	BW, 21d	ADG	ADF I	FCR	BW, 42	AD G	ADFI	FCR	Reference
Lignocellulose	0.05	nd	nd	3.4-3.8	↑3		↓1	↓5	↑2		↓2	↓4	(Sozcu, 2019)
Lignocellulose	0.1	nd	nd	3.4-3.8	↑**7**		↑3	↓3	↑**7**		↑2	↓**5**	(Sozcu, 2019)
Lignocellulose	0.2	nd	nd	3.4-3.8	↑1		↓2	↓3	↓1		↓2	↓2	(Sozcu, 2019)
Lignocellulose	0.25	nd	nd	3.3						↑**9**	↑2	↓**7**	(Sharikan et al., 2010)
Lignocellulose	0.5	nd	nd	3.3						↑**8**	↑0.3	↓**7**	(Sharikan et al., 2010)
Lignocellulose	0.75	nd	nd	3.3						↑**12**	↑3	↓**8**	(Sharikan et al., 2010)
Lignocellulose	0.8	13.4-15.4	nd	4.5-6.7					↓2	↓2	↓2		(Zeitz et al., 2019)
Lignocellulose	1.0	nd	nd	1.9-2.0						↓1	↑0.2	↑1	(Kheravii et al.,2017)
Lignocellulose	2.0	nd	nd	1.9-2.0						↓1	↑1	↑2	(Kheravii et al.,2017)
Fermentable lignocellulose	0.8	13.4-15.4	nd	4.5-6.7					↑2	↑2		↓2	(Zeitz et al., 2019)
Oat hulls	3.0	5.3-5.7	nd	1.5						↑**9**	↑2	↓**6**	(González Alvarado et al., 2010)
Oat hulls	3.0	5.2	nd	1.5		↑**6**	↑0.5	↓**6**					(Jiménez- Moreno et al., 2009)
Rice hulls	3.0	nd	nd	3.5-3.7						↓**6**	↓**8**	↓3	(Saadatmand et al., 2019)
Rice hulls	3.0	nd	nd	3.9-4.7							↓1	↑1	(Sabour et al., 2019)
Rice hulls	3.0	nd	nd	3.6-3.8						↓2	↑2	↑4	(Sadeghi et al., 2015)
Sunflower hulls	3.0	10	nd	nd		↓1	↓0.4	↑1					(Kimiaeitalab et al., 2018)
Wheat bran	3.0	11-11.4	13.5-14	nd	↑**5**	↑**7**	↓3	↓**9**	↑4	↑5	↓1	↓**6**	(Shang et al., 2020b)

nd, no data reported; [1]Significant differences (P<0.05) are marked in bold; [2] Neutral detergent fiber content of the control diet; [3] Total dietary fiber content of the control diet; [4] Crude fiber content of the control diet.

Table 6 (cont). The effect of fiber sources inclusion on body weight (BW) and performance: average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) during initiation (1-19/21d of age) and grower (1-35/42d od age) periods in broilers, compared to non-supplemented animals (%)[1].

					1-19/21d			1-35/42d			
	Inclusion level, %	NDF feed[2], %	TDF feed[3], %	CF feed[4], %	ADG	ADFI	FCR	ADG	ADFI	FCR	Reference
Soybean hulls	4.0	nd	nd	2	↑5	↑4	↓0.6				(Tejeda and Kim, 2020)
Soybean hulls	6.0	nd	nd	2	↓11	↓2	↑11				(Tejeda and Kim, 2020)
Soybean hulls	8.0	nd	nd	2	↓17	↑5	↑**27**				(Tejeda and Kim, 2020)
Carboxymethyl Cellulose	2.0	nd	nd	0.05	↓**54**	↓**33**	↑**47**				(Rajmatnejad and Saki, 2015)
Carboxymethyl Cellulose	4.0	nd	nd	0.05	↓**57**	↓**38**	↑**47**				(Rajmatnejad and Saki, 2015)
Sugar beet pulp	3.0	5.2	nd	1.5	↑4	↓0.2	↓**5**				(Jiménez- Moreno et al., 2009)
Sugar beet pulp	1.5	5.3-5.7	nd	1.5				↑2	↓3	↓4	(González Alvarado et al., 2010)
Sugar beet pulp	3.0	nd	nd	3.5-3.7				↓**6**	↓**7**	↓0.6	(Saadatmand et al., 2019)
Sugar beet pulp	3.0	nd	nd	3.9-4.7				↑2	↑0.8		(Sabour et al., 2019)
Sugar beet pulp	3.0	nd	nd	3.6-3.8				↓5	↑3	↑**9**	(Sadeghi et al., 2015)
Inulin	0.5	nd	nd	nd	↑0.4	↑**2**	↑2	↑4	↑1	↓1	(Huang et al., 2015)
Inulin	1.0	nd	nd	nd	↑3		↓3	↑3		↑3	(Huang et al., 2015)
Inulin	1.5	nd	nd	nd	↓3	↓1	↑3	↑0.9		↑1	(Huang et al., 2015)
Fructooligosaccharides	0.02	nd	nd	2.3	↑**58**	↑44	↓18	↑28	↑34	↓12	(Yang et al., 2021)
XOS	0.02	nd	nd	2.3	↑47	↑33	↓1	↑8	↑8	↑10	(Yang et al., 2021)

nd, no data reported; [1]Significant differences (P<0.05) are marked in bold; [2] Neutral detergent fiber content of the control diet; [3] Total dietary fiber content of the control diet; [4] Crude fiber content of the control diet.

2.3.5. Effect of DF on Post-Weaning Diarrhea

The weaning is a critical phase in pig farming, characterized by a transition from sow's milk to solid feed, and exposition to social and environmental stressors, affecting the intestinal health of piglets. These changes reduce the barrier function of intestine and increase the expansion of enteric pathogens (Gresse et al., 2017; Modina et al., 2019; Huting et al., 2021).

A high-protein diet was associated with greater expression of BCFAs and ammonia, which can promote the growth of pathogenic bacteria (Gao et al., 2019). This, together with lack of fermentable carbohydrates in the large intestine, may increase concentration of biogenic amines, which are implicated in the etiology of post weaning diarrhea (Jha et al., 2019).

To face this problem, IDF inclusion may provide advantages due to different function. Firstly, it may counteract the negative effects of protein fermentation and optimize intestinal health (Kim et al., 2008; Bikker et al., 2014; Jha and Berrocoso, 2015). Lignocellulose (1.5%) or oat hulls (2.0%) supplementation effectively decreased biogenic amines concentration (Kim et al., 2008; Slama et al., 2020), whereas 0.8% of lignocellulose reduced levels of cadaverine, phenol4-ethylphenol and 3-methylindole in the distal colon (Pieper et al., 2014).

Secondly, the HC of IDF may impact the fecal moisture content. Supplementation with IDF was suggested to decrease the unbound water of colonic digesta in piglets (Molist et al., 2009). However, in this case the PS of the fiber may also play an important role. Piglets fed coarse wheat bran, showed better fecal score after inoculation with *E. coli* than those fed finely milling and the control diet (Molist et al., 2012). These differences may be related with higher HC of coarse grinding durum wheat bran, which decreased with the PS reduction, as has been confirmed through *in vitro* studies (Espósito et al., 2005). It seems that holding substantial water amount in the matrix, improves water reuse, which may enhance the feces quality and decrease the diarrhea rate in post weaning piglets. Previous investigations suggested the SCFAs, and butyrate in particular, are antidiarrheal agents due to stimulation of sodium and water absorption (Hamer et al., 2008).

Another interesting strategy to control the diarrhea incidence is by the modification of the microbial colonization by highly fermentable oligosaccharides supplementation. It contributes to lower pathogen counts and enhances the barrier function due to increase of SCFA levels (Xu et al., 2005). In postweaning piglets sensitized with soybeans (Chang et al., 2018), and suckling piglets challenged with *S. typhimurium* (Correa-Matos et al., 2003), FOS was observed as an effective tool to prevent diarrhea. Also, feeding inulin-supplemented diets improved the fecal consistency and reduced the incidence of post weaning diarrhea in *Escherichia coli* challenged piglets (Halas et al., 2009).

Alternatively, supplementation of combination of SDF and IDF sources has become of growing interest. Piglets supplemented with inulin and lignocellulose shown lower diarrhea rate (Chen et al. 2019a), whereas supplementation with wheat bran and sugar beet pulp in a 20% crude protein diet, improved the fecal score and decreased the antibiotic intervention (Hermes et al., 2009).

2.3.6. Effect of DF on Litter Quality

In poultry, one of the aims of the farms is the maintenance of the low moisture of the litter, which is relevant for animal production, welfare and quality of the meat. The litter is the combination of bedding material, excreta, feathers, wasted feed and wasted water. If contains excessive moisture, it has a direct influence on skin condition and carcass quality, promoting pathogenic bacteria, and ammonia emissions (Ritz et al., 2004; Francesch and Brufau, 2014).

High fiber inclusion increases water intake, which may impact negatively the moisture content of excreta (Jiménez-Moreno et al., 2016). However, low and moderate IDF supplementation have been proposed as a strategy to control the litter quality, speculating the importance of higher HC (Amerah et al., 2009). Lignocellulose addition (0.4-2.0%), which is characterized by high HC, lowered the water amount in the litter (Kheravii et al., 2017; Makivich et al., 2018). The mechanism, on the contrary to the generally accepted of "sponge" fiber effect, may be associated with longer digesta retention time, and greater water absorption (Kheravii et al., 2017). The "sponge effect" lacks validity, as the high content of DF in feces retains a significant amount of water. Consequently, fecal moisture should be higher, producing the opposite effect than what is suggested by this concept. Also, SCFA production, and butyrate in particular, may take part in water and electrolytes reused (Guilloteau et al., 2010; Jha and Berrocoso, 2015), pointing out the cecum as the most important segment for absorption (Svihus, 2014).

Moreover, fiber may also interact with microbial end-products, lowering biogenic amines production. High levels of histamine and cadaverine were associated with proventriculus dilatation, and increased incidence and severity of gizzard erosion and proventricular ulcers, and a decreased of the prominence of gastric papillae, leading to poorer performance (Barnes et al., 2001). In broilers, supplementation of 0.5 and 1% lignocellulose decreased cadaverine and putrescine content in the ileum (Bogusławska-Tryk et al., 2020), confirming its utilization by resident microorganisms as an energy source over proteins for their growth. Lower ammonia was a consequence of decomposition of nitrogenous compounds assimilated into microbial biomass (Santos et al., 2019).

In case of undigested protein reaches the large intestine, sources of slowly fermentable carbohydrates contribute to redirect the fermentation, providing a balance of gut environment, and enhancing the barrier effect. Therefore, the correct design of the diet, based on adequate fibrous ingredients, may provide advantages by acting at different levels of the GIT.

OBJECTIVES

3.1. General Objective

The main goal of this PhD was the development of a new line of fibrous concentrates based on mixtures of high-fiber sources from agricultural by-products, combining IDF and prebiotic SDF, according to actual European strategy on sustainability and circular economy. Wastes such as almond shells, hazelnut shells, or olive kernel have not already been valorized as a feed ingredient due to their large size and hardness. The hypothesis is that, once these raw materials are properly ground, mixed and balanced the IDF and SDF, they can be administered in the diets of chickens and piglets as a fiber sources. This strategy would allow the substitution of commonly used fiber sources (wheat bran, soybean hulls, sunflower hulls) which often have several limitations, such as difficult to handle, variability, microbial or mycotoxin contamination, enhancing the animals gut health, and contributing to the circular economy.

3.2. Specific Objectives

1- Characterize the chemical composition and physical properties of the selected fiber sources.

2- Evaluate the dietary inclusion of single fiber sources characterized by different PS and HC, finely ground or not, at low level of inclusion (1.5%) on performance, gastrointestinal tract development, cecal fermentation (*in vivo* and *in vitro*) and digestive transit time in caged-housed broilers from 1 to 23 d of age (Experiment 1).

3- Evaluate the dietary inclusion of complex fibrous concentrates based on different composition, combined with a prebiotic fraction from fructooligosaccharides (characterized by a medium hydration capacity) on performance, nutrient digestibility, gut health and *in vitro* caecal fermentation were carried out in broiler chickens during the whole rearing period, 0-42d (Experiment 2).

4- Evaluate the dietary inclusion of complex fibrous concentrates differing on fermentability and hydration capacity on performance, nutrient digestibility and cecal fermentation (*in vivo* and *in vitro*) in post weaned piglets from 28 to 61 d of age. (Experiment 3).

5- Promote a circular economy model through the micronization of highly lignified by-products, such as almond shells, olive kernels, nutshells, and grape pomace, to produce novel fiber-rich feed materials.

Overall, the aim of this PhD it that the results obtained contribute to improve the knowledge about the impact of DF inclusion in piglets and broilers diets, and to develop a new fiber concentrates based on the mixture of different fiber sources, combining IDF with prebiotic fermentable fraction. Moreover, it has been proposed to promote the circular economy model and minimizing environmental impact and the waste of resources.

METHODOLOGY

4.1. Experiment 1: Effect of type of fiber and its physicochemical properties on performance, digestive transit time, and cecal fermentation in broilers from 1-23 days of age.

This study was performed in the experimental facilities of the Universidad Politécnica de Madrid (Spain). Animals were handled according to Spanish guidelines for experimental animal protection (BOE, 2013) and experimental protocols were approved by the Ethics Committee of the Polytechnic University of Madrid (approvement date: the 19th of November 2019).

Husbandry

A total of 550 1-d-old Ross-308 male broilers with an initial BW of 41.1±1.22 g was used. The birds were obtained from a commercial hatchery (UVESA, Tudela, Spain). The animals were weighed and distributed according to a completely randomized design into 50 wire flooring battery cages (1 m × 0.36 m), equipped with 2 nipple drinkers and 1 open trough feeder (65 cm length) in a windowless house. An additional first-age feeder and drinker were used during the first 7 d. During the first day, room temperature was set at 33ºC and then was reduced gradually to reach 22ºC at the end of the study (Ross, 2018). Mortality was recorded daily.

Diets and Experimental Design

The experimental diets in form of mash and based on corn and soybean meal were formulated according to FEDNA (2018) to meet or exceed the nutritional recommendations for broilers from 1 to 21 d of age. There were 5 dietary treatments: a Control diet was formulated to contain 2,944 kcal AMEn/kg and 1.28% standardized ileal digestible (SID) Lys. It was diluted with 1.5% of IDF sources: finely ground wood-lignocellulose (LC), finely-ground straw (FS), coarse-ground straw (CS), and finely ground almond shell (AS) to obtain 4 IDF-based experimental treatments. The straw was a mixture of wheat and barley straw, and it was ground in a hammer mill (Hosokawa Alpine 40/20, 11Kw, 1460 rev/min, Germany) to pass 0.5 and 3mm sieves for FS and CS, respectively. The wood pellet (*Pinus spp.,* decorticated) and AS (*Prunus dulcis* var. marcona) were finely ground using a micronizer (Hosokawa Alpine ACM 10 mill; 7.5Kw; Hosokawa Alpine AG, Augsburg, Germany), providing very finely ground final products. For preparation of the experimental diets, all IDF sources were homogenized with the control diet in a mixer (MMG 316, 250 L, Murcia, Spain) for 150 s. The ingredient composition of the control diet, and chemical composition of all experimental diets are shown in Tables 7 and 8, respectively.

Table 7. Ingredient composition of control diet. Experiment 1.

Ingredients, %	Control diet
Corn	55.65
Soybean meal 47%	37.75
Soy oil	2.23
Calcium carbonate	1.56
Monocalcium phosphate	1.01
L-Lysine 50% LQ	0.43
Metionine-OH	0.38
L-treonine	0.14
L-valine 96.5%	0.07
Salt	0.33
Vitamin mineral premix[1]	0.30
Sodium bicarbonate	0.10
Choline chloride 60%	0.05

[1] Provided per kilogram of the diet: Vitamin A (retinyl acetate) (3a672a) 9,000 U.I., Vitamin D3 (cholecalciferol) (3a671) 3,000 U.I., Vitamin E (all-rac-tocopherol acetate) (3a700) 30 mg, Vitamin K (bisulphate menadione complex) (3a711) 2.5 mg, Vitamin B1 (tiamine mononitrate) (3a821) 2.175 mg, Vitamin B2 (riboflavin) 6 mg, Calcium D-pantothenate (3a841) 10 mg, Vitamin B6 (pyroxydine hydrochloride) (3a831) 2.5 mg, Vitamin B12 (cyanocobalamin) 15 µg, Niacin (nicotinic acid) (3a314) 40 mg, Folic acid (3a316) 1.5 mg, Biotin (3a880) 0.12 mg, Betaine (betaine hydrochloride) (3a925) 250 mg, Iron (iron sulfate (II), monohydrate) (3b103) 30 mg, Copper (copper sulfate (II) pentahydrate) (3b405) 8 mg, Manganese (manganese oxide (II)) (3b502) 80 mg, Zinc (zinc sulfate, monohydrate) (3b605) 60 mg, iodine (coated granulated anhydrous calcium iodate) (3b203) 0.8 mg, Selenium (sodium selenite) (E8) 0.25 mg, citric acid 0.225 mg, Butylhydroxytoluene (BHT) (E321) 0.9 mg, propyl gallate (E310) 0.075mg, Sepiolite (E562) 3,000 mg, 6-phytase EC 3.1.3.26 (4a18) 667 FYT/g.

Table 8. Chemical composition of experimental diets (%, as-fed basis). Experiment 1.

Item	Control	LC	FS	CS	AS
Dry matter	89.7	89.5	89.5	89.8	89.0
Organic matter	82.2	82.5	81.7	82.3	83.6
Ash	7.53	7.06	7.84	7.50	7.39
Crude protein	22.6	22.4	22.3	22.1	22.3
Ether extract	5.28	4.83	4.91	5.02	5.05
Neutral detergent fiber	14.1	16.4	16.4	16.8	16.3
Acid detergent fiber	2.51	4.28	3.72	4.44	4.50
Acid detergent lignin	0.32	0.83	0.62	0.73	0.81

[1] Control: standard diet based on corn and soybean meal; LC, FS, CS, AS: control diet supplemented with 1.5% of lignocellulose, fine-ground straw, coarse-ground straw, and almond shell, respectively.

Growth Performance

Chickens were randomly assigned to each dietary treatment (10 cages/treatment; 11 birds/cage), and the experimental unit was the cage. Animals BW and feed consumption were determined by cage at 7, 14 and 21 d of age, and the data were used to calculate ADFI, ADG, and feed conversion ratio (FCR) in each period and cumulatively (1-21 d).

Gastrointestinal Traits

At 21 d of age, 3 birds per cage (10 cages/treatment; 30 birds/treatment) were selected randomly, individually weighed, and slaughtered by CO_2 asphyxiation. The GIT from the proventriculus to cloaca was removed and weighed. Then, the proventriculus, gizzard, and cecum were separated and weighed individually. The weight of the sum of the small intestine, colon, and rectum (SICR) was calculated as the difference between the GIT weight and the weight of the proventriculus, gizzard and cecum. The pH of the content of the proventriculus, gizzard and cecum were immediately measured *in situ* using a digital pH meter (model 507, Crison Instruments S.A., Barcelona, Spain). After that, the proventriculus and gizzard were emptied of digesta and weighed again. The cecal content of the 3 birds per cage was pooled and immediately frozen (−20ºC) until analysis of short chain fatty acids (SCFA) concentration. Sample processing was adapted from Kimiaeitalab et al. (2017). Briefly, samples were defrosted at 4ºC, 3 g were weighed, mixed with 5 mL of 0.5 *M* HCl, homogenized, and centrifuged (13,000 × *g*, 15 min, 4ºC). One mL of the supernatant was mixed with 0.5 mL of a deproteinizing solution (20 g metaphosphoric acid and 0.6 g of crotonic acid per L of 0.5 *M* HCl) and left overnight at 4ºC. Samples were centrifuged (13,000 × *g*, 15 min, 4ºC) and the supernatant was transferred to chromatography vials. Total SCFA concentration was determined by gas chromatography as described by García-Martínez et al. (2005), using a Shimadzu GC 2010 chromatography (Shimazdu Europa GmbH, Duisburg, Germany) provided with a TR-FFAP column (30 m × 0.53 mm × 1 µm; Supelco, Madrid, Spain).

The length of the small intestine was measured (1 mm precision) from the pylorus to the ileocecal valve. To evaluate the effect of the dietary treatments on the small intestine morphology, sections from distal jejunum (about 5 cm length) of 6 birds randomly selected per treatment were excised, and immediately placed in 10% formalin solution. Ten cross sections of the intestinal longitudinal axis were obtained, dehydrated by passage through ethanol of increasing concentrations, and embedded in liquid paraffin. Finally, 2 histological sections (2.5 µm thick) were obtained from each sample and stained with haematoxylin-eosin and periodic acid-schiff for goblet cells counting. Microphotographs (Leica ICC50W™ attached to a microscope Leica DM1000™) were taken from all samples to carry out measurements and assess sample preservation and suitability. The histopathologic and morphometric study of the samples were carried out by The ImageJ (Fiji, USA) and used for the following measurements: villous height, crypts depth and mucosal thickness (10 measurements / bird sample); villous and crypts density per mm (3 measurements/bird), and goblet cells count in 10 villi areas per bird. The ratio villous heigh to crypt depth was calculated. In all these digestive traits the bird was the

experimental unit.

Digestive Transit Time and Excreta Moisture

At 23 d of age, a total of 120 birds from 8 cages/treatment (3 birds/cage; cage as experimental unit) were fasted for 8 h. Then, 40 mg of titanium dioxide (TiO_2) in a gelatin capsule were introduced into the oro-pharynx cavity of each bird and the time of marker administration for each cage was recorded. Total excreta were collected at 8 and 12 h post capsule ingestion, and frozen (-20℃) until later analysis. Cages had a mesh floor, and a clean plastic pan was placed beneath the cage. After defrosting, samples were weighed, dried at 60°C for 72 h, and weighed to calculate DM content. Dried excreta were ground using a centrifugal mill (Retsch Model Z-I, Stuttgart, Germany) fitted with a 1-mm screen. The determination of the marker in the excreta was performed according to Short et al. (1996). Briefly, 100 mg of dried excreta was ashed at 580°C for 13 h. Ten mL of sulfuric acid (7.4 M) was added to each crucible upon cooling and samples were then gently boiled for 3 min. After cooling, the solutions were poured into 100 mL volumetric flask containing 25 mL distilled water through Whatman filter paper (WHA1443185). A total of 20 mL of hydrogen peroxide (30%) was added to each flask, and finally the content was diluted up to 100 mL with distilled water. The absorbance was measured in triplicate using a spectrophotometer (Epoch, BioTek®, USA) at 410 nm. The total amount of TiO_2 recovered at 8 h and cumulative at 12 h were expressed as the percentage of the total TiO_2 administered for each pen.

In Vitro Fermentation

An in vitro fermentation trial was carried out using the cecal content of 22-d old birds as inoculum and the IDF sources (LC, FS, CS and AS) as substrates. Samples of 200 mg DM of each substrate were weighed into 60-mL glass vials. A total of 16 birds per dietary treatment were slaughtered by asphyxiation with CO_2, the ceca was immediately removed, and the content of each 4 birds was pooled to make 4 different inocula per diet. Each inoculum (1.5 g of cecal content) was mixed with 100 mL of the culture medium described by Goering and Van Soest (1970), homogenized with a hand blender for 10 s, and filtered through 2 layers of cheesecloth. Vials were filled up with 20 ml of the mixture using a peristaltic pump (Watson-Marlow 520UIP31; Watson-Marlow Fluid Technology Group, United Kingdom), under continuous flushing with CO_2. Vials were sealed with rubber stoppers, and incubated at 40°C for 50 h. For each inoculum, a vial without substrate (blank) was included to correct for endogenous gas production. Gas production was measured at 3, 5, 7, 10, 13, 24, 28, 32, 37, and 50 h of incubation using a digital pressure gauge (HD 2304.0, Delta OHM, Italy) and a plastic syringe.

The inoculum from Control animals was used to incubate all IDF sources, whereas the inoculum from animals fed the other dietary treatments was used to incubate the IDF source included in the corresponding diet. In addition, LC and FS were fermented with all inocula to test the potential effects of the experimental diets on their in vitro cecal fermentation (Figure 3).

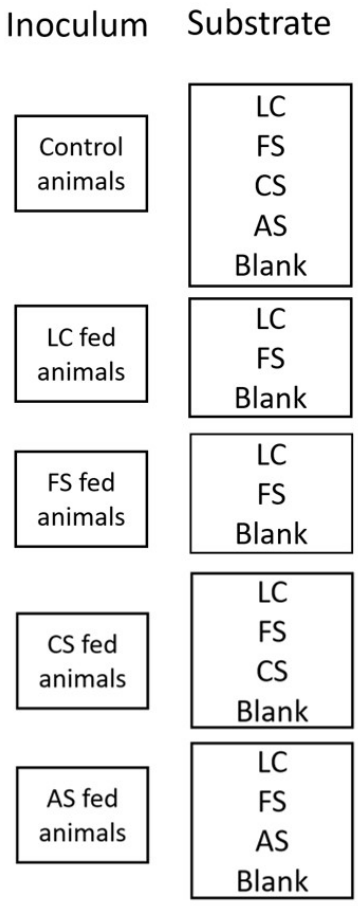


Figure 3. Experimental design of the in vitro trial indicating the substrates (lignocellulose (LC), fine straw (FS), coarse straw (CS), almond shell (AS)) that were fermented with inocula from birds fed each of the experimental diets (Control, LC, FS, CS and AS) for 50 h. Four different inocula were used for each experimental treatment, and each inoculum was a pooled cecal content from 4 birds.

Laboratory Analysis

Procedures of the AOAC (2005) were used to determine DM (method 934.01), ash (method 942.05), ether extract (EE, 920.39), and total dietary fiber (TDF, 985.29). The N content was analyzed by the Dumas method (method 968.06) using a Leco analyzer (model FP-528; Leco Corp., St. Joseph, MI, USA). The CP content was determined by multiplying N content by 6.25. Dietary NDF, acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially using the filter bag system (Ankom Technology, New York, USA) by adapting the method of Mertens et al. (2002) and Horwitz et al. (2006). Dietary NDF was determined using a thermo-stable amylase and without sodium sulphite, and values were corrected for ash and CP.

The mean PS, expressed as geometric mean diameter (GMD), and the PS distribution of the IDF sources were determined in 100 g samples using a sieve shaker (FTS-0200, Filtra, Badalona, Spain) with 5 sieves ranging in mesh from 62 to 1.000 μm (>1.000, 500-1.000, 250-500; 250-105; 105-62; <62 μm; ASAE, 1995). The HC of the IDF sources was measured by determining the water binding capacity (WBC, g/g) and the swelling capacity (SC, g/ml) by adapting the methods described by Slama et al.

(2019), Berrocoso et al. (2020) and Priester et al. (2020). Briefly, 0.4 g of each IDF source were hydrated during 22 h with 10 g of water, then centrifuged (3100 × g, 20 min, Centrifuge, 5810R, Eppendorf, Wesseling-Berzdorf, Germany). The unabsorbed water was weighed, and the WBC was calculated as the difference between the weight of water added and that of the supernatant (g) divided by the initial sample weight. The SC was measured using 1 g of IDF sample that was incubated with 20 mL of water stirring gently, and then left in graduated metric scale cylinders for 22 h. The SC was calculated as the final volume (mL) of the sample divided by the sample weight (g).

Statistical Analysis

The effect of IDF on the measured in vivo parameters was analyzed by one-way analysis of variance (ANOVA) with the diet as the main effect. In addition, two orthogonal contrast comparisons were performed to determine the effects of IDF supplementation: C1: control vs. IDF-containing diets (LC+FS+CS+AS), and to compare the IDF with different HC: C2: high HC (LC+FS+CS) vs. low HC (AS) IDF sources. In vitro gas production data were analyzed within each sampling time and independently for each preplanned comparison. Differences among substrates in gas production were tested when cecal inoculum from Control-fed broilers was used. The effects of including different IDF sources (LC, FS, CS and AS) in broilers diet was studied by analyzing the gas production from LC and FS substrates when they were incubated with cecal inoculum from broilers fed the IDF-containing diets. Finally, the gas production from each substrate (LC, FS, CS and AS) when it was fermented with inoculum from broilers fed either Control diet or the corresponding IDF-diet was compared. There were 4 replicates (inocula) for each experimental treatment, and each inoculum was a pooled cecal content from 4 birds. Significance was declared at $P < 0.05$, whereas $P < 0.10$ values were considered as a trend. When a significant effect of diet was detected, means multiple comparisons were carried out by using the Tukey test. Statistical analyses were performed with SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp).

4.2. Experiment 2: Effect of dietary supplementation of two fiber sources differing on fermentability and hydration capacity on performance, nutrient digestibility and cecal fermentation in broilers from 1 to 42 d of age.

This study was performed in the experimental facilities of the Universidad Politécnica de Madrid (Spain). Animals were handled according to Spanish guidelines for experimental animal protection (BOE, 2013) and experimental protocols were approved by the Ethics Committee of the Polytechnic University of Madrid (approvement date: 19th of November 2019).

Husbandry

A total of 378 one-day-old male broilers (Cobb-500) with an initial BW of 38.5±1.02g were obtained from a commercial hatchery. The birds were weighed in groups and distributed according to a completely randomized design into 27 floor pens (150 cm long x 100 cm wide x 50 cm high) equipped with first and second-age feeders and drinkers. All first-age equipment was retired at 7 d. The animals were bedded on pressed straw. The temperature was controlled automatically throughout the trial according to the animal´s age needs (Cobb, 2018). Bird's mortality was recorded daily.

Diets and Experimental Design

The diets were formulated to meet or exceed the nutritional recommendations for broilers from 1 to 42 d (FEDNA, 2018). There were 3 experimental diets. The control (CON) diets consisted of a starter diet (1 to 21 d) based on wheat and soybean meal (SBM), containing (as-fed basis) 3,066 kcal AMEn/kg and 1.15 % standardized ileal digestible (SID) lysine, and a finishing diet (21 to 42 d of age) based on wheat, corn and SBM containing 3,090 kcal AMEn/kg and 1.05% SID lysine (Table 9). The nutritional content of the CON starter diet for moisture, ash, ether extract, and neutral detergent fiber was 10.6%, 6.25, 4.48, and 10.2, respectively, whereas for the CON finishing feed was 10.9, 5.93, 4.17, and 9.94, respectively. The other experimental diets were prepared by diluting the CON diet with 1.5% of two different fiber sources: finely ground wood lignocellulose (LC) characterized by high hydration capacity and low-fermentability (LC diet), or a fiber (ISFC) consisting in a mixture of medium-hydration capacity IDF sources with a soluble prebiotic fiber fraction (ISFC diet). The wood lignocellulose came from wood pellets (*Pinus spp*., decorticated). The ISFC consisted of a mixture of micronized almond shells, grape skin by-products, olive kernel, wood-lignocellulose, nuts shells, and straw as IDF sources, and FOS as soluble-prebiotic source. Both LC and ISFC diets were prepared by mixing the CON diet with the corresponding fiber source for 150 s in a mixer (MMG 316, 250L, Murcia, Spain). In addition, titanium dioxide (TiO2, 0.5%) was included in the finishing diets fed from 38 to 42 d as a marker to determine apparent ileal digestibility. All diets were administered in mash form.

Table 9. Ingredient and chemical composition of control diets (%, as-fed basis). Experiment 2.

Items	Starter	Finishing
Ingredients, %		
Wheat	59.90	55.13
Soybean meal	32.40	27.92
Corn	0	10.00
Soybean oil	3.17	2.67
Calcium carbonate	1.56	1.74
Monocalcium phosphate	0.96	0.58
L-Lysine	0.39	0.40
DL-Metionine	0.36	0.32
L-Threonine	0.12	0.11
L-Valine	0.09	0.08
Salt	0.31	0.31
Sodium bicarbonate	0.10	0.10
Vitamin and mineral premix[1]	0.30	0.30
PX Maxiban[2]	0.20	0.20
ENP Enzyme Dry[3]	0.05	0.05
Choline chloride	0.05	0.07
Calculated nutritional content		
AMEn (kcal/kg)	3,066	3,090
SID Lys	1.15	1.05
Moisture	10.6	10.9
Total ash	6.25	5.93
Ether extract	4.48	4.17
Neutral detergent fiber	10.16	9.94

[1]per kg of diet: Vitamin A (Retinyl acetate) (3a672a) 9,000 I.U., Vitamin D3 (Cholecalciferol) (3a671) 3,000 I.U., Vitamin E (all-rac-α-tocopheryl acetate) (3a700) 30 mg, Vitamin K3 (menadione nicotinamide bisulfite) (3a711) 2.5 mg, Vitamin B1 (thiamine mononitrate) (3a821) 2.175 mg, Vitamin B2 (riboflavin) 6 mg, Calcium D-pantothenate (3a841) 10 mg, Vitamin B6 (pyridoxine hydrochloride) (3a831) 2.5 mg, Vitamin B12 (cyanocobalamin) (3a835)15 mg, Niacin (nicotinic acid) (3a314) 40 mg, Folic acid (3a316) 1.5 mg, Biotin (3a880) 0.12 mg, Betaine (Betaine hydrochloride) (3a925) 250 mg, Iron (Iron (II) sulfate, monohydrate) (3b103) 30 mg, Copper (Copper (II) sulfate pentahydrate) (3b405) 8 mg, Manganese (Manganese (II) oxide) (3b502) 80 mg, Zinc (Zinc sulfate, monohydrate) (3b605) 60 mg, Iodine (Coated granulated anhydrous calcium iodate) (3b203) 0.8 mg, Selenium (Sodium selenite) (E8) 0.25 mg, citric acid 0.225 mg, Butylhydroxytoluene (BHT) (E321) 0.9 mg, Propyl gallate (E310) 0.075mg, Sepiolite (E562) 3,000 mg, digestibility improvers 6-Phytase EC (3.1.3.26) 667 FYT/g

[2] per kg of diet: 160ppm of narasin and 160ppm of nicarbacin

[3] per kg of diet: 4a7 Endo-1,4-beta-xylanase EC 3.2.1.8 5,600 TXU, 4a7 Endo-1,4-beta-glucanase EC 3.2.1.4 2,500 TGU

Growth Performance

Each treatment was replicated 9 times, and the experimental unit was the pen (14 birds/pen). At d 1, all the birds were weighted in groups of 14, and the individual BW per each pen was calculated. Both, birds BW and feed intake were determined at 7, 21 and 42 d of age, and the data were used to calculate ADFI, ADG and feed conversion ratio (FCR) for each period.

Gastrointestinal Traits

At 7, 21, and 42 d of age, 3 birds per pen (27 birds /treatment) were randomly selected, slaughtered by CO_2 asphyxiation, and individually weighted. The GIT from the proventriculus to the cloaca was removed and weighted. Then, the different organs (proventriculus, gizzard, and cecum) were separated and weighed individually. The pH of the proventriculus, gizzard, and cecum content was measured using a digital pH meter (model 507, Crison Instruments S.A., Barcelona, Spain), the organs were emptied of digesta, and finally were weighed again to calculate the empty weight.

Short Chain Fatty Acids Analysis

At 42 d, 3 birds per pen were randomly selected and slaughtered as described before. Then, the cecal content was pooled per pen and immediately frozen (−20 ºC) until analysis of short-chain fatty acids (SCFA) concentration. The mixture of the 3 cecal contents per pen was used as a replicate. There were 9 replicates (pens) per treatment. Sample processing was adapted from Kimiaeitalab et al. (2017). Briefly, samples were defrosted at 4ºC, 3 g were weighed, mixed with 5 mL of 0.5 *M* HCl, homogenized, and centrifuged (13,000 × *g*, 15 min, 4 ºC). One mL of the supernatant was mixed with 0.5 mL of a deproteinizing solution (20 g metaphosphoric acid and 0.6 g of crotonic acid per L of 0.5 *M* HCl) and left overnight at 4ºC. Samples were centrifuged (13,000 × *g*, 15 min, 4 ºC) and the supernatant was transferred to chromatography vials. Analysis of SCFA concentrations was performed by gas chromatography using a Shimadzu GC 2010 chromatography (Shimazdu Europa GmbH, Duisburg, Germany) fitted with a TR-FFAP column (30 m × 0.53 mm × 1 µm; Supelco, Madrid, Spain) as described by García-Martínez et al. (2005).

Apparent Ileal digestibility (AID)

From 38 to 42 d, birds were fed the finishing diets containing TiO_2. On d 42, birds were slaughtered by CO_2 asphyxia, and ileal digesta was collected from the last third part of the ileum. The contents were gently squeezed into plastic containers, pooled by pen (3 birds/pen), frozen at −80 ˚C, and freeze-dried. Samples of feed and ileal content were ground using a centrifugal mill (Retsch Model Z-I, Stuttgart, Germany) provided with a 1-mm screen, and the AID of dry matter (DM), organic matter (OM), CP, and ether extract (EE) was determined by using TiO_2 as a marker. The determination of TiO_2 in the ileal digesta and diets was performed in accordance with Short et al. (1996). Briefly, 100 mg of freeze-dried sample was ashed at 580°C for 13 h. Ten mL of sulfuric acid (7.4 M) was carefully added to each

crucible upon cooling, and samples were then gently boiled for 3 min. After cooling, the solutions were poured into a 100 mL volumetric flask containing 25 mL distilled water through Whatman filter paper (90 mm). A total of 20 mL of hydrogen peroxide (30%) was added to each flask, and finally, the content was diluted up to 100 mL with distilled water. The absorbance was measured in triplicate using a spectrophotometer (Epoch, BioTek®, USA) at 410 nm**.** The AID was calculated by the equation as follows:

$$AID\ [\%] = [1 - (TiO_{2diet} \cdot Nutrient_{feces})/(TiO_{2feces} \cdot Nutrient_{diet})] \cdot 100\%,$$

where TiO_{2diet}: insoluble marker content in diet; TiO_{2feces}: insoluble marker content in feces; $Nutrient_{feces}$: nutrient content in feces; $Nutrient_{diet}$: nutrient content in diet. The results were expressed in percentage.

In Vitro Cecal Fermentation

An *in vitro* fermentation trial was carried out using the cecal content of 42-d-old birds as inoculum and the two fiber sources (LC and ISFC) as substrates. The inoculum from CON birds was considered as non-adapted to fiber sources, whereas that from birds fed either LC or ISFC treatments was considered adapted to the corresponding fiber source. Samples of 200 mg DM of each fiber source were accurately weighed into 60-mL glass vials. A total of 16 birds per dietary treatment were slaughtered by asphyxiation with CO_2, and the ceca were immediately removed. Within each dietary treatment, the cecal content of 4 birds was pooled to make 4 different inoculums per diet (4 replicates/treatment). Each inoculum was prepared by mixing 1.5 g of cecal content with 100mL of the culture medium described by Goering and Van Soest (1970). The mixture was homogenized for 20s using a hand blender and strained through a double layer of cheesecloth. Vials were filled up with the mixture using a peristaltic pump (Watson-Marlow 520UIP31; Watson-Marlow Fluid Technology Group, United Kingdom) under continuous flushing with CO_2, before being sealed with rubber stoppers and incubated at 40 ºC for 96 h. For each inoculum, 2 vials with each fiber source (LC and ISFC) and 2 vials without substrate (blank) were incubated, making a total of 72 vials (48 with substrate and 24 blanks). The blanks were included to correct the gas production values for the gas produced by the fermentation of the substrates added with the cecal content used as inoculum. Gas production was measured at 2, 7, 12, 48, 72, and 96 h, by using a digital pressure gauge (HD 2304.0, Delta OHM, Italy) and a plastic syringe. The gas values of the 2 vials for each inoculum and substrate were averaged before statistical analysis.

Laboratory Analysis

Procedures of the AOAC (2005) were used to determine DM (934.01), ash (942.05), EE (920.39), total dietary fiber (TDF, 985.29), and Nitrogen by Dumas (968.06), using a Leco analyzer (model FP-528; Leco Corp., St. Joseph, MI, USA). The CP content was determined by multiplying the N content by 6.25. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially using the 25-µm particle retention filter bags (F57; Ankom Technology, New York, USA) by adapting the method of Mertens et al. (2002) and Horwitz et al. (2006). Analysis of NDF was performed using amylase and without any sodium sulphite added, and values were corrected for ash and CP. The soluble fiber content was calculated TDF minus NDF. All analyses were performed in duplicate.

The PS, expressed as geometric mean diameter (GMD), and the PS distribution of the two fiber sources, were determined in 100 g samples using a sieve shaker (FTS-0200, Filtra, Badalona, Spain) provided with 5 sieves ranging in mesh from 62 to 1,000 μm (>1,000, 500-1,000, 250-500; 250-105; 105-62; <62 μm; ASAE, 1995). The hydration capacity was estimated by determining the water binding (WBC, g/g) and swelling capacities (SC, g/ml) by adapting the methods from Slama et al. (2019), Berrocoso et al. (2020) and Priester et al. (2020). Briefly, 0.4 g of each fiber source was hydrated with 10 g of water for 22 h and the mixture was centrifuged (3100 x g, 20 min, Centrifuge, 5810R, Eppendorf, Wesseling-Berzdorf, Germany). The unabsorbed water was weighed, and the WBC was calculated as the difference between the weight of the water added (10 g) and that of the supernatant divided by the initial weight of the sample. The SC was measured using 1 g of sample that was mixed with 20 ml of water under gently stirring and left in a metric cylinder for 22 h. The SC was calculated as the final volume (mL) of the sample divided by the initial sample weight (g).

Statistical Analysis

The effects of dietary treatments on the measured *in vivo* parameters were analyzed by one-way analysis of variance (ANOVA) with the diet as the main effect. Additionally, orthogonal contrasts were applied to study differences between CON and fiber-supplemented animals (C1: CON *vs.* LC+ISFC), and between both fiber concentrates (C2: LC *vs.* ISFC). The pen was an experimental unit for all the statistical analysis, with the only exception of the GIT trait were performed by using 3 birds per pen (n=27).

In vitro gas production data were analyzed for each measurement time and independently for each preplanned comparison. Differences between fiber sources (LC and ISFC) in gas production were tested when the cecal content from CON-fed broilers was used as inoculum (non-adapted inoculum). Potential effects of the experimental diets on gas production were tested independently for each fiber source (LC and ISFC) used as substrate in the *in vitro* trial. For all statistical analyses, effects were considered significant at $P < 0.05$, and trends were considered at $P < 0.10$. When a significant effect of diet was detected, multiple comparisons were carried out by using the Tukey test. Statistical analyses were performed with SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp).

4.3. Experiment 3: Different physiochemical properties of novel fiber sources in the diet of weaned pigs influence animal performance, nutrient digestibility, and cecal fermentation.

Animal Ethics Statement

The experimental protocols were approved by the Ethics Committee of Animal Experimentation of Castilla y León Agriculture Technology Institute (2022/53/CEEA). Animal care and handling procedures were performed according to RD53/2013 (BOE, 2013).

Animals and Housing

The trial was conducted at the Porcine Testing Center of Castilla y León Agriculture Technology Institute in Hontalbilla (Segovia, Spain). A total of 192 piglets (Landrace x Duroc), 50 % males and 50% females, weaned at 28±1 d, came from a commercial farm (La Parilla, Valladolid), and were individually weighed (6.9 ± 1.12 kg) and marked before starting the experiment.

There was a total of 32 pens (6 piglets/pen) distributed in 4 rooms with natural and artificial illumination (40 lux during 8h daily). Each pen (2.46 × 1.54 m) had a plastic slotted floor and was equipped with a nipple bowl drinker and a metal feeder. The ambient temperature was maintained at 28˚C during the first week and was then gradually decreased by 2˚C every week.

Experimental Design and Diets

Four experimental diets were prepared: a control diet (CON) with no additional fiber supplementation, and three diets based on the inclusion of 1.5% fiber sources differing in HC and fermentability. Fiber sources LHC and MHC were designed as a mixture of finely-ground (micronized to a target size below 100 µm) IDF from agricultural by-products. The low HC (LHC) diet was formulated using only low hydration properties sources such as almond shell, olive kernel and nutshell (75%), and a non-fermented grape pomace (15%). The medium HC (MHC) diet incorporated 50% of wood (*Pinus spp*., decorticated), 25% of a mixture of low hydration properties fiber sources (almond shell, olive kernel and nutshell), and 15% of non-fermented grape pomace. Both mixtures included chicory root (10%) to provide a fermentable fiber fraction. The high HC (HHC) diet contained only wood IDF, characterized as high-HC non-fermentable fiber. Fiber mixtures were added to both prestarter and starter diets. All the sources used in the trial were finely ground, presenting a geometric mean diameter (GMD) of 13, 28 and 97 µm for LHC, MHC and HHC, respectively. The chemical composition and physicochemical properties of the fiber sources included in the diets are summarized in Table 10.

Table 10. Fiber composition (%, as fed basis) and hydration properties characterized by water binding (WBC, g/g) and swelling (SC, g/ml) capacities of fiber sources included in the experimental diets[1]. Experiment 3.

	Fiber sources used in experimental diet		
	LHC	MHC	HHC
Chemical composition			
Dry matter	92.3	92.6	92.3
Total dietary fiber	79.5	82.7	90.5
Soluble fiber	2.5	2.7	1.0
Insoluble fiber	77.0	80.0	89.5
Neutral detergent fiber (NDF)	72.4	72.9	87.8
Acid detergent fiber (ADF)	49.9	55.5	75.8
Acid detergent lignin (ADL)	18.5	21.0	25.2
Hemicellulose[2]	22.5	17.4	18.6
Cellulose[3]	31.4	34.5	43.2
Crude protein	4.95	4.72	1.22
Hydration capacity			
WBC, g/g	2.55	3.97	6.54
SC, ml/g	3.99	5.51	7.17
Geometric mean diameter (GMD), µm	12.9	28.0	97.0

[1]LHC: low hydration capacity (HC) insoluble fiber with fermentable fraction, MHC: medium-HC insoluble fiber with fermentable fraction, and HHC: high-HC insoluble fiber as 100% micronized wood; [2]As NDF – ADF; [3]As ADF – ADL

The composition and nutrient content of the diets are shown in Table 11. All diets were formulated to be isocaloric and isonitrogenous, according to FEDNA (2013) to meet or exceed the nutrient recommendations for weaned piglets and were manufactured in pellet form (30 mm). The net energy (MJ/kg) content of the experimental diets was estimated based on the incorporation of each ingredient. The diets provided 11.1MJ/kg in prestarter, and 11.4 for starter diets, respectively.

Table 11. Ingredients (%, as fed basis) and calculated chemical composition of experimental diets[1]. Experiment 3.

	Prestarter diet				Starter diet			
Ingredients	CON	LHC	MHC	HHC	CON	LHC	MHC	HHC
Barley	20.3	20.0	20.0	20.0	---	---	---	---
Wheat	19.8	17.6	17.6	17.6	8.70	5.80	5.80	5.80
Corn	---	---	---	---	44.9	44.9	44.90	44.90
Extruded corn	15.0	15.0	15.0	15.0	---	---	---	---
Cookie meal	16.5	16.5	16.5	16.5	20.0	20.0	20.0	20.0
Sweet whey	10.0	10.0	10.0	10.0	---	---	---	---
Soy protein concentrate	4.40	4.90	4.90	4.90	1.30	0.85	0.85	0.85
Fish meal, CP 64%	4.00	4.00	4.00	4.00	1.50	1.50	1.50	1.50
Soybean meal, CP 48%	2.50	2.50	2.50	2.50	17.0	18.25	18.25	18.25
Spray-dried porcine plasma	2.00	2.00	2.00	2.00	---	---	---	---
Low HC fiber source	---	1.50	---	---	---	1.50	----	----
Medium HC fiber source	---	---	1.50	---	---	---	1.50	----
High HC fiber source	---	---	---	1.50	---	---	---	1.50
Celite®	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Calcium formate	0.79	0.79	0.79	0.79	---	---	---	---
Calcium carbonate	---	---	---	---	0.93	0.93	0.93	0.93
Monocalcium phosphate	0.70	0.70	0.70	0.70	1.12	1.12	1.12	1.12
Vitamin/mineral premix [2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
MCFA monoglycerides mix[3]	0.40	0.40	0.40	0.40	0.30	0.30	0.30	0.30
Soybean oil	0.32	0.85	0.85	0.85	0.80	1.45	1.45	1.45
Organic acids mix[4]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Benzoic acid	0.25	0.25	0.25	0.25	---	---	---	---
Salt	0.25	0.25	0.25	0.25	0.43	0.44	0.44	0.44
L-Lysine HCl, 78.8%	0.48	0.47	0.47	0.47	0.57	0.55	0.55	0.55
L-Threonine, 98%	0.23	0.23	0.23	0.23	0.26	0.26	0.26	0.26
DL-Methionine, 99%	0.18	0.18	0.18	0.18	0.21	0.21	0.21	0.21
L-Valine, 98%	0.09	0.09	0.09	0.09	0.11	0.11	0.11	0.11
L-Tryptophan, 98%	0.06	0.06	0.06	0.06	0.08	0.08	0.08	0.08
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Nutritive value, % DM								
dLys[5]	1.40	1.40	1.40	1.40	1.40	1.39	1.39	1.39
Estimated net energy (MJ/kg)	11.1	11.1	11.1	11.1	11.4	11.4	11.4	11.4
Analyzed composition, % DM								
Organic matter	92.8	92.3	91.9	92.1	92.8	92.2	92.4	93.1
Crude protein	20.7	20.9	21.0	20.7	20.0	20.4	20.2	19.9
Neutral detergent fiber	12.2	13.6	13.9	13.8	12.0	13.5	13.5	13.9
Acid detergent fiber	3.7	4.2	4.1	4.4	3.5	3.9	4.0	4.2
Acid detergent lignin	0.4	0.6	0.6	0.6	0.3	0.5	0.5	0.9

[1]CON: basal diet with no additional fiber inclusion, LHC: low hydration capacity (HC) insoluble fiber with fermentable fraction, MHC medium-HC insoluble fiber with fermentable fraction, and HHC: high-HC non-fermentable insoluble fiber.

[2]Provided per 1 kg of the diet: Vitamin A (3a572a) 15,000IU, Vitamin D3 (3a671) 2,000IU, Vitamin E (3a700) 250mg, Vitamin K3 (3a711) 2mg, Folic acid (3a316) 50mg, Niacinamide (3a315) 10mg, Calcium D-pantothenate (3a341) 10mg, Vitamin B1 (3a521) 10mg, Vitamin B2 (3a625) 10mg, Vitamin B6/pyridoxine hydrochloride (3a831) 10mg, Vitamin B12/Cyanocobalamin 0.05mg, Biotin (3a880) 0.2mg, Iron sulphate (II) monohydrate (3b103) 375mg, Copper oxide (I) (3b412) 131mg, Manganese oxide (II) (3b502) 77.6mg, Zinc oxide (3b603) 150mg, Sodium selenite (3b801) 0.3mg, Anhydrous calcium iodate-I (3b202) 1.5mg.

[3]ENTERO-Nova MTB 400G+, Eastman-3F Feed and Food

[4]Formic and propionic mixture, Eastman-3F Feed and Food

[5]Standarized ileal digestible lysine. Rest of amino acids were adjusted to: Met 38%, Met+cys: 60%, Thr: 65%, Trp: 20%, Val: 69%

The feed and fresh water were provided *ad libitum*. For preparation of experimental treatments, all raw materials were homogenized in a mixer (MMG 316, 250 L, Murcia, Spain) for 150 s. All diets contained 0.9% of diatomaceous earth (Celite ®) as an indigestible marker to determine total tract apparent digestibility of nutrients (Coca-Sinova et al., 2011;). Prestarter and starter diets were administrated from 28 to 42 d, and 42 to 61 d of age, respectively.

Growth Performance Trial

A total of 96 males and 96 females were randomly assigned to one of the four dietary treatments according to homogeneity in the initial body weight (BW) among 32 pens (6 piglets/pen) distributed in 4 rooms. The distribution of the pigs was performed using the individual weight of the animals at weaning, grouped to keep similar animals in each box. All experimental animals were distributed between four rooms with 8 pens (4 for males and 4 for females) each one: two rooms with small pigs (on average 5.7 kg), and two with large pigs (on average 7.8 kg). Within each room, the experimental treatments were randomly assigned between males and females to reach similar initial body weight among treatments. Each treatment was replicated eight times, and the experimental unit was the pen of six piglets per pen. Individual BW of piglets and pen feed consumption were measured at 28 (weaning age), 42 and 60 d of age. The feed was provided from the 25 kg bags. The feeders were filled up twice a day to ensure enough feed throughout the day. The number of bags used, and their weight were recorded daily. The consumption was calculated as a sum of the feed that disappeared during the prestarter (28-42), starter (42-61), and overall (28-61d) and divided between a number of piglets in the box, and the days in each period. Data were used to calculate ADFI, ADG and FCR. The morbidity and mortality were recorded daily.

Measurement of Total Tract Apparent Digestibility

The total tract apparent digestibility (TTAD) of dry matter (DM), organic matter (OM) and crude protein (CP) was measured at 42d and 61 d. The fecal samples were obtained by spontaneous defecation from three piglets per pen or the piglet chosen for slaughter at the end of prestarter and starter periods, respectively; feces were then gently squeezed into plastic containers, and frozen at −80˚C before being freeze-dried. Samples were ground using a centrifugal mill (Retsch Model Z-I, Stuttgart, Germany), and the concentration of acid-insoluble ash was measured in both feed and feces by the sequential method of Coca-Sinova et al. (2011). Briefly, DM was analyzed by drying at 103◦C for 24 h, and then the samples were ashed by incineration at 600◦C for 12 h to determine the OM content. Then, 40 mL of 2 N HCl was added to each Erlenmeyer flask and gently boiled for 5 minutes. The TTAD was calculated by the following equation:

TTAD [%] = [1 – (Celite diet x Nutritional constituent feces)/(Celite feces x Nutritional

constituent diet)] x 100

where ‘Celite diet’ and ‘Celite feces’ represents the insoluble marker content in the diets and feces, respectively. ‘Nutritional constituent feces’ and ‘nutritional constituent diet’ represents the DM, OM and CP content in feces and diets, respectively.

Gut Sampling and In Vitro Cecal Fermentation

At 61 d of age, one piglet per pen was pre-stunned with a captive-bolt gun and slaughtered by exsanguination via jugular vein for intestinal digesta and tissue sampling. The pH of the digestive content of ileum, caecum and colon was measured immediately using a digital pH meter (model 507, Crison Instruments S.A., Barcelona, Spain). The whole caecum was weighed, placed in individual bags, and maintained at 4°C until the *in vitro* fermentation trial was performed within the next 22 h. Samples of the ileum, colon and feces were dried at 70°C for 72 h to determine the DM content.

The cecal contents of two piglets from the same dietary treatment were pooled, and 5 g were weighed, mixed with 5 mL HCl 0.5 N and frozen at −20°C until the determination of the concentration of short chain fatty acids (SCFA). The rest of the cecal content was used as inoculum for the *in vitro* trial to analyze the fermentative capacity of the piglet microbiota at 61 d.

In Vitro Cecal Fermentation

The three fiber sources used in LHC, MHC and HHC diets and their individual constituents (almond shell, olive kernel, wood, nutshell, grape pomace and chicory root) were used as substrates for the *in vitro* incubations using the inoculum from piglets fed the CON diet. Additionally, LHC, MHC and HHC fiber sources were used as substrates to determine the impact of the adaptation of the cecal microbiota to ferment fiber. The inoculum from piglets fed the CON diet was considered as ‘non-adapted microflora’ and those from animals fed each fiber source were considered as a ‘fiber adapted microflora’.

The protocol was performed according to Ocasio-Vega et al. (2018). Briefly, samples of 200 mg of DM of each substrate were weighed and placed into 60 mL glass vials. Three different inocula were used for each dietary treatment, each constituted by the pooled cecal content of two piglets, making a total of 12 inocula. Although initially four inocula per experimental treatment were collected, due to an accident in the laboratory, four inocula (one per treatment) were lost, and only three inocula per treatment were used. Two grams of each inoculum were mixed with 100 mL of the culture medium of Goering and Van Soest. (1970). The mixture was homogenized with a blender (BP4570, 750W, Ufesa, Slovenia) for 20 s and filtered through a double piece of clean cheesecloth. Vials were filled with 50 mL of the mixture using a peristaltic pump (Watson-Marlow 520UIP31; Watson-Marlow Fluid Technology Group, United Kingdom), under continuous flushing with CO_2, sealed with rubber stoppers, and incubated at 40°C for 72 h. All substrates were incubated with inocula from all dietary treatments.

A total of 120 vials, 108 with substrate and 12 without substrate (blanks; one per inoculum) were incubated. Blanks were incubated to correct gas production for gas generated by the inoculum. Total gas production was quantified by measuring the gas produced using a digital pressure gauge (HD 2304.0, Delta OHM, Italy) and a plastic syringe, at 3, 9, 24, 33, 48, and 72 h of incubation. The gas produced was released after each measurement.

Laboratory Analysis

Chemical Composition

Diets and feces were ground through a 1-mm screen using a centrifugal mill (Retsch Model Z-I, Stuttgart, Germany) and then analyzed using the AOAC. (2005) procedures to determine dry matter (DM, method 934.01), ash (method 942.05), ether extract (EE, 920.39), and nitrogen by Dumas (method 968.06) using a Leco analyzer (model FP-528; Leco Corp., St. Joseph, MI, USA). The CP content was determined by multiplying the nitrogen content by 6.25. Insoluble and soluble fiber were determined according to AOAC 991.43 protocols using Fibertec® 1023 (FOSS System, Hilleroed, Denmark), and both values were added to obtain total dietary fiber (TDF) content. Dietary neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined sequentially using the filter bag system with F57 bags with a pore size of 25 μm (Ankom Technology, New York, USA) by adapting the method of Horwitz. (2006) and Mertens. (2002). Thermo-stable alpha-amylase (Ankom Technology, New York, USA) and sodium sulphite (PanReac Appli Chem, 131717.1211, Germany) were used, and the value was corrected for ash content.

Physicochemical Properties

The particle size, expressed as GMD, and the particle size distribution of different fiber sources were determined in 100 g samples using a sieve shaker (FTS-0200, Filtra, Badalona, Spain) provided with 5 sieves ranging in mesh from 62 to 1,000 μm (>1,000, 500-1,000, 250-500; 250-105; 105-62; <62 μm; ASAE, 1995 (American Society of Agricultural Engineers., 1995)). The hydration capacity of fiber sources was measured by determining the water binding capacity (WBC, g/g) and the swelling capacity (SC, g/ml) by adapting the method described by Slama et al. Berrocoso et al. and Priester et al. (Slama et al., 2019; Berrocoso et al., 2020; Priester et al., 2020). Briefly, 0.4 g of each fiber source was hydrated for 22 h with 10 g of water, then centrifuged (3100 × g, 20 min, Centrifuge, 5810R, Eppendorf, Wesseling-Berzdorf, Germany). The unabsorbed water was weighed, and the WBC was calculated as the difference between 10 g of water and the supernatant (g) divided by the starting weight of the sample. The SC was measured using 1 g of the sample, gently stirring it and incubating with 20 mL of water, then left in graduated metric scale cylinders for 22 h. The final volume (mL) of the sample was divided by the starting weight (g). All analyses were performed in triplicate.

Short Chain Fatty Acids Determination

Cecal content processing for determining SCFA concentrations was adapted from Kimiaeitalab et al. (2017). A 5 g sample of the cecal mixture was mixed with 5 mL of 0.5 M HCl, homogenized, and centrifuged (13,000 × g) for 15 min at 4°C. One mL of the supernatant was mixed with 0.5 mL of a deproteinizing solution (20 g metaphosphoric acid and 0.6 g of crotonic acid/L) and left overnight at 4°C. Total SCFA concentration was determined by gas chromatography, using a Shimadzu GC 2010 chromatograph (Shimazdu Europa GmbH, Duisburg, Germany) provided with a TR-FFAP column (30 m × 0.53 mm × 1 μm; Supelco, Madrid, Spain). Individual SCFAs were identified according to the procedure described by García-Martínez et al. (García-Martínez et al., 2005).

Statistical Analysis

Statistical analyses were performed using SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). The effects of the dietary treatments were analyzed by a one-way analysis of variance (ANOVA), and the model included the diet (D) as the main effect. The model for growth traits also included the average BW at weaning (BW0) of the pen as a covariate.

$$Y_{ij} = \mu + D_i + \beta\ (BW0_{ij} - \text{average of BW0}) + \varepsilon_{ij}$$

The residues of the model for each trait were analyzed to confirm they were normally distributed (Shapiro-Wilk or Kolmogorov-Smirnov tests), and the homoscedasticity was evaluated from the graph of residues distribution and confirmed with the Levene's test. Furthermore, orthogonal contrasts were applied to study: C1: differences between CON and fiber-supplemented animals (CON vs. LHC + MHC + HHC), C2: differences between high and low fermentable fiber sources (LHC + MHC vs. HHC), and C3: differences between both fiber sources containing a fermentable fraction (LHC vs. MHC). Linear and quadratic regressions between the WBC of the fiber sources and growth performance were determined. Morbidity and mortality rate were analyzed by Pearson`s chi-square test.

In vitro gas production data were analyzed individually for each measurement time and independently for each preplanned comparison. Differences among dietary fiber sources (LHC, MHC and HHC) in gas production were tested using the cecal content from CON-fed piglets as inoculum, which was considered an inoculum non-adapted to fiber. Potential effects of the experimental diets on gas production were tested independently for each fiber source used as substrate in the *in vitro* trial.

Piglets performance data were expressed as least-squares means, whereas all other variables were reported as means with standard error of the mean (SEM). For all statistical analyses, effects were considered significant when $P < 0.050$, whereas a trend was declared when $0.050 \leq P \leq 0.100$. When a significant effect was detected, means multiple comparisons were carried out using the Tukey test.

RESULTS

5.1 Experiment 1: Effect of type of fiber and its physicochemical properties on performance, digestive transit time, and cecal fermentation in broilers from 1-23 days of age.

The chemical composition and physicochemical properties of IDF sources are presented in Table 12. As expected, all IDF sources had high dietary fiber and NDF content, but ADL content was about 4 times higher in both LC and AS than in straw. The lowest PS and HC (WBC and SC) were observed for AS, whereas the rest of the IDF samples had greater WBC (>5.0 g/g) and SC (>6.0 g/ml) values. The LC presented low PS (GMD: 97 µm), and PS was high for CS (GMD: 875µm) and low for FS (GMD: 147 µm).

Growth Performance

The mortality rate was low (1.9%) and not related to any dietary treatment (data not shown). The influence of diet on performance from 0-21d is shown in Table 13. Compared with control birds, the dietary inclusion of IDF had no effect on broilers BW excepting a trend to decreased BW (P = 0.058) observed at 14 d. Similarly, ADFI was unaffected ($P \geq 0.14$) along the experiment compared with the control group, but ADG was decreased by IDF from 7-14 d of age (P = 0.016), with no effect in the whole experimental period (P = 0.16). The dietary inclusion of IDF sources also impaired FCR from 0 to 14 d of age by 4% ($P \leq 0.023$), and a trend to impaired FCR in the whole experimental period (P = 0.053) was observed.

More important differences in performance were observed among IDF sources differing in HC. The inclusion of AS tended to increase ADFI from 0 to 7 d of age (P = 0.10) and increased it from 7 to 21 d ($P \leq 0.012$), and in the whole experimental period by 6% (P = 0.005) compared to the average value of birds fed LC, FS and CS diets. This led to an 8% higher ADG in the AS-fed group than in the other IDF-fed groups along the experiment ($P \leq 0.026$). However, FCR was similar among all IDF supplemented groups until 14 d of age, and only tended to be lower in the AS group from 14 to 21 d, and 0 to 21 d of age ($P \leq 0.095$) than in the other IDF-fed groups. No differences were observed in growth traits between Control and AS groups. The PS of the straw had no impact on performance.

GIT Traits and Intestinal Morphology

As shown in Table 14, the dietary inclusion of IDF sources had no effect on the weight of the whole digestive tract (either expressed in g or as % of BW; $P \geq 0.14$), or of its parts when they were expressed relative to the weight of digestive tract ($P \geq 0.24$). In contrast, the type of IDF influenced the weight of digestive segments. The AS group showed a heavier full digestive tract, proventriculus (full and empty), full gizzard and full SICR (expressed in g) than the average value of the other three

IDF-fed groups (by 8, 8, 7, 6 and 10%. $P \leq 0.049$). When the weight of the digestive organs was expressed as proportion of the weight of the whole digestive tract, the AS group showed again a heavier full SICR ($P = 0.015$), and the cecum tended to be lighter ($P = 0.060$) than in Control group. No differences were observed between AS and Control group on these traits, but the SICR weight that was 9% higher in AS than in Control group ($P = 0.003$). Treatments had no influence on the length of the small intestine (in cm), but when expressed as cm / g of BW or as cm / 100 g digestive tract, the AS group showed lower values than the other three IDF-fed groups ($P \leq 0.015$).

Dietary treatments did not modify the pH of proventriculus, gizzard nor cecum ($P \geq 0.32$). The inclusion of the IDF sources reduced the moisture in the excreta compared with Control group by 2% ($P = 0.006$), with no difference between AS and the other IDF sources. Excreta moisture content was lower in LC than in Control and CS groups ($P = 0.004$). There were no differences among diets in the moisture of cecal digesta ($P = 0.46$). The reduction of PS in the straw had no impact on any digestive trait.

The effect of IDF sources on the morphology of jejunal mucosa in broilers at 21d is shown in the Table 15. No effects of treatment were observed on morphometric parameters ($P \geq 0.20$).

SCFA Production and In Vitro Fermentation

The effects of IDF sources on cecal total SCFA concentration and molar proportions of individual SCFA in broilers at 21d of age are presented in Table 16. Dietary treatments did not modify the cecal total SCFA concentration ($P \geq 0.23$), but the inclusion of IDF tended to increase the molar proportion of propionate ($P = 0.072$) and isobutyrate ($P = 0.059$) compared with Control group. The inclusion of AS tended to reduce the molar proportion of acetate ($P = 0.080$) with respect to the other IDF sources. The reduction of PS in the straw had no impact on the measured in vivo fermentation traits.

The cumulative gas production curves of the different IDF sources when they were fermented with the inoculum from Control animals are shown in Figure 4. No differences on gas production were observed during the first 7 h of in vitro fermentation for any substrate ($P > 0.05$). In contrast, from 10 h onwards both FS and CS produced more gas than LC and AS. In fact, after 15 h, no differences on the cumulative gas production were observed either between FS and CS or between LC and AS substrates.

As shown in Figure 5, feeding the different IDF sources to broilers had no effect ($P > 0.10$) on gas production from either LC (Figure 5A) or FS (Figure 5B) substrates when cecal content from IDF-fed broilers was used as inoculum for the in vitro incubations. As previously observed with the cecal inoculum from Control broilers, gas production at 50 h of fermentation from LC substrate was lower than that for FS substrate (< 11.0 and > 20.0 ml/g DM, respectively) for all inocula.

The potential adaptation of cecal microbiota to ferment each IDF source when it was included in the

diet was tested by comparing the fermentation of the IDF source when it was incubated with cecal inoculum from Control and from IDF-fed animals (Figure 6). Although in general gas production was numerically higher with the inoculum from IDF-fed animals compared with that from Control animals, there were either no significant or only minor differences for LC, FS and AS substrates. For CS substrate, the inoculum from broilers fed the CS-diet resulted in higher gas production ($P < 0.05$) at 10 and 13 h of incubation than that from Control animals.

Digestive Transit Time

The effect of IDF sources on digestive passage rate at 8 and 12 h is shown in Table 17. The inclusion of IDF sources did not affect the total TiO_2 recovery at 8 or 12 h post marker ingestion ($P \geq 0.28$). The TiO_2 recovery was lower in AS group at 8 and 12 h in comparison to the average of the other three IDF-fed groups (by 29 and 20%, respectively, $P \leq 0.008$), and it was also lower than that for control group at 12 h (by 20%; $P = 0.010$).

Table 12. Chemical composition (%, as-fed basis) and physico-chemical properties of the insoluble fiber sources included in the experimental diets. Experiment 1.

Chemical composition	Lignocellulose	Straw		Almond shell
Dry matter	94.4	94.0		92.0
Ash	0.41	9.13		1.42
Crude protein	1.82	2.89		1.45
Total dietary fiber	90.3	82.9		88.2
Neutral detergent fiber (NDF)	79.5	67.9		81.8
Acid detergent fiber (ADF)	63.4	38.2		51.8
Acid detergent lignin (ADL)	23.4	5.22		22.5
Hemicellulose[1]	16.1	29.7		30.0
Cellulose[2]	40.0	33.0		29.3
Physico-chemical properties		Fine-ground	Coarse-ground	
GMD[3] (µm)	97±1.0	147±1.8	875±1.8	44±3.6
Particle size distribution (%)				
< 63 µm	9.25	9.15	0.40	27.4
63-106 µm	21.6	9.44	1.00	17.2
106-150 µm	39.1	12.1	1.60	15.1
150-250 µm	26.0	25.1	3.37	25.3
250-500 µm	1.60	42.2	10.5	13.6
500-1000 µm	0	0.58	25.7	0.95
> 1000 µm	0	0.41	57.5	0.44
Hydration capacity				
WBC[4] (g/g)	6.4±0.1	5.1±0.2	6.3±0.1	3.2±0.4
SC[5] (g/ml)	8.4±0.2	6.1±0.2	8.3±0.1	3.2±0.2

[1] As NDF-ADF
[2] As ADF-LAD
[3] Geometric mean diameter
[4] Water binding capacity
[5] Swelling capaci

Table 13. Influence of various insoluble fiber sources differing in physicochemical properties on broiler's performance from 0-21d. Experiment 1.

Time	Item	Dietary treatments[1]					SEM[2]	*P*-value		
		Control	LC	FS	CS	AS		Treatment	C1[3]	C2[4]
0-7 d	BW at 7 d (g)	150	145	150	150	154	2.15	0.12	0.85	0.038
	ADG (g/d)	15.6	14.9	15.6	15.6	16.2	0.30	0.10	0.94	0.026
	ADFI (g/d)	14.6	14.6	15.4	15.3	15.7	0.35	0.049	0.14	0.10
	FCR[5] (g/g)	0.938	0.962	0.991	0.981	0.972	0.015	0.12	0.023	0.75
7-14 d	BW at 14 d (g)	417ab	393b	396ab	406ab	420a	5.96	0.007	0.058	0.003
	ADG (g/d)	38.1a	35.4b	35.2b	36.59ab	38.0a	0.64	0.003	0.016	0.003
	ADFI (g/d)	44.5	43.3	43.2	44.0	45.7	0.71	0.096	0.55	0.010
	FCR (g/g)	1.17a	1.223ab	1.227b	1.205ab	1.204ab	0.014	0.044	0.006	0.38
14-21 d	BW at 21 d (g)	847ab	806b	796b	824ab	870a	14.7	0.005	0.16	0.001
	ADG (g/d)	61.6ab	59.1ab	57.1b	59.8ab	64.3a	1.57	0.029	0.40	0.003
	ADFI (g/d)	81.6	79.3	78.2	81.5	84.3	1.55	0.072	0.66	0.012
	FCR (g/g)	1.331	1.343	1.372	1.368	1.316	0.023	0.37	0.47	0.095
0-21 d	ADG (g/d)	38.4ab	36.5b	35.9b	37.3ab	39.5a	0.70	0.005	0.16	0.001
	ADFI (g/d)	46.9ab	45.7ab	45.6b	46.9ab	48.6a	0.74	0.040	0.79	0.005
	FCR (g/g)	1.223	1.252	1.269	1.259	1.232	0.013	0.11	0.053	0.083

a,b: Means in column not sharing a common letter differ (P<0.05)

[1] Control: standard diet based on corn and soybean meal; LC, FS, CS, AS: control diet supplemented with 1.5% of lignocellulose, fine-ground straw, coarse-ground straw, and almond shell, respectively.

[2] SEM, n = 10

[3] Contrast 1: Control vs. (LC + FS + CS + AS)

[4] Contrast 2: AS vs. (LC + FS + CS)

[5] Feed conversion ratio

Table 14. Influence of various insoluble fiber sources differing in physicochemical properties on BW and gastrointestinal traits of broilers at 21d of age. Experiment 1.

	Dietary treatments[1]					SEM[2]	*P*-value		
	Control	LC	FS	CS	AS		Treatment	C1[3]	C2[4]
BW, g	950ab	897b	897b	907b	983a	16.6	<0.001	0.12	< 0.001
Weight of digestive segments (g)									
Whole digestive tract	118ab	115ab	113b	117ab	124ab	2.37	0.025	0.70	0.002
Full proventriculus	5.81a	5.17b	5.77a	5.70ab	5.97a	0.15	0.005	0.36	0.016
Empty proventriculus	5.51b	4.99a	5.44ab	5.40ab	5.67b	0.13	0.008	0.35	0.012
Full gizzard	38.2	37.1	37.1	37.5	39.4	0.98	0.39	0.72	0.049
Empty gizzard	23.5	22.5	22.1	22.9	23.4	0.61	0.48	0.28	0.23
Full cecum	8.20	8.41	7.68	8.18	7.72	0.42	0.67	0.67	0.45
Full SICR[5]	64.6b	63.9b	62.7b	65.4ab	70.7a	1.50	0.003	0.50	< 0.001
Small intestine length (cm)	137	136	133	139	139	2.00	0.25	0.74	0.23
Whole digestive tract (% BW)	12.4	12.8	12.7	12.9	12.5	0.18	0.36	0.14	0.23
Relative weight (% digestive tract weight)									
Full proventriculus	4.93	4.53	5.10	4.89	4.82	0.11	0.42	0.42	0.88
Empty proventriculus	4.68ab	4.37a	4.82b	4.64ab	4.58ab	0.090	0.013	0.44	0.77
Full gizzard	32.7	32.4	32.7	32.0	31.9	0.48	0.64	0.38	0.41
Empty gizzard	19.9	19.7	19.5	19.7	19.0	0.42	0.57	0.33	0.18
Full cecum	7.02	7.32	6.84	7.05	6.28	0.36	0.34	0.72	0.060
Full SICR[5]	55.3	55.78	55.3	56.0	57.1	0.59	0.20	0.24	0.050
Small intestine length (cm/100 g)	117	119	119	120	113	2.19	0.18	0.86	0.015
pH									
Proventriculus	2.37	2.10	2.27	2.27	2.27	0.16	0.82	0.43	0.75
Gizzard	2.73	2.73	2.56	2.54	2.72	0.095	0.40	0.38	0.32
Cecum	6.38	6.43	6.42	6.47	6.41	0.072	0.93	0.49	0.73
Moisture (%)									
Excreta	82.6b	79.6a	80.9ab	81.9b	80.9ab	0.53	0.004	0.006	0.84
Cecum	86.6	88.9	87.2	86.6	86.6	0.91	0.46	0.46	0.46

Means in column not sharing a common letter are significantly different (P<0.005); [1] Control: standard diet based on corn and soybean meal; LC, FS, CS, AS: control diet supplemented with 1.5% of lignocellulose, fine-ground straw, coarse-ground straw, and almond shell, respectively; [2] SEM, n = 30; [3] Contrast 1: Control vs. (LC + FS + CS + AS); [4] Contrast 2: AS vs. (LC + FS + CS); [5] Weight of full small intestine, colon and rectum calculated by difference.

Table 15. Influence of various insoluble fiber sources differing in physicochemical properties on the morphology of the distal jejunum mucosa of broilers at 21d of age. Experiment 1.

	Dietary treatments[1]						*P*-value		
	Control	LC	FS	CS	AS	SEM[2]	Treatment	C1[3]	C2[4]
Villous height (µm)	523	516	538	580	574	40.7	0.72	0.52	0.53
Crypth depth (µm)	146	162	161	160	170	11.8	0.70	0.20	0.52
Villous height to crypth depth	3.67	3.31	3.50	3.78	3.62	0.32	0.91	0.66	0.75
Mucosal thickness (µm)	673	663	697	723	714	52.2	0.91	0.66	0.75
Villi density (per-mm)	3.32	3.31	3.04	3.44	2.99	0.21	0.24	0.64	0.28
Crypth density (per-mm)	13.2	13.0	11.3	11.6	13.0	0.89	0.36	0.31	0.30
Goblet cells count (nº)	134	122	147	143	139	20.0	0.91	0.88	0.93

[1] Control: standard diet based on corn and soybean meal; LC, FS, CS, AS: control diet supplemented with 1.5% of lignocellulose, fine-ground straw, coarse-ground straw, and almond shell, respectively; [2] SEM n = 6; [3]Contrast 1: Control vs. (LC + FS + CS + AS); [4]Contrast 2: AS vs. (LC + FS + CS).

Table 16. Influence of various fiber sources differing in physicochemical properties on the short chain fatty acids production (SCFA), expressed in µmol/g and as molar proportions of SCFA (mol/100mol) in broilers at 21d of age. Experiment 1.

	Dietary treatments[1]						*P*- value		
	C	LC	FS	CS	AS	SEM [2]	Treatment	C1[3]	C2[4]
Total SCFA, µmol/g	60.7	72.0	55.3	63.1	58.1	5.08	0.23	0.81	0.37
Molar proportions of SCFA (mol/100mol)									
Acetate	74.1	73.9	74.6	74.8	72.2	1.09	0.47	0.86	0.080
Propionate	4.76	5.39	5.39	5.96	5.50	0.37	0.30	0.072	0.85
Butyrate	19.2	18.6	17.8	16.9	19.9	1.13	0.39	0.50	0.12
Isobutyrate	0.50	0.60	0.69	0.79	0.77	0.09	0.20	0.059	0.51
Valerate	0.86	0.94	0.84	0.91	0.95	0.04	0.21	0.28	0.26
Isovaleriate	0.56	0.54	0.63	0.70	0.72	0.08	0.47	0.380	0.32

[1] Control: standard diet based on corn and soybean meal; LC, FS, CS, AS: control diet supplemented with 1.5% of lignocellulose, fine-ground straw, coarse-ground straw, and almond shell, respectively; [2] SEM, n = 10; [3]Contrast 1: Control vs. (LC + FS + CS + AS); [4]Contrast 2: AS vs. (LC + FS + CS).

Table 17. Influence of various fiber sources differing in physicochemical properties on cumulative TiO_2 recovery (%) at 8 h and 12 h after TiO_2 administration in broilers of 23d of age. Experiment 1.

	Dietary treatments[1]						*P*-value		
Time	Control	LC	FS	CS	AS	SEM [2]	Treatment	C1[3]	C2[4]
8 h	78.0	86.2	83.3	88.1	61.0	7.4	0.10	0.83	0.008
12 h	96.8a	92.7ab	98.0a	99.1a	77.8b	4.3	0.010	0.28	0.001

Means in column not sharing a common letter are significantly different (P<0.005)

[1] Control: standard diet based on corn and soybean meal; LC, FS, CS, AS: control diet supplemented with 1.5% of lignocellulose, fine-ground straw, coarse-ground straw, and almond shell, respectively; [2] SEM, n = 8; [3]Contrast 1: Control vs. (LC + FS + CS + AS); [4]Contrast 2: AS vs. (LC + FS + CS).

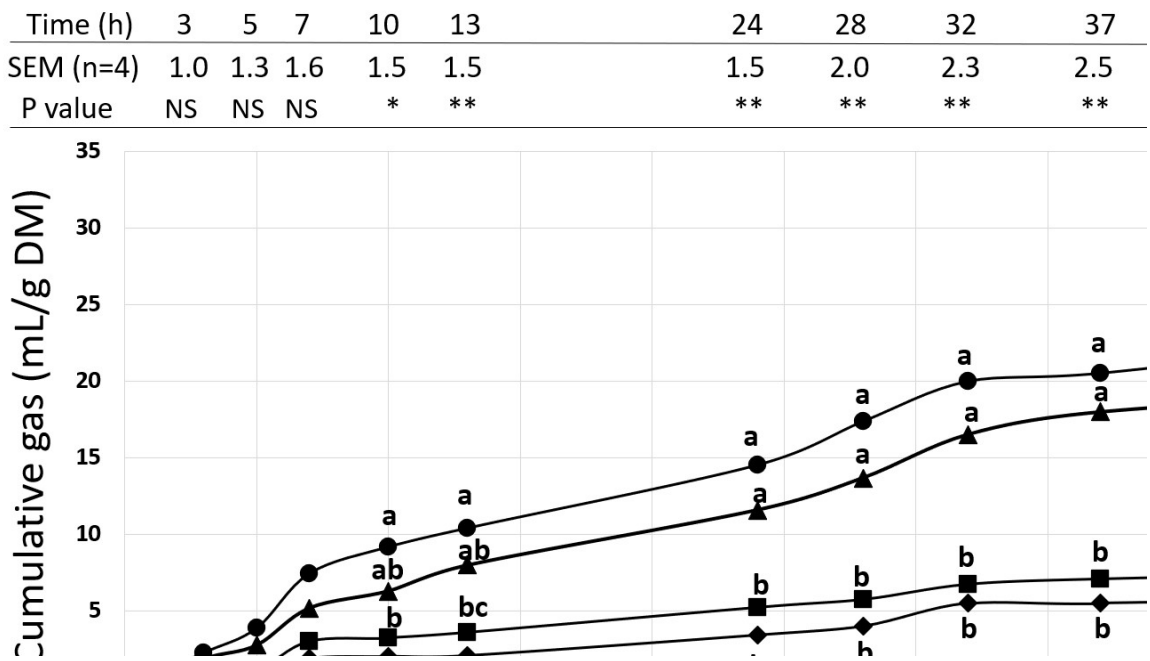
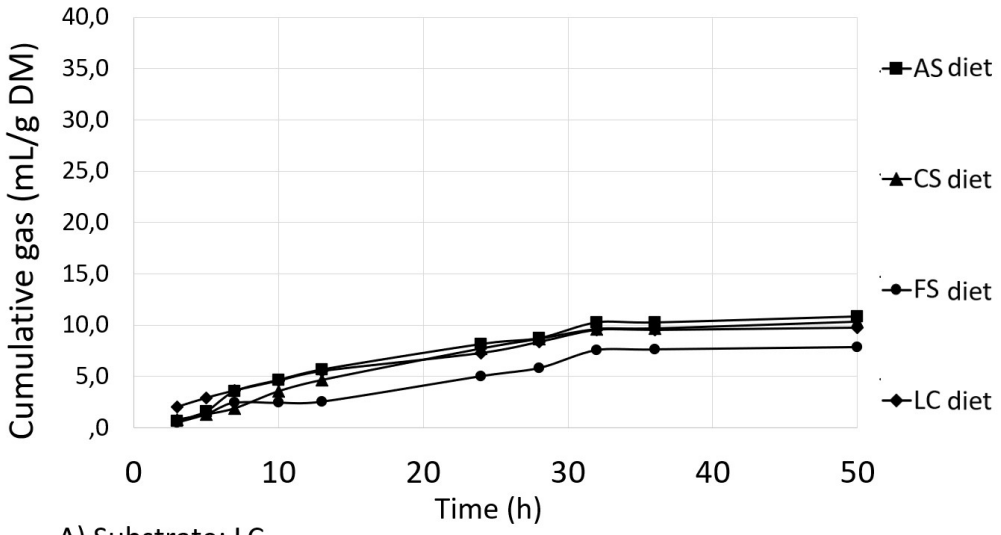


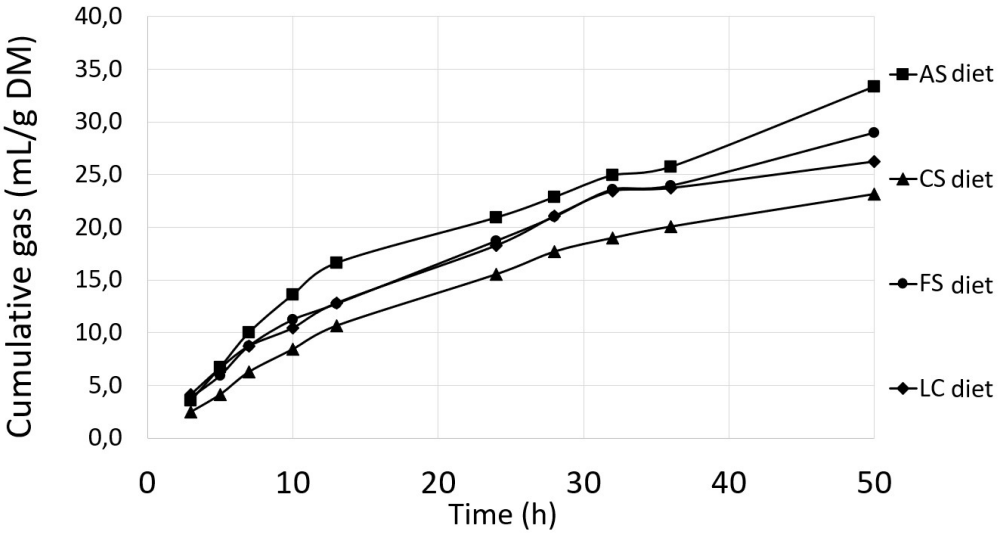
Figure 4. Cumulative gas production curve (mL/gDM) of lignocellulose (LC), fine-ground straw (FS), coarse-ground straw (CS), and almond shell (AS) after incubation with cecal content from birds fed a control diet based on corn and soybean meal. Four different inocula were used, and each inoculum was a pooled cecal content from 4 birds. Within each incubation time, means not sharing a common letter differ (P<0.05). Values in the Table indicate the SEM and *P*-value of the ANOVA analyzing potential differences in gas production at each measurement time.

Time(h)	3	5	7	10	13	24	28	32	37	50
SEM(n=4)	0.9	0.9	0.9	0.9	1.1	1.8	1.8	2.0	2.0	1.8
P value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS



A) Substrate: LC

Time(h)	3	5	7	10	13	24	28	32	37	50
SEM(n=4)	1.2	1.7	2.0	1.8	1.7	1.4	1.8	2.3	2.1	3.6
P value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS



B) Substrate: FS

Figure 5. Cumulative gas production curves (mL/gDM) obtained by fermenting A) lignocellulose (LC) or B) fine-ground straw (FS) when inocula from birds fed the experimental diets containing the 4 insoluble fiber sources (LC, FS, CS (coarse-ground straw), and AS (almond shell)) were used. Four different inocula were used for each experimental treatment, and each inoculum was a pooled cecal content from 4 birds. Values in the Tables indicate the SEM and *P*-value of the ANOVA analyzing potential differences in gas production at each measurement time. NS: $P > 0.10$.

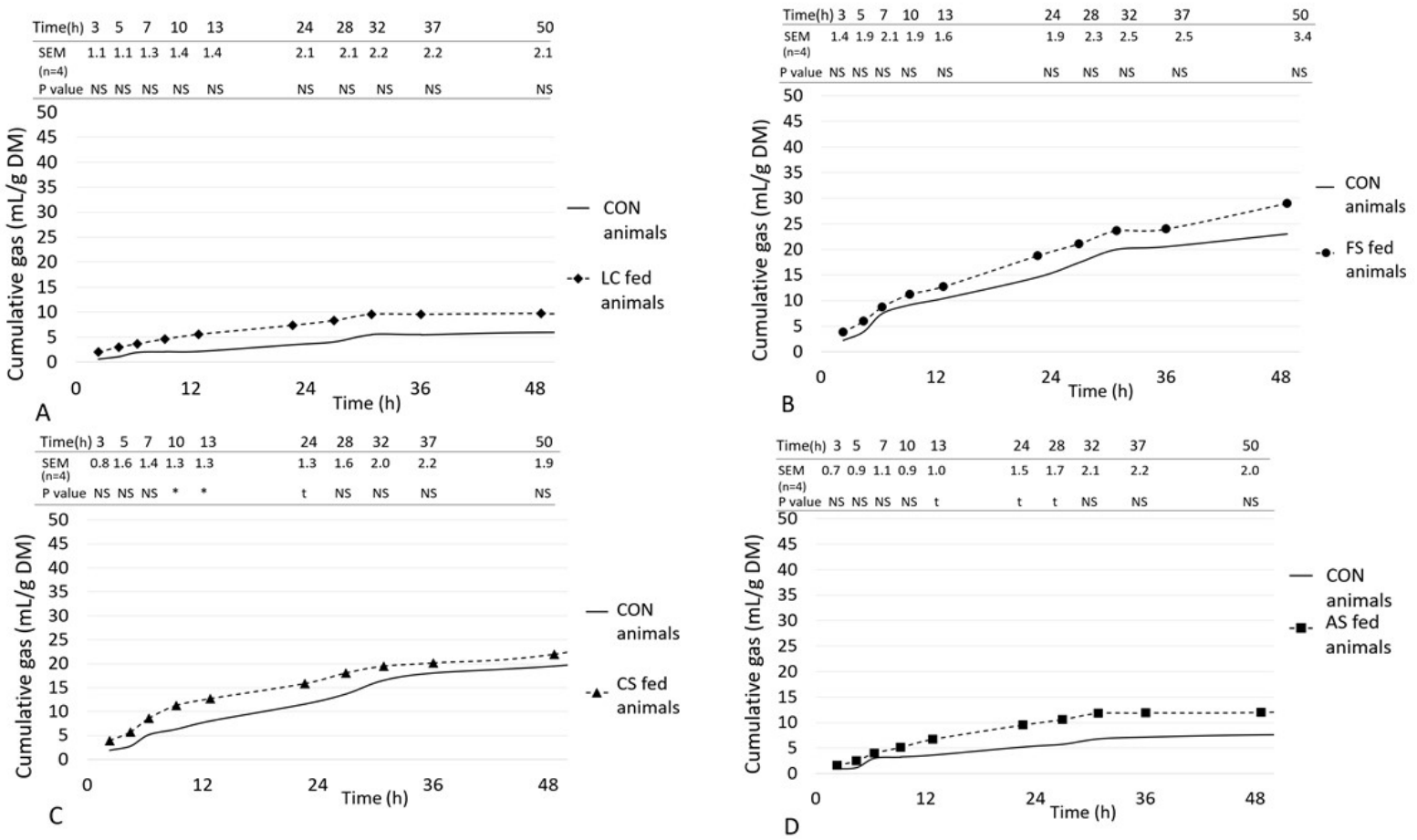


Figure 6. Cumulative gas production curves (mL/gDM) from different insoluble fiber sources: A) lignocellulose (LC), B) fine-ground straw (FS), C) coarse-ground straw (CS), and D) almond shell (AS) when they were fermented either using inocula from birds fed a diet including the incubated fiber source (adapted microbiota) or a control diet (CON; non-adapted microbiota). Four different inocula were used for each experimental dietary treatment, and each inoculum was a pooled cecal content from 4 birds fed the same diet. Tables indicate the SEM and *P*-value of the ANOVA analyzing potential differences in gas production at each measurement time. NS= P>0.05.0; t=0.10<P<0.05; * P<0.05

5.2. Experiment 2: Effect of dietary supplementation of two fiber sources differing on fermentability and hydration capacity on performance, nutrient digestibility and cecal fermentation in broilers from 1 to 42 d of age.

The fiber composition, and physical properties of fiber sources are presented in Table 18. Both LC and ISFC had high amounts of TDF, but the proportions of hemicellulose, cellulose, and lignin were different (16.1, 40.0, 23.4% in LC *vs.* 26.3, 26.1, 17.9% in ISFC, respectively). Moreover, ISFC contained FOS which would indicate greater fermentability due to higher soluble fiber content (10.8% vs. 16.0 for LC and ISFC, respectively). The GMD of fiber sources was 97 *vs.* 68µm for LC and ISFC, respectively. The water binding and swelling capacities of ISFC were 47 and 36% of those of LC.

The chemical composition of the experimental diets was close to expected from feed ingredients composition (Table 19). The GMD of the starter and finishing diets were 652±1.77µm and 616±1.76µm, respectively, confirming that the experimental diets were finely ground to avoid the additional effect of PS of other ingredients. The addition of fiber sources produced low impact of the PS of the diets (646±1.78 and 645±1.80µm in a starter, and 610±1.77 and 610±1.78µm in a finisher diet, for LC and ISFC, respectively).

Growth Performance and Nutrient Digestibility

The mortality rate was low (2.9% on average) and was not related to dietary treatments (data not shown). The influence of dietary treatments on broiler performance is shown in Table 20. From 1 to 7 d of age, dietary supplementation with both fiber sources impaired FCR by 4% ($P = 0.003$) but this effect disappeared thereafter.

In the finishing period (21 - 42 d of age), ISFC-fed broilers had greater ADG than CON animals, presenting LC intermediate values ($P = 0.039$). This effect remained as a trend in the overall period ($P = 0.094$). Feeding ISFC also tended to result in greater BW than CON at 42 d ($P = 0.076$) but no effect of dietary treatments on ADFI was detected in any period ($P \geq 0.23$).

Birds fed ISFC showed 4% higher AID of both DM ($P = 0.013$) and OM ($P = 0.016$) compared with LC-fed birds, although values were similar to those in CON group. Feeding ISFC tended to increase AID of CP in comparison to CON, presenting LC-fed birds with intermediate values ($P = 0.099$; Table 21).

GIT Traits

No effects on GIT traits were observed at 7 or 21d ($P \geq 0.17$). At 42 d, the full weight of both the whole digestive tract ($P = 0.019$) and gizzard ($P = 0.030$) were increased by fiber inclusion (Table 22). Compared with CON birds, fiber supplementation increased the full weight of gizzard ($P = 0.030$) and tended to increase the full weight of gizzard+proventriculus ($P = 0.054$).

When the weights of the digestive organs were expressed relative to broilers BW, the full digestive tract

remained heavier in LC-fed birds than in CON birds, whereas the ISFC group showed intermediate values ($P = 0.041$; Table 23). The empty weight of the gizzard was greater in ISFC than in CON group, expressed both in absolute and relative numbers, with LC broilers having an intermediate value ($P < 0.05$).

At 42 d, ISFC-fed birds had greater gizzard pH than those fed LC but similar to CON animals ($P = 0.021$).

Cecal Fermentation

A trend of higher weight of cecum was observed in broilers supplemented with ISFC in comparison to CON ($P = 0.083$), but cecal pH was not modified ($P = 0.27$; Table 24). Both, total SCFA and acetate concentrations in the cecum were decreased ($P = 0.048$ and 0.040, respectively) with fiber sources addition (Table 24). Only the molar proportion of valerate in the cecum was modified by fiber inclusion, with greater values in ISFC-fed broilers compared with control ones, presenting LC intermediate values ($P = 0.039$).

As shown in Figure 7, gas production from ISFC started slightly earlier and had greater values from 7 h of incubation onwards in comparison to LC substrate ($P < 0.050$).

The gas production curves for LC and ISFC substrates when using cecal inoculum from broilers fed CON diet (without exposition to fiber sources) and LC or ISFC diets (with previous exposition to the substrate in the diet) are presented in Figures 8A and 8B.

Only small differences between control and substrate-adapted inoculum were observed for LC substrate. The inoculum from LC-fed broilers tended to produce more gas at 12 h ($P = 0.063$) and produced more gas at 24 h ($P = 0.017$) than the inoculum from CON-fed broilers, but differences disappeared thereafter. In contrast, there were no differences ($P > 0.10$) between CON and ISFC inoculums in the amount of gas produced from ISFC fermentation at any incubation time.

Table 18. Fiber fractions composition and physicochemical properties of the two experimental fiber sources. Experiment 2.

	Fiber sources[1]	
Fiber composition (%; as-fed basis)	LC	ISFC
Total dietary fiber (TDF)	90.3	86.4
Neutral detergent fiber (NDF)	79.5	70.4
Acid detergent fiber (ADF)	63.4	44.0
Acid detergent lignin (ADL)	23.4	17.9
Hemicellulose[2]	16.1	26.3
Cellulose[3]	40.0	26.1
Soluble dietary fiber[4]	10.8	16.0
Physico-chemical properties		
Geometric mean diameter (GMD) (µm)	97	68
Particle size distribution (%)		
>1000 µm	0	6.67
500-1000 µm	0	10.8
250-500 µm	1.6	16.8
150-250 µm	26.0	15.7
106-150 µm	39.1	12.0
63-106 µm	21.6	13.1
<63 µm	9.2	24.9
Hydration capacity		
Water binding capacity (WBC) (g/g)	5.8	2.7
Swelling capacity (SC) (g/mL)	8.4	3.0

[1] LC: insoluble high-hydration capacity fiber; ISFC: medium-hydration capacity and partially fermentable prebiotic fiber
[2] As NDF - ADF
[3] As ADF - AFL
[4] As TDF - NDF

Table 19. Chemical composition (%, as-fed basis) and geometric mean diameter (GMD) of starter and finishing experimental diets[1]. Experiment 2.

	Starter			Finishing		
Item	CON	LC	ISFC	CON	LC	ISFC
Organic matter	84.3	85.0	85.2	84.3	84.6	84.7
Dry matter	90.6	90.5	90.7	90.1	89.9	90.1
Crude protein	22.1	22.4	22.6	21.0	20.5	20.2
Ether extract	4.29	4.21	4.40	4.72	4.31	4.33
Neutral detergent fiber	11.4	13.8	13.7	11.8	13.0	12.7
Acid detergent fiber	3.35	4.28	4.39	3.61	4.56	4.42
Acid detergent lignin	0.81	1.04	1.29	0.72	1.00	1.32
GMD[2] (μm)	652	646	645	616	610	610

[1]CON: standard non-fiber supplemented diet; LC: CON diet diluted with 1.5% of insoluble high hydration properties fiber source; ISFC: CON diet diluted with 1.5% of insoluble medium hydration properties and partially fermentable prebiotic fiber source.
[2]Geometric mean diameter

Table 20. Influence of fiber sources differing in hydration capacity and fermentability on broiler's performance from 1 to 42 d. Experiment 2.

		Dietary treatments[1]				*P*-value		
Time	Item	CON	LC	ISFC	SEM[2]	Treatment	C1[3]	C2[4]
	BW at 7 d (g)	161	159	158	1.96	0.59	0.31	0.89
	ADG (g/d)	17.5	17.2	17.2	0.27	0.58	0.30	0.95
	ADFI (g/d)	17.0	17.5	17.5	0.24	0.60	0.32	0.89
1-7 d	FCR (g/g)	0.981a	1.017b	1.022b	0.0096	0.012	0.003	0.75
	BW at 21 d (g)	857	838	841	10.3	0.38	0.17	0.86
	ADG (g/d)	49.8	48.6	48.8	0.70	0.46	0.22	0.88
	ADFI (g/d)	65.4	64.5	65.1	1.28	0.88	0.72	0.75
7-21 d	FCR (g/g)	1.316	1.327	1.336	0.0250	0.86	0.63	0.81
	ADG (g/d)	38.9	38.1	38.2	0.48	0.37	0.17	0.84
	ADFI (g/d)	49.3	48.8	49.3	0.83	0.91	0.78	0.73
1-21 d	FCR (g/g)	1.267	1.283	1.290	0.0217	0.76	0.48	0.83
	BW at 42 d (g)	3035	3046	3136	34.1	0.095	0.195	0.076
	ADG (g/d)	103.7b	105.2ab	109.3a	1.49	0.039	0.068	0.063
	ADFI (g/d)	163.2	164.5	167.9	1.95	0.24	0.23	0.23
21-42 d	FCR (g/g)	1.575	1.564	1.536	0.0150	0.18	0.18	0.20
	ADG (g/d)	71.4	71.6	73.7	0.81	0.094	0.19	0.075
	ADFI (g/d)	106.3	106.6	108.6	1.23	0.39	0.39	0.28
1-42 d	FCR (g/g)	1.490	1.490	1.472	0.0117	0.45	0.50	0.29

a,b: Means in the same row not sharing a common letter differ ($P < 0.05$)

[1]CON: standard non-fiber supplemented diet; LC: CON diet diluted with 1.5% of insoluble high hydration properties fiber source; ISFC: CON diet diluted with 1.5% of insoluble medium hydration properties and partially fermentable prebiotic fiber source.

[2]SEM, n=9.

[3]C1: CON vs. LC+ISFC.

[4]C2: LC vs. ISFC.

Table 21. Influence of fiber sources differing in hydration capacity and fermentability on apparent ileal digestibility (%) of dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) in broilers at 42 d of age. Experiment 2.

	Dietary treatments[1]				*P*-value		
Item	CON	LC	ISFC	SEM[2]	Treatment	C1[3]	C2[4]
DM	83.5ab	82.7b	86.1a	0.90	0.033	0.41	0.013
OM	85.7ab	85.0b	87.8a	0.78	0.043	0.49	0.016
CP	87.8	87.9	89.9	0.73	0.099	0.25	0.066
EE	91.0	91.2	92.1	0.77	0.55	0.50	0.39

a,b: Means in the same row not sharing a common letter differ ($P < 0.05$)

[1]CON: standard non-fiber supplemented diet; LC: CON diet diluted with 1.5% of insoluble high hydration properties fiber source; ISFC: CON diet diluted with 1.5% of insoluble medium hydration properties and partially fermentable prebiotic fiber source.

[2]SEM, n=9.

[3]C1: CON vs. LC+ISFC.

[4]C2: LC vs. ISFC.

Table 22. Influence of fiber sources differing in hydration capacity and fermentability on BW and gastrointestinal traits of broilers at 42 d[1]. Experiment 2.

	Dietary treatments[1]				*P*-value		
Item	CON	LC	ISFC	SEM[2]	Treatment	C1[3]	C2[4]
BW (g)	3,016	3,089	3,137	64.2	0.41	0.22	0.61
Full weight (g)							
Whole digestive tract	233b	260a	249ab	7.3	0.038	0.019	0.30
Gizzard	62.3	72.1	68.4	2.95	0.065	0.030	0.38
Proventriculus	13.4	17.8	14.4	2.34	0.39	0.35	0.31
Cecum	21.2	22.3	24.9	1.17	0.083	0.11	0.12
Gizzard+Proventiculus	75.7	89.9	82.8	4.43	0.085	0.054	0.26
SICR[5]	136	148	142	5.3	0.30	0.19	0.40
Empty weight (g)							
Gizzard	34.8b	37.3ab	39.9a	0.98	0.002	0.002	0.070
Proventriculus	9.8	10.6	10.6	0.56	0.49	0.23	0.96
Gizzard+Proventiculus	44.5b	47.9ab	50.5a	1.31	0.005	0.003	0.15
Content weight (g)							
Gizzard	27.5	34.8	28.5	2.37	0.069	0.16	0.065
Proventriculus	3.71	3.25	3.79	1.872	0.32	0.43	0.19
pH							
Gizzard	3.12ab	2.81b	3.29a	0.119	0.021	0.64	0.006
Proventriculus	3.07	3.23	3.25	0.230	0.83	0.55	0.94
Cecum	6.36	6.47	6.54	0.081	0.27	0.14	0.53

Means in the same row not sharing a common letter differ ($P < 0.05$)

[1]CON: standard non-fiber supplemented diet; LC: CON diet diluted with 1.5% of insoluble high hydration properties fiber source; ISFC: CON diet diluted with 1.5% of insoluble medium hydration properties and partially fermentable prebiotic fiber source.

[2]SEM, n=27.

[3]C1: CON vs. LC+ISFC.

[4]C2: LC vs. ISFC.

[5] SICR: weight of full small intestine, colon and rectum calculated by difference.

Table 23. Influence of fiber sources differing in hydration capacity and fermentability on gastrointestinal tract development relative to BW of broilers at 42 d[1]. Experiment 2.

	Dietary treatments[1]				*P*-value		
Item	CON	LC	ISFC	SEM[2]	Treatment	C1[3]	C2[4]
Full weight (% BW)							
Whole digestive tract	7.75b	8.41a	7.99ab	0.174	0.032	0.041	0.093
Gizzard	2.07	2.34	2.20	0.096	0.16	0.10	0.32
Proventriculus	0.44	0.58	0.45	0.079	0.40	0.44	0.26
Cecum	0.70	0.72	0.80	0.036	0.13	0.20	0.12
Gizzard+Proventiculus	2.51	2.91	2.65	0.145	0.15	0.13	0.21
SICR[5]	4.54	4.77	4.53	0.125	0.31	0.46	0.18
Empty weight (% BW)							
Gizzard	1.16b	1.21ab	1.28a	0.034	0.038	0.034	0.15
Proventriculus	0.32	0.34	0.34	0.016	0.71	0.42	0.85
Gizzard+Proventiculus	1.48b	1.55ab	1.62a	0.039	0.049	0.030	24
Content weight (% BW)							
Gizzard	0.92	1.12	0.92	0.081	0.10	0.27	0.06
Proventriculus	0.12	0.24	0.11	0.062	0.31	0.47	0.18

Means in the same row not sharing a common letter differ ($P < 0.05$)

[1]CON: standard non-fiber supplemented diet; LC: CON diet diluted with 1.5% of insoluble high hydration properties fiber source; ISFC: CON diet diluted with 1.5% of insoluble medium hydration properties and partially fermentable prebiotic fiber source.

[2]SEM, n=27.

[3]C1: CON vs. LC+ISFC.

[4]C2: LC vs. ISFC.

[5] SICR: weight of full small intestine, colon and rectum calculated by difference.

Table 24. The influence of insoluble fiber concentrates differing in hydration capacity and fermentability on the cecal short chain fatty acids (SCFA) concentration in broilers at 42d[1]. Experiment 2.

	Dietary treatments[1]				*P*-value		
Item	CON	LC	ISFC	SEM[2]	Treatment	C1[3]	C2[4]
SCFA concentration (µmol/g of cecal content)							
Acetate	92.8	76.0	73.9	6.77	0.11	0.040	0.83
Propionate	13.9	11.3	11.2	1.59	0.39	0.17	0.97
Butyrate	20.3	17.5	16.6	1.75	0.32	0.15	0.70
Isobutyrate	1.07	1.34	1.04	0.11	0.73	0.82	0.44
Valerate	1.46	1.41	1.49	0.12	0.19	0.95	0.64
Isovalerate	1.01	1.11	1.04	0.09	0.73	0.73	0.99
Total SCFA	134.0	114.1	108.5	8.97	0.13	0.048	0.66
Molar proportion of individual SCFA (mol/100mol)							
Acetate	69.3	66.5	67.6	1.64	0.48	0.27	0.64
Propionate	10.4	9.9	10.0	0.95	0.91	0.67	0.90
Butyrate	15.3	15.5	16.0	1.36	0.94	0.80	0.81
Isobutyrate	0.84	1.2	0.96	0.12	0.085	0.17	0.36
Valerate	1.1b	1.2ab	1.4a	0.10	0.039	0.037	0.13
Isovalerate	0.81	1.045	1.00	0.11	0.28	0.17	0.91

Means in the same row not sharing a common letter differ ($P < 0.05$)

[1]CON: standard non-fiber supplemented diet; LC: CON diet diluted with 1.5% of insoluble high hydration properties fiber source; ISFC: CON diet diluted with 1.5% of insoluble medium hydration properties and partially fermentable prebiotic fiber source.

[2]SEM, n=9.

[3]C1: CON vs. LC+ISFC.

[4]C2: LC vs. ISFC.

Time (h)	2	4	7	12	24	48	72	96
SEM	0.5	0.5	0.6	0.6	0.9	1.4	1.3	1.4
P	NS	NS	*	***	***	***	***	***

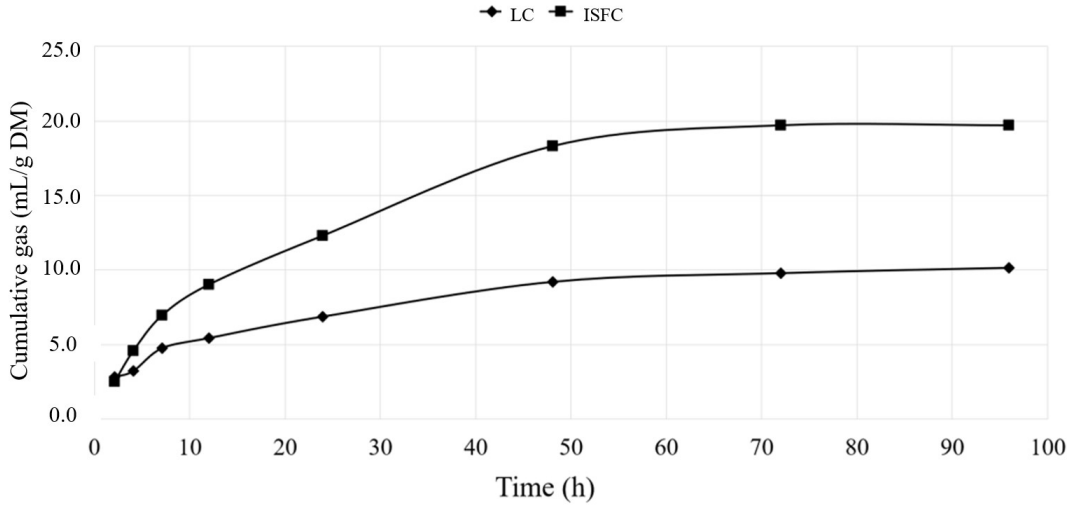
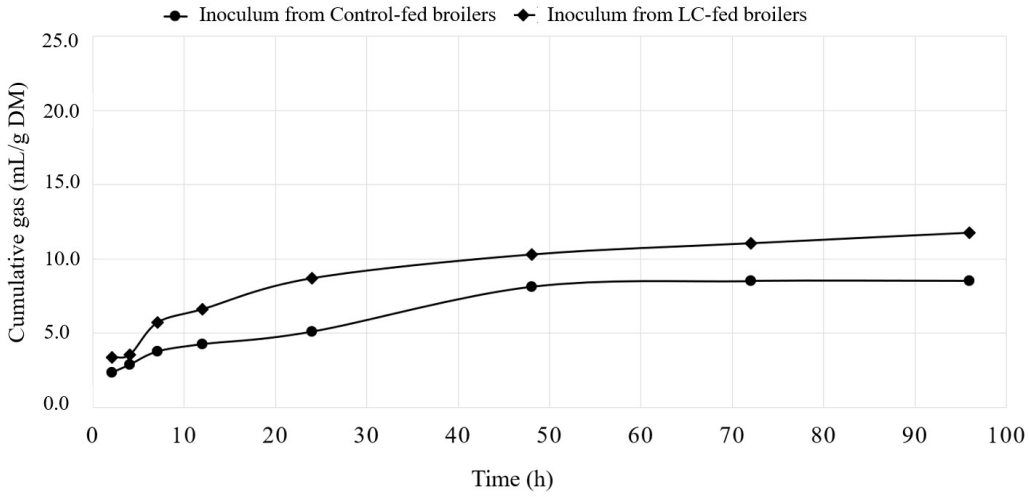


Figure 7. Cumulative gas production curves (mL/gDM) of LC (insoluble and high hydration properties fiber) and ISFC (insoluble and medium hydration properties with partially fermentable prebiotic fiber) substrates after their incubation with cecal content from 42-d birds fed a control diet based on cereals and soybean meal. Four different inocula were used, and each inocula was a pooled cecal content from 4 birds fed the control diet. Values in the Table indicate the SEM and P value of the ANOVA analyzing potential differences in gas production at each measurement time. NS= $P > 0.10$; t = $0.10 < P < 0.05$; * $P < 0.05$; *** $P < 0.001$

A) LC

Time (h)	2	4	7	12	24	48	72	96
SEM	0.9	0.7	0.8	0.7	0.8	1.6	1.6	1.8
P	NS	NS	NS	t	**	NS	NS	NS



B) ISFC

Time (h)	2	4	7	12	24	48	72	96
SEM	0.4	0.7	0.9	0.9	1.2	2.3	2.2	2.1
P	NS	NS	NS	NS	NS	NS	NS	NS

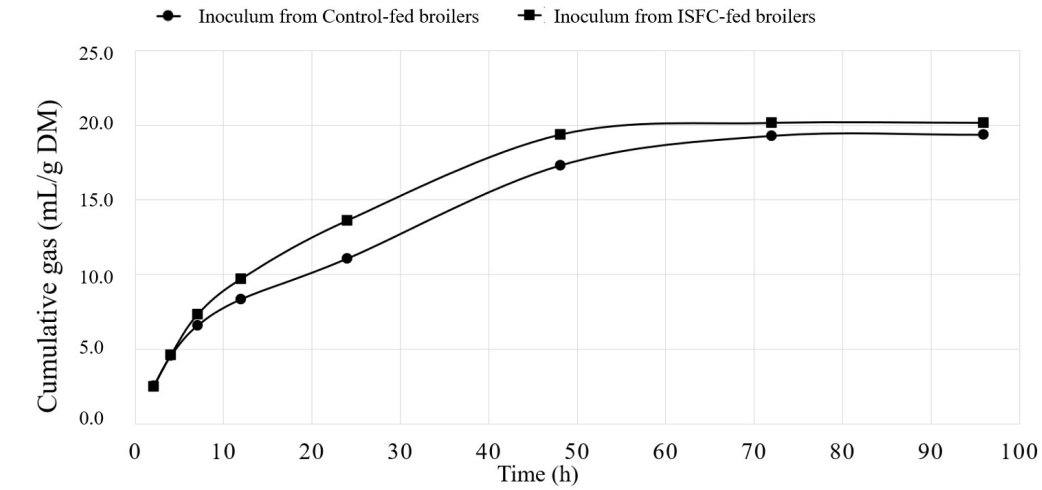


Figure 8. Cumulative gas production curves (mL/gDM) of A) insoluble and high hydration capacity fiber substrate (LC), and B) insoluble and medium hydration capacity with partially fermentable prebiotic fiber substrate (ISFC) when they were fermented either using cecal inoculum from 42-d birds fed a diet including the incubated fiber source (adapted microbiota) or a control diet without LC and ISFC (CON; non-adapted microbiota). Four different inoculums were used for each experimental dietary treatment, and each inocula was a pooled cecal content from 4 birds fed the same diet. Tables indicate the SEM and P value of the ANOVA analyzing potential differences in gas production values at each measurement time. Significance was declared at $P < 0.05$, whereas $P < 0.10$ values were considered as a trend. NS= $P > 0.10$; t = $0.10 < P < 0.05$; * $P < 0.05$.

5.3. Experiment 3: Different physiochemical properties of novel fiber sources in the diet of weaned pigs influence animal performance, nutrient digestibility, and cecal fermentation.

Growth Traits

From 28 to 42 d of age, piglets fed LHC and MHC diets grew on average 10% faster than those of the HHC group and consumed 7% more feed ($P \leq 0.036$; Table 25). During this period, the MHC group tended to have a higher ADG than the LHC ($P = 0.052$), but ADG and ADFI were similar to the CON group. Dietary treatments had no effect on FCR during this period ($p \geq 0.15$). From 42 to 60 d of age, both LHC and MHC groups grew on average 12% faster and consumed 10% more feed ($P \leq 0.025$) than piglets fed the HHC diet. Moreover, the MHC group tended to have higher ADFI ($P = 0.064$) and similar ADG to LHC-fed piglets but had higher ADG and ADFI than the CON group (by 7 and 12%, respectively; $P < 0.05$). In contrast, diet had no effect on FCR ($P \geq 0.11$) during this period. When the entire experimental period (28-60 d of age) was considered, piglets fed LHC and MHC diets had a 10% increase in both ADG and ADFI compared with those fed the HHC diet ($P \leq 0.019$) and tended to have a reduced FCR ($P = 0.087$). Piglets fed the MHC diet had 9% higher ADG and ADFI than the CON group and grew 6% faster than LHC group ($P < 0.05$).

In the current trial, piglets did not exhibit severe digestive disorder. However, during the starter period, some cases of meningitis and lameness were reported (affecting to 23.4, 8.3, 12.5 and 27.7% of piglets of for CON, LHC, MHC and HHC groups, respectively, $P = 0.047$), and were treated individually with amoxycillin and glucocorticoids (Bivamox® 150 mg/mL, Caliercortin® 4 mg/mL). All the animals remained in the experiment.

Total Tract Apparent Digestibility

At 42 d, the apparent total tract CP digestibility of LHC and MHC piglets was 5% higher than that in the HHC group ($P = 0.035$), and a similar trend was observed for DM and OM digestibility ($P \leq 0.098$), but values did not differ from those in the CON group (Table 26). Dietary treatments had no effect on the apparent total tract digestibility of DM, OM and CP at 61 d of age ($P \geq 0.20$).

Gut Traits and Short Chain Fatty Acids Concentration

The pH of ileal digesta in the fibre-supplemented groups (LHC, MHC and HHC) was 5% higher compared to that of the CON group ($p = 0.045$; Table 27). Piglets fed the MHC diet tended to have a lower ileal pH compared to the LHC group ($p = 0.087$). Dietary treatments had a low impact on caecal SCFA concentrations at 61d of age. Piglets fed MHC diet tended to have lower total SCFA concentrations than those fed the LHC diet ($P = 0.080$), which was due to a reduction of propionate ($P = 0.044$) and butyrate ($P = 0.076$). No other effects of dietary treatments were observed on caecal SCFA concentrations, either when expressed as µmol/g or as molar proportions ($P \geq 0.12$). Dietary treatments

had no effect on the pH of the caecum and colon ($P \geq 0.15$), caecal weight ($P \geq 0.36$), or moisture content of the digesta ($P \geq 0.17$. Figure 9).

The cumulative gas production curves of different micronized raw materials, and the experimental fibre sources (LHC, MHC and HHC) when the caecal content of CON-fed piglets was used as inoculum are presented in Figures 10A and 10B, respectively. As expected, chicory roots showed the highest gas production, whereas grape pomace, olive kernel, almond shell, nutshell, and wood generated lower amounts of gas at all incubation times ($P < 0.001$). Differences among these IDF sources were detected only at 9 h, when grape pomace produced more gas than the rest of substrates (Figure 10A). Fibre sources included in both LHC and MHC diets showed higher gas production than HHC ($P \leq 0.003$; Figure 10B) from 24 h of incubation onwards, but no differences were detected at shorter incubation times (3 and 9 h). Generally, gas production reached the maximal value at 33 h, with the only exception being chicory roots, which continued generating gas until the end of the incubation at 72 h.

The gas production curves of the fibre sources included in LHC, MHC and HHC diets when caecal inoculum from piglets fed CON, LHC, MHC and HHC diets were used are presented in Figures 11A, 11B and 11C, respectively. A tendency for greater gas production from 33 h onwards in non-adapted microbiota in LHC ($P = 0.082$) and MHC ($P = 0.061$) was observed. No effect of adaptation was observed in the HHC ($P \geq 0.25$).

Table 25. Effect of dietary treatments on body weight (BW) and performance of piglets during the prestarter, starter and overall postweaning period. Experiment 3.

	Dietary treatment (DT)[1]					*P*-value[3]				
Item	CON	LHC	MHC	HHC	SEM [2]	COV	DT	C1	C2	C3
Prestarter (28-42 d)										
ADFI, g	356a	334ab	352a	319b	10.2	<0.001	0.033	0.119	0.036	0.112
ADG, g	316a	307ab	332a	291b	9.9	<0.001	0.027	0.670	0.018	0.052
FCR	1.125	1.092	1.064	1.098	0.02	0.48	0.401	0.149	0.551	0.502
BW 42 d	11.1ab	11.0ab	11.4a	10.8b	0.14	<0.001	0.028	0.915	0.017	0.053
Starter (42-60 d)										
ADFI, g	812b	836ab	912a	792b	21.3	<0.001	0.028	0.291	0.025	0.064
ADG, g	539bc	561ab	578a	510c	12.0	<0.001	0.002	0.431	<0.001	0.301
FCR	1.510	1.493	1.512	1.561	0.03	0.083	0.386	0.729	0.108	0.660
BW 60 d	20.8bc	21.1b	22.0a	20.00c	0.31	<0.001	0.001	0.561	<0.001	0.049
Overall (28-60 d)										
ADFI, g	632b	636ab	691a	603b	15.6	<0.001	0.029	0.610	0.019	0.056
ADG, g	455bc	464b	493a	429c	9.98	<0.001	0.001	0.561	<0.001	0.049
FCR	1.388	1.372	1.358	1.411	0.02	0.278	0.355	0.753	0.087	0.647

a, b, c: Within the same row, means with different letter differ ($p < 0.05$; Tukey test)
[1]CON: basal diet with no additional fibre inclusion, LHC: basal diet including 1.5% of low hydration capacity insoluble fibre with fermentable fraction, MHC: basal diet including 1.5% of medium hydration capacity insoluble fibre with fermentable fraction, and HHC: basal diet including 1.5% of high hydration capacity insoluble fibre; [2]n = 8; [3]COV: Weaning body weight (6.7±1.12 kg) was used as a covariate; C1: CON vs. LHC, MHC and HHC; C2: LHC and MHC vs. HHC; C3: LHC vs. MHC.

Table 26. Effect of dietary treatments on total tract apparent digestibility (TTAD) of dry matter (DM), crude protein (CP), and organic matter (OM) in piglets at 42 and 61 d of age. Experiment 3.

	Dietary treatment (DT)[1]					*P*-value[3]			
Item	CON	LHC	MHC	HHC	SEM[2]	DT	C1	C2	C3
TTAD, 42 d									
DM	82.4	81.4	83.6	80.3	0.98	0.152	0.596	0.098	0.541
CP	80.9ab	80.0ab	83.9a	78.3b	1.26	0.040	0.936	0.035	0.802
OM	84.7	83.8	85.7	82.7	0.87	0.139	0.555	0.076	0.789
TTAD, 61 d									
DM	82.5	82.7	82.0	82.8	0.65	0.712	0.207	0.59	0.440
CP	77.4	76.7	78.3	78.9	1.17	0.566	0.697	0.34	0.337
OM	84.1	85.2	84.7	84.9	0.58	0.557	0.200	0.95	0.538

a, b: Within the same row, means with different letter differ ($p < 0.05$; Tukey test)

[1]CON: basal diet with no additional fibre inclusion, LHC: basal diet including 1.5% of low hydration capacity insoluble fibre with fermentable fraction, MHC: basal diet including 1.5% of medium hydration capacity insoluble fibre with fermentable fraction, and HHC: basal diet including 1.5% of high hydration capacity insoluble fibre; [2]n = 8; [3]C1: CON vs. LHC, MHC and HHC; C2: LHC and MHC vs. HHC; C3: LHC vs. MHC

Table 27. Effect of dietary treatments on intestinal traits and caecal short chain fatty acids concentration of piglets at 61d. Experiment 3.

	Dietary treatment (DT)[1]					*P*-value			
Item	CON	LHC	MHC	HHC	SEM[2]	DT	C1	C2	C3
pH of ileum	6.45	6.95	6.63	6.67	0.13	0.064	0.045	0.443	0.087
pH of colon	6.48	6.57	6.46	6.50	0.12	0.929	0.838	0.920	0.534
pH of caecum	5.83	5.92	5.66	5.88	0.12	0.467	0.912	0.550	0.146
Caecal weight, g	147	166	178	149	20.3	0.672	0.477	0.363	0.670
Caecal weight, % body weight	7.24	7.31	8.23	7.33	0.96	0.868	0.727	0.713	0.504
Caecal short chain fatty acids (SCFA), μmol/g									
Total, SCFA	239	246	209	235	14.4	0.297	0.590	0.664	0.076
Acetate	143	145	130	145	8.41	0.521	0.714	0.453	0.218
Propionate	63.1	64.0	51.1	59.7	4.33	0.160	0.339	0.695	0.040
Isobutyrate	0.72	0.69	0.76	0.70	0.15	0.990	0.972	0.913	0.757
Butyrate	28.0	32.5	24.3	25.9	3.13	0.303	0.914	0.522	0.080
Isovalerate	0.86	0.74	0.64	0.91	0.11	0.340	0.471	0.123	0.515
Valerate	3.26	3.82	2.47	3.21	0.79	0.690	0.917	0.943	0.237
Individual SCFA, mol/100 mol									
Acetate	60.1	59.2	62.0	61.7	1.50	0.502	0.623	0.535	0.191
Propionate	26.4	25.8	24.5	25.2	0.86	0.421	0.213	0.967	0.269
Isobutyrate	0.30	0.30	0.36	0.32	0.07	0.925	0.794	0.887	0.544
Butyrate	11.5	12.9	11.7	10.9	0.90	0.487	0.733	0.237	0.345
Isovalerate	0.36	0.31	0.30	0.41	0.05	0.465	0.745	0.124	0.950
Valerate	1.33	1.50	1.20	1.41	0.34	0.936	0.923	0.894	0.538

[1]CON: basal diet with no additional fibre inclusion, LHC: basal diet including 1.5% of low hydration capacity insoluble fibre with fermentable fraction, MHC: basal diet including 1.5% of medium hydration capacity insoluble fibre with fermentable fraction, and HHC: basal diet including 1.5% of high hydration capacity insoluble fibre; 2n = 8; 3C1: CON vs. LHC, MHC and HHC; C2: LHC and MHC vs. HHC; C3: LHC vs. MHC.

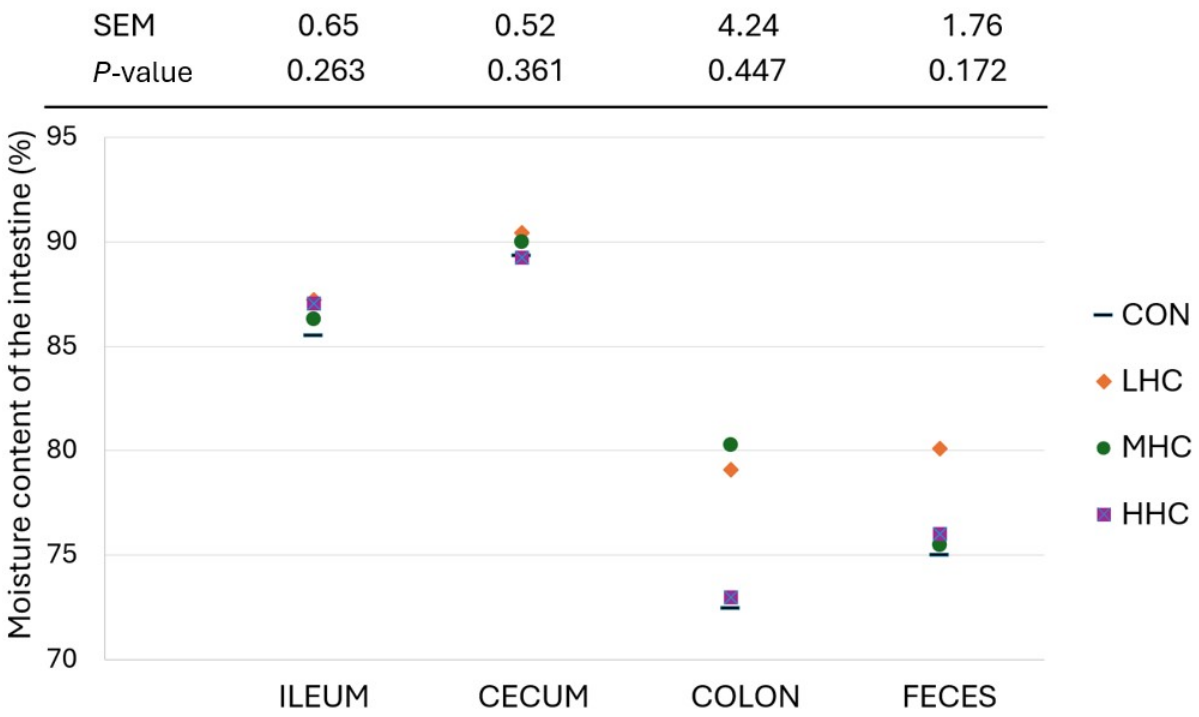


Figure 9. Effect of dietary treatments on the moisture content of the intestinal digesta (ileum, caecum, colon and faeces) of 61d old piglets. CON: basal diet with no additional fibre inclusion, LHC: basal diet including 1.5% of low hydration capacity insoluble fibre with fermentable fraction, MHC: basal diet including 1.5% of medium hydration capacity insoluble fibre with fermentable fraction, and HHC: basal diet including 1.5% of high hydration capacity insoluble fibre; n = 8 for all treatments.

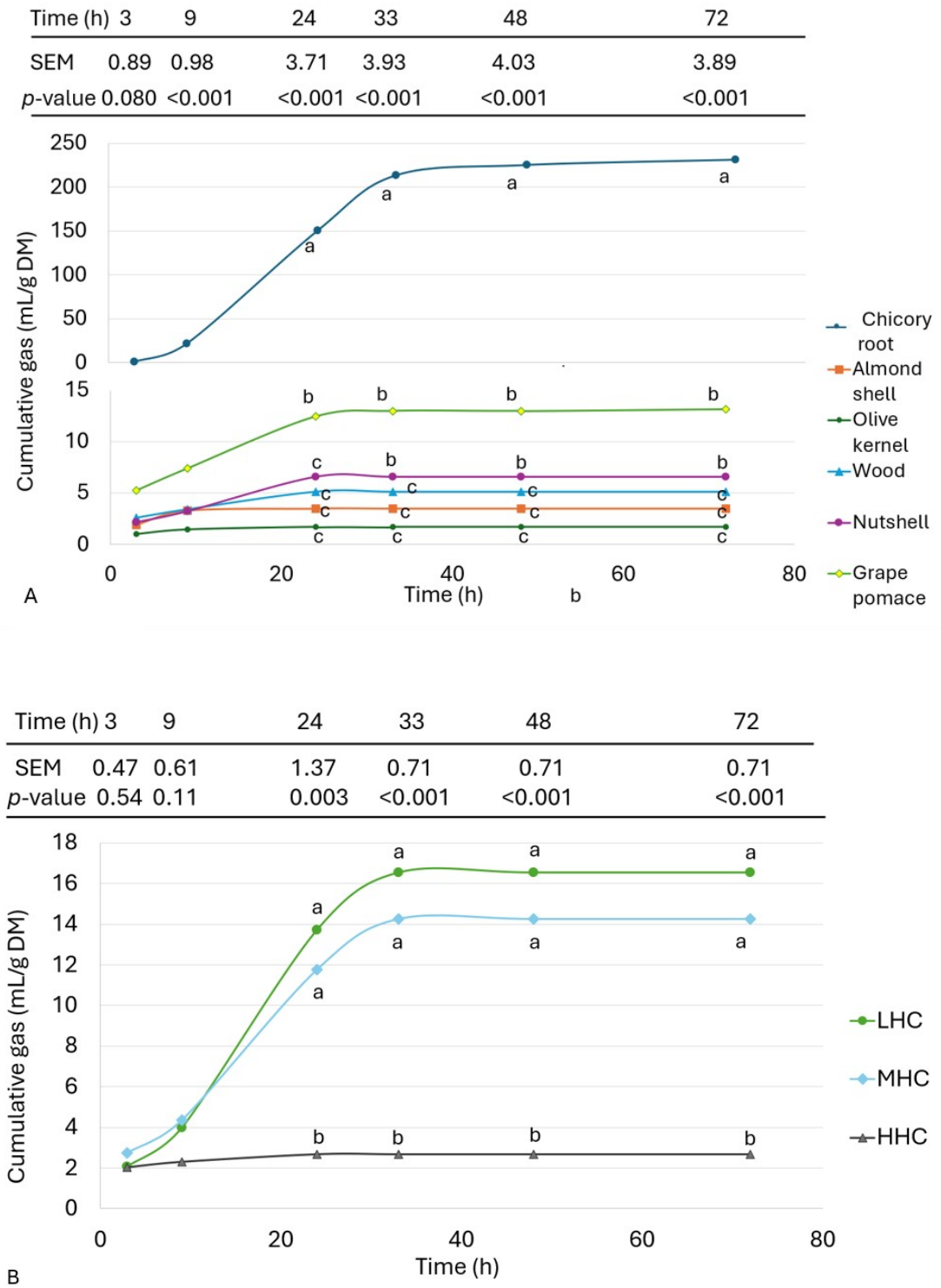


Figure 10. Cumulative gas production curves (mL/g DM) of A) LHC, MHC, HHC, and B) different micronized fibre sources (chicory root, almond shell, olive kernel, wood, nutshell, and grape by-product) after their incubation with caecal content from 61-d piglets fed the control diet. Three different inocula were used, and each inoculum was pooled from caecal content of 2 piglets fed the control diet. a, b, c: Within the same row, means with different letters differ ($p < 0.05$; Tukey test). Values in the Table indicate the SEM (n=3) and P-value of the ANOVA analysing potential differences in gas production at each measurement time.

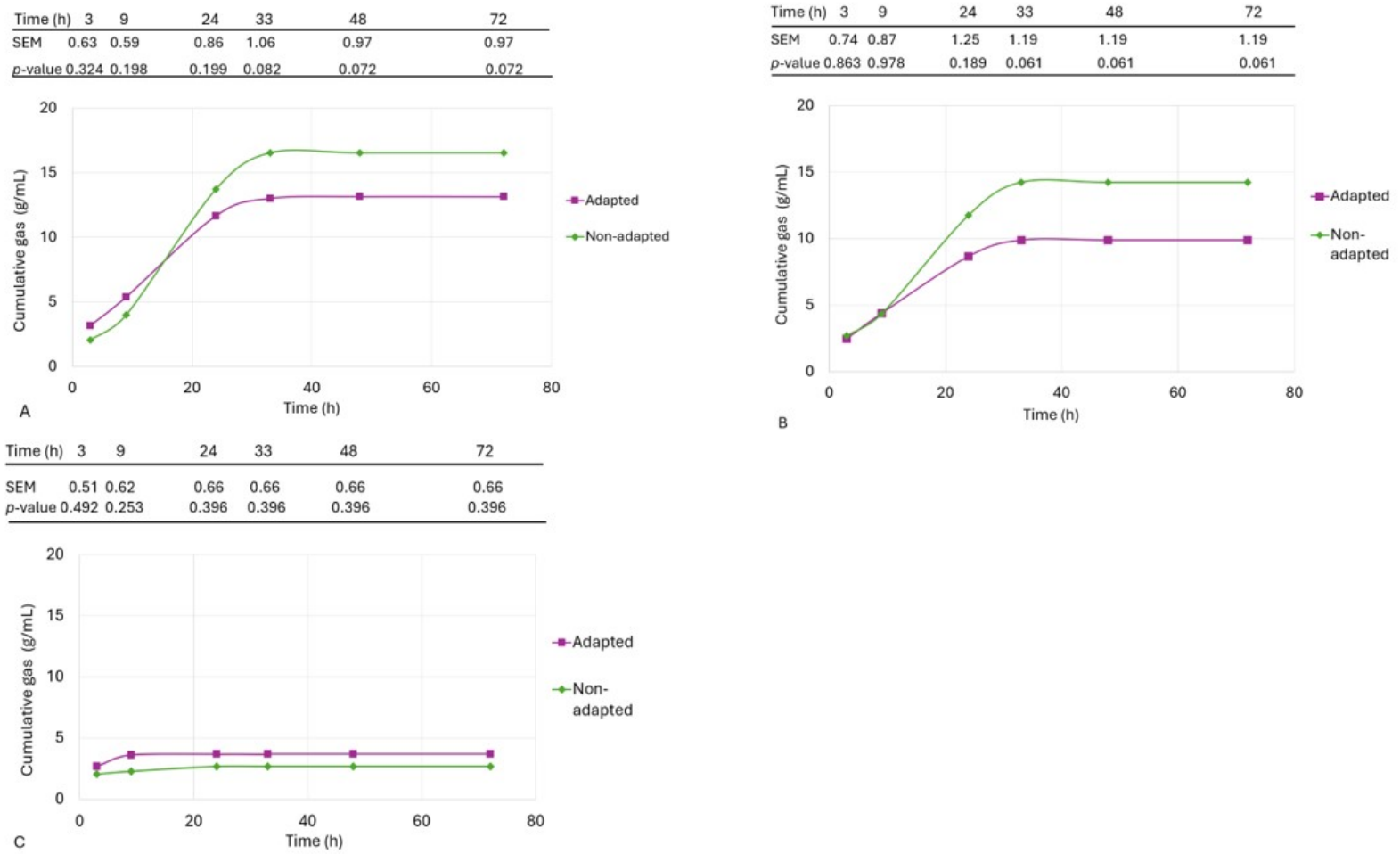


Figure 11. Cumulative gas production curves (mL/g DM) of A) LHC: low hydration capacity insoluble fibre with fermentable fraction, B) MHC: medium hydration capacity insoluble fibre with fermentable fraction, and C) HHC: high hydration capacity but non-fermentable insoluble fibre, when they were fermented either using caecal inoculum from 61-d piglets fed a diet including the incubated fibre source (adapted microbiota) or a control diet without fibre sources (non-adapted microbiota). Three different inocula were used for each experimental dietary treatment, and each inoculum was a pooled caecal content from 2 piglets fed the same diet. Tables indicate the SEM (n=3) and P-value of the ANOVA analysing potential differences in gas production values at each measurement time

DISCUSSION

4

6.1. Experiment 1: Effect of type of fiber and its physicochemical properties on performance, digestive transit time, and cecal fermentation in broilers from 1-23 days of age.

Growth Traits, GIT Development and Digestive Transit Time

The impact of IDF on growth performance of broilers is influenced by the inclusion level, diet composition, and physicochemical characteristics of the IDF source (Mateos et al., 2012; Jiménez-Moreno et al., 2013). The fiber sources used in this trial differ in multiple ways depending on the grinding process needed to reach desirable PS, nutritional content, and HC. All the differences came from the original properties of agricultural by products. Straw was easily ground in the conventional hammer mill, whereas grinding process in the hammer mill of almond shell and wood in the same conditions as straw led to coarse and hard final product, and their addition to the broilers diet might probably harm the digestive system of young animals. For that reason, reduction of the PS under 100µm was applied to decrease the hardness of the lignocellulosic endocarp which was indispensable to make possibly its ingestion by young broilers. Therefore, all the effects for each IDF observed herein were linked specifically to its PS.

The capacity of IDF to retain water is related to the PS of the fiber source, but the inconsistent results reported by previous investigation suggest that it strongly depended on the fiber source. The HC of coconut residue, cellulose, carrot insoluble fiber and black kidney bean powder were increased with PS reduction that increased the surface area (Raghavendra et al., 2006; Chou et al., 2008; Dubey et al., 2018; Sun et al., 2019). The opposite effect was observed for grape pomace, outer leaves of cabbage, and wheat bran (Jongaroontaprangsee et al., 2007; Zhu et al., 2010; Sheng et al., 2017; Beutinger Bender et al., 2019b). It was suggested that in DF sources with capillary structure, excessive grinding may collapse the fiber structure conducting to decrease of HC (Raghavendra et al., 2006). In this case, the HC of straw was decreased with grinding, whereas the finely grinded LC showed high values. This might be partially explained by the structure of the straw and wood by products which allow water transportation within the plant, whereas the almond shell most probably exert hydrophobic function for the kernal protection. Therefore, it seems that the capacity to retain water of the fiber matrix is more dependent on DF structure and its original function in the plant than only the PS.

In the current research broilers were raised in cages to avoid a possibly interaction with fiber in the litter, since the amount of the fiber ingested from the litter cannot be controlled, and the inclusion level of the experimental fibers was low (1.5%). Previous investigations were carried out in similar conditions (Gonzalez Alvarado et al., 2007, 2010; Jiménez Moreno et al., 2009, 2010) to assess the effect of different fiber sources on performance. In spite of the common commercial practice in which birds have access to high lignocellulosic litter, all the effects observed herein reflect only the impact of the experimental design.

In the present study, the dilution of a Control diet based on corn and soybean meal with 1.5% of

different IDF sources had a moderate impact on the broiler's growth performance from 0-21 d, and the effect differed depending on the HC of the IDF. Sources characterized with high HC (LC, CS and FS) tended to impair FCR more than the low-HC AS. Due to the same inclusion level, the results might indicate the importance of the IDF physicochemical properties, especially the HC. During the trial, chicks fed AS performed like those of Control group, in spite of the diet dilution with IDF, and had better ADG than those fed LC and FS. To our best knowledge, there is no previous information on the effects of supplementing broilers diets with AS. However, microcrystalline cellulose is an IDF source similar to AS, with low PS, WHC, and SC (Jiménez-Moreno et al., 2010). Feeding birds with microcrystalline cellulose produced variable results depending on its inclusion level, and the NDF content of the control diet (Jimenez-Moreno et al., 2010; Rezaei et al., 2011; 2014; 2018). Low levels of micronized cellulose supplementation to a corn and soybean meal-based diet for broilers (0.3-0.5%) and quails (0.5, 1 and 1.5%) improved both growth and FCR (Rezaei et al., 2011, 2018). In contrast, higher levels (2, 3 and 4%) had no effect on performance when added to low-fiber diets based on either broken rice or corn starch (Jiménez-Moreno et al., 2010; Rahmatnejad and Saki, 2015). However, supplementing cellulose at 10% decreased the BW and N utilization in chickens (Cao et al., 2003), which may be related to greater ileal endogenous losses reported when increasing dietary cellulose levels from 2.5 to 7.5% (Khali et al., 2022).

Our results may indicate that including 1.5% of high-HC IDF sources (LC, FS and CS) may impair feed intake in young broilers compared with a low-HC IDF source (AS). High HC IDF sources can bind water to single particles and this may produce distention of the digestive tract wall, leading to stimulation of vagal afferents that create fullness perception and decreased feed intake in broilers (González-Alvarado et al., 2007; Wang et al., 2008). Accordingly, the better performance of AS group compared with the other three IDF-fed groups was based on the positive influence exerted on feed intake. This is in good agreement with the heavier digestive tract segments (expressed in g) observed in AS group, except for the cecum. In fact, in this study positive correlations ($P \leq 0.030$; n = 5) were found between ADFI at 0-21 d and the weights of the whole digestive tract (r = 0.96), full gizzard (r = 0.92), and full SICR (r = 0.92). In contrast, no significant correlations were found between ADFI and the weight of full proventriculus and of cecum or the length of the small intestine. However, when the weight of digestive organs was expressed as relative to the whole digestive tract weight, to evaluate whether feed intake influenced similarly all segments of the digestive tract, differences between AS and the other IDF-fed groups were noticed only for SICR. The relative heavier SICR and relative shorter small intestine in birds from AS group might suggest an accumulation of digesta, most probably in the small intestine, compared with those fed the other IDF-diets.

Feed intake was not correlated with faecal TiO_2 recovery, as it might be expected according to the relationship between feed intake and rate of passage (Almirall and Esteve-García, 1994; Lázaro et al., 2003). Faecal TiO_2 recovery was negatively correlated with the weight of the SICR (r = -0.89; $P = 0.043$; n= 5). A hypothesis for the lowest TiO_2 recovery observed in the AS group is that AS might be stimulating the reverse peristalsis along the small intestine, especially considering that the length of small

intestine of birds fed AS was not longer than that of the other IDF-fed groups, but it had lower relative cecum weight (Angel et al., 2013). This hypothesis assumes that TiO_2 was probably accumulated in the cecum (Almirall and Esteve-García, 1994), which might be another possible explanation due to the small PS of AS (Svihus, 2014). However, the dietary factors potentially implicated in the reverse peristalsis have been scarcely studied and still remain unclear. The reverse peristalsis along the small intestine and gizzard is a physiological process in broilers that favors the nutrient digestion and absorption in the upper small intestine (Sacranie et al., 2007; Rodrigues and Choct, 2018; Svihus and Itani, 2019). The dilution of the diet with high levels of IDF (15% of finely ground or unground oat and barley hulls) seems to promote this phenomenon (Sacranie et al., 2012). Recently, the addition of 15.0% of finely-ground oat hulls to a laying hens' diet resulted in increased digesta retention time in the crop (Habibi et al., 2023), where digesta is moistured, fermented in some extend, and stored before entering the gizzard (Rodrigues and Choct, 2018). However, in the current experiment no crop traits were measured.

The difference in PS between FS and CS treatments did not affect broilers performance in the present trial. This agrees with previous studies showing that the use of *Miscanthus giganteus* with fine (108 μm) or coarse (294 μm) PS, and oat hulls with fine (386 μm) or coarse (462 μm) PS did not affect broilers performance (Jiménez-Moreno et al., 2010; Donadelli et al., 2019).

In the current study no positive effect of LC on growth traits was observed. In contrast, LC supplementation up to 0.75%, did not affect ADFI and ADG in broilers from 0-21d, but it decreased FCR (Sarikhan et al., 2010). Similarly, an increase of ADG and FCR was reported with 1% LC inclusion compared to no or 2% supplementation in young broilers (Rahmatnejad and Saki, 2015). Other authors have reported a reduction of FCR at much lower level of LC inclusion (0.1%) in broilers from 1-35 d (Sozcu, 2019). On the opposite, the inclusion of 1% LC had negative results in broilers fed a finely-ground wheat-based diet, impairing the FCR from 1-21 d of age (Abdollahi et al., 2018). The effect of LC inclusion in broilers is not consistent between studies, probably due to differences in the inclusion level, diet composition or even in the LC source evaluated (Rohë and Zentek, 2021).

The positive effect of reducing the moisture of excreta observed for the average of IDF- groups, and especially for LC-fed broilers, compared with Control broilers supports previous results in broilers fed 0.3-2% of LC (Rezaei et al., 2011; Farran et al., 2013; Kheravii et al., 2017; Makivic et al., 2018). However, Jiménez-Moreno et al. (2016) reported that increasing the dietary inclusion of IDF from 2.5 to 5% resulted in higher excreta moisture content, which was attributed to the greater water intake observed in broilers fed IDF at 5%. Therefore, the favorable effect observed in our study might be related with the low inclusion level of IDF used. Although in this study the chickens were housed in wire cages in batteries, the moisture of excreta is usually positively correlated with litter moisture (Hoeven-Hangoor, 2014). In agreement with the lack of differences on excreta moisture due to straw PS observed in our study, Jiménez-Moreno et al. (2016) reported no effect of grinding oat hulls on excreta moisture. A positive relationship between rate of passage and the excreta moisture content has been hypothesized (Kheravii et al., 2017), but it was not observed in the present study. Another possible explanation for the

reduced moisture excreta observed in LC might be that the drying effect of micronized fiber was associated with mechanical stimulation on the mucosal layer of the intestine (Rezaei et al., 2011, 2014). The results of this study indicate no effect of IDF sources or their HC on moisture of cecum content, despite it has been speculated that IDF with high HC may reduce cecal moisture content (Amerah et al., 2009). In spite of the data available, the mechanisms by which IDF can decrease the excreta moisture content are currently still not well understood.

Cecal pH, SCFA Concentration and In Vitro Fermentation

Generally, the influence of fiber on cecal fermentation is expected to be low in poultry due to the limited extent of IDF fermentation (Walugembe et al., 2015; Bautil et al., 2023). Since no measurements of passage of fibrous particles to the ceca was assessed in this study, eventual differences in fermentation properties cannot be related specifically to IDF fermentability but may have been due to side-effects of the IDF on the digestibility of dietary components (Pourazadi et al., 2020) or on the specific endogenous losses (Ravindran, 2021). The inclusion of low levels of LC (0.25-1%) increased the counts of *Bifidobacterium* spp. and decreased those of *E. coli* and *C. perfringens* (Boguslawska-Tryk et al., 2015; Makivic et al., 2018) and increased the cecal SCFA concentration when included at 0.5 but not at 1% (Boguslawska-Tryk et al., 2015). These authors hypothesized a role of the phenolic compounds from lignin on these effects. In contrast, in this study the IDF sources had no effect either on cecal pH or in the cecal SCFA concentration in agreement with other authors (Zeitz et al. 2019). There was only a trend to increase the molar proportion of isobutyrate in fiber fed groups. It is well known the positive influence of fiber on the secretion of endogenous substances (Kluth and Rodehutscord, 2009; Khalil et al., 2022) with a relevant content of valine, leucine and isoleucine (Ravindran, 2021). In fact, the increase of dietary NDF (with barley) increased endogenous losses of leucine in broilers (Cerrate et al., 2018). The fermentation of these amino acids is associated to an increase of the isobutyrate and isovalerate concentration in ruminal fermentation (Jouany, 1991), and suggest the influence of IDF sources on cecal fermentation through their potential stimulating effect on the production of endogenous substances.

The gas production technique has been extensively used to investigate ruminal and cecal fermentation in several species, but studies in poultry are scarce. The amount of gas produced in the in vitro fermentation is positively correlated with the amount of organic matter fermented (Menke and Steingass, 1988), and therefore the amount of gas is indicative of the extent of fermentation. Both FS and CS substrates produced more gas than LC and AS when they were fermented with the same inoculum (Control birds), which was probably due to the lower lignin concentration of straw. Previous studies have reported LC as a very low fermentable IDF in different animals' species, including broilers and turkey (Youssef and Kamphues, 2018; Zeitz et al., 2019; Pi et al., 2020). To our best knowledge, cecal fermentation of AS had not been previously studied in broilers, but our results indicate a gas production pattern similar to that of LC, which is consistent with the similar NDF and lignin content in both IDF sources. The in vitro experiment was designed to analyze the effect of straw PS used as substrate on its

cecal fermentation, and it was expected that decreasing PS would increase the in vitro cecal fermentation of straw by facilitating microbial colonization (Dubey et al., 2018; Speroni et al., 2019). In fact, a previous study (Vermeulen et al., 2017) showed greater total SCFA production when finely-ground (280 μm) wheat bran was incubated in vitro with cecal content from broilers compared with coarse-ground (1,690 μm) wheat bran. However, no differences due to straw PS in gas production were observed in our study at any sampling time (Figure 4), although average values for FS were numerically higher at most sampling times.

Two substrates of different fermentability (LC and FS) were chosen to assess potential changes in the in vitro cecal fermentative capacity of broilers fed the different IDF sources. The lack of differences among cecal inocula in gas production from both LC and FS substrates indicate that either there were not changes in cecal microbiota induced by feeding different IDF sources, or that potential changes were not reflected in gas production. Although differences among inocula did not reach the significance level, the numerically greater amounts of gas observed when FS was fermented with the inoculum from AS-fed broilers might indicate that dietary AS supplementation stimulated the cellulolytic activity in the cecum.

Finally, the comparison of the amount of gas produced when each IDF source (LC, FS, CS and AS) was fermented in vitro either with the cecal inoculum of Control birds or with the cecal inoculum of birds fed the corresponding IDF source (Figure 6), revealed no differences for any IDF source at any sampling time. Nevertheless, the average values were numerically higher, especially after 24 h of incubation, when the inoculum from the birds fed the IDF was used. The results suggest that feeding the IDF sources for 22d did not produce a significant modification of the cecal microbiota that could be detected by measuring in vitro gas production.

6.2. Experiment 2: Effect of dietary supplementation of two fiber sources differing on fermentability and hydration capacity on performance, nutrient digestibility and cecal fermentation in broilers from 1 to 42 d of age.

The analysis of PS confirmed that both fiber sources were finely ground but had different GMD and PS distribution. The current methodology was performed due to its common application for the feed PS determination, and previously was used with different fiber sources such as oat hulls, cellulose, pea hulls, rice hulls, sunflower hulls and sugar beet pulp (Jiménez-Moreno et al., 2009; 2010; 2011; 2016; Berrocoso et al., 2020). However, clumping of particles was observed on the top sieves of ISFC sample, which may impact in both particle distribution and GMD, since this method is mostly suitable for spherical and cuboid shapes particles. Also, the FOS addition may have increased the aggregates formation.

It also should be highlighted that the Ankom filter bags used in this trial to analyze fiber fractions content of fiber sources were able to retain particles > 25μm and given the fine particle size of ISFC it is possible that NDF content had been underestimated due to loss of the finer particles (Hall and Mertens, 2023).

Growth Performance and Nutrient Digestibility

The increase of FCR between 1-7d was most probably a result of the energetic and nutritional dilution produced by 1.5% of fiber inclusion. In this case, several adaptation mechanisms may take place to maintain the growth rate. The modern highly selected birds can compensate the dietary reduced nutrient concentrations due to fiber addition by increasing feed intake (Jha and Misha, 2022), although ADFI differences in our trial were not significant. Changes in FCR disappeared with time, and FCR values were similar in all treatments from 7 d onwards and in the whole period.

Different fiber sources were reported to have a negative effect on FCR in young chicks. Dietary lignocellulose inclusion at 1 and 2% increased FCR in broilers at 7 d, although 2% supplementation resulted in a significant recover of FCR at 35 d (Sozcu, 2019). Sugar beet pulp or rice hulls supplemented at 3% to the basal diet impaired FCR from 1-14 d in chicks but had no effect in the overall period (1-42 d) (Saadatmand et al., 2019). These findings support that dietary fiber inclusion produces digestive tract modifications in young broilers.

In previous investigations performed using a standard corn-soybean meal diet, the administration of different micronized IDF sources (cellulose or lignocellulose) at low levels (0.25-0.75%) increased ADG of broilers between 1-42 d (Sarikhan et al., 2010; Rezaei et al., 2011; Makivic et al., 2018). In contrast, higher levels (1-2%) of lignocellulose inclusion had limited effects on animal growth in the finishing period (Kheravi et al., 2017). Besides, different supplementation levels of prebiotic compounds as FOS (0.15%) or inulin (0.12-4%) have been reported to increase broilers' growth in the finishing period (Yang et al., 2016; Xia et al., 2019). Likewise, chicory root powder supplemented at 0.1-1.0%

improved performance in male chickens at 42 d (Khoobani et al., 2019; Gurram et al., 2021). These positive effects were probably due to an increase of beneficial microbiota in the GIT, due to the prebiotic activities of the supplements, which contributed to gut health.

Values of AID of CP were positively correlated with ADG from 1 to 42 d (r = 0.998; *P* = 0.036; n=3) and with BW at 42 d (r = 0.997; *P* = 0.050, n=3). Nutrient digestibility is normally depressed by high dietary fiber levels, but moderate amounts of fiber have been considered as one of the alternatives proposed to improve nutrient digestibility and performance in broilers (Khempaka et al., 2009; Tejeda and Kim, 2020; Jha and Misha, 2022). Finely ground fiber sources have shown a positive influence on nutrient digestibility in broilers (Jiménez Moreno et al., 2010; Tejeda and Kim, 2021a). Greater AID in ISFC than LC is in agreement with results of Experiment 1, where finely ground almond shells retained more time in the small intestine and may lead to more efficacious digestibility than LC, hypothesizing a stimulation of reverse peristalsis and digestive enzyme secretion (González-Alvarado et al., 2007). The highly fermentable fraction of FOS may be partially fermented before reaching the cecum, resulting in better AID of DM and OM compared to LC.

Highly fermentable oligosaccharides can improve nutrient digestibility due to beneficial modifications of gastrointestinal microbiota and increased immune function (Huang et al., 2005; Yadav and Jha, 2019). Dietary addition of FOS at 0.05% improved DM, crude fiber, CP, and EE digestibility in broilers, which was associated with an increased activity of digestive enzymes in the small intestine (Saleh et al., 2014). Similarly, Xu et al. (2003) reported that the administration of 0.4% of FOS enhanced protease, amylase, and lipase secretion and improved the morphology of intestinal mucosa leading to increased growth of broilers. In addition, intestinal morphology improvements may be associated with better nutrient digestibility, and several studies have reported changes in intestinal morphology by fiber supplementation. In the present trial, the intestinal morphology could not be reported due to autolysis of samples. However, in Experiment 1 we observed that LC supplementation at 1.5% produced no effect on intestinal morphology in 21-d broilers, whereas in the current experiment, the same level of LC supplementation had a negative effect on AID of DM and OM. Therefore, the positive effects of ISFC on digestibility and broiler growth may be associated with the FOS fraction or its interaction with IDF. These results indicate that despite of the very fine PS of both fiber sources, including a highly fermentable fraction (such as FOS) may be relevant to improve nutrient digestibility, as fermentability of high-lignified IDF sources cannot be improved by reducing their PS.

GIT Traits

The heavier weight of the whole digestive tract was probably associated with high bulking properties of LC. Particles of IDF can incorporate water into their matrix and swell during their passage through the GIT, resulting in a bulkier digesta (González-Alvarado et al., 2007). Indeed, the greater capacity to retain water of LC compared with ISFC may have a direct impact on the full weight of the GIT.

On the opposite, the empty weight of the gizzard was greater in ISFC than in CON group. Proventriculus and gizzard are usually considered a functional unit taking part in the digestion process, due to the bidirectional digesta flow between both organs that regulates its flow throughout the GIT (Jiménez-Moreno et al., 2019; Liermann et al., 2019). The enhancement effect of gizzard development produced by ISFC was unexpected, due to its low PS and hydration capacity. Generally, a lack of effect, or even a reduction of gizzard development, were reported by dietary inclusion of finely ground fiber, despite some studies not indicating if full or empty gizzard weights were given (Rezaei et al., 2011; Makiavic et al., 2018). However, a greater gizzard was observed in 28-d Japanese quails due to the dietary addition of 1.5% of micronized cellulose (Rezaei et al., 2018). In fact, it was suggested that dietary supplementation of modified cassava pulp at 1 or 1.5% induced thickening of the gizzard muscular wall due to the hardness of its IDF (Okrathok and Khempaka, 2020). This may suggest that there are more factors involved in the gizzard development, than only the PS. For example, in this trial all animals had straw bedding that is a common practice in commercial conditions. Thus, a potential additional source of coarse fiber, cannot be discarded which might have some influenced on gizzard development, although no apparent presence of straw or feathers were observed in the digesta.

Higher gizzard pH of ISFC-fed than LC birds at 42 d agrees well with results of Sadeghi et al. (2015), who observed greater gizzard pH in broilers supplemented with a 50:50 mixture of IDF and soluble fiber from rice hulls and sugar beet pulp, than in those fed only IDF from rice hulls. Other studies have supplemented the diet with LC, but the results are inconclusive. In broilers, Makiavic et al. (2018) reported a gizzard content acidification by LC supplementation at 0.4 and 0.6% with no effect on gizzard weight, whereas Sozcu (2019) observed no effect on gizzard pH by lignocellulose inclusion at 0.5, 1 or 2% of the total diet. These inconsistent results suggest that for finely ground fiber sources, the effect on the gizzard pH could be affected by additional factors, such as the inclusion level, bird's age, diet composition and structure, and buffer capacity of fiber sources. Normally, the increase in proventriculus and gizzard weight is associated with a pH decrease, due to higher HCl secretion that was commonly reported for diets containing structural components, such as coarse fibers or coarse grains (Svihus, 2011; Mateos et al., 2012; Okrathok and Khempaka, 2020). However, in the present study the feed was finely ground, and no pH decrease was observed. Despite of higher values of gizzard's pH in ISFC birds, no negative effects were detected on performance or nutrient digestibility.

Cecal Fermentation

Cecum weight is presumably influenced by PS and the amount of fermentable substrate, which should be fine enough to enter in the cecum (Röhe and Zentek, 2021). In the current study, both fiber sources were finely ground, thus allowing their pass into the cecum. In contrast to the current results, in Experiment 1, inclusion of 1.5% finely ground almond shell, an inert source with low hydration capacity, tended to decrease the weight of cecum in 21-d broilers. Therefore, the positive effects on cecal weight observed in the current study might be related to the FOS content of ISFC and its high fermentability,

which may stimulate the growth of GIT microbiota affecting also the cecum due to peristaltic and antiperistaltic movements (Wedegaertner, 2021).

Lower total SCFA and acetate concentrations in the cecum by fiber addition support the limited cecal fermentation of highly lignified IDF sources in poultry (Bautil et al., 2023). Results of the current trial are in line with Experiment 1 that showed no effect of dietary supplementation of LC and other IDF sources on cecal total SCFA concentration in 21-d broilers. Previous studies from other groups reported that LC is slightly fermentable in the cecum of swine and turkey (Youssef and Kamphus, 2018), and had a low capacity to modulate cecal SCFA production (Zeitz et al., 2019) and cecal pH (Bogusławska-Tryk et al., 2015). Also, supplementing higher doses (2 and 4%) of LC had no effect on cecal concentrations of SCFA in chickens (Hou et al., 2020).

The question of whether prebiotic sources are able to improve cecal SCFA concentrations in broilers still remains unresolved (Worawang et al., 2022). Feeding FOS during either the whole rearing period or the finishing phase of broilers did not modify cecal concentrations of total SCFA, acetate, propionate, or butyrate (Cao et al., 2005; Ao and Choct, 2013). A metanalysis carried out by Worawong et al. (2022) showed that prebiotic supplementation increased cecal concentrations of propionate and butyrate, with no effects on concentrations of total SCFA and acetate. These results suggest that these easily fermentable prebiotic fiber sources might partially disappear before reaching the cecum. However, prebiotics are widely known to modify the GIT microbiota and to stimulate the growth of cecal beneficial microorganisms (Teng and Kim, 2018; Kumar et al., 2019; Xia et al., 2019). For that reason, ISFC was designed as a mixture of fibers to maintain the properties of IDF, due to its physical effects on GIT development, and a highly fermentable source in form of FOS was included.

In the current study, only the molar proportion of valerate was higher in ISFC-fed broilers compared with control ones. This is well in accordance with the greater valerate concentration in the cecal content of 42-d broilers fed FOS (4 g/kg) reported by Cao et al. (2005), which also selectively promoted the growth of favorable microbes and inhibited phenol, ethylphenol, cresol, and indole production. Higher levels of valerate were associated with beneficial modification of the microbiota by *L. acidophilus* supplementation in broilers (Li et al., 2017).

The lower content in ADL and greater content in total soluble fiber of ISFC (17.9 and 16.0%, respectively) than in LC (23.4 and 10.8%) can help to explain the greater gas production from ISFC, as gas production is positively related to the OM fermented (Menke and Steingass, 1988). These results are, in general, in good agreement with those reported in Experiment 1 in 21-d broilers, confirming the low fermentability of LC and that it does not seem to change with broilers aging.

The capacity to ferment fiber did not seem to increase by 42-d exposition to the substrate. Values of gas production were in accordance with those of Experiment 1 in 21-d old broilers.

It is generally accepted that the ceca are the main fermentation chambers in the avian GIT and had the highest bacterial density in broilers (Rehman et al., 2007; Wedegaertner, 2021). The FOS used as a fermentable fiber source are not digested in the small intestine (Hajati and Rezaei, 2010), but are

selectively and fast fermented by *Lactobacillus*, *Enterococcus,* and *Pediococcus* spp. strains that were also isolated in other parts of the GIT including the crop (Greppi et al., 2019; Reuben et al., 2019). Despite of the higher fermentability of ISFC determined *in vitro* (as indicated by the relatively high gas production) compared with LC substrate, the low *in vivo* effects observed on cecal SCFA concentrations and pH may be related to low amounts of ISFC reaching the cecum due to partial fermentation in previous sections of GIT. More studies should be carried out with different types of fiber to characterize the fiber fermentative capacity in the cecum of broilers.

6.3. Experiment 3: Different physiochemical properties of novel fiber sources in the diet of weaned pigs influence animal performance, nutrient digestibility, and cecal fermentation.

The TDF value of the analysed samples may be underestimated for LHC and MHC, as the SDF content came mostly from inulin, which cannot be precipitated with ethanol by the SDF method (Prosky and Hoebregs, 1999). Instead, the NDFom analysis was performed using F57 Ankom filter bags that retain only particles > 25μm. Therefore, the loss of smaller particles from the bags was possible (Hall and Mertens, 2023), and these losses may vary depending on the fibre source. The analysis of water binding and swelling properties confirmed that LHC and MHC sources were properly designed with low and medium HC, respectively.

Growth Performance and Diet Digestibility

Animals fed HHC performed similarly to those fed the control diet, but both control and HHC performed lower than MHC. This may be associated with the lower feed intake registered in these groups during the experimental period, and lower CP digestibility at 42 d, although FCR was not significantly affected. In general, the result of this trial seems to confirm that a combination of the physical effects of insoluble fibre with highly fermentable sources may be advantageous for performance. It partially aligns with what was previously reported by Chen et al. (2019a) who observed improvement in the FCR supplemented with 0.5% inulin and 0.5% lignocellulose at 38 and 52 d compared to control animals. However, Molist et al. (Molist et al., 2009) also supplemented piglets diets with insoluble and soluble fibre sources, based on wheat bran and sugar beet pulp, but no effect was observed on performance. This may indicate that the inclusion of fermentable fibrous sources that do not increase intestinal viscosity may be more recommended for young piglets.

On the other hand, micronized wood-lignocellulose has been widely used in recent investigations as an IDF source with generally positive effects, although some contradictory results have also been reported. Supporting our results, supplementation with 1% of non-fermentable lignocellulose (Chen et al., 2019a) or 1.5% of non-fermentable or partially fermentable lignocellulose (Slama et al., 2020) produced no effects on ADG or ADFI of weaned piglets. Other authors reported that 1% supplementation of lignocellulose increased both ADFI and ADG in piglets from 31 to 52 d, leading to higher final body weight compared to the control diet (Superchi et al., 2017). These inconsistent results suggest that a dosage, the interaction with other dietary constituents, the age and the timing of ingestion of highly lignified fibre sources may affect the performance of weaned piglets.

Better ADG and ADFI in MHC animals compared to IDF lignocellulose align with previous results observed in piglets fed both inulin and lignocellulose, suggesting synergistic effects of a combination of IDF and prebiotic sources on piglets' performance in the post weaning period (Chen et al., 2019a). The inclusion of 0.5% inulin has been reported to improve growth rate, which was associated

with an increase in serum levels of IGF-1, regulating growth hormone and stimulating cells proliferation in both weaning and growing pigs (Wang et al., 2019, 2020).

In the current trial, MHC increased CP digestibility at 42 d compared to HHC, which likely had a direct impact on growth performance at this stage. All fibre sources used in our study were highly lignified, suggesting low fermentability. However, the inclusion of a fermentable fraction in LHC and MHC diets was designed to provide a usable substrate to the resident microbiota. It was hypothesized that the combined supplementation of IDF with a soluble fraction may improve gut health by beneficial modifications of microbial colonization and fermentation patterns (Molist et al., 2009), leading to more efficient nutrient utilization.

The positive correlation observed between fermentability and nutrient digestibility may be attributed to longer retention time of digesta and greater enzymatic secretion, both leading to increased subsequent nutrient absorption (Zhao et al., 2020). In weaned piglets, 0.5% of inulin supplementation has increased the expression levels of GLUT2 and DMT1, associated with higher digestive capacity in the proximal intestinal mucosa (Wang et al., 2020).

In addition, the particle size of all experimental treatments in our study was particularly low and provided a high number of inert particles in contact with the intestinal mucosa, potentially benefiting gut physiology or morphology. In fact, previous studies reported that wheat bran supplemented at 3% increased both the villus height to crypt depth ratio in the ileum and the sucrase and maltase activity in the intestine of piglets (Shang et al., 2019). Similarly, increasing levels of inulin supplementation (0.5-2 g/d) during the suckling period increased the villus height and the villus height to crypt depth ratio in the jejunum and ileum of piglets after 28 d (Li et al., 2018b). Although previous studies reported none or even negative effects on CP digestibility, depending on the inclusion levels of fibre sources (Ivarsson et al., 2012; Metzler-Zebeli et al., 2017), a higher absorption surface due to possibly changes on the mucosa morphology may explain the greater digestibility observed in our study for the MHC diet.

Dietary treatment showed no effects on caecal weight or the moisture content in different intestinal compartments. The moisture content of digesta observed in this research generally aligns with previous reports (Anguita et al., 2007). The moisture of digestive contents is a balance between secretion and absorption of water from the duodenum to the distal colon, crucial for maintaining epithelium hydration and correct functionality (Farré et al., 2020).

Higher CP digestibility observed for the MHC diet at 42 d may be associated with potential improvements in digestive efficiency, leading to better performance in the postweaning period compared to lignocellulose inclusion, although no changes were observed in digestive traits or moisture levels of digestive contents. In the current trial, hydration capacity of treatments had no linear or quadratic effect on performance ($p \geq 0.237$, r = 0.215, n = 24).

In Vitro Fermentation and Caecal Content Characteristics

Dietary fibre plays a crucial role in maintaining gut microbiota balance and gut health (Liu et al., 2018; Farré et al., 2020). Various pathogenic bacteria species such as *Escherichia coli, Klebsiella spp., Campylobacter spp., Streptococcus spp., Clostridium perfringens, Clostridium difficile and Bacteroides fragilis,* produce harmful metabolites that can impair barrier function, leading to colonic diseases and diarrhoea problems (Macfarlane and Macfarlane, 2012; Williams et al., 2019). Conversely, a higher abundance of *Roseburia*, *Prevotella* and genera belonging to *Ruminococcaceae,* which are adapted to metabolize complex oligosaccharides and polysaccharides, may protect the host from pathogen infections (Dou et al., 2017).

Fibre supplementation in the diet alters the richness of the microbiota, although the proportions and diversity of fibre-degrading bacteria depend on the structure and composition of the fibre sources (Hald et al., 2016; Bedford et al., 2024). For instance, feeding 1% inulin resulted in greater microbial diversity and richness compared to the same level of lignocellulose (Chen et al., 2019b). A combination of 4% wheat bran and 3% sugar beet pulp reduced enterobacteria counts in the digesta, indicating a synergistic effect of the two different sources on the microbial population (Molist et al., 2009). Additionally, dietary supplementation with 0.5% lignocellulose and 0.5% inulin increased the relative abundance of the phylum Bacteroidetes and the genus *Selenomonas*, *Phascolarctobacterium*, *Sharpea*, and *Alloprevotella* (Chen et al., 2019b).

In this study, the microbiota was not analysed directly. However, all the fibre sources were highly lignified, but a finely grinding were performed to increase the surface area for microbial colonization (Jiang et al., 2022). Furthermore, the inclusion of inulin from chicory root would likely have a positive impact on the fibre-degradation microbiota in LHC and MHC piglets.

The individual fibre sources - almond shell, olive kernel, wood, and nutshell - showed cumulative gas production bellow 10 mL/g DM after 72 h of incubation with CON inoculum. Only grape pomace had slightly greater gas production at short incubation times, reaching 14 mL/g DM, likely due to its moderate protein content (Ahmed et al., 2020), although no increase in gas production was observed after 24 h of incubation. In contrast, chicory root which has a high content of inulin (Guo et al., 2018), demonstrated much greater and prolonged gas production, suggesting its potential use as a prebiotic source (Uerlings et al., 2019).

The HHC fibre source showed low gas production (< 3 mL/g DM), consistent with previously reported results using faeces or caecal digesta of pigs as inoculum (Youssef and Kamphues, 2018; Bachmann et al., 2021). The greater gas production of LHC and MHC fibre sources (> 14 mL/g DM) confirmed the potential fermentation capacity of their fermentable fraction. These results confirm the low fermentability of lignocellulosic fibre sources. All these results are in line with our previous study in 21-d (Experiment 1 and 42-d (Experiment 2) broilers, confirming the low fermentability of lignocellulosic fibre sources.

Unexpectedly, the fibre sources used in the current experiment had no impact on caecal SCFA concentration, despite differences observed in their fermentation in the *in vitro* trial. A lack of effects on caecal characteristics was also previously reported with the supplementation of cellulose at 0.5% or 2% (Hanczakowska et al., 2008) or finely ground wheat bran at 4% (Molist et al., 2012) to piglets. However, lignocellulose addition at 1, 1.5, 2 and 3% improved caecal butyrate formation in another study (Slama et al., 2020; Silva-Guillen et al., 2022). Also, a combination of insoluble and soluble fibre based on wheat bran and sugar beet pulp, or lignocellulose with inulin, promoted a beneficial shift in microbial colonization, with a higher production of butyric acid in the large intestine (Molist et al., 2009; Chen et al., 2019a). Therefore, the lack of effects may be related to diet composition, fibre inclusion level, transit time, fibre composition, or the interaction among all of them.

In our study, it was expected that the prebiotic fraction from chicory root would decrease the caecal pH and increase SCFA production. Chicory root contains inulin and fructans and was included in LHC and MHC to create the properties of an ideal fibre source, given its potential to modify the intestinal microbial profile (Ivarsson, 2012). Surprisingly, the results showed no impact of inulin addition on fermentation patterns in the caecum.

The effects of inulin supplementation on SCFA are ambiguous. Supplementation with 0.25% inulin did not affect SCFA levels in the caecum and colon, although ileal propionate levels were increased compared to the same level of cellulose inclusion (Yang et al., 2023). High dietary inulin (3%) resulted in lower acetate, and higher proportion of propionate and butyrate levels compared to pigs fed wheat bran (Loh et al., 2006). According to a metanalysis (Metzler-Zebeli et al., 2017), digesta pH in the ileum, caecum, colon and faeces, as well as SCFA concentrations in the gastrointestinal tract and the faeces, appeared to be largely unaffected by dietary inulin, which is consistent with our results.

The lack of differences in *in vivo* caecal fermentation may be attributed to factors such as the source of inulin, the level of supplementation, or the degree of polymerization, which suggest extensive pre-caecal fermentation (Böhmer et al., 2005; Eberhard et al., 2007). Short-chain inulin may be degraded in the jejunum, whereas long-chain inulin has been detected in all intestinal segments (Loh et al., 2006; Paßlack et al., 2012). Studies involving post-weaned piglets fed different types of inulin at 4% of the diet reported an absence of inulin in digesta samples from the caecum as well as the proximal, mid, and distal colon (Yasuda et al., 2009). The loss of fructans during passage through the small intestine could be due to hydrolysis by acid or enzymes degradation by the microflora present in the small intestine (Hedemann and Bach Knudsen, 2010). The discrepancies observed among studies may also relate to factors such as the inclusion level of fibre sources, the composition of the basal diet, and the characteristic of the target animals, including age and the maturity stage of the intestinal tract development (Yan et al., 2017; Lv et al., 2022).

Since the fibre sources used in our study might influence the microbiota profile due to their adaptation to ferment different types of fibres (Cronin et al., 2021), it was hypothesized that exposure to each fibre source during the post-weaning period would increase the fermentative capacity of the

microbiota. Unexpectedly, both LHC and MHC tended to produce more gas when incubated with inoculum from piglets fed the control diet than with inoculum from piglets fed the corresponding diet ('adapted inoculum'). This finding suggests that, as indicated by *in vivo* results, the inulin was likely fermented in the proximal intestine and did not reach the caecum. Consequently, the caecal inocula were probably not truly 'adapted', as the fibre had already been fermented in previous part of the intestine.

On the other hand, gas production from HHC substrate was unaffected by microbiota exposure. The results suggest that inulin was easily fermentable, and prior adaptation of microbiota is not necessary for efficient degradation. However, this appears to differ for other types of substrates. When wheat starch was incubated with faecal inoculum from weaned piglets, it showed greater fermentation (faster gas production and more SCFA production) using an inoculum from animals fed a control diet than using an inoculum previously 'adapted', although sugar beet pulp was better fermented using the adapted inoculum (Awati et al., 2005).

In summary, the high fermentability of LHC and MHC observed in the *in vitro* fermentation trial confirms a synergetic effect between insoluble fibre with a prebiotic fraction, which positively influenced performance and crude protein digestibility. Fibre fermentation is generally a beneficial process, mediated by the proliferation of saccharolytic bacteria (Ivarsson et al., 2012), which helps to prevent the growth of facultative pathogens (Jha et al., 2019). The synergetic supplementation of insoluble and highly fermentable fibre may provide the optimal balance of gut microbiota, which could be crucial for modulating intestinal permeability and maintaining gut barrier integrity.

Due to several limitation of this study, further research is needed to explore: i) the impact of different combinations of fibre sources, ii) varying levels of hydration capacity or fermentability, iii) different levels of fibre inclusion, iv) additional physiological parameters or markers that could enhance our understanding of fibre`s role in physiology, digestibility, fermentation and gut health.

This investigation supports the idea that micronization of highly lignified agricultural by-products such as almond shell, olive kernel, nutshell or grape pomace, may provide an alternative fibre source for animal nutrition. Achieving an optimal balance between soluble and insoluble fibre with medium hydration properties could be a practical strategy for maintaining post-weaning performance in piglets. In this case, the combination of insoluble fibre with a prebiotic fraction from chicory root provided advantages in performance and crude protein digestibility compared with lignocellulose at 1.5% inclusion in the diet. Further research is needed to elucidate the impact of the hydration capacity of insoluble fibre sources in piglets.

6.3. General Discussion

The objective of this PhD was the evaluation of the type of fiber in diets for piglets and broilers, and how mixtures of finely ground agricultural by-products can be used as a fiber source in these diets. Results confirm that either the hydration capacity (HC) and the relation insoluble-soluble fraction, play a key role in the impact of the fiber inclusion on the physiology, fermentability and performance. Also, it has been demonstrated that raw materials such as almond shell, nutshell, or olive kernel were suitable for inclusion as fiber sources in the diets of weaned piglets and broilers. The results confirm that functionality of fiber fraction in the diet varies according to its physicochemical properties, and those should be adapted to the specific species and age requirements.

Performance

The results of trials with broilers and piglets, point out that the effect of fiber inclusion would be greatly affected by the intrinsic properties of the fiber (particle size, fermentability and hydration properties), and the species (including age, and maturity stage of the digestive tract development). Most of the DF sources used (almond shell, nutshells, olive kernel, grape pomace) were characterized by moderate low binding-water values and swelling properties, except for the straw and wood, that had high values.

In Experiment 1, broiler's performance from 1-21d was negatively affected by the high HC-IDF sources (lignocellulose-LC, fine straw- FS, and coarse straw- CS), showing lower ADFI and ADG than those fed the AS (almond shells) diet (Figure 12).

Figure 12. Change of ADFI and ADG (%) in the period 1-21d with respect to non-supplemented birds. Experiment 1.

ADFI- average daily feed intake, ADG- average daily gain, LC-lignocellulose, FS-fine straw, CS-coarse straw, AS-almond shell.

In this case, LC and FS addition decreased ADFI by 2.6 – 2.8%, impairing ADG by near 5.0-6.5% when compared to control animals. Lower negative impact was observed in CS, whereas AS improved ADFI by 3.6% and ADG by 2.9%, suggesting that more moderate HC is more beneficial for young birds. This finding agrees with Nascimento et al. (2020), who reported the increases in intake of bulky feeds, which achieves the maxima with HC between 2.51 and 2.93 g water/g feed DM. The AS presented more equivalent values of HC at 3.5g/g DM, whereas LC, FS and CS had much higher values(5.4-6.8g/g DM), which probably explain the depression of the feed intake and growth.

However, the correlation analysis performed with fiber sources used in Experiment 1 and 2, showed a low relationship in the starter period between HC and feed intake (r=0.35, *P* = 0.027, n=10) and growth (r=0.36, P = 0.020, n=10; Figure 13). Only a slight tendency to decrease performance was observed with increase of WBC.

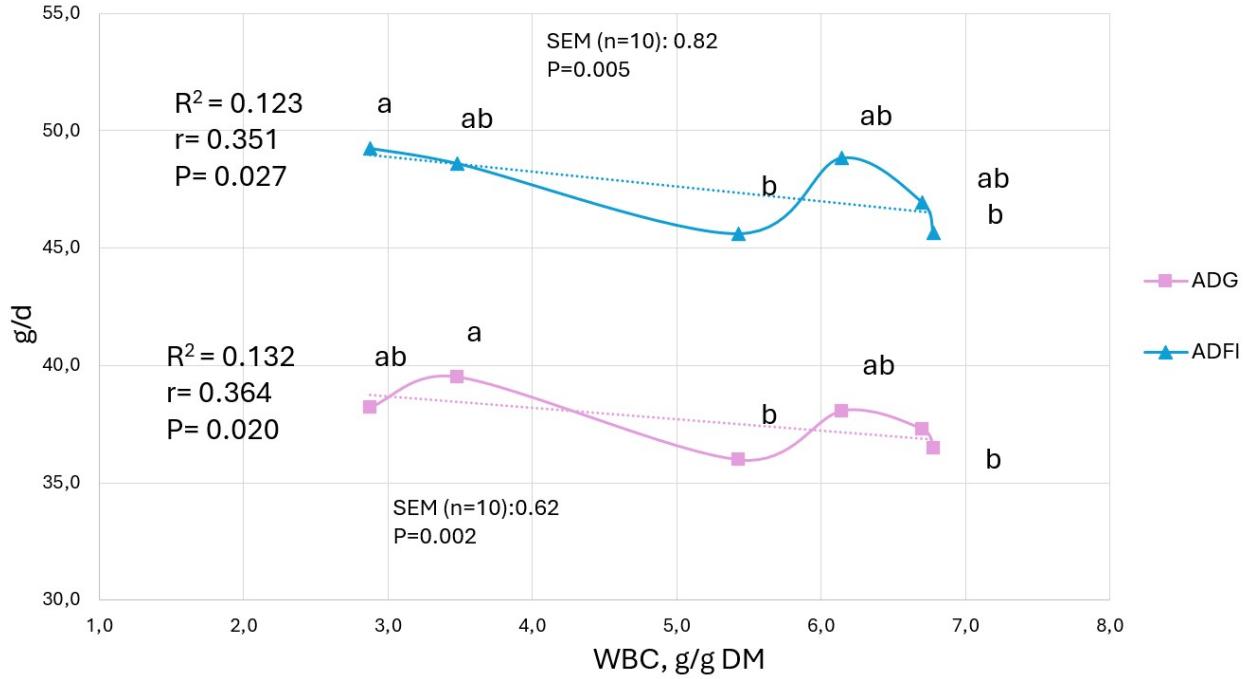


Figure 13. Correlation between hydration capacity (WBC, g/g DM) of the fiber sources on ADFI and ADG expressed in g/d in the period 1-21d. Experiment 1 and 2.a, b, within the same row, means with different letter differ (P < 0.05; Tukey test). ADFI- average daily feed intake, ADG- average daily gain, WBC-water binding capacity.

This analysis may not be accurate due to substantially different condictions between both studies. In fact, performance was not affected between 1-21d of Experiment 2.

This discrepancy may be due to differences in the properties of the experimental fibers. Almond shell used in Experiment 1 was an insoluble and inert material, whereas the complex mixture of fibers (ISFC) used in Experiment 2 was design based on a mixture of mostly insoluble fiber sources, but also included a prebiotic fraction from FOS, that might exert a positive influence on metabolic health by modifying the gut microbiota (Cronin et al., 2021). Another possibility is the slightly different

composition and particle size in both starter diets, since the first one was based in corn, and the second one on wheat, with slightly higher NDF content of control feed in Experiment 1 than Experiment 2 (14% vs 11.5%, respectively). Also, the feed used in Experiment 2, based on wheat instead of corn, was more finely ground to avoid selection of the coarse particle by the birds. The last potential reason is consumption of bed-straw. In Experiment 1 broilers were raised in cages, whereas in Experiment 2 on pressed straw litter, and the amount of the fiber ingested from the litter was not controlled. All above-mentioned differences between trials, or their interactions may have produced different answers to fiber inclusion in the starter period.

Nevertheless, in the growing period birds fed ISFC grew faster than LC and tended to present higher final BW at 42d. The fiber inclusion during the growing period (21-42d), increased ADG by 1.5 and 5.4% for LC and ISFC, respectively, as compared to non-supplemented birds (Figure 14).

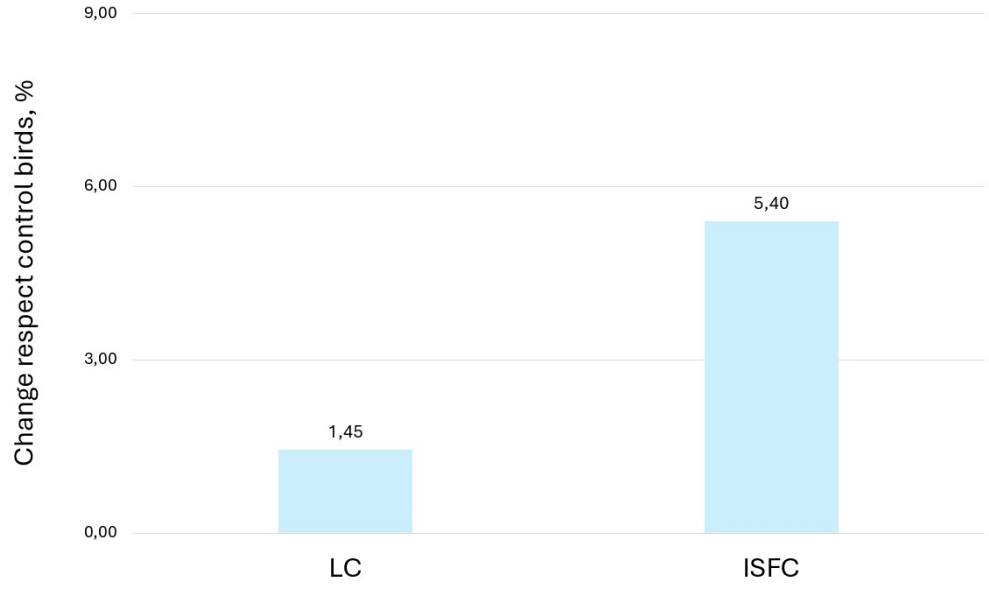


Figure 14. Change of ADG (%, 21-42d) with respect to non-supplemented broilers. Experiment 2. ADG- average daily gain, LC-lignocellulose, ISFC- Insoluble fiber concentrate with prebiotic fraction.

Higher growth was probably due to a correct balance of the physical presence of the inert fiber, and likely due to the modification of the intestinal ecosystem produced by the highly fermentable and prebiotic fraction.

In piglets, results of Experiment 3 are in line with Ndou et al. (2013), who reported an HC of 4.53 g/g DM for maximizing the consumption. Beyond this level the feed intake decreases, because the capacity of the gut for feed was filled up with the water held within the fiber matrix. In our Experiment, animals fed medium HC (MHC) had higher ADG and ADFI compared to high HC lignocellulose (HHC, Figure 15). Piglets supplemented with low HC fiber (LHC) increased feed consumption by 0.6% resulting in 2% better growth than control piglets. Inclusion of MHC increased ADFI by 9.3% and ADG by 8.4, whereas HHC impaired both, by 4.6 and 5.7% respectively, as compared to non-supplemented piglets. In

this case, the WBC of MHC fiber was 4.28g water/ g DM, suggesting that higher HC may be not recommended for maximize performance. However, the correlation analysis performed with data of Experiment 3, showed no linear or quadratic of hydration capacity on performance ($P \geq 0.237$, r = 0.215, n = 24). It needs to be consider the limitation of the analysis due to low number of different hydration levels of fiber sources, or the interaction with fermentability.

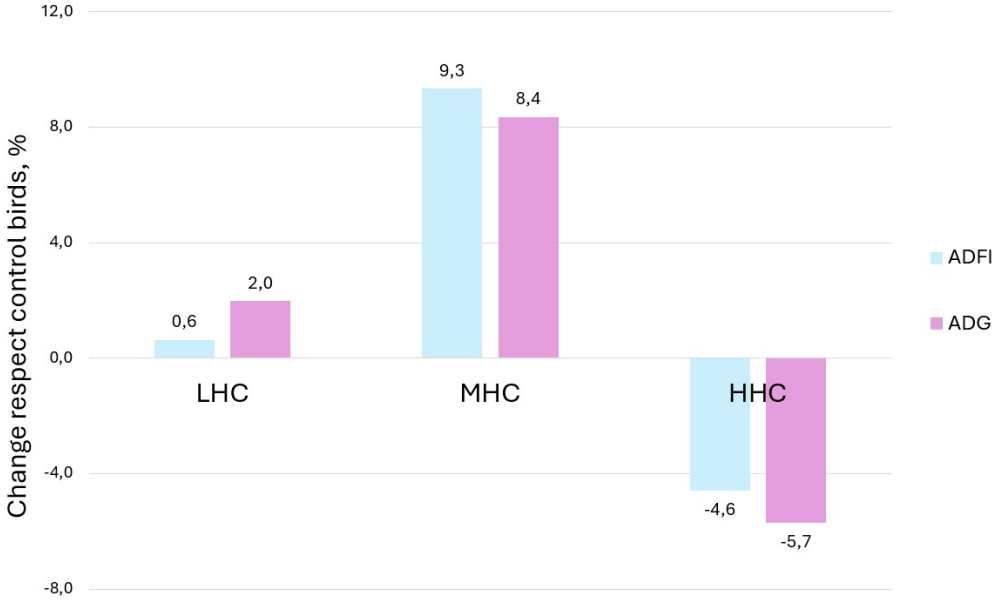


Figure 15. Change of ADFI and ADG (%) in the overall period with respect to non-supplemented animals. Experiment 3.ADFI - average daily feed intake, ADG - average daily gain, LHC - low hydration capacity fiber, MHC - medium hydration capacity fiber, HHC - high hydration capacity fiber (lignocellulose).

On the other hand, these results align with data of Experiment 2 in broilers, and previous results observed in piglets fed both inulin and lignocellulose, confirming that a combination of insoluble fiber and prebiotic sources improves performance in the post weaning period (Chen et al., 2019a).

Nutrient Digestibility

Values of CP digestibility were positively correlated with the final BW of birds (r = 0.997; P = 0,050, n = 3. Experiment 2), and piglet's BW at 42d (r = 0.999; P = 0.001, n=4. Experiment 3). Results of Experiment 2 and 3 also confirms a positive impact of a moderate amounts of DF on digestibility and performance in broilers and piglets as previously reported by several authors (Gresse et al., 2017; Tejeda and Kim, 2020; Jha and Misha, 2022).

All fiber sources used in these studies were highly insoluble to potentiate the positive physical effect on the GIT (Mateos et al., 2012; Jha et al., 2019). However, a prebiotic fraction was added to create the fiber source with appropriate properties. Several previous works tested a combination of insoluble and

soluble fiber sources with satisfactory results (Molist et al., 2009; Chen et al., 2019a). In addition, the particle size of the fiber sources in these studies was particularly low, providing a huge number of inert particles in contact with the intestinal mucosa, potentially benefiting gut physiology and morphology (Jiménez-Moreno et al., 2010; Tejeda and Kim, 2021a). It was hypothesized that the combined supplementation of insoluble inert fiber with a fermentable and prebiotic fraction, may provide a synergetic effect achieved through physical enhancement, and adjustments in the colonization and fermentation patterns of specifically selected microbes, leading to more efficient nutrient utilization.

In broilers diets, higher nutrient digestibility observed in the ISFC than in LC broilers (Experiment 2) agrees with the results of Experiment 1, that finely ground almond shells slower the transit in the small intestine, which may be related to higher reverse peristalsis. A stimulation of reverse peristalsis and digestive enzyme secretion may impact positively performance (González-Alvarado et al., 2007). Higher transit time was only observed in broilers fed micronized almond shell and might be associated to more reduced PS than LC, and therefore, produce more efficacious digestibility. Moreover, addition of a fermentable fraction may enhance this effect, since even low FOS inclusion (0.4%) was able to increase the amylase and protease activity in male broilers (Xu et al., 2003). Also, presence of Lactobacilli community in the crop and ileum of birds (Classen et al., 2016; Lv et al., 2021) may explain partial degradation of the fructans, leading to higher AID of DM and OM observed in Experiment 2.

In piglets, insoluble fiber has been widely reported to improve intestinal mucosa morphology through digestive tract, suggesting an increase in the absorption surface. Insoluble sources, LC and cellulose inclusion (1.5-2%) increased the villus heigh and crypt dept in the small intestine (Hanczakowska et al., 2008; Sun et al., 2021). Also, inulin supplementation (0.25 and 0.5%) enhanced the villus height to crypt depth in the duodenum and ileum (Wang et al., 2020). Dietary supplementation with 6% wheat bran increased the sucrase and maltase activities in the jejunum (Shang et al., 2019), and inulin added at 1% the sucrase activity in the ileum (Wang et al., 2020). In the case of piglets fed MHC, we hypothesized that the positive results on digestibility may be associated with a higher digestive capacity induced by the correct balance between insoluble and soluble fiber, that in one hand improves digesta physiology, and in the other hand may modify fermentation patterns before and after the cecum.

The research performed by Chen et al. (2019a) had similar design to Experiment 3, since they used LC and inulin, as insoluble and soluble fiber sources, respectively, which were used in different combination to create two additional treatments based on the mixture between both. However, in this case, authors observed no effects on CP TTAD between LC and a mixture of LC with inulin, although 1% inulin increased CP TTAD compared to control. The discrepancy between both investigations may be related with a different composition of the experiment diets, amount of the fiber included (1% vs. 1.5%), or the composition of the fiber concentrates.

The positive effects of ISFC and MHC on digestibility may be associated with the interaction between insoluble fiber and the fermentable fraction (such as FOS or chicory root), leading to potential improvements in digestive efficiency compared to insoluble and non-fermentable lignocellulose in

poultry and piglets.

Moisture of Intestinal Content

High HC of fiber sources, in particular of LC, has been suggested as an important factor to control the moisture content of digesta (Amerah et al., 2009), which may impact the litter quality in broilers, or the feces moisture in piglets. However, the drying effect associated with HC seems to be contradictory, since if the water is retained in DF matrix, humidity in the digesta would be increased. Specially in the feaces, where the quantity of DF is maxima, since the rest of the nutrients have been absorbed previously. Generally, insoluble fiber sources with high HC absorb water and expand the size, with the advantage of no increase of digesta viscosity.

Previous investigations have reported better fecal consistency in broilers and piglets, using different fiber sources with moderate to high water holding capacity, such as wheat bran, oat hulls, lignocellulose or wheat straw (Mateos et al., 2006; Gerritsen et al., 2012; Makivic et al., 2018; Shang et al., 2021). Within the experiments of this thesis, drying effect of fiber on excreta was observed only in Experiment 1 for the average of IDF- groups, and especially for LC-fed broilers, compared with Control. However, in Experiment 1 the positive effect of high HC was not detected. Moreover, results of the Experiment 3 showed a lack of relation between the HC of DF and moisture content of digesta and feces in piglets.

Few theories have been proposed to explain this phenomenon. Micronized fiber was associated with mechanical stimulation on the intestinal mucosal layer (Rezaei et al., 2011, 2014), and balanced gut microbiome, controlling the overgrowth of harmful bacteria and its toxic compounds by SCFAs (Hanczakowska et al., 2008; Pieper et al., 2014), modulating fluid and electrolyte absorption (Molist et al., 2012; Jha and Berrocoso, 2015). All these assumptions point out the importance of the proper intestinal barrier integrity maintenance by insoluble fiber (Pluske et al., 2014; Gresse et al., 2017), which may be crucial to improve the water reuse in the intestine. For instance, dietary lignocellulose addition increased the intestinal barrier function in lipopolysaccharide-induced intestinal injury, inhibiting the expression of proinflammatory cytokines or decrease of biogenic amines (Barnes et al., 2001; Sun et al., 2021).

It is also important to indicate, that the moisture of digestive contents is a balance between secretion and absorption of water through the GIT (Farré et al., 2020). Insoluble fiber sources are characterized by ‘bulking’ the stools, which refers to an increase of the volume and mass of feces, due to a relative increase of fiber content in relation to a decrease of all the nutrients that have been digested and absorbed. The increasing stool volume, reduce the relative concentration of solutes over a larger mass, lowering the osmolarity of the intestinal contents, which contributes to water absorption.

Overall, DF may decrease the moisture content of the digestive compartments due to a multifactorial effect associated with the presence of fiber in the intestine. However, the HC of DF *per se*, does not seem to be particularly involved in this process.

Cecal Characteristics and SCFA Concentration

Cecum is generally considered as the main fermentation chamber with the highest bacterial density in piglets and broilers (Jha et al., 2019; Wedegaertner, 2021). For that reason, cecal characteristics, such as weight or pH, and the concentration of SCFA, were studied to assess the potential effects of fiber source on fermentable patterns.

Cecum weight is presumably influenced by particle size and the amount of fermentable substrate (Röhe and Zentek, 2021). However, in broilers, contradictory effects were observed, and in piglets, was unaffected. Inclusion of finely ground almond shell, an inert source with moderate HC, tended to decrease the weight of cecum in 21-d broilers. In contrast, in Experiment 2, the cecal weight tended to be increased by ISFC, which also has lower HC than LC. Thus, the positive effects on cecal weight might be related to high fermentability of ISFC.

Fiber sources used in these trials had a limited impact on the cecal SCFA production in poultry at 21 or 42d, and in piglets at 61d, which is consistent with lack of effects on the cecal pH.

In Experiment 1, inert sources were used, and the results agree with those reported using LC, which has been used in all the experiments as a negative control, according to low capacity to ferment in the cecum of swine and poultry (Youssef and Kamphus, 2018), modulate cecal SCFA production (Zeitz et al., 2019), or cecal pH (Bogusławska-Tryk et al., 2015).

Experimental fiber sources used in Experiment 2 and 3 were mainly designed based on highly lignified IDF sources, but a highly fermentable and prebiotic fraction was added. Prebiotics are widely known to modify the GIT microbiota, and to stimulate the growth of cecal beneficial microorganisms (Kumar et al., 2019; Xia et al., 2019). Fermentable source used in Experiment 2 to design ISFC was FOS, based on a linear chain of fructose with a low degree of polymerization (Steward et al., 2008). Chicory root used in Experiment 3 contained inulin and fructans and was included into LHC and MHC to create the potential to modify the intestinal microbial profile (Ivarsson, 2012).

Only minor in vivo effects were observed in trials performed with broilers. Lower total cecal SCFA and acetate concentrations by fiber addition reported in Experiment 2, supports the limited cecal fermentation of highly lignified IDF sources in poultry at 42d (Bautil et al., 2023), and is consistent with Experiment 1 in 21-d broilers.

On the other hand, inclusion of a fermentable fraction in fiber sources of Experiment 2 and 3 produced only higher molar proportion of valerate in ISFC-fed broilers compared with control, and no effects in piglets. It may suggest that these easily fermentable fructans might partially disappear before reaching the cecum in poultry and pigs. In poultry, FOS may be potentially fermented by *Lactobacillus*, *Enterococcus,* and *Pediococcus* spp. strains which were also isolated in proximal parts of the GIT, mostly in the crop (Greppi et al., 2019; Reuben et al., 2019). In case of piglets, the inulin degree of polymerization may impact the fermentation site (Böhmer et al., 2005; Eberhard et al., 2007), since the short-chain may be degraded already in the jejunum. The contradictory results observed among studies

may also be related to factors such as the inclusion level of fiber sources, the composition of the basal diet, and the characteristic of the target animals, including age and the maturity stage of the intestinal tract development (Yan et al., 2017; Lv et al., 2022).

In Vitro Fermentation and Microbiota Adaptation

In vitro fermentation trials assessed the amount of gas produced, using the cecal content of experimental animals as *inoculum*, and were performed to evaluate the potential of individual fiber source or its complex mixture, to be fermented by the resident microbiota.

Individual fiber sources were evaluated using the cecal content of control animals as *inoculum*. In Experiment 1 with broilers, both lignocellulose and almond shell produced lower gas than fine straw and coarse straw. Similarly in piglets, almond shell, olive kernel, wood, and nutshell presented very low gas production in the Experiment 3. These effects were associated, generally, to high lignin content. In contrast, chicory root which has a high content of inulin (Guo et al., 2018), demonstrated much greater and prolonged gas production, confirming its potential as a prebiotic source (Uerlings et al., 2019).

Results of Experiment 2 and Experiment 3 confirmed that the fiber mixtures with fermentable fraction (ISFC, LHC and MHC) produced higher gas than LC in 42d-broilers, and 61d-piglests, suggesting their prebiotic potential. Low fermentability of lignocellulose is also consistent with previously reported results using feces or cecal digesta of pigs as inoculum (Youssef and Kamphues, 2018; Bachmann et al., 2021). These results confirm the low fermentability of lignocellulosic fiber sources, which does not seem to change with aging in broilers.

Since the fiber sources used in Experiment 1, 2 and 3 might affect the microbiota profile by their adaptation to ferment different fibers (Cronin et al., 2021), it was hypothesized that exposure during the experimental period would increase the fermentative capacity of the cecal microbiota.

In Experiments 1 and 2, the comparison of the amount of gas produced when each fiber source was fermented *in vitro,* either with the cecal *inoculum* of Control birds or with the cecal *inoculum* of birds fed the corresponding fiber source, revealed no differences at any sampling time. Nevertheless, the average values were numerically higher, when the inoculum from the birds fed the IDF was used. Similar observation was found in piglets using lignocellulose (HHC substrate) in Experiment 3. The results suggest that feeding the insoluble fiber sources did not produce a significant modification of the cecal microbiota, which could be detected by measuring *in vitro* gas production.

Unexpectedly in Experiment 3, both LHC and MHC tended to produce more gas when incubated with *inoculum* from control piglets, than from piglets fed the corresponding diet (‘adapted *inoculum’*). Since the jejunal degradation of inulin in piglets was estimated between 20-50% (Loh et al., 2006), the lack of effect either on the cecal pH, or SCFA production, and lower gas production by the ‘adapted’ microbiota, suggests its partial fermentation before reach the cecum. It confirms the inulin as an easily fermentable source which may be highly fermentable by non-adapted bacteria. Moreover, it seems to

point out that the gas production is more dependent on the nature of the substrate than the potential exposure of the microbiota.

Despite of the higher fermentability of ISFC, LHC and MHC determined *in vitro* compared with LC substrate, the low *in vivo* effects observed on cecal SCFA concentrations and pH may be related to low amounts of fermentable substrates reaching the cecum due to partial fermentation in previous sections of GIT. More studies should be carried out with different types and levels of fiber to characterize the fiber fermentative capacity before the cecum of broilers and piglets.

CONCLUSIONS

Conclusions

- This investigation achieved the objective of promoting a circular economy model by minimizing environmental impact of agricultural wastes. The technological process of micronization of highly lignified agricultural by-products such as almond shell, olive kernel, nutshell or grape pomace, provides novel feed materials rich in fiber.

- The physicochemical properties of insoluble fiber sources play role on animal's physiology and performance. However, the effects vary depending on the species, ageing and management. Broilers supplemented with 1.5% of low hydration capacity almond shell produced similar performance (0-21d) to non-supplemented group, is spite of the diet dilution, and performed better than the other insoluble fiber sources with high hydration capacity. In the grower period, dietary supplementation of a mixture of insoluble fiber and prebiotic soluble fiber improved broilers performance due to an increase of the apparent ileal digestibility of nutrients. In piglets, an optimal balance between soluble and insoluble fibre with medium hydration properties seems to be a practical strategy for maintaining post-weaning performance.

- The cecal fermentability of IDF sources mostly depend on the lignin content. In *in vitro* studies, assessed with cecal inocula of broilers and piglets classified the wood-lignocellulose, almond shells, olive kernel, nutshells, as mostly inert sources. Chicory root or fructooligosaccharides were considered as a highly fermentable sources by cecal microbiota, whereas straw and grape pomace were intermediate.

- Although the high fermentability of the complex mixtures of insoluble fiber with prebiotic sources was confirmed in the *in vitro* trial, the differences found in the *in vivo* measurement of SCFA production were low, which might indicate partial pre-cecal fermentation of fructans through the gastrointestinal tract in pigs and poultry.

- A complex mixture of insoluble fiber sources from agricultural wastes combined with highly fermentable fiber with prebiotic activity fiber enhances performance in pigs and poultry compared with only insoluble and non-fermentable fiber.

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