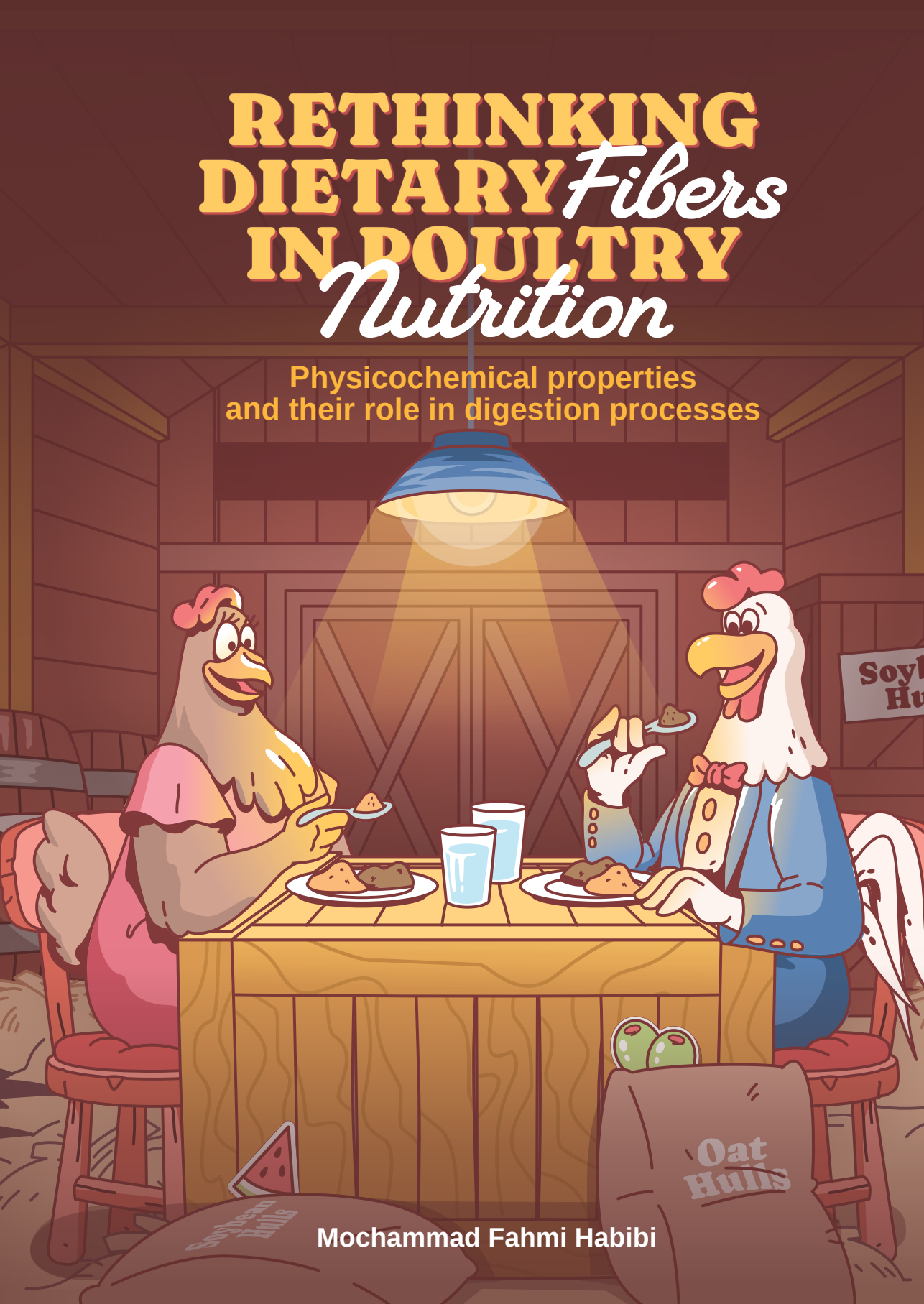
RETHINKING DIETARY *Fibers* IN POULTRY *Nutrition*

Physicochemical properties and their role in digestion processes



Mochammad Fahmi Habibi

Propositions

1. Solubility is an obsolete category in the classification of physicochemical properties of dietary fibers.
   (this thesis)
2. At high inclusion level of insoluble dietary fibers, particle size is irrelevant.
   (this thesis)
3. Application of ethical standards in animal research depends on economic development of a country.
4. Artificial intelligence strengthens the creativity of researchers.
5. It is unacceptable to provide free nutritious meal programs for pupils that shift food-related costs from households to the public sector.
6. A time-based work-life balance is a myth.

Propositions belonging to the thesis, entitled
Rethinking dietary fibers in poultry nutrition: Physicochemical properties and their role in digestion processes.

Mochammad Fahmi Habibi
Wageningen, 8 July 2026

Rethinking dietary fibers in poultry nutrition: physicochemical properties and their role in digestion processes

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Rethinking dietary fibers in poultry nutrition: physicochemical properties and their role in digestion processes

Mochammad Fahmi Habibi

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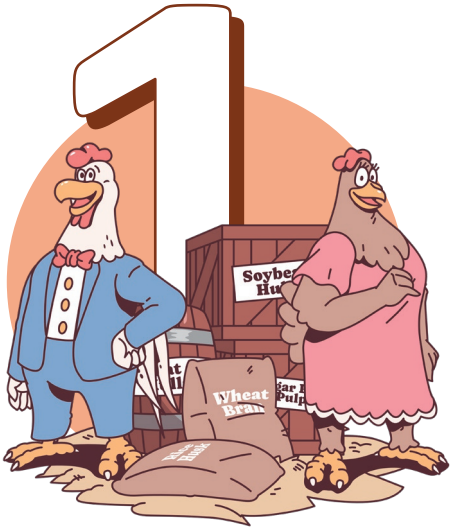
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TABLE OF CONTENTS

Chapter 1	General introduction	7
Chapter 2	Effect of adding soluble viscous fibers to diets containing coarse and finely ground insoluble fibers on digesta transit behavior and nutrient digestibility in broiler chickens	33
Chapter 3	Quantification of total endogenous protein losses in broilers using a minimally invasive ^{15}N-isotope dilution method	59
Chapter 4	Effects of dietary fibers varying in physicochemical properties on total endogenous protein losses and protein digestibility in broilers	95
Chapter 5	Nutrient digestibility, total endogenous protein losses, and digesta mean retention time in broilers fed dietary insoluble fibers varying in particle size and hydration properties	123
Chapter 6	Gastrointestinal tract morphology, nutrient digestibility, and digesta mean retention time in laying hens of two different breeds fed with fine or coarse oat hulls	153
Chapter 7	General discussion	183
Summary		219
Acknowledgements		225
About the author		231


Soybean Hu
Wheat Bran

Chapter 1

General Introduction

BACKGROUND

Challenges of the global chicken industry

The global population is expected to reach 9.7 billion by 2050 (FAO, 2018), leading to a substantial increase in demand for animal-based protein (Hamad and Tayel, 2025; Boland et al., 2013). In response to this growing demand, poultry production is expected to expand considerably, largely due to its high efficiency in converting feed into edible protein for human consumption (Mottet and Tempio, 2017). Additionally, compared with other livestock industries, such as cattle and swine, the poultry industry plays a crucial role in meeting the global protein demand because of its relatively low production costs, rapid growth cycles, year-round availability, and limited cultural or religious constraints (Castro et al., 2023; Mottet and Tempio, 2017; Vaarst et al., 2015).

To meet the increasing global demand for poultry products, intensive genetic selection for productivity traits in both broilers and laying hens has resulted in modern genotypes characterized by high growth rates or elevated egg production (Hartcher and Lum, 2020; Kidd and Anderson, 2019). As a result, over the past decade, global egg production has increased by 24%, and chicken meat now accounts for nearly 40% of total meat production worldwide (FAO, 2023). However, these genetic improvements are typically associated with increased nutritional requirements, demanding the use of highly digestible diets to fully support the physiological potential of modern birds (Sell-Kubiak et al., 2017).

Despite its rapid expansion, the global poultry industry faces significant sustainability challenges. Feed availability is a major concern, particularly due to the increasing competition between poultry production, human food consumption, and biofuel production for high-quality feed ingredients (Muscat et al., 2020). Furthermore, the cultivation of the important feed ingredients, including maize and soybean, is associated with serious environmental impacts, including deforestation and biodiversity loss, especially in tropical rainforest regions (Bist et al., 2024; Gasparri et al., 2013; Mekonnen and Hoekstra, 2012; Morton et al., 2006). These challenges highlight the urgent need to identify more sustainable feed strategies for poultry production.

One of the promising approaches is the increased use of agricultural by-products that are not suitable for human consumption but still provide nutritional value or functional properties for animals. Such by-products have been proposed as partial substitutes for conventional feed ingredients, offering a potential source to reduce environmental pressure and improve the sustainability of poultry production systems (Sandström et al., 2022; Scholey et al., 2020; Jiménez-Moreno et al., 2013; Mateos et al., 2012).

Agricultural by-products are widely available worldwide, although their type and availability vary according to regional food production systems; for example, rice hulls are abundant in Asia, whereas wheat bran is commonly available in Europe. A shared characteristic of these by-products is their relatively high content of fibrous components, which requires careful evaluation before their inclusion in poultry diets. Dietary fibers (**DF**) differ markedly in their physicochemical properties, such as solubility, viscosity, and fermentability, which can strongly influence digestion processes along the gastrointestinal tract (**GIT**). Therefore, DF can affect nutrient digestibility and overall performance in chickens (Khalil et al., 2022; Morgan et al., 2022; Tejeda and Kim, 2021; Kheravii et al., 2018; Mateos et al., 2012; van Krimpen et al., 2011).

This challenge is further compounded in modern chicken breeds. Compared with traditional chicken breeds, modern high-performance breeds exhibit a limited capacity to cope with diets high in DF (Krás et al., 2013). This reduced adaptability often leads to impaired nutrient digestibility and decreased productive performance when high DF ingredients are included in the diet (Desbruslais et al., 2021; Pauwels et al., 2015; Barros de Carvalho et al., 2012; Havenstein et al., 1994). Understanding how DF with different physicochemical properties influence digestion processes is therefore essential to optimize the use of fiber-rich ingredients in sustainable poultry nutrition.

Understanding of dietary fibers in poultry nutrition

Dietary fibers consist of a heterogeneous group of plant cell wall components, primarily non-starch polysaccharides (**NSP**) and lignin (Bach Knudsen, 1997). These structural carbohydrates are inherently resistant to hydrolysis by endogenous digestive enzymes in chickens (Buchanan et al., 2007). As a result, DF are largely indigestible along most

segments of the GIT, although limited microbial fermentation may occur in the crop, small intestine, and hindgut (Classen et al., 2016; Kim et al., 2021; Ali et al., 2022). Consequently, these plant-derived components have traditionally been regarded as nutrient diluents and antinutritional factors that can impair nutrient digestibility (reviewed by Jha and Mishra, 2021). For instance, 3 g/kg guar gum added in the broiler diets decreased apparent total tract digestibility of protein by 5%-units, starch by 2%-units, and fat by 12%-units, when compared to the control diet (Maisonnier et al., 2001). In contrast, recent studies have found that the inclusion of 5 g/kg of oat hulls in broiler diets has been shown to increase gizzard weight and improve ileal apparent amino acids and metabolizable energy digestibility (Naeem et al., 2023).

Such different effects are largely attributable to differences in the physicochemical properties of DF (Vivares et al., 2025), which can greatly influence digestion processes in chickens but are often insufficiently considered in current feed formulation strategies. These properties include solubility, viscosity, hydration capacity, particle size, fermentability, and bulking effects (Guillon and Champ, 2000), and they vary substantially among DF sources, as illustrated in Table 1.1.

Table 1.1. Typically values of solubility and hydration properties of different sources of dietary fibers, as reported in literature[1].

Ingredients	Insoluble DF (%)	Soluble DF (%)	Water-holding capacity (g/g)[2]	Water-swelling capacity (mL/g)[3]
Oat hulls	94–99	1–6	3–5	2–4
Rice hulls	97–100	0–3	3–5	1–3
Soybean hulls	77–79	21–23	3–5	1–7
Sugar beet pulp	65–80	20–35	6–11	5–6
Sunflower hulls	89–96	4–11	4–7	2–5
Wheat bran	89–98	2–11	4–5	2–12
Pea hulls	89–90	10–11	2–3.	6–10
Rapeseed hulls	83–91	9–17	5–6	1
Purified wood lignocellulose	> 90.0	N/A	5–7	5–10
Purified wheat arabinoxylans	N/A	> 90.0	13–16	N/A

[1] Some information are N/A (not available).

[2] The ability of dietary fibers to absorb and retain the water within their structure.

[3] The ability of dietary fibers to absorb water and expand in volume.

(Auffret et al., 1994; Hori et al., 2000; Dust et al., 2004; Esposito et al., 2005; Fischer et al., 2012; Brachet et al., 2015; Jacometti et al., 2015; Kimiaeitalab et al., 2017; Slama et al., 2019; Shen et al., 2020; Berrocoso et al., 2020; Kaur et al., 2021; Lynch et al., 2021; Ramirez et al., 2021; Hussein et al., 2023; van Hees et al., 2023).

Among these characteristics, DF are most commonly classified based on their solubility in water into insoluble dietary fibers (**iDF**) and soluble dietary fibers (**sDF**) (Capuano, 2017). Although this binary classification is widely used, it represents a considerable simplification of the complex nature of DF physicochemical properties (Williams et al., 2019). Insoluble DF are typically poorly dispersible in water and, in practical feed formulation, are often used to provide dietary structure, thereby stimulating gizzard development and enhancing gut motility. In contrast, sDF exhibit hydrophilic properties that allow them to modulate digesta characteristics along the GIT. These effects include alterations in digesta viscosity, gel formation, bulking capacity, and nutrient-binding capacity, which can ultimately influence nutrient hydrolysis and absorption (Reviewed by Souffrant, 2001; Capuano, 2017).

The Influence of dietary fibers on the digestion processes in chickens

During digestion, feed materials are mechanically and chemically broken down into absorbable monomers as they pass through the GIT, forming a complex mixture known as digesta (Svihus, 2014; Lentle and Janssen, 2010). Digesta consists of hydrolyzed feed components together with endogenous materials, and its physical and chemical characteristics continuously change during transit through the different GIT segments. Typically, in chickens, as in pigs, the passage of digesta along the GIT is characterized by a separation into liquid and solid phases (Garçon et al., 2023; de Vries et al., 2014).

Liquid digesta, composed mainly of soluble and fine particles, generally pass more rapidly through the foregut than solid, fibrous, or coarse materials, whereas liquid digesta tend to be retained for longer time in the hindgut (Garçon et al., 2023). This digesta phase separation occurs because of the unique digestive system in chickens, which includes reflux mechanisms at three main sites: (1) from the gizzard to the proventriculus, (2) from the duodenum to the gizzard and proventriculus, and (3) from the cloaca to the ceca (reviewed by Svihus, 2014; Frei et al., 2017).

The presence of DF, particularly those differing in physicochemical properties such as solubility, can markedly alter digesta kinetics along the GIT. These alterations may affect endogenous enzyme secretion, microbial fermentation, interactions between digesta, enzymes, and the mucus layer, as well as nutrient hydrolysis and absorption. Ultimately, these processes influence nutrient digestibility and endogenous protein losses in chickens, as schematically illustrated in Figure 1.1.

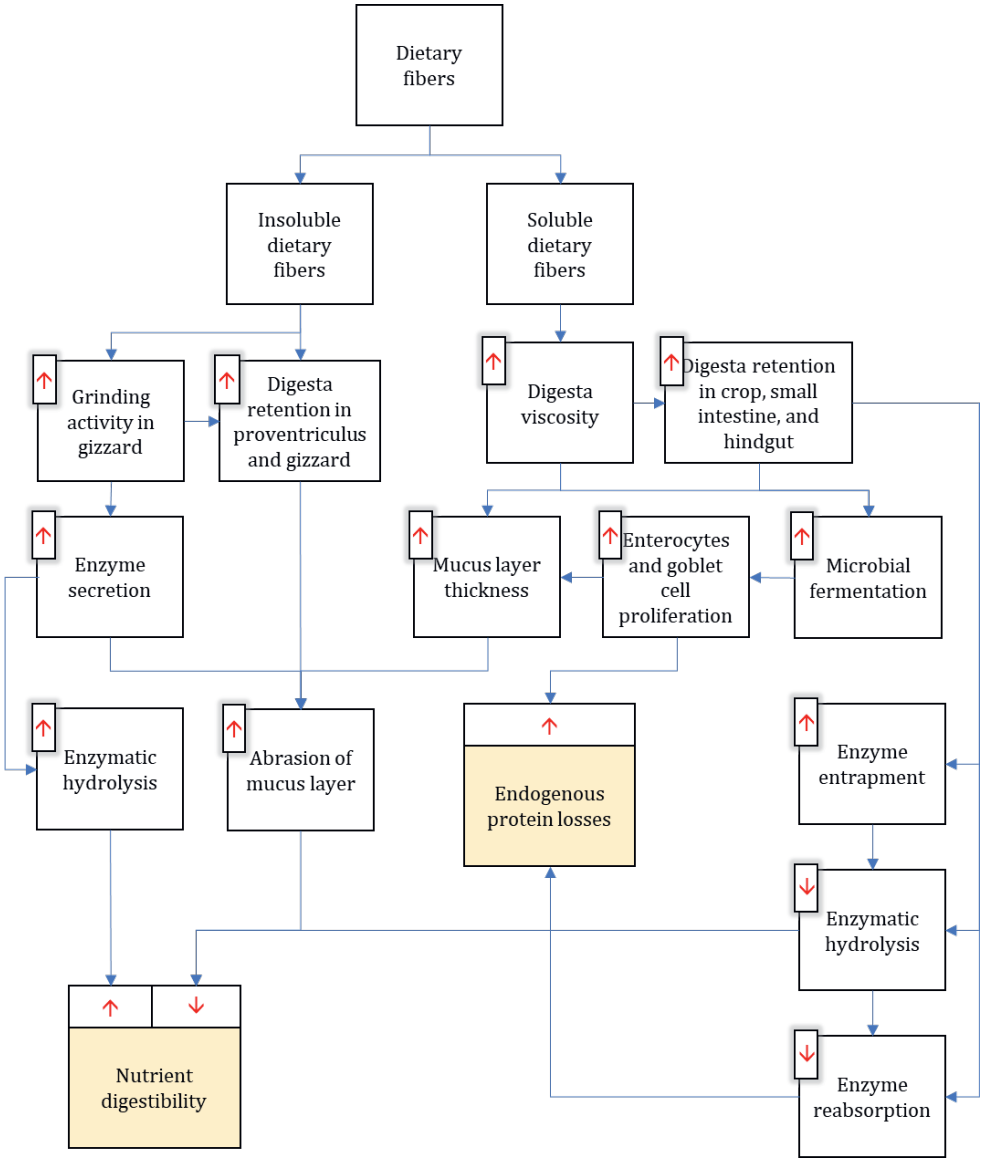


Figure 1.1. Theoretical framework illustrating the effects of soluble and insoluble dietary fibers on digestion processes in chickens. Blue arrows represent mechanistic interactions among digestion-related processes along the gastrointestinal tract. Red arrows indicate the direction of change (increase or decrease) in response to dietary fiber inclusion.

Digesta passage and nutrient digestibility

When feed is first ingested, it enters the crop, which serves as a temporary storage organ. In broilers and laying hens fed maize-based and insoluble fiber-rich diets, the retention time of solid digesta can be in a range between 1–67 min, whereas liquid digesta can be in a range between 6–88 min (Garçon et al., 2023; Rougière and Carré, 2010; Shires et al., 1987). The inclusion of sDF may promote gel formation and viscosity of the digesta that may prolong the digesta in this segment and enhance microbial fermentation of macronutrients. It has been reviewed that the crop plays a role in softening digesta and facilitating initial microbial degradation (Classen et al., 2016), which may influence the nutrient digestibility (Svihus et al., 2013). *Lactobacillus* spp. are the predominant microbes inhabiting the crop and contribute to starch hydrolysis during this early phase of digestion (Champ et al., 1983; Koenen et al., 2004).

After leaving the crop, digesta enter the proventriculus and are exposed to hydrochloric acid and pepsinogen, initiating protein hydrolysis through enzymatic digestion, before passing into the gizzard for mechanical grinding (Svihus, 2011). These two organs function synergistically as a single entity to process and prepare digesta for intestinal digestion (Rodrigues and Choct, 2018). Through the contraction mechanism, the gizzard acts as a sieve-like organ, allowing only digesta particles smaller than approximately 180 μm to pass into the duodenum (Hetland et al., 2002, 2003). During these contractions, some digesta may be refluxed into the proventriculus, further enhancing enzymatic exposure (Svihus, 2014). Consequently, solid digesta of broilers fed maize-based diets may be retained in the proventriculus and gizzard for 10 to 84 min (Garçon et al., 2023; Shires et al., 1987). The inclusion of iDF may stimulate grinding activity, prolong digesta retention, and increase gizzard weight compared with finer iDF or control diets (Amerah et al., 2007; Svihus, 2011; Garçon et al., 2023). The prolonged retention enhances the exposure of digesta to gastric and pancreatic enzymes, thereby promoting more extensive enzymatic hydrolysis and, ultimately, improving nutrient digestibility (Kheravii et al., 2017; Svihus, 2014; Washburn, 1991).

The small intestine is the primary site where the digestion and absorption of all macronutrients take place. Upon entering the duodenum, the acidic digesta is mixed with bile and pancreatic secretions, partly through a reflux mechanism (Svihus, 2014),

before nutrients are absorbed in the jejunum and ileum. The retention of solid digesta of broilers fed maize-based or insoluble fiber-rich diets ranges from 110 to 158 min and liquid digesta ranges from 87 to 129 min (Garçon et al., 2023). The increased digesta viscosity due to the presence of viscous sDF may slow down digesta passage through the small intestine (Blackwood et al., 2000; Lentle and Janssen, 2008; Wanders et al., 2013, 2014; Low et al., 2020) and impair nutrient digestibility. Viscous digesta can encapsulate nutrients within the plant cell matrix, inhibit digestive enzyme activity, reduce the interaction between nutrients and enzymes, and hinder nutrient diffusion across the absorptive surface of the small intestine (Grundy et al., 2016; Capuano, 2017; Karim et al., 2024; Henn and Alim, 2025).

Following passage through the ileocecal junction, digesta flows into the colon and cloaca. Upon reaching the cloaca, the liquid digesta is partially refluxed into the colon and ceca (Duke, 1989; Vergara et al., 1989). This reflux is driven by a slow-wave mechanism that facilitates the recovery of electrolytes, water, and nitrogenous sources (Björnhag, 1989; Vergara et al., 1989; Lai and Duke, 1978). Due to their narrow entrances, the ceca selectively retain mainly liquid or soluble digesta fractions (Vanderghinste et al., 2025; Björnhag, 1989; Vergara et al., 1989). Indeed, only ~13 to 50% of liquid digesta is transported into the ceca (Garçon et al., 2023; Vergara et al., 1989), where it may be retained for up to ~26 hours (Garçon et al., 2023). In the hindgut, especially in the ceca, however, studies on digesta behaviour influenced by the DF remain limited, whereas DF can serve as an energy source for cecal microbes for fermentation (Qiu et al., 2022). The viscous digesta due to the presence of sDF may limit liquid or soluble digesta to enter the ceca and prevent cecal microbial fermentation.

As noted, DF with varying solubility influence digesta passage and nutrient digestibility through different mechanisms. Insoluble DF, with a more structural role, are generally associated with improved digestibility of starch, protein, and fat, whereas sDF tend to reduce their digestibility due to increased hydration properties and viscosity of digesta. Limited information, however, is available regarding how solubility of DF affect the retention of liquid digesta and its subsequent relationship with nutrient digestibility in chickens, especially in laying hens.

Endogenous protein losses

The passage of digesta through the GIT of chickens inherently stimulates the secretion of endogenous proteins (Adeola et al., 2016). When these endogenous proteins are not reabsorbed, they contribute to endogenous protein losses (**EPL**), which may also be influenced by the present of DF (Ravindran, 2021). Endogenous protein losses originate from digestive enzymes, mucin protein, and sloughed intestinal epithelial cells, and depending on the definition, the bacterial proteins (Miner-Williams et al., 2009; Nyachoti et al., 1997), and can be classified into basal and specific EPL. Basal EPL represent the inevitable losses of endogenous protein within the GIT that are directly affected by dry matter intake, independent of the dry matter composition (Adeola et al., 2016). In contrast, diet-specific EPL are induced by the presence of particular dietary compounds, such as antinutritional factors or DF (Ravindran, 2016; Boisen and Moughan, 1996). Quantifying the effect of DF on specific EPL is complex; therefore, total EPL, which encompasses both basal and diet-specific losses, is more commonly measured in animals. An overview of the methodology used in this study to estimate total EPL is presented in Text Box 1.1.

Text Box 1.1: Quantification of total endogenous protein losses

In feed evaluation studies, basal EPL are frequently measured by using protein free diet method, but this method may result in the underestimation of basal EPL due to providing protein-deficient diets and does not allow to estimate diet-specific EPL (Ravindran, 2021). An alternative method involves enzymatically hydrolyzed casein-ultrafiltration, which uses a highly digestible protein source to more accurately quantify basal EPL and addresses limitations of the nitrogen-free diet method (Ravindran et al., 2004). Nevertheless, this method relies on a single purified protein source and is therefore restricted to the estimation of basal EPL, making it unsuitable for quantifying diet-specific EPL in animals fed a mix of feed ingredients.

One of the methods to measure total (basal and specific) EPL has predominantly been done in pigs using the ^{15}N-isotope dilution method (Schulze et al., 1995; Simon et al.,

1987). In principle, this method distinguishes endogenous protein from unabsorbed dietary protein by isotopically labeling either the animal's body or the diet with ^{15}N and subsequently measuring ^{15}N-enrichment in intestinal digesta or feces. This has been achieved by administering ^{15}N-leucine intravenously or orally, with blood plasma ^{15}N-enrichment serving as a reliable proxy for endogenous protein present in the gut lumen (Steendam et al., 2004; Gabert et al., 1997; Schulze et al., 1995).

Despite its accuracy, the ^{15}N-isotope dilution method requires frequent blood sampling, resulting in invasive and complex experimental designs, as well as limited practical applicability, particularly in poultry. As an alternative, Dänicke et al. (2000) proposed a minimally invasive ^{15}N-isotope dilution method using urinary ^{15}N-enrichment as a proxy for endogenous protein in the gut lumen, based on its similarity with ^{15}N-enrichment in tissues contributing to endogenous protein found in digesta, including the liver, pancreas, and small intestine. However, the relation between the ^{15}N-enrichment in urine and in those tissues requires further validation, especially when chickens offered diets with fiber-rich ingredients.

Both iDF and sDF may influence EPL, but the underlying mechanism can differ due to their different physicochemical properties (Reviewed by Montagne et al., 2003). The longer retention time in the proventriculus and gizzard due to the presence of iDF (Svihus, 2011) allows an increase pancreatic and bile acid secretion (Langlois et al., 1987; Hetland et al., 2003). Previous studies reported that a diet containing 10 g/kg iDF increased pancreatic secretion by 24% in laying hens (Yokhana et al., 2016), while the inclusion of 30 g/kg iDF increased bile acid secretion by 32–33% in broilers (Hetland et al., 2003), compared to control diets. Furthermore, due to the abrasive action, it was expected that iDF may influence the scraping mucin from the mucosa throughout the GIT (Reviewed by McRorie and McKeown, 2017).

Contrastingly, the increased digesta viscosity due to the presence of sDF may promote microbial fermentation (Reviewed by Annison, 1993), which increases enterocytes and goblet cell proliferation and finally increase mucus layer thickness (Reviewed by Ali et al., 2022). Additionally, viscous digesta may bind secreted enzymes and reduce their reabsorption in the small intestine (Jiménez-Escrig & Sánchez-Muniz, 2000; Wang et al.,

2021). A previous study showed that the inclusion of 15 g/kg wheat pentosanes in broiler diets increased endogenous total amino acids by ~44% (Angkanaporn et al., 1994), while replacing 10 g/kg cellulose with guar gum increased intestinal cell proliferation by ~35% (Gee et al., 1996).

Apparent and true protein digestibility

Digestibility measurements are essential tools to identify proteins and amino acids available in the poultry feed ingredients. Apparent digestibility coefficients are commonly used to assess the nutritional value of feedstuffs because they are relatively straightforward to determine. This approach, however, is based solely on the net disappearance of ingested protein or amino acids along GIT and does not account for total EPL. As a consequence, apparent digestibility often underestimates the true digestibility of dietary protein, particularly in diets that stimulate endogenous secretions, including fiber-rich ingredients (Adedokun et al., 2011). Under such conditions, the change in apparent protein or amino acids digestibility may be reflected by two processes, which the change in EPL and/or the change in enzymatic digestion or absorption of dietary protein. Therefore, correcting apparent digestibility values for total EPL allows a distinction to be made between dietary effects on endogenous protein secretion and effects on the true digestibility of dietary protein.

This distinction is illustrated by Steendam et al. (2004), reported that pigs fed a quebracho-containing diet exhibited 16%-units lower apparent protein digestibility and a 67% increase in total EPL compared with a control diet. After correcting for total EPL, however, true protein digestibility did not differ between treatments, indicating that the dietary effect was primarily mediated through increased EPL rather than reduced digestion of dietary protein. These findings highlight the importance of quantifying total EPL when evaluating protein utilization in poultry diets, particularly when dietary interventions, such as the inclusion of fiber-rich ingredients, are expected to influence endogenous secretions. Distinguishing between apparent and true protein digestibility is therefore essential to correctly interpret the nutritional consequences of DF.

KNOWLEDGE GAP

Current evidence indicates that the inclusion of fiber-rich ingredients in chicken diets may influence nutrient hydrolysis and absorption. Nevertheless, information remains limited on the effects of DF varying in physicochemical properties on retention time of solid and liquid digesta, total EPL, and nutrient digestibility in chickens. This gap is particularly relevant given the increasing interest in incorporating agricultural by-products, many of which are rich in fiber, into poultry diets. A clearer understanding of the role of DF physicochemical properties in digestion processes would enable to consider their inclusion in feed formulation strategies and provide a clearer biological explanation for evaluating fiber-rich ingredients in poultry nutrition.

AIM AND OUTLINE OF THE THESIS

The aim of this research was to study the effects of DF varying in physicochemical properties on digesta passage, EPL, and nutrient digestibility of the chickens. To address this aim, **Chapter 2** examined the effects of viscous sDF inclusion, in the form of purified arabinoxylans, in diets containing fine or coarse soybean hulls, as iDF source, on GIT development, retention time of solid and liquid digesta, and apparent nutrient digestibility in broilers. **Chapter 3** focused on evaluating a minimally invasive ^{15}N-isotope dilution methods in broilers fed diets used in **Chapter 2**, as well as a diet containing fine sugar beet pulp as a substitution for fine soybean hulls, or a semi-synthetic, highly digestible diet containing enzymatically hydrolyzed casein. In this chapter, ^{15}N-enrichment in the urine was verified for ^{15}N-enrichment in the gut lumen, as a basis to calculate the proportion of total EPL. Additionally, because the absence of a gold-standard method in the quantification of total EPL, ^{15}N-isotope dilution was compared with enzymatically hydrolyzed casein and -ultrafiltration methods. **In Chapter 4**, the effects of the diets used in **Chapter 2**, as well as a diet containing fine sugar beet pulp, were investigated with respect to total EPL and true protein digestibility. **Chapter 5** further explored the effect of coarse or fine hulls, or purified lignocellulose, iDF source with with high hydration capacity on total EPL, apparent nutrient digestibility, true protein digestibility, and retention time of solid digesta in broilers. Furthermore, **in Chapter 6**, the effects of coarse vs. fine oat hulls on GIT

development, retention time of solid and liquid digesta, and apparent nutrient digestibility were evaluated in two laying hen breeds. Finally, **Chapter 7** synthesizes the main findings of this thesis, providing an integrated perspective on how fiber-rich ingredients influence digestion processes in chickens, how these insights can inform current feeding strategies, and how future research on DF may further improve sustainable poultry nutrition. The overall structure and interconnection of the experimental chapters are graphically summarized in Figure 2.1.

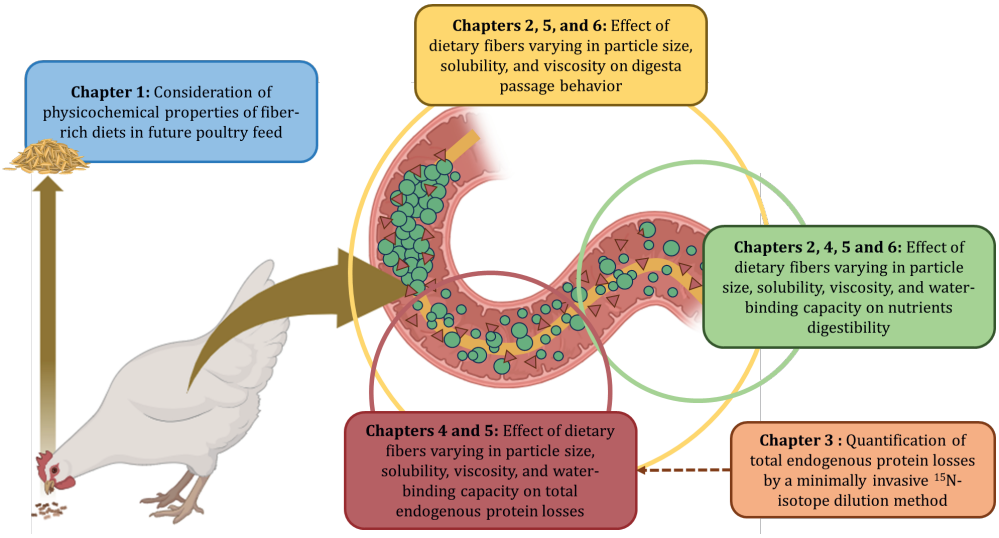


Figure 2.1. Overview of the topics and chapters of this thesis (images were generated from Biorender.com).

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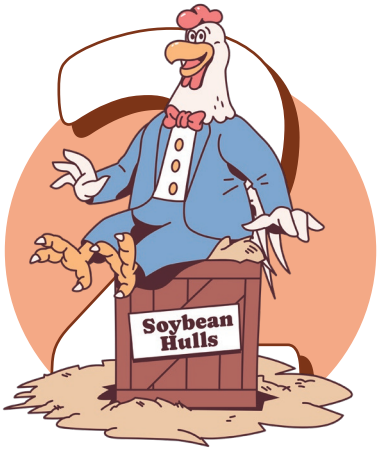
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Soybean
Hulls

Chapter 2

Effect of adding soluble viscous fibers to diets containing coarse and finely ground insoluble fibers on digesta transit behavior and nutrient digestibility in broiler chickens

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ABSTRACT

This paper aimed to study the interactive effects of the addition of soluble arabinoxylans (AX) and the particle size (PS) of soybean hulls (SBH) on digesta mean retention time (MRT) and nutrient digestibility in broiler chickens. A total of 288 one-day old Ross 308 female chicks were assigned to 32 pens (9 birds/pen) and fed a commercial starter diet for ten days. At 10 days of age, pens were assigned to one of four dietary treatments (8 pens/diet) containing 120 g/kg coarse or fine SBH, with or without addition of 50 g/kg of soluble wheat AX, substituting maize starch. Titanium dioxide (4 g/kg) and cobalt-EDTA (1 g/kg) were added as inert markers. Excreta were quantitatively collected from day 22 - 25. Gastrointestinal tract and digesta were collected on days 28, 29, or 30. Arabinoxylans reduced the weight of the gizzard relative to body weight (RW) by 0.07% units ($P = 0.005$), and increased ceca RW (0.28 vs. 0.34%, $P < 0.001$) and length (10.45 vs. 11.21 cm/kg BW, $P < 0.001$). Arabinoxylans increased digesta MRT in the crop (solids/liquids: +12 min, $P < 0.05$), small intestine (solids/liquids: +17 min, $P < 0.01$), and hindgut (liquids: +77.5 min, $P < 0.05$); and reduced apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of DM (-5.4 and -3.9%, $P < 0.001$, respectively) and starch (-1.35 and -0.7%, $P < 0.001$, respectively). Particle size of SBH only affected the ATTD of non-starch polysaccharides, presenting higher digestibility values with fine SBH (-4.3%-units, $P = 0.034$). The addition of AX reduced AID of protein by 4.3%-units, only in presence of fine SBH (interaction, $P < 0.05$). In conclusion, arabinoxylans greatly influenced digestion in the chicken GIT, while PS of SBH had marginal effects. Arabinoxylans reduced AID of protein only with fine SBH, suggesting coarse SBH counteracted AX effects on protein digestion, speculatively by modifying digesta viscosity.

INTRODUCTION

The increased use of by-products from agricultural and food industries in animal diets has stimulated extensive research to comprehend the impact of dietary fibers (**DF**) on digestion processes. Dietary fiber has the potential to influence the digestive physiology of monogastric animals by modulating the physicochemical properties of the digesta (Smits et al., 2000), digesta transit behavior along the gastrointestinal tract (**GIT**)

(Svihus et al., 2002), and by serving as a substrate for microbial fermentation (reviewed by Williams et al., 2019). However, due to significant peculiarities in the chemical and structural characteristics of DF (Bach Knudsen, 1997), their effects on digestion processes and, ultimately, nutrient digestibility, may vary among sources, leading to either beneficial or detrimental outcomes for the animals.

In chickens, the addition of insoluble DF (**iDF**) has been shown to have positive effects on the development of the gizzard (Hetland et al., 2003; Sadeghi et al., 2020), consequently allowing a better grinding and mixing of digesta with digestive enzymes (reviewed by Svihus, 2011). Several studies have reported no effect or even improvements in nutrient digestibility and productive performance when iDF were added to the diets of broilers and laying hens, even though the degradation of iDF in poultry is negligible (Jørgensen et al., 1996; Hetland et al., 2003; González-Alvarado et al., 2007; Jiménez-Moreno et al., 2011; Kalmendal et al., 2011; Yokhana et al., 2016; Tejeda and Kim, 2020; Zhang et al., 2023). Furthermore, there is evidence that particle size (**PS**) of iDF is partially responsible for the iDF effects on gastrointestinal tract development (Jiménez-Moreno et al., 2010), digesta retention time (Moore, 1999), and nutrient digestibility (Donadelli et al., 2019; Pourazadi et al., 2020) in poultry. For instance, Sacranie et al. (2012) reported that adding 150 g/kg of a 50:50 mixture of oat and barley hulls to broiler diets resulted in a fuller and larger gizzard, with birds fed the coarse hulls presenting the largest gizzards. Moreover, Hetland et al. (2005) observed that about 30% of coarse (unground) oat hulls were still found in the gizzard 48 hours post feeding, while 90% of fine (ground using a 0.5 mm sieve) oat hulls had passed the gizzard after 2 hours.

Although iDF may be partly beneficial for broilers, the addition of soluble DF (**sDF**) has been mainly associated with negative effects. Several studies have reported that the addition of sDF increase digesta viscosity, modulates digesta retention time, and has negative effects on nutrient digestibility and productive performance (van der Klis and van Voorst, 1993; Van Der Klis et al., 1993; Saki et al., 2011).

However, there is limited understanding regarding how the addition of sDF may impact the digestion process in the presence of various PS of iDF, as well as the potential interactions between these factors. Since digesta consists of a combination of solid

particles suspended in a liquid, alterations in viscosity, which is a natural property of liquids, may impact the digestion processes differently when particle size is modified. Therefore, the objective of this paper was to study the effects of adding soluble viscous fibers, in form of arabinoxylans (**AX**), to diets containing coarse versus finely ground insoluble fibers, in the form of soybean hulls (**SBH**), on digesta mean retention time (**MRT**), and nutrient digestibility in broiler chickens. We hypothesized that coarse iDF will prolong solid digesta MRT in the proximal GIT, and improve nutrient digestibility, while AX addition will prolong liquid digesta MRT and reduce nutrient digestibility. Moreover, the negative effect of viscous fibers over nutrient digestibility is expected to be reduced in the presence of fine PS of the iDF.

MATERIAL AND METHODS

Ethics declaration

The experiment was approved by the National Central Committee of Animal Experiments (CCD) under the permit number AVD1040020197324, in accordance with the Dutch Act on Animal Experimentation and EU Directive 2010/63/EU; and performed at the research facility Carus, Wageningen University & Research, (Wageningen, The Netherlands).

Animals and housing

A total of 288 female one-day-old Ross 308 broiler chicks (initial body weight: 40.7 ± 3.22 g) were randomly distributed over 48 pens, and pens were assigned to 1 of 4 treatments following a randomized block design with a 2 x 2 factorial arrangement of treatments. Each treatment had 8 replicate pens of 9 birds each. Under controlled environmental conditions, each pen was equipped with plastic slatted floor covered with cardboard matting and wood shavings, 6 nipple drinkers, 1 hopper feeder and 1 perch. Birds were vaccinated at 14 days of age against Newcastle disease.

Dietary treatments and diets

During the first 10 days, all birds were fed a commercial pelleted maize-soybean based starter diet. From day 10 and until the end of the experiment (day 30), the pelleted

experimental growing diets were offered (Table 1.2). A maize-based diet with soy protein concentrate, fish meal, potato protein, and 120 g/kg of SBH was formulated to meet or exceed the requirements for growing broiler chickens (Aviagen, 2019a; CVB, 2018). We formulated 4 dietary treatments, where SBH were included in coarse (**C-SBH**: unground; geometric mean diameter (**GMD**) 1134 µm ± 144 µm) or fine (**F-SBH**; ground with a 1 mm screen, GMD 459 µm ± 85 µm) form; and 50 g/kg soluble wheat arabinoxylans (Naxus®, BioActor BV, Maastricht, The Netherlands) were substituted for maize starch (**C-SBH-AX** and **F-SBH-AX**) or not (C-SBH and F-SBH). All the experimental diets contained 4 g/kg of titanium dioxide (TiO_2) as inert markers to represent the solid digesta phase and 1 g/kg of cobalt-EDTA (Co-EDTA) to represent the liquid phase. Feed and water were available ad libitum during the whole experiment.

Table 1.2. Ingredient and nutritional composition of experimental diets fed to female growing broiler chickens from 10 to 30 days of age.

Item	C-SBH / F-SBH[1]	C-SBH-AX / F-SBH-AX[2]
Ingredient composition (g/kg)		
Maize	450.0	450.0
Maize Starch	175.1	126.1
Soy protein concentrate	126.0	126.0
Soybean hulls	120.1	120.1
Arabinoxylans	-	50.0
Sugar	28.4	28.4
Potato protein	1.5	1.5
Fish meal	24.8	24.8
Soybean oil	15.0	15.0
Potassium carbonate	3.9	3.9
Sodium bicarbonate	2.0	2.0
L-Lysine	5.0	5.0
D-Methionine	4.1	4.1
L-Threonine	2.7	2.7
L-Tryptophane	0.2	0.2
L-Isoleucine	1.2	1.2
L-Arginine	3.2	3.2
L-Leucine	0.5	0.5
L-Valine	2.4	2.4
Mineral and vitamin Premix[3]	5.0	5.0
Monocalcium phosphate	11.7	11.7
Salt	1.2	1.2
Calcium carbonate	9.0	8.0

Cobalt-EDTA	1.0	1.0
Titanium dioxide	4.0	4.0
Polyethylene glycol	2.0	2.0
Total composition (g/kg)	1000.0	1000.0
Calculated nutritional composition[4] (g/kg)		
AME[5] (MJ/kg)	12.7	-
Digestible lysine[6]	11.1	-
Digestible methionine + cysteine[6]	7.2	-
Digestible threonine[6]	7.6	-
Calcium	7.2	-
Phosphorus	2.9	-
Analysed nutritional composition (g/kg)		
Dry matter	902.1	900.7
Crude Protein[7]	171.8	180.7
Crude Fat	35.5	37.7
Crude ash	50.6	51.4
Total non-starch polysaccharides	116.5	139.6

[1] C-SBH: soybean hulls coarse; F-SBH: soybean hulls fine.

[2] Arabinoxylans (AX) (Naxus®, BioActor BV, Maastricht, The Netherlands).

[3] Premix provided per kilogram of diet: Vitamin A (retinyl acetate), 10.000 IU; Vitamin D_3 (cholecalciferol), 2.500 IU; Vitamin E (dl-a-tocopherol), 50 mg; Vitamin K_3 (menadione), 1.5 mg; Vitamin B_1 (thiamin), 2.0 mg; Vitamin B_2 (riboflavin), 7.5 mg; Vitamin B_6 (pyridoxin-HCl), 3.5 mg; Vitamin B_{12} (cyanocobalamin), 20 µg; Niacin, 35 mg; D-pantothenic acid, 12 mg; Choline chloride, 460 mg; Folic acid, 1.0 mg; Biotin, 0.2 mg; Iron, 80 mg, as $FeSo_4$; Copper, 12 mg, as $CuSO_4$; Manganese, 85 mg, as MnO; Zinc, 60 mg, as $ZnSO_4$; Iodate, 0.8 mg, as KJ; Selenium, 0.15 mg, as Na_2SeO_3.

[4] Calculated nutritional composition based on the composition and nutritional value of feed materials (CVB, 2018). Digestible amino acids and metabolizable energy values were calculated based on values of standardized ileal digestibility and apparent metabolizable energy for broilers.

[5] Apparent metabolizable energy for broilers.

[6] Standardized ileal digestible amino acids for broilers.

[7] Nitrogen × 6.25.

Sampling and data collection

Birds were individually weighed on day 1, 10, 21, and immediately prior to being euthanized. Feed consumption per pen was measured at day 10, 21, and 25. On day 18, the cardboard matting and wood shavings were removed to prevent ingestion of bedding. From day 22 to 25, excreta were quantitatively collected and weighed per pen twice a day (at 8:00 and 14:00). A subsample of clean excreta was collected and frozen immediately in the freezer at –20°C. All the daily collected excreta subsamples from each pen during these 4 days were then homogenously mixed in a bucket and a portion was taken for the analysis of apparent total tract retention.

At day 28, 29, or 30, we euthanized 6 birds/pen (192 in total), according to a dissection schedule designed to obtain digesta flowing curves for the liquid and solid phase, after a pulse dose of markers (data not shown). An injection of 0.5 mL sodium pentobarbital (20% or 500 mg/mL) was applied to each bird at the base of the back edge of the skull. The whole gastrointestinal tract was removed, and divided into 7 segments (crop, proventriculus, gizzard, first half of small intestine (**SI**) until Meckel's diverticulum, second half of SI until ileo-ceca junction, ceca, and colon) using tie wraps. Each segment was weighed full and empty, and digestive contents were quantitatively collected and frozen at -20°C per bird. Moreover, the thickest part of the gizzard wall was measured with a caliper (mm), and the length of each cecum per bird was measured using a measuring tape. The measurement of GIT traits was conducted as supplementary data to provide further understanding of the potential effects of fibers on the digestion process.

The contents from the gizzard and proventriculus were combined and treated as a unified compartment (hereafter referred to as Stomach) for analysis of digesta MRT. Also, the MRT of ceca and colon was analyzed as one compartment (hereafter referred to as hindgut). The reasoning for this is the occurrence of antiperistatical movements in the passage of digesta between these organs (Duke, 1982), which invalidates the assumptions made for measuring digesta retention time using markers (de Vries and Gerrits, 2018). Crop and stomach samples were pooled and analyzed per pen, while SI, ceca and colon samples were analyzed per bird (for another experiment). Subsamples were obtained from the distal SI and pooled per pen to analyze apparent ileal digestibility of nutrients. The rest of digesta content from the distal SI segment was then mixed with a subsample of digesta from the proximal SI, both with the same weight proportion, to create a representative sample of all the small intestine per bird.

During the experimental period, approximately 500 g of each experimental diet was randomly collected from different feeders at a single time point, and the samples were stored in sealed and labelled plastic bags at 4°C until their chemical composition was analyzed.

Analyses and calculations

Particle size analysis

Particle size distribution, geometric mean diameter **(GMD**), and geometric standard deviation (**GSD**) of C-SBH and F-SBH were analyzed in duplicate using dry sieving analysis (ASABE, 2008). Geometric mean diameter and GSD of the fibrous ingredients were calculated as described by Lyu et al. (2020).

Water-binding capacity

Samples were mixed with deionized water inside a conical tube (50 ml) using a vortex (2500 rpm) for 5 s to ensure thorough mixing of the components. The WBC of fibers and diets was analyzed in duplicate based on Jacobs et al. (2015).

Digesta mean retention time

Mean retention time of solid and liquid digesta (min) was calculated using Ti and Co as inert markers. Under continuous dosing (steady state) condition, the marker input equals the output in each segment of the GIT. Thus, MRT was calculated using the following equation (de Vries and Gerrits, 2018):

$$\text{MRT} = 1440 \times \frac{T_{sample} \times Q_{sample}}{T_{diet}\,(intake/day)}$$

where T_{sample} is the concentration of the marker Ti (g/kg) or Co (mg/kg) in the sample (digesta of each segment), T_{diet} is the concentration of the marker in the feed, and Qsample is the quantity of the sample (kg). To calculate the marker consumption, an average feed intake was calculated from the data collected from day 21 to 25 and extrapolated to the day of euthanasia for each bird. Digesta phase segregation (min) was calculated as the difference between the MRT of the digesta phases and presented as absolute values.

Nutrient digestibility and retention

Samples of ileal digesta and excreta were freeze-dried prior to analyses, diet samples were analyzed on as-fed basis. All samples were ground using a centrifugal mill with a 1 mm sieve (Retsch ZM 200). Feed, ileal digesta, and excreta were analyzed for dry

matter (DM; ISO 6496:1999), starch (ISO 15914:2004), nitrogen (N; ISO 5983:2005), non-starch polysaccharides (NSP; Englyst et al., 1994), Ti and Co (van Bussel et al., 2010). Feed were additionally analyzed for fat after acid hydrolysis (ISO 6492:1999) and ash (ISO 5984:2002). The apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) of DM, starch, protein, and non-starch polysaccharides (**NSP**) (%) were calculated using the following equation:

$$\text{AID or ATTD} = 100\left[1 - \left(\frac{T_{diet}}{T_{sample}}\right) \times \left(\frac{Nutrient_{sample}}{Nutrient_{diet}}\right)\right]$$

where T_{diet} is the concentration of Ti in the feed, and T_{sample} in the ileal digesta or in the excreta. Moreover, $Nutrient_{diet}$ is the concentration of nutrient (DM, nitrogen, starch, NSP) in the feed and $Nutrient_{sample}$ in the ileal digesta or in the excreta. All the units are in g/kg.

Statistical analyses

Pen (6 birds/pen for all variables, except for ATTD, 9 birds/pen) was considered the experimental unit for all the analysis. Data were analyzed using a general linear model (PROC GLM, SAS 9.4) with GIT traits, digesta MRT, digesta phase segregation, and digestibility or retention values as dependent variables, and AX, PS, and their interaction as fixed effects. Model residuals were visually assessed to verify model assumptions. Crop relative weight (**RW**), proventriculus RW, and the MRT for both solid and liquid digesta in the crop and stomach did not meet the assumption of normal distribution of the model residuals. Hence, these parameters were logarithmic transformed, prior to statistical analyses. Differences among means were tested using type III least squares statistics, using Tukey adjustments for multiple comparisons. Data are presented as (back transformed) estimated means and pooled SEM. Differences among means were considered to be statistically significant when $P < 0.05$. A Pearson's correlation analysis (PROC CORR, SAS 9.4) was conducted to study the correlations among DM and starch disappearance in the hindgut (ATTD-AID), ATTD of NSP, ceca length and ceca RW.

RESULTS

The grinding procedure decreased the GMD of SBH by almost 2.5 times, reaching a contrast in particle size of 675 µm (1134 ± 144 µm vs 459 ± 85 µm) (Table 2.2). Particles larger than 630 mm, accounting for approximately 95% of the total C-SBH, were reduced to only 20% of the total F-SBH.

Table 2.2. Particle size distribution (%), geometric mean diameter (GMD, µm), and geometric standard deviation (GSD, µm) of coarse and fine soybean hulls (SBH), measured using dry sieving method[1,2,3]

Feed ingredient	Nominal sieve aperture, µm							GMD, µm	GSD, µm
	2500	1250	630	315	160	71	<71		
Coarse soybean hulls	0.75	39.15	55.59	2.79	0.46	0.37	0.99	1134	144
Fine soybean hulls	0.00	0.02	20.27	55.78	12.21	5.52	6.44	459	85

[1] Coarse hulls offered intact.
[2] Fine hulls ground in a hammermill with a screen size of 1.0 mm.
[3] GMD (n) = 2 replicates.

Gastrointestinal tract traits

The addition of AX reduced gizzard (1.42 vs. 1.36%, P = 0.005) and stomach (2.01 vs. 1.90%, P = 0.017) weight relative to body weight (Table 3.2). However, ceca weight (0.34 vs. 0.28%, P < 0.001), and ceca length relative to body weight (11.21 vs. 10.45%, P = 0.001) increased with the addition of AX. Particle size of the SBH did not have any impact on the GIT traits (P > 0.05) and no interactions between particle size and AX were observed.

Mean retention time of digesta phases along the gastrointestinal tract

The addition of AX in the diets resulted in an increase in MRT for both digesta solids and liquids (+12 min for both) in the crop (P < 0.05) (Table 4.2). Neither the particle size of SBH nor the addition of AX affected the MRT of solid or liquid digesta in the stomach (P > 0.05). Segregation of digesta phases in the stomach, however, tended to be reduced by F-SBH by 6 minutes (P = 0.061) compared with C-SBH.

In the small intestine, the addition of AX led to a 17-minute increase in MRT for digesta solids ($P = 0.007$) and a 22-minute increase for digesta liquids ($P < 0.001$), while the particle size of SBH did not affect digesta MRT. In the hindgut, the addition of AX resulted in a prolonged MRT of liquid digesta (220 vs. 297 min, $P = 0.020$), causing a significant increase in digesta phase segregation of 68 min ($P = 0.032$). No interactions between particle size and AX were observed among treatments.

Nutrient digestibility

The addition of 50 g/kg AX in the diet decreased the AID and ATTD of DM and starch ($P < 0.05$), regardless of the particle size of SBH (Table 5.2). The AID of protein was reduced by 4%-points when AX was added in the diet with F-SBH but not with C-SBH (interaction, $P = 0.024$). Additionally, the ATTD of NSP increased by 2.4% units with F-SBH ($P = 0.034$) and by 4.3% units with the addition of AX ($P < 0.001$)

Table 3.2. Effect of arabinoxylan (AX) addition (50 g/kg) to diets with different particle size of soybean hulls (SBH) on body weight, feed intake, and gastrointestinal traits of broiler chickens measured at 28, 29, or 30 days of age[1,2,3]

Particle size	Coarse		Fine		Pooled	Particle	AX	Particle
AX (g/kg)	0	50	0	50	SEM	size		size*AX
Daily feed intake (g/day)	96 ± 4.2	96 ± 6.9	97 ± 3.6	96 ± 5.6	-	-	-	-
Body weight (g)	1360 ± 71.7	1404 ± 50.8	1323 ± 46.3	1347 ± 63.6	-	-	-	-
Relative weight (RW, %)								
Crop	0.27	0.29	0.29	0.29	0.011	0.263	0.706	0.554
Proventriculus	0.57	0.60	0.55	0.55	0.030	0.739	0.218	0.562
Gizzard	1.43	1.36	1.41	1.35	0.026	0.981	0.005	0.517
Stomach	2.01	1.89	2.01	1.90	0.046	0.862	0.017	0.989
Ceca	0.27	0.34	0.29	0.33	0.010	0.757	<0.001	0.357
Gizzard thickness (mm)	9.0	9.0	8.9	9.0	0.26	0.811	0.837	0.753
Ceca length (cm/kg BW)	10.3	11.1	10.6	11.3	0.20	0.165	0.001	0.635

[1] n = 8 replicate pens (6 birds/pen) for all treatments.

[2] Stomach: Proventriculus + Gizzard.

[3] Coarse: hulls offered unground; Fine: hulls ground in a hammermill with a screen size of 1.0 mm.

Table 4.2. Effect of arabinoxylan (AX) addition (50 g/kg) to diets with different particle size of soybean hulls (SBH) on the mean retention time (MRT, min) of the solid and liquid digesta phase in the crop, proventriculus and gizzard (stomach), small intestine, caeca and colon (hindgut) in broiler chickens, measured at 28, 29, or 30 days of age[1,2,3,4]

Segment	Digesta fraction	Particle size	Coarse		Fine		Pooled SEM	Particle size	AX	Particle size*AX
		AX (g/kg)	0	50	0	50				
Crop	Solids		16	30	18	29	4.8	0.819	0.014	0.691
	Liquids		15	29	17	27	4.5	0.899	0.010	0.745
	Phase segregation (min)		1	1	2	1	0.5	0.533	0.921	0.332
Stomach	Solids		77	75	72	59	8.6	0.218	0.375	0.504
	Liquids		45	45	44	40	4.9	0.509	0.763	0.640
	Phase segregation (min)		32	29	28	19	4.1	0.061	0.107	0.371
Small Intestine	Solids		235	247	221	243	6.0	0.142	0.007	0.422
	Liquids		200	218	190	216	5.4	0.264	<0.001	0.429
	Phase segregation (min)		34	29	31	27	3.0	0.340	0. 169	0.856
Hindgut	Solids		49	65	57	61	5.2	0.749	0.080	0.238
	Liquids		189	297	250	297	31.3	0.331	0.020	0.337
	Phase segregation (min)		140	232	193	237	30.0	0.338	0.032	0.425
Total GIT	Solids		380	428	380	399	18.1	0.429	0.077	0.436
	Liquids		451	594	509	587	35.2	0.478	0.004	0.357
	Phase segregation (min)		71	167	130	188	30.2	0.198	0.017	0.541

[1] n = 8 replicate pens (6 birds/pen) for all treatments.

[2] Coarse: hulls offered unground; Fine: hulls ground in a hammermill with a screen size of 1.0 mm.

[3] GIT: gastrointestinal tract.

[4] Phase segregation (min) presented as absolute values.

Table 5.2. Effect of arabinoxylan (AX) addition (50 g/kg) to diets with different particle size of soybean hulls (SBH) on the apparent ileal digestibility (AID, %) and apparent total tract digestibility (ATTD, %) of dry matter (DM), protein, starch, and total non-starch polysaccharides (NSP) in broiler chickens[1,2]

Particle size	Coarse		Fine		Pooled SEM	Particle size	AX	Particle size* AX
AX (g/kg)	0	50	0	50				
AID								
DM	68.9	64.8	68.6	62.0	0.88	0.088	<0.001	0.165
Starch	96.2	95.1	96.2	94.6	0.18	0.185	<0.001	0.172
Protein	76.4[a]	75.1[a]	76.6[a]	72.3[b]	0.61	0.045	<0.001	0.024
ATTD								
DM	72.0	68.2	72.0	68.0	0.26	0.641	<0.001	0.608
Starch	97.6	96.9	97.7	97.0	0.09	0.258	<0.001	0.957
NSP	4.8	8.3	6.4	11.4	1.05	0.034	<0.001	0.484

[1] n = 8 replicate pens (6 birds/pen for AID, 9 birds/pen for ATTD) for all treatments.

[2] Coarse: hulls offered unground; Fine: hulls ground in a hammermill with a screen size of 1.0 mm.

[a, b] Means within a row lacking a common superscript differ ($P < 0.05$).

DISCUSSION

The aim of this study was to elucidate how incorporating soluble, viscous fibers (AX) into diets containing iDF, in the form of SBH with different PS, affects digesta mean retention time and nutrient digestibility in broiler chickens. Our results reveal that the addition of AX modified some GIT traits, prolonged digesta MRT throughout the GIT, and reduced nutrient digestibility, mostly regardless of the PS of SBH. Moreover, PS of SBH only had an effect on ATTD of NSP, increasing NSP degradation when F-SBH was used; and had no effects on GIT traits or digesta MRT. Finally, AID of protein was reduced when adding AX to the diets with F-SBH but not with C-SBH.

In the current study, the addition of 50 g/kg AX had a considerable impact on digestion processes in the proximal and distal GIT. In the proximal GIT, it reduced the relative weight of the gizzard and prolonged digesta MRT in the crop and the SI. The prolonged MRT coincided with reduced AID of nutrients as indicated by negative correlations between the MRT of liquid digesta in the SI and AID of DM ($r = -0.37$, $P = 0.036$), protein ($r = -0.36$, $P = 0.036$), and starch ($r = -0.34$, $P = 0.060$). This correlation illustrates that prolonged MRT is not inherently associated to improved nutrient digestibility. Previous studies have similarly reported that the addition of sDF into the diets of chickens (van der Klis and van Voorst, 1993) and pigs (Schop et al., 2020) prolongs digesta MRT and reduces nutrient digestibility (Choct and Annison, 1992; Langhout et al., 2000; Lin and Wright, 2018). These phenomena can be explained by the increase in digesta viscosity that arises in the presence of sDF (Bedford and Classen, 1993; Carré et al., 1994; Chen et al., 2020). The rise in digesta viscosity has been associated with reduced contractile activity (Smulikowska et al., 2002), leading to a slower digesta transit. Moreover, elevated viscosity of the digesta imposes greater restrictions on the enzyme-substrate interaction (Lentle and de Loubens, 2015), resulting in changes in mixing, diffusion, and absorption of nutrients; ultimately, affecting overall digestion efficiency (Smits et al., 1997). In such circumstances, it is plausible to attribute the lower values of DM AID and starch AID to the structural and chemical impairments caused by the AX added.

Interestingly, our findings indicate that the addition of AX into the diets led to a reduction in AID of protein by 4.3%-units only in the presence of F-SBH. The fact that we did not see effects of PS on AID of protein for the diets without AX, suggests that AX

affects the digestibility of proteins from ingredients other than SBH. Moreover, if the digestibility of SBH proteins was different between F-SBH and C-SBH diets, we would expect the F-SBH to have higher AID of protein compared with F-SBH, not lower. One potential explanation for this phenomenon may be linked to the interplay between the particles and the viscosity of the digesta. In model studies, it has been reported that the presence of particles can modulate the viscosity of a suspension, depending on the viscosity of the liquid phase of the suspension and the particle size (Senapati et al., 2010; Konijn et al., 2014). Therefore, it is reasonable to speculate that C-SBH may mitigate the adverse effects of AX on digesta viscosity compared to F-SBH. This reduction in AID of protein then might be related to a digesta matrix effect, to the hindrance of enzymes, or to incomplete absorption.

In the distal GIT, the addition of AX also significantly affected the length and weight of the ceca, digesta transit behavior, and nutrient ATTD-AID (disappearance in hindgut). The ceca play a crucial role in chickens for nutrient fermentation and fermentation-products absorption (Svihus et al., 2013). As a positive correlation was observed between nutrient disappearance in the hindgut, ATTD of NSP, and ceca traits (Table 6.2), it is possible to suggest that enhanced fermentation processes may promote development of the ceca.

Table 6.2. Pearson correlation coefficients of nutrient disappearance in the hindgut (ATTD-AID), ATTD of NSP, and ceca traits [1,2,3]

Parameters	DM disappearance	Starch disappearance	ATTD of NSP	Ceca RW	Ceca length
DM disappearance	1.00				
Starch disappearance	0.75*	1.00			
ATTD of NSP	0.47*	0.52*	1.00		
Ceca RW	0.34†	0.43*	0.57*	1.00	
Ceca length	0.28	0.48*	0.45*	0.81*	1.00

[1] Parameters n = 32 replicate pens (6 or 9 birds/pen).
[2] ATTD: apparent total tract digestibility; AID: apparent ileal digestibility; NSP: non-starch polysaccharides.
[3] * $P \leq 0.05$; † $P \leq 0.10$.

Furthermore, our results suggest that addition of AX may impact the flow of solids and/or liquids into or out of the ceca. We observed that limited amount of solids (represented by Ti) entered the ceca (2.96 mg in the ceca vs. 6.17mg in the colon), regardless of viscosity and fiber particle size. Other studies (Vergara et al., 1989;

Rougière and Carré, 2010; de Vries et al., 2014; Garçon et al., 2023) have reported similar observations. Nevertheless, insoluble marker:soluble marker ratios were significantly higher in AX diets (1.09 vs 1.66 g/g, $P < 0.001$), indicating that there is a reduced digesta phase separation in the ceca with these diets. It is possible that the high viscosity of the digesta influences cecal mixing and emptying processes (Clench, 1999). Further investigation is necessary to better understand the underlying factors influencing cecal entry and evacuation behavior of solid and liquid digesta.

The decrease in PS of SBH resulted in greater ATTD of NSP, but it had no effect on GIT traits, digesta transit, and nutrient AID. The improvement in ATTD of NSP could be explained by the increase in surface area that occurs when larger fiber particles are ground, facilitating more effective microbial colonization (Wong and Jenkins, 2007). This improved accessibility subsequently promotes NSP fermentation by gut microbiota. Although SBH are scarcely studied (Griffith, 1969; Tejeda and Kim, 2021), several studies have demonstrated an effect of PS of iDF on proventriculus (Jiménez-Moreno et al., 2011), gizzard (Hetland and Svihus, 2001; Hetland et al., 2003, 2005; Sacranie et al., 2012) and small intestine (Jørgensen et al., 1996) development. Also, it has been reported that PS of iDF affects digesta passage rate (Svihus et al., 2002). However, most of these findings are in relation to iDF other than SBH. Some of these materials, like oat hulls, can be considered rather hard or resistant to grinding. The harder the iDF, the longer the retention time of the material in the gizzard, as observed by others (Ferrando et al., 1987; Nishii et al., 2016). Another possible reason for the lack of PS effects on digesta transit could be related to the insoluble marker used to represent the particulate digesta. Given that titanium dioxide has a small particle size, it may not accurately reflect the retention of the whole digesta, but rather mainly the retention of fine particles, as noted by Svihus et al. (2002). This underlines the importance of using mordanted markers, particularly when using fibrous diets, as done by others (Ferrando et al., 1987; Solà-Oriol et al., 2010; Dorado-Montenegro et al., 2023), to track the transit behavior of the iDF.

In conclusion, adding sDF, in the form of purified wheat AX, considerably influenced digestion processes in the proximal and distal GIT. In the proximal GIT, addition of AX led to a decrease in the RW of the gizzard, prolonged MRT of digesta in the crop and

small intestine, and reduced nutrient digestibility. In the hindgut, addition of AX increased ceca size and prolonged MRT of digesta; coinciding with increased disappearance of starch. In addition, our data suggest that addition of AX may influence filling or emptying processes of the ceca, and increased retention of solids and liquids in the hindgut. Reducing particle size of SBH increased ATTD of NSP by 4.3%-units but had no impact on GIT development, digesta MRT, or digestion of other nutrients. Furthermore, the addition of AX reduced AID of protein only in the presence of F-SBH, but not C-SBH. This suggests that coarse particles may, to some extent, counteract the negative effects of soluble, viscous, fibers on nutrient digestion.

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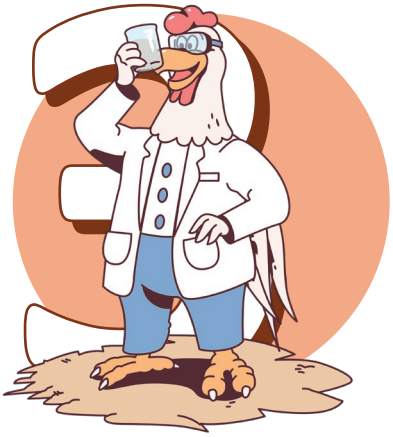
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Chapter 3

Quantification of total endogenous protein losses in broilers using a minimally invasive ^{15}N-isotope dilution method

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ABSTRACT

Total endogenous protein losses (**EPL**) measurement in broilers is important for quantifying dietary true protein digestibility. Basal EPL are commonly quantified using enzymatically hydrolyzed casein (**EHC**) or EHC-ultrafiltration (**EHC-UF**), whereas diet-specific EPL require regression methods. Total (**tEPL**; basal + diet-specific) EPL can be measured using the ^{15}N-isotope dilution (**ID**) method. This study aimed to quantify tEPL using a minimally invasive ID method based on urinary ^{15}N-enrichment as proxy for ^{15}N-enrichment in the precursor pool for endogenous protein synthesis, and to compare ID-derived tEPL with estimates from EHC and EHC-UF methods. A total of 432 day-old female broilers were randomly assigned to 48 pens (9 birds/pen), and fed one of five maize-based diets varying in fibrous ingredients (intact or ground soybean hulls with or without purified arabinoxylans, or ground sugar beet pulp), or a semisynthetic diet containing EHC (molecular weight < 5,000 Da) as the only protein source. Titanium dioxide (4 g/kg) was added as inert digestibility marker. At day 26, three birds per pen received a daily oral dose of ^{15}N-isotope solution containing 2.4 g ^{15}N-milk protein concentrate (atom-% = 2.27%) and 0.12 g $^{15}NH_4Cl$ (atom-% ≥ 98%); between days 27–31, the milk protein dose was reduced to 1.2 g. From days 31–37, excreta were collected, and at day 37, birds were euthanized for ileal digesta and tissues sampling. Nitrogen (**N**) and tracer concentrations in digesta and excreta, ^{15}N-enrichment in samples, and high-molecular weight N in digesta (EHC-diet) were quantified. Urinary ^{15}N-enrichment dropped within 2 days post-dosing, then reached a steady state. Total EPL were calculated from ^{15}N-enrichment in digesta or feces, relative to ^{15}N-enrichment in the precursor pool, assuming that it can be reflected by (1) the weighted average of ^{15}N-enrichment in liver-, pancreas-, and ileum tissues, or (2) urinary ^{15}N-enrichment. Estimates of tEPL based on these assumptions positively correlated (r = 0.84–0.90, P < 0.001). Ileal tEPL estimates were smaller with the ID compared with EHC (-75.6%, P < 0.001) and EHC-UF (-70.3%, P < 0.001) methods, resulting in lower true ileal protein digestibility estimates (-1 to -4%-units, P < 0.001). In conclusion, the minimally invasive ID method can be used for quantifying tEPL in broilers under close-to-practical conditions. Using urinary ^{15}N-enrichment to represent ^{15}N-enrichment in the precursor pool enabled tEPL quantification across diets varying in fiber and protein sources. The

ID method provides a useful tool to compare total EPL and dietary true protein digestibility.

INTRODUCTION

Digestibility assays are crucial tools to assess the bioavailability of proteins and amino acids in poultry feed ingredients. Apparent digestibility coefficients of proteins and amino acids – based on the net disappearance of ingested protein or amino acids along gastrointestinal tract– are routinely measured to assess the nutritional value of feedstuffs, but do not account for endogenous protein losses (**EPL**), which typically leads to an underestimation of true digestibility (Adedokun et al., 2011). Endogenous protein losses, which originate from digestive enzymes, mucins, sloughed-off intestinal epithelial cells, and, depending on the definition, bacterial proteins (Nyachoti et al., 1997; Miner-Williams et al., 2009; Noorman et al., 2024), can be classified into basal and diet-specific EPL. Basal EPL are defined as the inevitable losses of endogenous protein within the gastrointestinal tract that are directly influenced by DM intake, regardless of the composition of that DM (Adeola et al., 2016). In contrast, diet-specific EPL are defined as the losses induced by certain dietary compounds, such as antinutritional factors or fibers (Boisen and Moughan, 1996). To assess the true digestibility of proteins or amino acids in feed ingredients or diets, apparent digestibility should be corrected for total (**tEPL**; basal + diet-specific) EPL present in the digesta or feces (Dänicke et al., 2000; Hodgkinson and Darragh, 2000).

The most frequently used method to quantify basal EPL is the provision of an N-free diet, after which EPL can be quantified by measuring nitrogen (**N**) in digesta, which is - inherent to the method - of non-dietary origin. Although this approach is straightforward, it has been criticized for its effects of feeding protein-deficient diets on gut metabolism and the potential underestimation of basal EPL (Ravindran, 2021; Ravindran et al., 2004). To measure basal EPL under normal physiological conditions, a diet containing highly digestible proteins can be used, where all dietary proteins are assumed to be fully absorbed in the small intestine (Barua et al., 2021). A frequently used highly digestible protein source is enzymatically hydrolyzed casein (**EHC**), consisting of small peptides and free amino acids with a molecular weight < 5,000 Da

(Hodgkinson et al., 2003; Lemme et al., 2004; Deglaire et al., 2019). The validity of this method, however, depends on the assumption of complete disappearance of N in molecules of < 5,000 Da. In fact, data from pig studies indicate that ~3–10% of N originating from EHC may still be present in ileal digesta, resulting in an overestimation of EPL (Hodgkinson and Darragh, 2000). The separation of N in digesta by ultrafiltration (**EHC-UF**), considering N present as large polymers (molecular weight ≥ 10,000 Da) to be of endogenous origin and N present in the low molecular weight fraction (< 10,000 Da) to originate form EHC, can be a useful addition to the EHC method to quantify this fraction of undigested EHC (Ravindran et al., 2004; Steendam et al., 2004a). By design, these methods - relying on a single, purified, and hydrolyzed protein source - are suitable only for estimating tEPL of that specific protein source, which, due to its nature, are assumed to approximate basal EPL. In contrast, diet-specific EPL of (mixtures of) more complex feed ingredients can only be derived from the relation between amino acids digestibility and incremental dietary inclusion levels of the ingredient of interest, a procedure referred to as the regression-method.

The ^{15}N-isotope dilution (**ID**) method has been the predominant approach for measuring tEPL, especially in pig studies (Simon et al., 1987; de Lange et al., 1990; Schulze et al., 1995; Moughan et al., 1998). This method relies on distinguishing protein of endogenous origin from that of non-absorbed dietary sources by isotopically labeling either the feed or the precursor pool for endogenous protein synthesis, followed by the measurement of ^{15}N-enrichment in intestinal contents relative to ^{15}N-enrichment in the feed or body (Schulze, 1994). In pigs, tEPL have been reliably quantified by labeling endogenous protein through intravenous or oral dosing of ^{15}N-leucine for 9–10 days, followed by frequent blood collection via catheters to monitor ^{15}N-enrichment in plasma, which was assumed to reflect the enrichment in endogenous protein (Schulze et al., 1995; Gabert et al., 1997; Steendam et al., 2004b). However, this method requires repeated ^{15}N infusion and blood sampling, making it invasive and less practical for routine feed evaluation. Its application is particularly limited in chickens, where catheterization is technically more challenging. Dänicke et al. (2000) proposed measuring ^{15}N-enrichment in urine as a minimally invasive alternative to blood sampling, with the assumption that urinary ^{15}N-enrichment reflects that of endogenous N. They showed that urinary ^{15}N-enrichment closely mirrors ^{15}N-enrichment observed

in some body tissues contributing to the endogenous protein found in digesta, including the liver, pancreas, and small intestine (Simon et al., 1987; Dänicke et al., 2000).

Although the minimally invasive ID is promising, there is limited understanding regarding the reliability of this method to quantify tEPL in broilers fed induced-EPL diets. Therefore, the objective of this study was to quantify tEPL in broilers fed diets widely varying in fiber and protein sources using a minimally invasive ID method, to anticipating contrasts in diet-induced EPL. We hypothesized that by varying feed macrostructure, dietary viscous soluble fiber contents, and protein sources we would induce variation in tEPL and true protein digestibility in broilers fed different diets; where higher levels of viscous-soluble fibers will increase diet-specific, and thus tEPL and a highly digestible semi-synthetic diet will result in lower tEPL compared with compound diets with lower digestibility and greater structural complexity. The calculation of tEPL was based on the ^{15}N-enrichment in ileal digesta or feces, relative to ^{15}N-enrichment in the precursor pool, which was assumed to be reflected by (1) the weighted average of ^{15}N-enrichment in liver-, pancreas-, and ileum tissues, or (2) urinary ^{15}N-enrichment. Additionally, in the absence of a gold-standard method, the inclusion of a diet containing highly digestible EHC as the only protein source, allowed comparison of tEPL and true protein digestibility estimates obtained using the ID method with those obtained from EHC, and EHC-UF methods.

MATERIAL AND METHODS

Experimental design

This study was part of a larger animal experiment focusing on the effects of particle size of insoluble fibers and the addition of viscous soluble fibers on digesta retention time and apparent nutrient digestibility (Dorado-Montenegro et al., 2024). In this study, five fibrous diets were offered (Table 1.3), each containing either intact (coarse, **C**) or ground (fine, **F**) soybean hulls (**SBH**), as insoluble fiber source, with or without the inclusion of 50 g/kg arabinoxylans (**AX**), as viscous soluble source, replacing maize starch. Another diet contained ground sugar beet pulp (**F-SBP**), a fiber source with high a water-binding capacity, substituting ground SBH. These diets were designed to

anticipate the potential contrasts in diet-induced EPL. To compare methods (ID vs. EHC vs. EHC-UF), a diet with highly digestible EHC (169 g/kg), consisting of small peptides and free amino acids with a molecular weight < 5,000 Da, as the only protein source, was also included. Titanium dioxide (TiO_2; 4 g/kg) was included in the diet as inert tracer to calculate tEPL flow and true protein digestibility. All diets were formulated to be isonitrogenous and to meet or exceed the nutrient requirements for broilers (CVB, 2018).

Table 1.3. Ingredient and nutrient composition of experimental diets[1,2] (in g/kg as-fed, unless indicated otherwise) fed to female broilers between 10 and 37 days of age.

Item	C-SBH / F-SBH	C-SBH -AX / F-SBH-AX	F-SBP	EHC
Ingredient composition				
Maize	450.0	450.0	450.0	-
Maize Starch	175.1	126.1	176.1	634.0
Soybean hulls	120.1	120.1	-	-
Arabinoxylans	-	50.0	-	-
Sugar beet pulp	-	-	150.0	-
Arbocel[3]	-	-	-	35.0
Hydrolyzed casein	-	-	-	169.0
Soy protein concentrate	126.0	126.0	126.0	-
Sugar	28.4	28.4	-	80.0
Potato protein	1.5	1.5	-	-
Fish meal	24.8	24.8	24.8	-
Soybean oil	15.0	15.0	15.0	12.5
Potassium carbonate	3.9	3.9	3.9	17.0
Sodium bicarbonate	2.0	2.0	2.0	-
Magnesiumoxide	-	-	-	5.0
L-Lysine	5.0	5.0	5.0	-
D-Methionine	4.1	4.1	4.1	2.3
L-Threonine	2.7	2.7	2.7	0.2
L-Tryptophane	0.2	0.2	0.2	-
L-Isoleucine	1.2	1.2	1.2	-
L-Arginine	3.2	3.2	3.2	5.0
L-Leucine	0.5	0.5	0.5	-
L-Valine	2.4	2.4	2.4	-
Mineral and vitamin Premix[4]	5.0	5.0	5.0	5.0
Monocalcium phosphate	11.7	11.7	11.7	14.0
NaCl	1.2	1.2	1.2	4.0

Calcium carbonate	9.0	8.0	8.0	12.0
Cobalt-EDTA	1.0	1.0	1.0	1.0
Titanium dioxide	4.0	4.0	4.0	4.0
Polyethylene glycol	2.0	2.0	2.0	-
Calculated nutrient composition[5]				
AME (MJ/kg)	12.7	-	13.2	13.8
Digestible lysine	11.1	-	10.8	12.6
Digestible methionine + cysteine	7.2	-	7.1	7.6
Digestible threonine	7.6	-	7.3	6.7
Digestible tryptophan	1.6	-	1.5	2.0
Analyzed nutrient composition				
DM	902.1	900.7	902.2	901.1
CP[6]	171.8	180.7	168.7	166.4
Starch	433.5	392.4	434.1	520.8
Fat	35.5	37.7	33.6	10.2
Ash	50.6	51.4	55.5	54.6
Background ^{15}N-enrichment	0.3666	0.3665	0.3665	0.3679

[1] C-SBH = coarse soybean hulls; F-SBH = fine soybean hulls; F-SBP = fine sugar beet pulp; EHC = enzymatically hydrolyzed casein.
[2] Arabinoxylans (AX) (NAXus®, BioActor BV, Maastricht, The Netherlands).
[3] ARBOCEL®. J. Rettenmaier & Söhne GmbH + Co KG, Rosenberg, Germany
[4] Premix provided per kilogram of diet: Vitamin A (retinyl acetate), 10 000 IU; Vitamin D_3 (cholecalciferol), 2.500 IU; Vitamin E (dl-a-tocopherol), 50 mg; Vitamin K_3 (menadione), 1.5 mg; Vitamin B_1 (thiamin), 2.0 mg; Vitamin B_2 (riboflavin), 7.5 mg; Vitamin B_6 (pyridoxin-HCl), 3.5 mg; Vitamin B_{12} (cyanocobalamin), 20 µg; Niacin, 35 mg; D-pantothenic acid, 12 mg; Choline chloride, 460 mg; Folic acid, 1.0 mg; Biotin, 0.2 mg; Iron, 80 mg, as $FeSO_4$; Copper, 12 mg, as $CuSO_4$; Manganese, 85 mg, as MnO; Zinc, 60 mg, as $ZnSO_4$; Iodate, 0.8 mg, as KJ; Selenium, 0.15 mg, as Na_2SeO_3.
[5] Calculated nutritional composition based on the composition and nutritional value of feed materials (CVB, 2018). Digestible amino acids and metabolizable energy values were calculated based on values of apparent metabolizable energy and standardized ileal digestibility for broilers.
[6] Nitrogen × 6.25.

Birds and housing

The experiment was performed at the research facility Carus, Wageningen University & Research, Wageningen, The Netherlands. A total of 432 female (Ross 308) one-day-old broilers (initial BW: 40.7 ± 3.22 g) were obtained from a commercial hatchery. Each bird was individually weighed, wing-tagged, and randomly allocated to one of 48 pens (9 birds/pen). The pens were then randomly assigned to one of six dietary treatments, resulting in 8 replicate pens per dietary treatment. Pens (1.1 m × 1.85 m) were equipped with plastic floating slatted floors covered with rubber matting, bedded with wood

shavings, and equipped with a perch, feeder, and five drinking nipples connected to a 5-L water tank. Birds had *ad libitum* access to feed and water during the whole experiment. Room temperature was set at 32 °C at days 1 to 3, and thereafter gradually decreased to a constant temperature of 22 °C in week 4 and maintained until the end of the experiment.

Oral isotope administration and sampling procedures

The isotope administration procedure was adapted from Dänicke et al. (2000). Before ^{15}N-isotope administration, at 25 days of age, 6 randomly selected birds per pen were removed for digesta retention time and apparent nutrient digestibility measurements, reported elsewhere (Dorado-Montenegro et al., 2024). At 26 days of age, the remaining birds (3 per pen) were orally administered 2.4 g of ^{15}N-enriched milk protein concentrate (protein source; atom-% = 2.27%) and 0.12 g of $^{15}NH_4Cl$ (non-protein N source; atom-% ≥ 98%), which were diluted in 50 ml saline, between 08:00 and 12:00. The ^{15}N-enriched milk protein concentrate was prepared as described by van der Wielen et al. (2020). A syringe and curved tube feed drencher were used to administer the ^{15}N-isotope solution directly into the crop. We observed this dose to be too large for the broilers to receive within a reasonable amount of time. Thus, between 27 to 31 days of age, the dose was reduced to a total volume of 25 ml of saline solution, containing 1.2 g of ^{15}N-enriched milk protein concentrate and 0.12 g of $^{15}NH_4Cl$. Representative spot samples of excreta (combined feces + urine) at 0800 h and 1300 h from the day of the last isotope dose (day 31) until 37 days of age, and immediately frozen after collection. At 37 days of age, birds were individually weighed and euthanized by injection of 0.5 ml of sodium pentobarbital (500 mg/ml) at the back edge of the skull. The liver, pancreas, and intestinal tract were removed from the body. Digesta from the ileum - defined as the segment from the Meckel's diverticulum to the ileocolic junction - were gently flushed out with demineralized water and collected in plastic containers with a seal cap. The liver, pancreas, and ileum were removed from the body, cleaned, weighed, and immediately stored in plastic containers with a seal cap. All samples were immediately stored at -20 °C until further processing. A summary of the procedures in course of time is depicted in Figure 1.3.

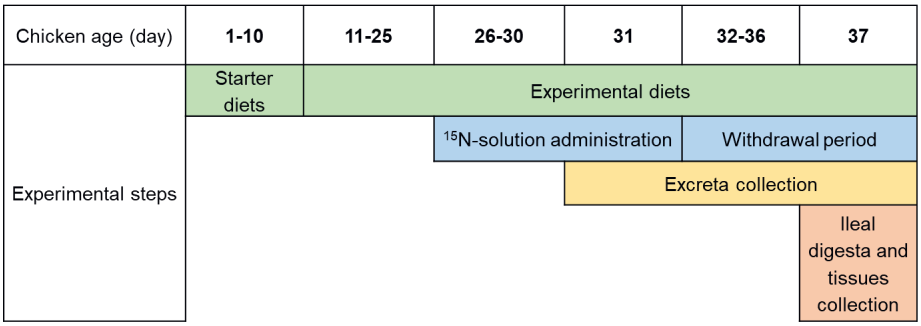


Figure 1.3. Broilers received a daily oral pulse dose of ^{15}N-solution between 26 and 31 days of age, followed by a 6-day withdrawal period (between 32 and 37 days of age) prior to dissection and collection of digesta and tissues (day 37). Excreta were collected from the last day of ^{15}N-solution administration (day 31) until the end of the experiment (day 37).

Analytical methods

Sample preparation

Prior to analyses, total mass of excreta and ileal digesta from each of the three animals per pen were pooled, freeze-dried, and ground using a rotor mill (Retsch ZM200, Haan, Germany) at 12,000 rpm with a 1 mm screen. Feed, freeze-dried ileal digesta, and excreta samples were subsequently finely ground using a mixer mill (Retsch MM200, Germany) for 5 min. Liver-, pancreas-, and ileal tissues were each pooled per pen and ground with a basic analytical mill (IKA A11, Werke GmbH and Co. KG, Staufen im Breisgau, Germany).

Chemical analyses

Uric acid was extracted from excreta following the procedure of (Baker, 1946) and Adeola and Rogler (1994) and subsequently quantified using a commercial test kit (Uric Acid liquicolor plus, 10694, Human GmbH, Wiesbaden, Germany). The precipitate remaining after uric acid extraction was collected and considered to represent feces and was finely ground using a mixer mill (Retsch MM200, Germany) for 5 min. Feed, ileal digesta, and excreta were analyzed for titanium concentration (van Bussel et al., 2010), DM (ISO 6496, 1999) and N (ISO 16634, 2008). ^{15}N-enrichment in feed, digesta, excreta, feces and tissue samples, was analyzed by combustion-isotope ratio mass spectrometry

using a Delta C continuous-flow isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany).

Ultrafiltration procedures

Endogenous protein in the ileal digesta of birds fed the EHC diet was separated from dietary protein based on molecular weight following the ultrafiltration procedure as described by Ravindran et al. (2004b). Subsamples of 0.16 g of freeze-dried digesta were re-suspended in 5 ml of deionized water and acidified to pH 3.5 with H_2SO_4 (9 M). Subsequently, samples were centrifuged at 1,500 x *g* for 45 min at 0 °C. The supernatant fraction was decanted and collected, and the precipitate was washed with 10 ml of deionized water, followed by centrifugation at 1,500 x *g* for 30 minutes at 0 °C. The second supernatant fraction was added to the first, and the combined supernatant fractions were ultrafiltered using an Amicon® ultra 15 ml centrifugal device (molecular mass cut-off filter 10,000 Da; Amicon Inc., Beverly, MA, USA). The precipitate from the first step was added to the precipitate from the ultrafiltration step, and the material was freeze-dried for analysis of N.

Calculations

^{15}N-enrichment above background enrichment (expressed as atom-% excess (**APE**)) was calculated from ^{15}N-enrichment in samples minus the background ^{15}N-enrichment as measured in the feed (Table 1.3).

Total EPL in ileal digesta and feces were calculated from ^{15}N-enrichment in digesta or feces, relative to ^{15}N-enrichment in the precursor pool for endogenous protein synthesis. The precursor pool was assumed to be reflected by (1) the weighted average of ^{15}N-enrichment in liver-, pancreas-, and ileum tissues, or (2) urinary ^{15}N-enrichment.

The weighted average of ^{15}N-enrichment ($APE_{digestive\ tissues}$, APE) in different digestive tissues (liver, pancreas, and ileum) was calculated, based on the assumption of their main contributions to endogenous protein secretions (Schulze, 1994):

$$APE_{Digestive\ tissues} = (APE_{Liver} \times 0.10) + (APE_{Pancreas} \times 0.19) + (APE_{Ileum} \times 0.71)$$

where $APE_{Digestive\ tissues}$ is the weighted average of the ^{15}N-enrichment (APE) in liver (APE_{Liver}), pancreas ($APE_{Pancreas}$), and ileum (APE_{Ileum}) tissue.

The ^{15}N-enrichment in urine (APE_{Urine}) was calculated as follows (Dänicke et al., 2000):

$$APE_{Urine} = \frac{(APE_{Excreta} \times N_{Excreta}) - (APE_{Feces} \times N_{Feces})}{N_{Uric\ acid} \times 1.2}$$

where $APE_{Excreta}$ and APE_{Feces} are the ^{15}N-enrichment (APE) in excreta and feces. Nitrogen content in feces (N_{Feces}; g/kg DM) was calculated as the contribution of feces in excreta:

$$N_{Feces} = N_{Excreta} - (N_{Uric\ acid} \times 1.2)$$

where $N_{Excreta}$, and $N_{Uric\ acid}$ are the N concentration (g/kg DM) in excreta and uric acid. A correction factor of 1.2 was used to account for urinary N contents others than uric acid, including ammonia, urea, and amino acids (Terpstra and de Hart, 1973; Dänicke et al., 2000).

Total EPL in ileum digesta or feces where then calculated using the ratio of ^{15}N-enrichment in the digesta or feces samples (APE_{Sample}) relative to the ^{15}N-enrichment in the precursor pool, as described by Dänicke et al. (2000):

$$tEPLP_{ij} = \frac{APE_{Sample}}{APE_{Precursor\ ij}}$$

where $tEPLP_{ij}$ (unitless) is the proportion of tEPL in the sample, calculated using ^{15}N-enrichment in (i) the weighted average of digestive tissues , or (j) urine as the precursor pool and $APE_{Precursor\ ij}$ is (i) $APE_{Digestive\ tissues}$ or (j) APE_{urine}. Endogenous protein losses (g/kg DM intake) in ileal digesta and feces were calculated using the ID, EHC, and EHC-UF methods as described by Deglaire et al. (2007, 2009). Briefly, tEPL based on the EHC ($EPL_{EHC\ x}$) and EHC-UF ($EPL_{EHC\ y}$) methods were calculated as follows:

$$tEPL_{EHC\ xy} = N_{Sample\ xy} \times \left(\frac{T_{Diet}}{T_{Sample}}\right)$$

and tEPL based on the ID method were calculated as:

$$tEPL_{ID} = N_{Sample\ x} \times \left(\frac{T_{Diet}}{T_{Sample}}\right) \times tEPLP_{ij}$$

where $N_{Sample\ xy}$ is the concentration of N (g/kg DM) in ileal digesta before (x; EHC and ID methods) or after ultrafiltration (y; EHC-UF method), or feces samples, T_{Diet}, and T_{Sample} are the concentrations of tracer (Ti) (g/kg DM) in diet and ileal digesta or excreta samples, and $tEPLP_{ij}$ is the proportion of tEPL in the sample calculated using (i) digestive tissues or (j) urine to reflect the precursor pool. Subsequently, the true ileal and total tract digestibility of protein (%) was calculated as follows:

$$True\ protein\ digestibility = \left(\frac{N_{Diet} - (N_{Sample\ xy} - tENL)}{N_{Diet}}\right) \times 100\%$$

where N_{Diet} is the concentration of N (g/kg DM) in diet and other parameters as defined above.

Statistical analyses

Observations at pen level (3 birds/pen) were considered the experimental unit for all analyses. The ^{15}N-enrichment in ileal digesta, excreta, feces, urine, liver, pancreas, ileum, and tEPL estimates in ileal digesta and feces in birds fed one of the six experimental diets was analyzed using ANOVA (PROC MIXED, SAS, version 9.4, SAS Institute Inc., Cary, NC), with diet as fixed effect. Differences among means were tested using type III least squares statistics, using Tukey adjustments for multiple comparisons.

The tEPL estimates in a subset of birds fed the EHC diet were analyzed using a general linear mixed model (PROC MIXED, SAS, version 9.4, SAS Institute Inc., Cary, NC), with method (ID vs. EHC vs. EHC-UF) as fixed effect. Method comparison was modelled as within-subjects random (R-side) effect, assuming an unstructured covariance structure, to account for repeated observations for the three methods within pen. Differences between true ileal protein digestibility estimated with the ID or EHC-UF methods and the EHC as the reference method, where protein digestibility is, by definition, 100%, were evaluated using a paired t-test (PROC TTEST, SAS version 9.4; SAS Institute Inc., Cary, NC) with method (ID vs. EHC-UF) as fixed effect. For true total tract protein digestibility, a one-sample t-test was used to determine whether estimates obtained by the ID method differed from 100% (EHC-method; PROC TTEST, SAS version 9.4).

Model assumptions and goodness of fit of models were evaluated through visual evaluation of histograms, quantile-quantile plots, and studentized residuals (for all models), and the null model Likelihood Ratio test, and Akaike and Bayesian information criteria (only models used for method comparison).

The relation between ^{15}N-enrichment in urine vs. in digestive tissues, tEPL estimates in ileal digesta and feces calculated using ^{15}N-enrichment in urine vs. in digestive tissues, and the relation between tEPL estimates obtained with various methods (ID, EHC, EHC-UF) were evaluated with Pearson correlation coefficients (PROC CORR, SAS, version 9.4, SAS Institute Inc., Cary, NC). Preliminary analysis of the relation between ^{15}N-enrichment in urine and ^{15}N-enrichment in digestive tissues (PROC GLM, SAS, version 9.4, SAS Institute Inc., Cary, NC), revealed no significant effects of diet, nor the interaction between diet × ^{15}N-enrichment in digestive tissues ($P > 0.1$, data not showed), verifying the assumption that the correlation between urinary- and digestive tissues ^{15}N-enrichment was diet independent.

^{15}N-enrichment values are expressed as ^{15}N-enrichment above background enrichment (atom percentage excess, APE), unless indicated otherwise. All data are presented as estimated means and pooled SEM, unless indicated otherwise. Differences were considered significant at $P < 0.05$.

RESULTS

In general, birds remained healthy and performed well during the course of the experiment. Due to the powderous, semi-synthetic nature of the EHC diet, however, birds were less eager to consume the EHC compared with the fibrous diets, resulting in lower daily feed intake (~30±6.3 g/bird/day for the EHC diet vs. ~70 to 110±13.3 g/bird/day for the fibrous diets; measured between days 22 and 25) and final BW (1024±103.6 g for the EHC diet vs. ~1850 to 2230±102.7 for the fibrous diets at day 37). Background ^{15}N-enrichment in feed samples was numerically smaller for the fibrous diets (0.3665–0.3666 atom-%) compared with the EHC diet (0.3679 atom-%) (Table 1.3).

Preliminary analyses (n = 2 replicate pens per treatment) showed a high initial ^{15}N-enrichment after the last day of ^{15}N-isotope administration at day 31 in excreta, urine, and feces in all treatments, followed by a decrease, with the strongest decline between day 32 and 33, reaching a baseline-plateau on day 34 or 35 (Figure 2.3). In birds fed the EHC diet, however, ^{15}N-enrichment in excreta, urine, and feces initially increased between day 31 and 32, before declining from day 32. Across all treatments, urinary ^{15}N-enrichment was consistently greater than fecal ^{15}N-enrichment, with the difference being ~+0.5–1.0 APE one day after isotope withdrawal, diminishing to ~+0.1–0.2 APE by day 37. Assuming the initial decline in ^{15}N-enrichment in feces – and thus excreta – is partially due to excretion of unabsorbed ^{15}N originating from the orally infused ^{15}N-isotope solution, ^{15}N-enrichment in feces and urine at baseline values (≥ day 34) was considered most representative for calculation of tEPL. Based on these results, excreta samples from day 34 to day 37 were pooled per pen (n= 8 replicate pens per treatment) for further analyses of ^{15}N-enrichment in excreta, feces, and urine.

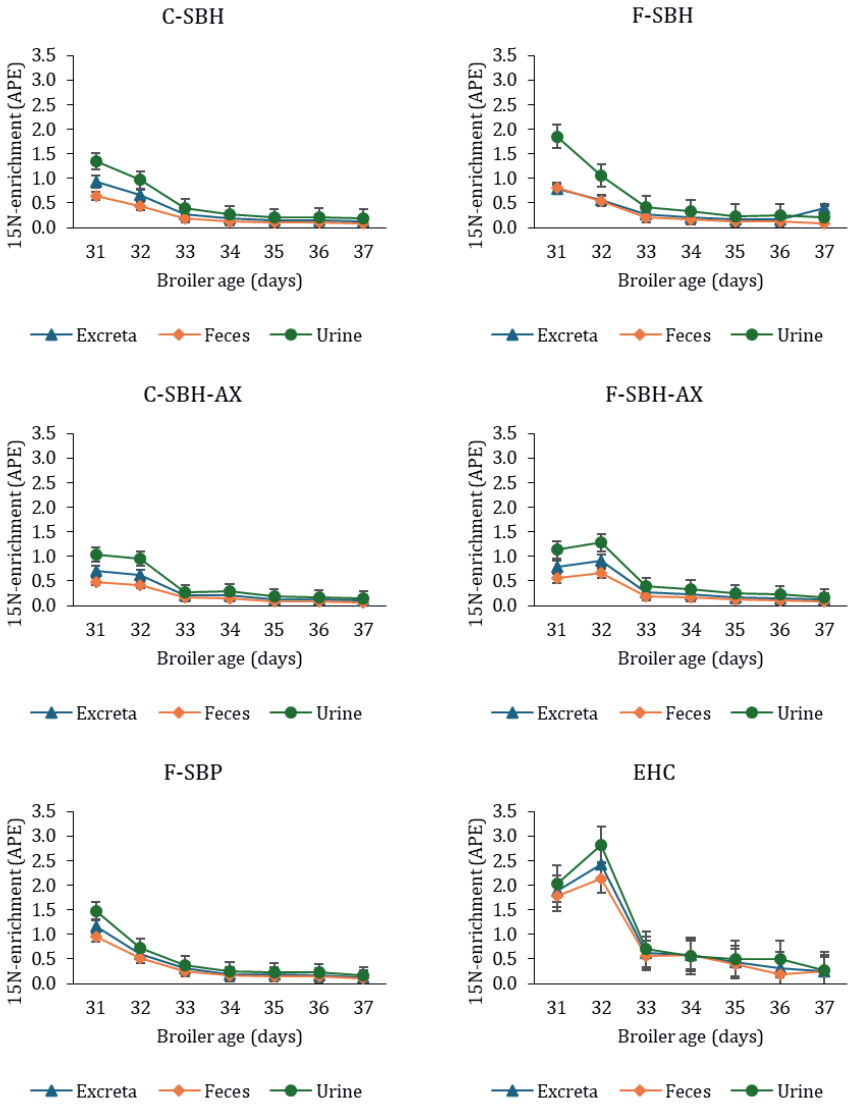


Figure 2.3. ^{15}N-enrichment in excess of background enrichment (atom-% excess, APE) in excreta, feces, and urine from the last day of ^{15}N-isotope administration at 31 days of age until dissection at 37 days of age, as measured in female broilers fed diets varying in fiber sources (intact (coarse, C) and ground (fine, F) soybean hulls (SBH), with or without the addition of 50 g/kg arabinoxylans (AX), and ground sugar beet pulp (F-SBP)) or a semisynthetic diet with enzymatically hydrolyzed casein as the only protein source (EHC). Data are presented as means of two replicate pens (3 birds/pen), error bars represent standard error of the mean.

The ^{15}N-enrichment in ileal digesta (+0.069 APE, $P < 0.001$), excreta (+0.202 APE, $P < 0.001$), feces (+0.208 APE, $P < 0.001$), urine (+0.097 APE, $P = 0.001$), liver (+0.114 APE, $P < 0.001$), pancreas (+0.014 APE, $P < 0.001$), ileum (+0.031 APE, $P < 0.001$), and the weighted average of digestive tissues (+0.026 APE, $P < 0.001$) were greater in birds fed EHC diet compared with birds fed the fibrous diets (Table 2.3). The ^{15}N-enrichment in urine positively correlated with ^{15}N-enrichment in digestive tissues (r = 0.71, $P < 0.001$; Figure 3.3). Consequently, tEPL estimates in ileal digesta (r = 0.90, $P < 0.001$) and feces (r = 0.84, $P < 0.001$) based on urinary ^{15}N-enrichment as a proxy for the precursor pool were positively correlated with those based on ^{15}N-enrichment in digestive tissues across diets (Figure 4.3). Estimates of tEPL in ileal digesta and feces were greater in birds fed F-SBP diet compared with birds fed the other diets (+0.32 and +0.75 g/kg DM intake, respectively, $P < 0.001$).

Table 2.3. ^{15}N-enrichment in excess of background enrichment (atom-% excess, APE) in ileal digesta, excreta, feces, urine, liver, pancreas, ileum[1], and the weighted average of digestive tissues, and total endogenous protein losses (EPL) in ileal digesta and feces (g/kg DM intake as measured in female broilers (n=8)[2] at 37 days of age, 6 days after oral dosing of a ^{15}N-solution[3].

^{15}N-enrichment (APE)	Diet[4]						SEM	*P*-values[5]
	C-SBH	F-SBH	C-SBH-AX	F-SBH-AX	F-SBP	EHC		
Ileal digesta	0.032^{bc}	0.031^{bc}	0.023^{c}	0.029^{bc}	0.043^{b}	0.101^{a}	0.0037	< 0.001
Excreta	0.172^{b}	0.170^{b}	0.150^{b}	0.148^{b}	0.180^{b}	0.366^{a}	0.0106	< 0.001
Feces	0.070^{c}	0.069^{c}	0.066^{c}	0.062^{c}	0.108^{b}	0.283^{a}	0.0075	< 0.001
Urine	0.352^{ab}	0.334^{ab}	0.291^{b}	0.298^{b}	0.317^{b}	0.415^{a}	0.0202	0.001
Liver	0.216^{b}	0.217^{b}	0.177^{c}	0.211^{b}	0.233^{b}	0.273^{a}	0.0079	< 0.001
Pancreas	0.219^{bc}	0.221^{bc}	0.185^{d}	0.198^{cd}	0.233^{b}	0.325^{a}	0.0080	< 0.001
Ileum	0.143^{bc}	0.150^{bc}	0.119^{d}	0.129^{cd}	0.159^{b}	0.225^{a}	0.0059	< 0.001
Digestive tissues[6]	0.165^{bc}	0.170^{bc}	0.137^{d}	0.151^{cd}	0.180^{b}	0.249^{a}	0.0062	< 0.001
Total EPL								
Ileal digesta	0.65^{bc}	0.68^{ab}	0.66^{bc}	0.91^{ab}	0.99^{a}	0.43^{c}	0.073	< 0.001
Feces	1.26^{b}	1.25^{b}	1.69^{ab}	1.51^{b}	2.17^{a}	1.40^{b}	0.154	< 0.001

[1] ^{15}N-enrichment in excreta, feces, and urine was measured in pooled samples from day 34 to 37.

[2] Number of replicate pens (3 birds/pen).

[3] An initial dose of 2.4 g ^{15}N-milk protein concentrate (atom-% = 2.27%) and 0.12 g of $^{15}NH_4Cl$ (atom-% ≥ 98%) at 26 days of age followed by 1.2 g ^{15}N-milk protein concentrate and 0.12 g $^{15}NH_4Cl$ per day between 27 and 31 days of age.

[4] Five fibrous diets containing intact (coarse, C) or ground (fine, F) soybean hulls (SBH), with or without the addition of 50 g/kg arabinoxylans (AX), or ground sugar beet pulp (SBP) as the main fiber source; and one diet containing enzymatically hydrolyzed casein as the only protein source (EHC).

[5] Model established *P*-values for the fixed effect of diet.

[6] Weighted average of ^{15}N-enrichment in liver-, pancreas-, and ileum tissue based on their contributions to endogenous protein losses in ileal digesta (Souffrant, 1991).

[a,b,c] Values within a row lacking a common superscript letter differ ($P < 0.05$).

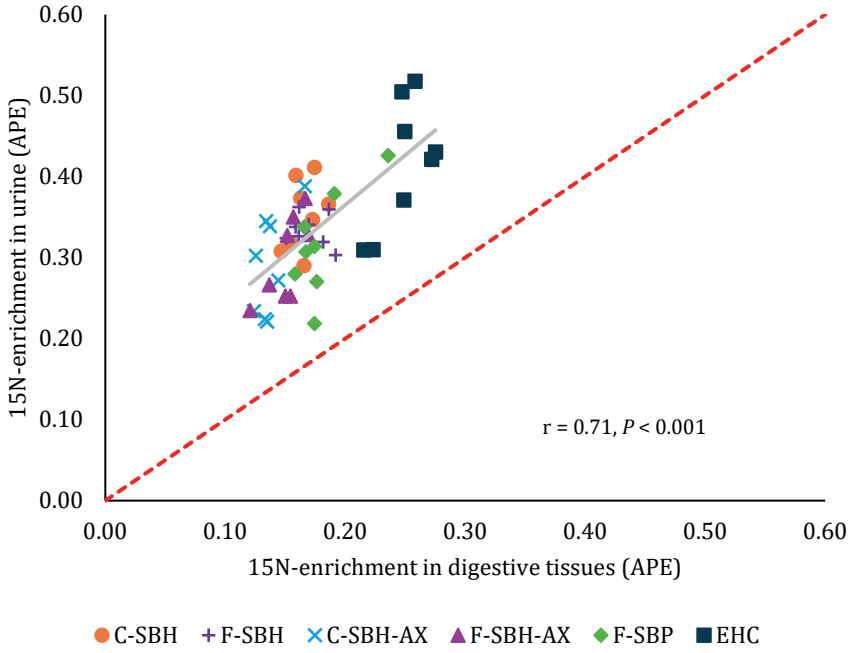


Figure 3.3. Correlation between ^{15}N-enrichment in excess of background enrichment (atom-% excess, (APE)) in urine (pooled samples from day 34 to 37) and digestive tissues (at day 37) in female broilers fed maize-based diets varying in fiber sources (intact (coarse, C) and ground (fine, F) soybean hulls (SBH), with or without the addition of 50 g/kg arabinoxylans (AX), ground sugar beet pulp (F-SBP)) or a semisynthetic diets containing enzymatically hydrolyzed casein (EHC). ^{15}N-enrichment in digestive tissues was calculated as the weighted average of ^{15}N-enrichment in liver-, pancreas-, and ileum tissues. Data are presented as individual observations, trendline y = a+bx, and corresponding correlation parameters. Red-dashed line represents reference line y=x.

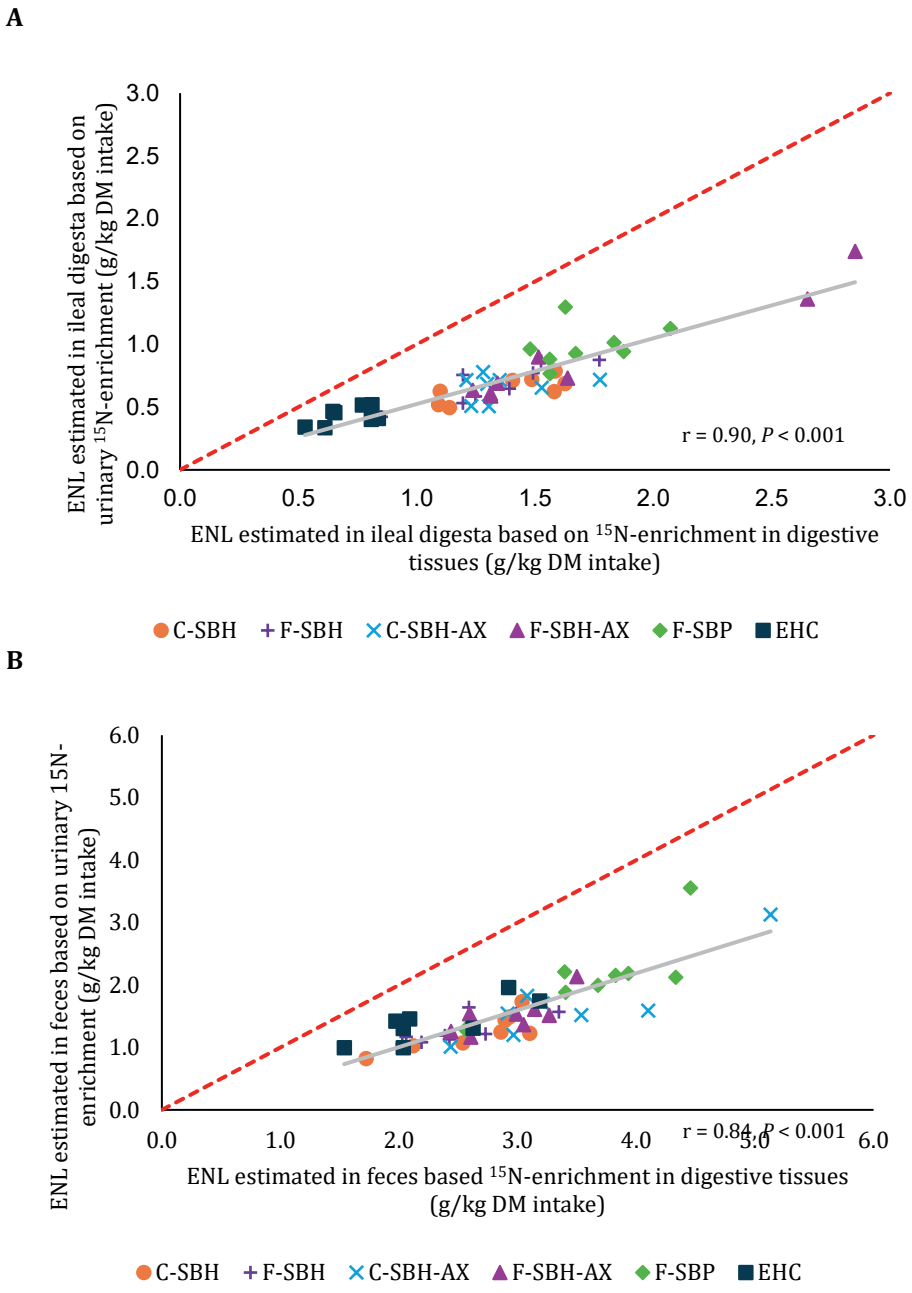


Figure 4.3. Correlation between total endogenous protein losses (EPL; g/kg DMI) in ileal digesta (A) and feces (B) measured using ^{15}N-enrichment in excess of background enrichment (atom-% excess, (APE)) in urine (pooled samples from day 34 to 37) and digestive tissues (at day 37) in female broilers fed maize-based diets varying in fiber

sources (intact (coarse, C) and ground (fine, F) soybean hulls (SBH), with or without the addition of 50 g/kg arabinoxylans (AX), ground sugar beet pulp (F-SBP)) or a semisynthetic diets containing enzymatically hydrolyzed casein (EHC). ^{15}N-enrichment in digestive tissues was calculated as the weighted average of ^{15}N-enrichment in liver-, pancreas-, and ileum tissues. Data are presented as individual observations, trendline y = a+bx, and corresponding correlation parameters. Red-dashed line represents reference line y=x.

The ID method resulted in smaller estimates of tEPL in ileal digesta compared with the EHC (-1.33 g/kg DM intake) and EHC-UF (-1.02 g/kg DM intake) methods (P < 0.001) and in feces compared with EHC (-0.62 g/kg DM intake, P < 0.001) method (Table 3.3). This resulted in 1 – 4%-units lower estimates of true ileal protein digestibility compared with the EHC- and EHC-UF methods (P < 0.001), and 1%-unit lower true total tract protein digestibility compared with the EHC method (P = 0.003). No significant correlations were found between tEPL estimates in ileal digesta obtained with the ID vs. EHC-UF methods (r = 0.01, P = 0.976; Figure 5.3) or ID vs. EHC methods (r = 0.44, P = 0.270; Figure 6.3). A positive correlation, however, was observed between tEPL estimates in feces obtained with the ID vs. EHC methods (r = 0.90, P = 0.003; Figure 6.3).

Table 3.3. Estimates of endogenous protein losses (EPL; g/kg DM intake) in ileal digesta and in feces, and true digestibility of protein (%) in female broilers (n=8)[1] fed a semisynthetic EHC diet at 37 days of age, as estimated with ^{15}N-isotope dilution (ID), enzymatically hydrolyzed casein (EHC), and enzymatically hydrolyzed casein-ultrafiltration (EHC-UF) methods[2].

	ID	RSD (%)	EHC	RSD (%)	EHC-UF	RSD (%)	*P-values*[3]
EPL							
Ileal digesta	0.43	5.8	1.76	2.6	1.45	4.4	< 0.001
Feces	1.40	8.5	2.02	7.2	-	-	< 0.001
True protein digestibility							
Ileal	95.6	0.1	100	-	99.0	0.1	< 0.001
Total tract	99.0	0.3	100	-	-	-	0.003

[1] Number of replicate pens (3 birds/pen).

[2] Data presented as Least Squares Means (LSMeans) and relative SD (RSD, %; calculated as pooled SD × 100/|LSMean|)

[3] Model established *P*-values for contrasts between methods. For true ileal protein digestibility, *P*-values were obtained by comparing the difference between the ID and EHC-UF methods against the reference value of

100% as measured by the EHC method. For true total tract protein digestibility, *P*-values were derived from testing whether the ID method differed from 100% based on the EHC method.

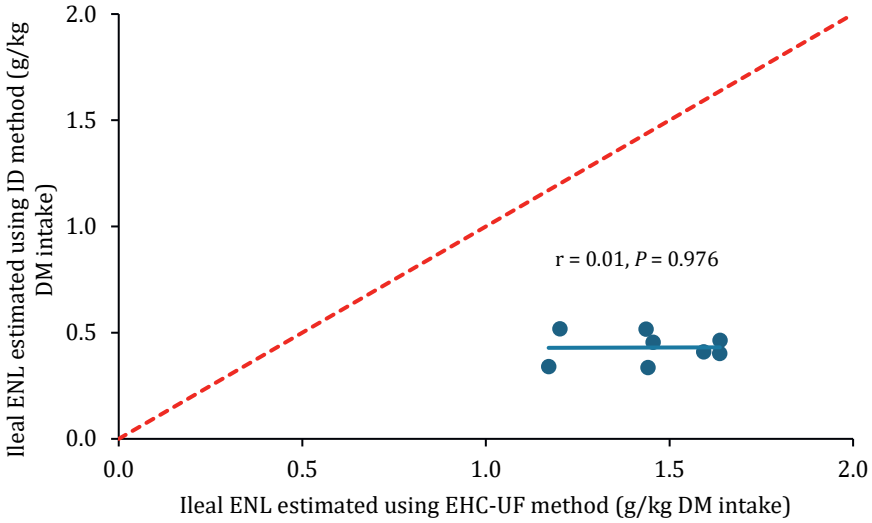


Figure 5.3. Correlation between endogenous protein losses (EPL, g/kg DM intake) in ileal digesta of female broilers fed a enzymatically hydrolyzed casein (EHC) diet, as measured with ^{15}N-isotope dilution- (ID) and enzymatically hydrolyzed casein-ultrafiltration (EHC-UF) methods. Data are presented as means of replicate pens (3 birds/pen), trendline (y = a+bx), and corresponding correlation parameters. Red-dashed line represents reference line y=x.

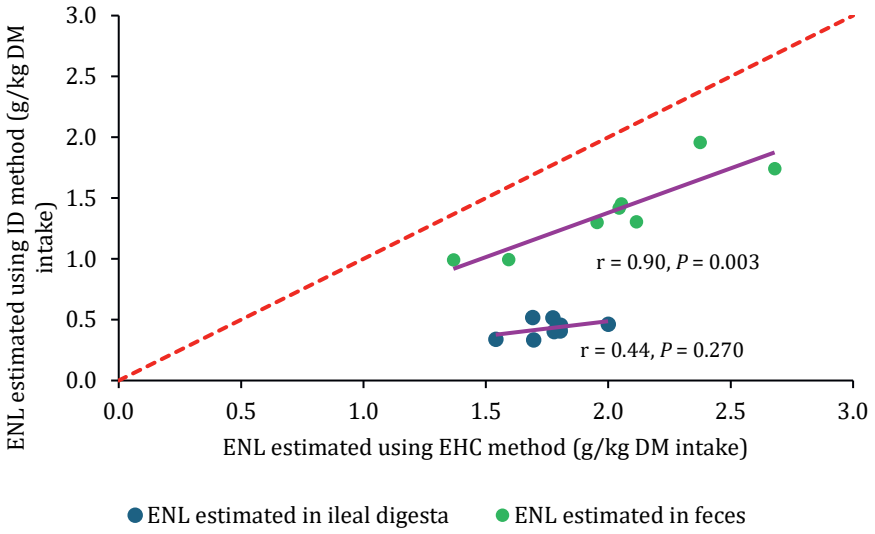


Figure 6.3. Correlation between endogenous protein losses (EPL, g/kg DM intake) in ileal digesta of female broilers fed a enzymatically hydrolyzed casein (EHC) diet, as measured with ^{15}N-isotope dilution- (ID) and enzymatically hydrolyzed casein (EHC) methods and relation between EPL estimates in feces measured with ID and EHC methods. Data are presented as means of replicate pens (3 birds/pen), trendline (y = a+bx), and corresponding correlation parameters. Red-dashed line represents reference line y=x.

DISCUSSION

The objective of this study was to quantify tEPL in broilers using a minimally invasive ID method in broilers fed diets varying in fiber- and protein sources. First, ^{15}N-enrichment in the urine of birds was verified to be a reliable proxy of ^{15}N-enrichment in in the precursor pool for endogenous protein synthesis, reflecting dietary variation in a similar way as the weighted average of ^{15}N-enrichment in digestive tissues (liver, pancreas, ileum). Based on this, tEPL were calculated by estimating the proportion of endogenous protein in the ileal digesta and feces using the ^{15}N-enrichment in urine. In the absence of a gold-standard method to quantify tEPL, we compared estimates of tEPL and true protein digestibility obtained with the ID vs. EHC- and EHC-UF methods, in broilers fed a semisynthetic EHC-based diet. Estimates of tEPL were greater in birds fed the F-SBP diet compared with those fed other diets. Estimates of tEPL obtained with the

ID method were smaller and the corresponding true protein digestibility estimates lower compared with EHC- and EHC-UF methods.

^{15}N-enrichment in urine as a proxy for ^{15}N-enrichment in the precursor pool

This study employed a minimally invasive ID method to quantify tEPL, using urinary ^{15}N-enrichment as a proxy for the precursor pool, instead of ^{15}N-enrichment in blood plasma or body tissues. The approach to use ^{15}N-enrichment in urine to quantify the proportion of endogenous protein in ileal digesta and feces was previously introduced based on the similarity of ^{15}N-enrichment in urine and body tissues (Dänicke et al., 2000). When the ^{15}N-isotope administration was stopped, urinary ^{15}N-enrichment declined rapidly from 1.2–1.9 APE to reach plateau values of ~0.2–0.3 APE within 3–4 days, in line with previous observations (Simon et al., 1987; Dänicke et al., 2000, 2001). Given the relative short total tract retention time of feed in broilers (mean retention time: ~3–8 h for solids, ~20–30 h liquids; Garçon et al., 2023), any unabsorbed ^{15}N from the administered ^{15}N-solution would be expected to be excreted within the first two days. Thus, it is assumed that ^{15}N-enrichment in excreta, feces, and urine during the plateau phase reflects the presence of ^{15}N from endogenous protein. For this reason, excreta from days 34 to 37 were pooled to determine urinary ^{15}N-enrichment and quantify the proportion of endogenous protein in ileal digesta and feces.

It should be noted that although the retention of the digesta in the gastrointestinal tract is less than two days, the ^{15}N from the administered solution may initially be incorporated into newly formed body protein or microbial biomass. The ^{15}N originating from these sources may thus appear in excreta after a certain lag time, or alternatively be reabsorbed prior to excretion through urine. Such phenomena may explain the initial increase in urinary ^{15}N-enrichment observed for the EHC diet (Figure 2.3), suggesting the plateau value of maximum enrichment was not yet reached for this diet after six days of ^{15}N-isotope administration. Additionally, this may imply that it takes longer to reach baseline-plateau values after ceasing the isotope administration. Indeed, for the EHC diet, urinary ^{15}N-enrichment only approached values comparable to the fibrous diets (~0.3 APE) at day 37. It remains to be elucidated why this was specifically observed for the EHC diet and, much less pronounced, for the AX diets. Speculatively, dietary properties that induce EPL or affect microbial composition and/or -activity

could elicit such diet-specific responses. These findings emphasize, the importance of the withdrawal period, that should be sufficiently long.

In contrast to our expectations, diet-related differences were found in ^{15}N-enrichment in the precursor pool, regardless of evaluated through urine or digestive tissues at day 37. Birds fed AX-containing diets showed lower enrichment compared with those fed SBH diets without AX, possibly due to the gelling properties of AX, which may hinder protein digestion and absorption (Zhang et al., 2015), and thus may have reduced absorption efficiency of the administered ^{15}N-isotope solution. For the EHC diet, ^{15}N-enrichment in digestive tissues was greater compared with the fibrous diets, corresponding to the above described greater ^{15}N-enrichment in urine and feces. Considering the lower BW and daily feed intake of EHC birds, this may result from the corresponding higher contribution of the administered ^{15}N-isotope solution relative to the total digestible protein intake, although the possibility that absorption efficiency of the administered ^{15}N-isotope solution was greater for the EHC diet compared with the fibrous diets cannot be excluded.

Urinary ^{15}N-enrichment was ~3–4-fold greater than values reported by Dänicke et al. (2000), whereas our ^{15}N-enrichments in digestive tissues were comparable to values found that study. Dänicke et al. (2000) administered ~55 mg ^{15}N/kg $BW^{0.75}$/day originating from $(NH_4)HCO_3$ for 7 days, whereas we administered between ~30 (fibrous diets) and ~50 (EHC diet) mg ^{15}N/kg $BW^{0.75}$/day originating from $(NH_4)Cl$ and ~4 (fibrous diets) to ~6 (EHC diet) mg ^{15}N/kg $BW^{0.75}$/day originating from milk protein concentrate for 6 days, with an initial double dose of milk protein concentrate at the first day. In both studies, ^{15}N-enrichment in body tissues was measured 6 days after the last day of ^{15}N-isotope administration. In contrast, in the study of Dänicke et al. (2000), excreta samples of the last 3 days prior to the dissection were pooled for analyses of ^{15}N-enrichment in excreta and feces, whereas we pooled excreta from the final four days. Despite these practical differences, total ^{15}N-enrichment in digestive tissues was similar, making the higher urinary ^{15}N-enrichment observed in our study remarkable.

In addition, we considered three methodological constraints that potentially impact the accuracy of urinary ^{15}N-enrichment estimates, and thus the tEPL estimates obtained with the proposed minimally invasive ID method. As the method relies on measuring

^{15}N-enrichment in whole excreta and fecal N, and calculating urinary enrichment by difference, the accurate quantification and separation of fecal and urinary N are critical elements contributing to the overall accuracy of the method.

First, incomplete extraction of urinary N during the uric acid extraction procedure may leave residual urinary N in the fecal pellet and contaminate fecal N-, as described by Dänicke et al. (2000), who showed fecal pellets were only slightly contaminated with uric acid, resulting in overestimation of fecal ^{15}N-enrichment of ~0.9%. We observed fecal ^{15}N-enrichment values in the same range as reported by Dänicke et al. (2000) and potential overestimation of fecal ^{15}N-enrichment would result in underestimation of urinary ^{15}N-enrichment rather than overestimation, and hence this phenomenon cannot explain the results observed.

Second, non-urinary N sources (e.g., undigested dietary protein, microbial protein, or endogenous protein) may solubilize during the extraction procedure and are then not recovered in the fecal pellet. Assuming these components have similar ^{15}N-enrichment as the fraction recovered in the fecal pellet, this would not influence the estimated urinary ^{15}N-enrichment. Only if the solubilized fraction has a different ^{15}N-enrichment, such as highly enriched endogenous protein, urinary ^{15}N-enrichment estimates would be overestimated.

Third, as only uric acid N is quantified, total urinary N is estimated based on assumptions on the contribution of uric acid N to total urinary N. Following the approach of Dänicke et al. (2000), we used a factor of 1.2 to calculate total urinary N from uric acid N. Reported values for the contribution of uric acid N to total urinary N vary widely, from ~60% to 80%, depending on diet and the physiological state of the animal (Selle et al., 2021; Terpstra and de Hart, 1973; Nesheim and Carpenter, 1967; O'Dell et al., 1960). For example, a previous study (O'Dell et al., 1960) showed a lower concentration of uric acid N in urinary N when feeding a semisynthetic maize diet compared with a soya-maize-based diet, speculatively explaining the higher urinary ^{15}N-enrichment for the EHC diet in our study. Provisional calculations indicate that variation in this correction factor would yield mean urinary ^{15}N-enrichment values ranging from 0.245 APE, using a factor of 1.67, to 0.321 APE, using factor of 1.19.

These provisional estimates indicate that inaccurate assumptions regarding the contribution of uric acid N to total urinary N may partly, but not fully, explain the higher urinary ^{15}N-enrichment found in our study. This highlights the need for future studies to further identify and quantify the origin of various proteon sources in excreta and their fate during the uric acid extraction procedure. Insights in the variation in fecal vs. urinary N present in excreta, and the contribution of uric acid to total urinary N under various conditions, will help to improve the accuracy of fecal and urinary ^{15}N-enrichment determination and the final tEPL estimates obtained with the minimally invasive ID method.

Despite these methodological considerations, the positive correlation between tEPL estimates obtained with ^{15}N-enrichment in urine vs. digestive tissues, indicates that both proxies represent the diet-induced variation in ^{15}N-enrichment in the precursor pool equally well. It should be noted that the absolute level of ^{15}N-enrichment, however, was, 2–3 fold greater in urine than in the selected digestive tissues. For future research and further refinement of the method, it would be of particular interest to compare the temporal changes in urinary ^{15}N-enrichment after ceasing the ^{15}N-isotope administration, to changes in body materials, such as blood plasma and digestive organs. This will allow further substantiation for the selection of appropriate proxies for the precursor pool and the duration of the withdrawal period.

Minimally invasive ^{15}N-isotope dilution versus hydrolyzed casein methods to estimate endogenous protein losses and protein digestibility

The estimates of tEPL measured with ID method in our study were ~2-fold smaller than values reported with this method in broilers, rats and pigs (Dänicke et al., 2000; Hodgkinson et al., 2003). In contrast, tEPL estimates derived from the EHC and EHC-UF methods (1.5–1.8 g/kg DM intake) aligned well with those reported in earlier studies across these species (Steendam et al., 2004b, 2024; Deglaire et al., 2007, 2008, 2019; Ravindran et al., 2004, 2009; Golian et al., 2008; Ravindran and Hendriks, 2004b, 2004a; Hodgkinson et al., 2003). In the current study, the ID method resulted in smaller tEPL estimates for ileal digesta, and corresponding lower true ileal protein digestibility, compared with EHC and EHC-UF methods. Similar discrepancies between these methods have been found with pigs and rats (Deglaire et al., 2008; Hodgkinson et al.,

2003). It has been postulated that this difference can be partly explained by the difference in protein turnover-rates of the various tissues that contribute to EPL in digesta and feces vs. all tissues that contribute to the precursor pool (Simon et al., 1987). From studies in pigs, it is known that the protein turnover rates of the liver (20%), pancreas (82%), and small intestine (38%) are higher than those of non-digestive organs such as muscle (3%) and heart (5%) that quantitatively explain a large portion of the precursor pool (Van Der Meulen and Jansman, 1997). This implies that after stopping the oral ^{15}N-isotope administration, the ^{15}N-enrichment in ileal digesta and feces declines more rapidly than that in urine, leading to an underestimation of assumed ^{15}N-enrichment in the precursor pool and thus an overestimation of tEPL. Supporting this interpretation, when we calculated tEPL using the weighted average of ^{15}N-enrichment in liver-, pancreas-, and ileum tissues to represent the precursor pool, tEPL estimates were ~2-fold higher than those obtained using urinary ^{15}N-enrichment (Figure 4.3).

For the EHC and EHC-UF methods, tEPL were quantified by assuming that all N in ileal digesta is of endogenous origin (the EHC method) or that only N remaining after ultrafiltration of ileal digesta is of endogenous origin (the EHC-UF method). The smaller estimates of tEPL obtained with the EHC-UF compared with EHC methods indicate that the general assumption that EHC is completely absorbed in the small intestine, may not be fully correct. These findings are consistent with observations in pigs, where it was reported that ileal protein digestibility of EHC was ~94–97% (Farrel et al., 1998; Kies et al., 1986). On the contrary, as endogenous proteins can also be hydrolyzed along the small intestine, part of the protein removed by ultrafiltration may actually be of endogenous protein origin, leading to potential underestimation of tEPL with the EHC-UF method.

Furthermore, we compared the precision of the methods based on their RSD, where we found that the RSD of tEPL estimates measured using ID was higher than the RSD for EHC and EHC-UF methods, indicating that the ID method is less precise than the EHC and EHC-UF methods. This resulted in poor correlations between tEPL estimates obtained with the ID method and estimates obtained with the EHC- and EHC-UF methods (Figure 5.3 and Figure 6.3). In contrast, for tEPL estimates in feces obtained

with the ID method and EHC method correlated well (Figure 6.3). Speculatively, violation of the assumption of complete EHC absorption in the small intestine is partially mitigated by further degradation of EHC in the hindgut. Additionally, incorporation of unabsorbed ^{15}N-tracer into microbial biomass in the hindgut may influence tEPL estimates obtained with the ID method and lead to an overestimation of tEPL in feces compared to ileal digesta, bringing the estimates of ID and EHC methods post-ileum closer together.

Taken together, the comparison of the ID, EHC, and EHC-UF methods presented in the current study highlights important limitations associated with each approach. In the absence of a gold-standard method to quantify diet-specific EPL, however, it remains uncertain which method yields the most biologically accurate estimates, and interpretations must therefore rely on methodological assumptions and the study aims. Given that the EHC and EHC-UF methods can only be used with a single, purified and hydrolyzed protein source, the ID method currently is the only alternative for the quantification of tEPL for diets containing a mix of feed ingredients under semi-practical conditions. The positive correlation between tEPL in ileal digesta and feces estimates obtained with ^{15}N-enrichment in urine vs. digestive tissues (Figure 4.3), indicates that both proxies represent diet-induced variation in ^{15}N-enrichment in the precursor pool equally well, substantiating the suitability of the minimally invasive methodology to compare tEPL of feed ingredients or compound feeds within studies. To further optimize methods for quantification of tEPL, future studies should distinguish between protein derived from bacterial biomass and that originating from host endogenous protein sources, as bacterial proteins substantially contribute to the tEPL estimates.

Total endogenous protein losses across dietary treatments

The differences in tEPL among diets as estimated with ID method were consistent with our expectations based on the dietary insoluble and soluble fibers present in these diets. Endogenous protein losses estimates were ~1.5–2.3-fold higher in ileal digesta and ~0.9–1.6-fold higher in feces for the high-fiber diets compared with the EHC diet, with the greatest values observed for diets containing SBP or AX. These effects are likely explained by the ability of dietary fibers to stimulate digestive enzyme secretion

(Langlois et al., 1987), increase the thickness of the epithelial mucus layer (Wang et al., 2020), promote the proliferation of enterocytes (Gee et al., 1996) and reduce endogenous protein reabsorption (Capuano, 2017; Langlois et al., 1987), which should contribute to increased EPL (de Lange et al., 1989). Additionally, specific antinutritional factors present in the various diet ingredients, such as the presence of trypsin inhibitors in soybean meal (Aderibigbe et al., 2021), may have further contributed to the higher tEPL observed in the fibrous diets.

CONCLUSION

The minimally invasive ID method presented in this study proves to be a practical and reliable approach to quantify total (basal + diet-specific) EPL in broilers. By using urinary ^{15}N-enrichment to represent ^{15}N-enrichment in the precursor pool for endogenous protein synthesis, we obtained estimates of total EPL for diets varying in fiber- and protein sources, consistent with expected contrasts in diet-specific EPL. Although - in absence of a gold-standard method for quantification of specific and total EPL - it remains uncertain which method yields the most biologically accurate estimates, the method provides a robust new approach to compare total EPL of feed ingredients or compound feeds within studies. It is concluded that the proposed method provides reliable estimates of true protein digestibility for diets contrasting in diet-specific EPL. For feed evaluation purposes, the ID method provides a useful tool to obtain insights in ingredient or diet induced EPL, and its consequences for true protein digestibility estimates. Future research should aim to further refine this method, for example by optimizing the duration of the withdrawal period after ceasing ^{15}N-isotope administration and by distinguishing between host-derived and microbial protein contributions.

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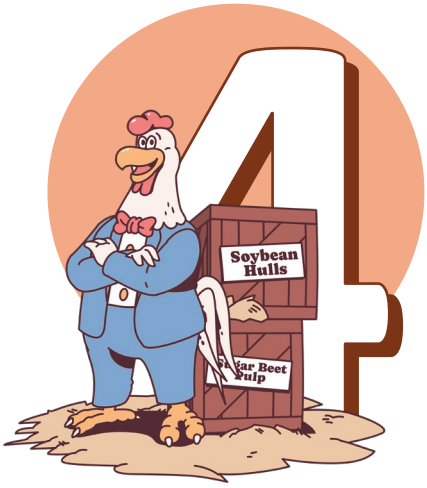
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Soybean
Hulls

Chapter 4

Effects of dietary fibers varying in physicochemical properties on total endogenous protein losses and protein digestibility in broilers

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ABSTRACT

The aim of this study was to quantify endogenous protein losses (**EPL**) and protein digestibility in broilers fed diets containing dietary fibers (**DF**) differing in particle size (**PS**) and solubility. A total of 360 female 1-day-old Ross 308 broilers were randomly allocated to 40 pens (9 birds/pen). From day 10, broilers received one of five dietary treatments. Four diets were tested in a 2 × 2 factorial design containing 120 g/kg coarse or fine soybean hulls (**SBH**) as an insoluble DF source, with or without 50 g/kg purified wheat arabinoxylans (**AX**) as a viscous soluble DF source. A fifth diet replaced fine SBH with 150 g/kg fine sugar beet pulp (**SBP**), a DF source with notable hydration properties. Between days 26 and 31, three birds per pen received a daily oral dose of a ^{15}N-isotope solution containing 1.2 g ^{15}N-enriched milk protein concentrate (2.27 atom-%) and 0.12 g $^{15}NH_4Cl$ (≥98 atom-%), with an initial dose of milk protein concentrate of 2.4 g at d 26. Six days after ^{15}N-solution withdrawal (day 37), excreta were collected, birds were euthanized, and ileal digesta were sampled. Arabinoxylans increased EPL in feces (+0.35 g/kg DM intake, P = 0.020) and reduced true ileal protein digestibility (−2.45 %-units, P < 0.001), regardless of SBH PS. Apparent ileal protein digestibility decreased only when AX was added to the fine SBH diet (PS × AX interaction, P = 0.024). Replacing fine SBH with fine SBP increased EPL in ileal digesta (+0.31 g/kg DM intake, P = 0.002) and feces (+0.92 g/kg DM intake, P = 0.004), without affecting true protein digestibility. Overall, both AX and SBP increased EPL in broilers, but only AX reduced true protein digestibility. The PS × AX interaction suggests that the viscous soluble DF impact depends on PS of the dietary matrix. These results highlight the importance of considering the counteracting DF effects on EPL and nutrient absorption, as apparent digestibility alone is insufficient to capture these responses.

INTRODUCTION

The use of fiber-rich by-products from agricultural and food industries has gained attention in the poultry feed sector as a strategy to enhance food security, sustainability, and animal welfare. Dietary fibers (**DF**) have been shown to modulate endogenous protein losses (**EPL**) and digestibility of nutrients in chickens (reviewed by Jha and Mishra, 2021) due to their physicochemical properties (reviewed by Eggum, 1995) and

understanding these effects is essential when considering inclusion of DF-rich ingredients into poultry diets. Inherent to the structural diversity of DF, the effects of DF-rich ingredients on EPL and nutrient digestion are, however, highly variable.

For example, the structural characteristics of insoluble DF (**iDF**) sources, including hardness or resistance to breakdown, have been known to influence digesta transit behavior and nutrient digestibility (Ferrando et al., 1987; González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009; Mateos et al., 2012; Kakhki et al., 2024). Particles that resist physical and chemical degradation in the upper gastrointestinal tract (**GIT**) stimulate gizzard contraction, promoting muscular development, prolonging digesta retention time, and enhancing enzyme–substrate interactions, which may ultimately improve nutrient digestibility (Hetland and Svihus, 2001; González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009; Svihus, 2011). The modification of particle size (**PS**) of iDF sources is a practical strategy to exploit these structural effects (Mosenthin et al., 1994; Lahaye et al., 2008; reviewed by Jha and Mishra, 2021). For instance, coarse oat hulls, which provide larger and harder particles, have been shown to increase gizzard weight, prolong digesta retention time, and finally may improve nutrient digestibility when compared with finely ground oat hulls (Hetland and Svihus, 2001; Svihus, 2011; Naeem et al., 2023). Nevertheless, whereas enhanced gizzard function may facilitate nutrient digestion, as reflected by increased true protein digestibility, iDF may also elevate EPL due to the increased secretion of digestive enzymes (Svihus, 2011; Yokhana et al., 2016), increased thickness of the gastrointestinal mucus layer, and the abrasive action of coarse particles on the epithelial mucosa (Leterme et al., 1998b; Oryschak et al., 2002).

Soluble DF (**sDF**) and DF source with noticeable hydration properties, including high water-binding capacity (**WBC**), may also increase EPL and decrease nutrient digestibility, although the mode of action may differ from iDF (Souffrant, 2001; reviewed by Dégen et al., 2007). Soluble DF typically increase digesta viscosity, which may enhance cell proliferation in the small intestine (Gee et al., 1996; Wanders et al., 2013, 2014), hinder the reabsorption of bile acids and digestive enzymes in the ileum (Souffrant, 2001; Adeola et al., 2016; reviewed by Jha and Mishra, 2021), resulting in increased EPL. Furthermore, computer simulations revealed that high digesta viscosity

may lower nutrient diffusion and hamper intestinal contractions along the small intestine (Henn and Alim, 2025), hence potentially reducing nutrient hydrolysis, limiting transport through the gut lumen, decreasing interactions with the epithelial surface, and ultimately impairing nutrient absorption.

Our current understanding of DF-induced effects on nutrient digestibility is largely based on apparent digestibility estimates (Mateos et al., 2012; Vivares et al., 2025), which are routinely evaluated to assess the nutritional quality of feed ingredients. By influencing EPL and true digestibility of protein, however, DF may affect apparent digestibility values in various ways, depending on their specific physicochemical properties such as PS, solubility, and gel forming- and hydration characteristics. Because apparent protein digestibility values reflect the combined effects of EPL and true protein digestibility, they provide no insights into the underlying mode of action and - when EPL and true digestibility change in opposite directions - may even mask the effects of DF.

To better interpret effects of DF on apparent protein digestibility reported in literature, we need to obtain insights in the diverse effects of DF on (1) EPL vs. (2) hydrolysis and absorption of protein, i.e. true digestibility. To address this, the objective of this study was to investigate how DF sources varying in physicochemical properties influence EPL and true digestibility of protein in broilers. As a model for cereal-derived soluble DF, we used isolated arabinoxylans from wheat, as soluble polysaccharides with strong gelling properties (Mendez-Encinas et al., 2018), to induce variation in digesta viscosity and passage behavior, anticipating effects on EPL and protein digestion. Arabinoxylans were added to maize-based diets containing either coarse or fine soybean hulls as the main iDF source, enabling evaluation of the effects of supplementing viscous sDF to iDF differing in PS. It was expected that the presence of viscous sDF in fine soybean hulls will change digesta properties as the viscosity of suspensions is very sensitive to the size of particles present (Senapati et al., 2010; Konijn et al., 2014). A fifth diet was added where ground soybean hulls were replaced with ground sugar beet pulp, a DF source containing a mix of heterogeneous water-insoluble (~60% of NSP) and soluble (~40% of NSP) polysaccharides linked within its plant cell wall matrix, with pectic polysaccharides, arabinans, and cellulose as the major constituents (Zykwinska et al.,

2006; Kakhki et al., 2024). Sugar beet pulp has low lignin-content, exerting noticeable hydration properties but low gelling properties and viscosity (Phatak et al., 1988). Hence, this diet allowed evaluation of the effects of an insoluble DF source with markedly different characteristics from cereal-derived arabinoxylans.

MATERIALS AND METHODS

The experiment was performed at the research facility Carus, Wageningen University & Research, Wageningen, The Netherlands in June 2021. Experimental procedures were approved by the Dutch Central Committee of Animal Experiments, the Netherlands, under the authorization number AVD1040020197324 and in accordance with the Dutch Act on Animal Experimentation and EU Directive 2010/63/EU. This study was part of a larger animal experiment on the use of minimally invasive ^{15}N-isotope dilution method and the effects of DF on digesta retention time and apparent nutrient digestibility (Dorado-Montenegro et al., 2024; Habibi et al., 2026).

Birds and housing

A total of 360 female (Ross 308) one-day-old broilers (initial BW: 40.7 ± 3.22 g) were obtained from a commercial hatchery. Each bird was individually weighed, wing-tagged, and randomly allocated to one of 40 pens (9 birds/pen). The pens were then assigned to one of five dietary treatments, resulting in 8 replicate pens per treatment. Pens (1.1 m × 1.85 m) were equipped with plastic floating slatted floors covered with rubber matting, bedded with wood shavings, and equipped with a perch, feeder, and five drinking nipples connected to a 5-L water tank. Birds had *ad libitum* access to feed and water. The room temperature was set at 32 °C from d 1 to d 3, and thereafter gradually decreased to 22 °C in week 4 and maintained until the end of the experiment. The health condition of the birds was assessed daily.

Experimental design and dietary treatments

In the first 10 days, all birds were fed a commercial pelleted maize-soybean based starter diet. From day 10 to 37, the pelleted experimental diets were offered. The effects of PS of soybean hulls (coarse vs. fine) and the addition of arabinoxylans (0 vs. 50 g/kg)

were tested using a 2×2 factorial arrangement of treatments. A fifth diet was used to compare iDF sources varying in physicochemical properties, including WBC, by substituting fine soybean hulls with fine sugar beet pulp.

Soybean hulls (120 g/kg) either in coarse (**C-SBH**: intact SBH;) or fine (**F-SBH**: ground to pass a 1 mm screen using LHM20/16, 1.5 kW, Condux International, Mankato, United States of America; 1,500 rpm) form, purified wheat arabinoxylans (**AX**) (50 g/kg) (NAXus®, BioActor BV, Maastricht, The Netherlands), and fine sugar beet pulp (150 g/kg) (**F-SBP**: ground twice to pass 4 mm and 1 mm screen using LHM20/16, 1.5 kW, Condux International, Mankato, United States of America; 1,500 rpm) were used as main DF sources. For the fifth diet, 150 g/kg F-SBP was substituted for 120.1 g/kg fine SBH, 28.4 g/kg sugar, and 1.5 g/kg potato protein (Table 1.4). All diets were pelleted and formulated to meet or exceed nutrient requirements for growing broiler chickens (CVB, 2018). Titanium dioxide (TiO_2; 4 g/kg) was added to the diets as inert tracer to measure EPL and protein digestibility.

Table 1.4. Ingredient and nutrient composition of experimental diets (in g/kg as-fed, unless indicated otherwise) fed to female broilers between 10 and 37 days of age.

Item	C-SBH / F-SBH[1]	C-SBH-AX / F-SBH-AX[2]	F-SBP[3]
Ingredient composition			
Maize	450.0	450.0	450.0
Maize starch	175.1	126.1	176.1
Soybean hulls	120.1	120.1	-
Arabinoxylans	-	50.0	-
Sugar beet pulp	-	-	150.0
Soy protein concentrate	126.0	126.0	126.0
Sugar	28.4	28.4	-
Potato protein	1.5	1.5	-
Fish meal	24.8	24.8	24.8
Soybean oil	15.0	15.0	15.0
Potassium carbonate	3.9	3.9	3.9
Sodium bicarbonate	2.0	2.0	2.0
L-Lysine	5.0	5.0	5.0
D-Methionine	4.1	4.1	4.1
L-Threonine	2.7	2.7	2.7
L-Tryptophane	0.2	0.2	0.2
L-Isoleucine	1.2	1.2	1.2

L-Arginine	3.2	3.2	3.2
L-Leucine	0.5	0.5	0.5
L-Valine	2.4	2.4	2.4
Mineral and vitamin premix[4]	5.0	5.0	5.0
Monocalcium phosphate	11.7	11.7	11.7
Salt	1.2	1.2	1.2
Calcium carbonate	9.0	8.0	8.0
Cobalt-EDTA	1.0	1.0	1.0
Titanium dioxide	4.0	4.0	4.0
Polyethylene glycol	2.0	2.0	2.0
Calculated nutrient composition[5]			
AME[6] (MJ/kg)	12.7	-	13.2
Dig lys[7]	11.1	-	10.8
Dig met + cys[7]	7.2	-	7.1
Dig thr[7]	7.6	-	7.3
Calcium	7.2	-	7.5
Phosphorus	2.9	-	3.0
Analyzed nutrient composition			
DM	902.1	900.7	902.2
CP[8]	171.8	180.7	168.7
Fat	35.5	37.7	33.6
Ash	50.6	51.4	55.5
Total non-starch polysaccharides	116.5	139.6	109.0
Background ^{15}N-enrichment	0.3666	0.3666	0.3665

[1] C-SBH: coarse soybean hulls (intact) and F-SBH: fine soybean hulls (ground by a hammer mill using a 1 mm screen).

[2] Soluble wheat arabinoxylans (AX) (NAXus®, BioActor BV, Maastricht, The Netherlands).

[3] F-SBP: fine sugar beet pulp (ground by a hammer mill using a 4 mm and subsequently a 1 mm screen).

[4] Premix provided per kilogram of diet: Vitamin A (retinyl acetate), 10,000 IU; Vitamin D_3 (cholecalciferol), 2.500 IU; Vitamin E (dl-a-tocopherol), 50 mg; Vitamin K_3 (menadione), 1.5 mg; Vitamin B_1 (thiamin), 2.0 mg; Vitamin B_2 (riboflavin), 7.5 mg; Vitamin B_6 (pyridoxin-HCl), 3.5 mg; Vitamin B_{12} (cyanocobalamin), 20 µg; Niacin, 35 mg; D-pantothenic acid, 12 mg; Choline chloride, 460 mg; Folic acid, 1.0 mg; Biotin, 0.2 mg; Iron, 80 mg, as $FeSo_4$; Copper, 12 mg, as $CuSO_4$; Manganese, 85 mg, as MnO; Zinc, 60 mg, as $ZnSO_4$; Iodate, 0.8 mg, as KJ; Selenium, 0.15 mg, as Na_2SeO_3.

[5] Calculated using data from (CVB, 2018).

[6] Apparent metabolizable energy for broilers.

[7] Standardized ileal digestible amino acids for broilers.

[8] Nitrogen × 6.25.

Oral isotope administration and sampling procedures

Before ^{15}N-isotope administration, at 25 day of age, 6 randomly selected birds per pen were removed and dissected for apparent nutrient digestibility and digesta retention time measurements, as reported elsewhere (Dorado-Montenegro et al., 2024). The

procedures of ^{15}N-isotope solution administration were adapted from Dänicke et al. (2000) as described by Habibi et al. (2026). In brief, between days 26 and 31, three birds per pen received daily an oral dose of a ^{15}N-isotope solution containing 1.2 g ^{15}N-enriched milk protein concentrate (atom-% = 2.27%) and 0.12 g $^{15}NH_4Cl$ (atom-% ≥ 98%), with an initial priming dose of milk protein concentrate of 2.4 g at day 26. Representative spot samples of excreta were collected daily at 08:00 and 13:00 from the d of the last isotope dose (day 31) until 37 days of age. At 37 d of age, birds were individually weighed and dissected by injection of 0.5 mL of sodium pentobarbital (500 mg/mL) at the back edge of the skull. Ileal digesta were gently flushed out with saline water and collected in the container. All samples were immediately stored at -20°C until further processing.

Sample preparation and analytical methods

Prior to analyses, excreta and ileal digesta were pooled per pen, freeze-dried, and ground using a rotor mill (Retsch ZM200, Haan, Germany) at 12,000 rpm with a 1 mm screen. All chemical and physical analyses were performed in duplicate.

Uric acid was extracted from excreta following the procedure of Baker (1946) and subsequently quantified using a commercial test kit (Uric Acid liquicolor plus, 10694, Human GmbH, Wiesbaden, Germany). The precipitate remaining after uric acid extraction was collected and considered feces. Feed, ileal digesta, and excreta samples were analyzed for titanium (van Bussel et al., 2010), DM (ISO 6496, 1999) and nitrogen (ISO 16634, 2008). ^{15}N-enrichment in feed, digesta, excreta, and feces was analyzed by combustion-isotope ratio mass spectrometry using a Delta C continuous-flow isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany).

Water-binding capacity and extract viscosity

Water-binding capacity of diets was analyzed with the following modifications of the method of Jacobs et al. (2015). Diets and deionized water were mixed in a conical tube (50 mL) using a vortex (2,500 × g) for 5 s to ensure thorough mixing the components. Values were reported as g water/g dry sample. Extract viscosity was analyzed as the viscosity of the buffer-soluble fraction of diets after extraction of the aqueous fraction,

following the modifications of the method of Dorado-Montenegro et al. (2025). All analyses were performed in duplicate.

Particle size distribution

Particle size distributions of C-SBH, F-SBH, and F-SBP ingredients were analyzed by dry sieving method (ASABE, 2008). Particle size distributions of C-SBH, F-SBH, and F-SBP diets were analyzed by wet sieving method (Wolf et al., 2010). The geometric mean diameter (**GMD**), and geometric standard deviation (**GSD**) of samples were calculated as described by Lyu et al. (2020).

Calculations

^{15}N-enrichment above background enrichment (expressed as atom percentage excess (**APE**)) was calculated from ^{15}N-enrichment in samples minus the background ^{15}N-enrichment as measured in the feed (Table 1.4). The ^{15}N-enrichment in excess of background enrichment in those samples were used to calculate total EPL proportion as follows (Dänicke et al., 2000):

$$EPL\ proportion = \frac{APE_{Sample}}{APE_{Urine}}$$

where APE_{Sample} is the ^{15}N-enrichments in excess of background enrichment in ileal digesta or feces. ^{15}N-enrichments in excess of background enrichment in urine (APE_{Urine}) was used to represent the ^{15}N-enrichment in endogenous nitrogen pool, which was extensively discussed in a previous study (Habibi et al., 2026) and was calculated as follows (Dänicke et al., 2000):

$$APE_{Urine} = \frac{(APE_{Excreta} \times N_{Excreta}) - (APE_{Feces} \times N_{Feces})}{N_{Uric\ acid} \times 1.2}$$

where $APE_{Excreta}$ and APE_{Feces} are the ^{15}N-enrichment in excess of background enrichment in excreta and feces. Nitrogen content in feces (N_{Feces}; g/kg DM) was calculated as the contribution of feces in excreta:

$$N_{Feces} = N_{Excreta} - (N_{Uric\ acid} \times 1.2)$$

where $N_{Excreta}$, and $N_{Uric\ acid}$ are the nitrogen contents (g/kg DM) in excreta and uric acid. The correction factor of 1.2 was used to account for urinary nitrogen contents others than uric acid, including ammonia, urea, and amino acids (Terpstra and de Hart, 1973; Dänicke et al., 2000). Subsequently, total EPL (g/kg DM intake) in ileal digesta and feces were calculated by correcting nitrogen flows (N_{Flows}, g/kg DM intake) in ileal digesta and feces for EPL proportion as previously calculated (Deglaire et al., 2009):

$$N_{Flows} = N_{Sample} \times \left(\frac{T_{Diet}}{T_{Sample}}\right)$$

$$EPL = N_{Flows} \times EPL\ proportion$$

where N_{Sample} is the nitrogen contents (g/kg DM) in ileal digesta or feces and T_{Diet} and T_{Sample} are the concentrations of tracers (Ti) (g/kg DM) in the diet and samples of ileal digesta and excreta. Finally, the apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) of protein (%) were corrected for EPL to quantify true ileal digestibility (**TID**) and true total tract digestibility (**TTTD**) of protein (%).

$$AID\ or\ ATTD\ of\ protein = \left(\frac{N_{Diet} - N_{flows}}{N_{Diet}}\right) \times 100\%$$

$$TID\ or\ TTTD\ of\ protein = \left(\frac{N_{Diet} - (N_{flows} - EPL)}{N_{Diet}}\right) \times 100\%$$

where N_{Diet} is the concentration of nitrogen (g/kg DM) in diet.

Statistical analyses

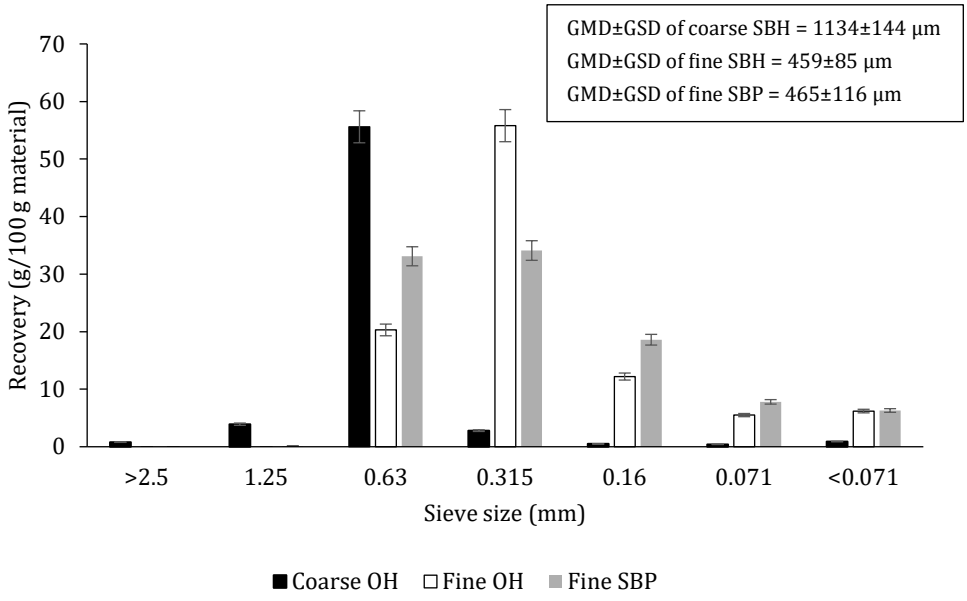
Observations at pen level (3 birds/pen) were considered the experimental unit for all statistical analyses. Effects of PS of SBH and addition of AX on daily FI, BW, EPL, and digestibility of protein were analyzed using a two-way ANOVA (PROC GLM, SAS, version 9.4, SAS Institute Inc., Cary, NC) with PS (coarse vs. fine) of SBH, AX inclusion (0 vs. 50 g/kg), and their interaction as fixed effects. Differences among means were tested using type III least squares, with Tukey adjustments for multiple comparisons. Substitution of F-SBP for F-SBH was tested using an independent t-test. Model assumptions for both analyses were visually verified using histograms, quantile-quantile plots, and studentized residuals. Homogeneity of variances was assessed using Levene's test. Data

are presented as estimated means and pooled SEM, unless indicated otherwise. Differences were considered significant at $P < 0.05$.

RESULTS

Grinding of SBH reduced GMD by 59.5% (C-SBH 1134±144 µm; F-SBH 459±85 µm, Figure 1.4) and GMD of F-SBP was 465±116 µm. However, the contrast in particle size of the resulting pelleted diets was only 2.3–2.4% (C-SBH diet 543±128 µm vs. F-SBH diet 532±125 µm vs. F-SBP diet 552±141 µm). The addition of AX to SBH diets resulted in a greater extract viscosity (+19%) and replacing F-SBH with F-SBP resulted in a greater WBC (+18%) (Table 2.4).

A



B

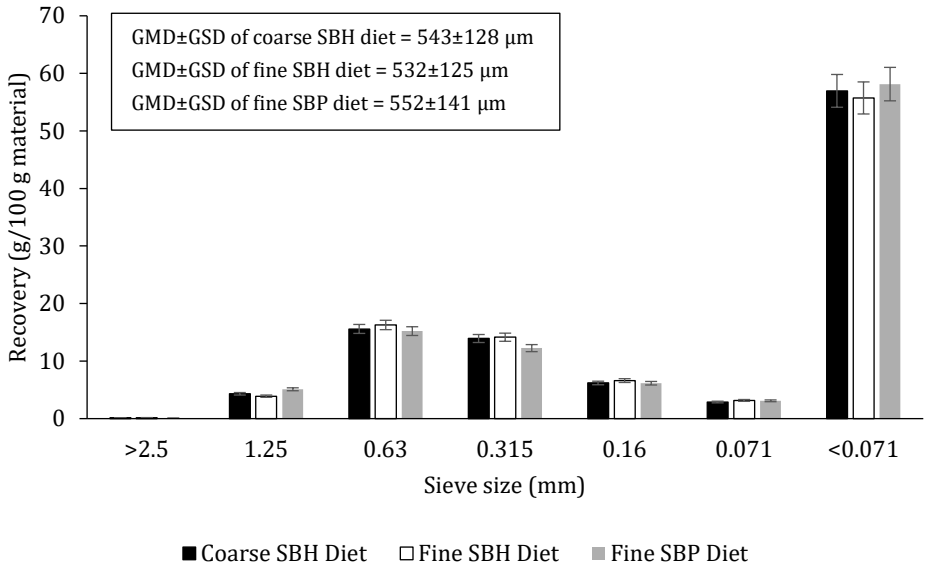


Figure 1.4. (A) Weight fraction of coarse or fine soybean hulls (SBH), or fine sugar beet pulp (SBP) recovered at sieves with various mash sizes during dry sieving analyses. (B) Weight fraction of coarse or fine SBH, or fine SBP diets recovered at sieves with various mash sizes during wet sieving analyses. Bars represent mean of two replicate measurements.

Table 2.4. Physicochemical characteristics of experimental diets fed to female broilers between 10 and 37 d of age[1].

	Diets[2]				
	C-SBH	F-SBH	C-SBH-AX	F-SBH-AX	F-SBP
Water-binding capacity (g/g)	1.6 ± 0.03	1.8 ± 0.04	1.5 ± 0.06	1.5 ± 0.00	2.2 ± 0.00
Extract viscosity (mPa.s)	1.3 ± 0.03	1.3 ± 0.01	1.6 ± 0.05	1.6 ± 0.08	1.3 ± 0.00

[1] Data are presented as mean and standard deviation of duplicate analyses.
[2] C-SBH = coarse soybean hulls (intact), F-SBH = fine soybean hulls (ground in hammer mill using a 1 mm screen), C-SBH-AX = coarse soybean hulls with additional 50 g/kg arabinoxylans, F-SBH-AX = fine soybean hulls with additional 50 g/kg arabinoxylans, F-SBP = fine sugar beet pulps (ground in hammer mill using 4 mm and 1 mm screen).

Body weight of broilers at the end of the experiment fed SBH diets with addition of AX was greater (+6 to 9%, $P = 0.002$) compared with SBH diets without addition of AX, but daily FI did not differ (Table 3.4). Daily FI of broilers fed F-SBP was greater (+8%, $P <$

0.001) compared with F-SBH diet, while BW at the end of experiment did not differ between these birds.

Table 3.4. Body weight and daily feed intake of female broilers fed diets varying in fiber source (soybean hulls (SBH) vs. sugar beet pulp (SBP)), particle size of SBH, and addition of arabinoxylans (AX), measured at 37 d of age.

Dietary treatment[1]	n[2]	Daily feed intake (g/d)[3]	Body weight (g)
Coarse SBH	8	96	2058
Fine SBH	8	97	2008
Coarse SBH + AX	8	96	2234
Fine SBH + AX	8	96	2131
Pooled SEM		1.9	44.7
P-values[4]			
Particle size (PS)		0.771	0.098
Arabinoxylans (AX)		0.706	0.002
PS × AX		0.617	0.556
Fine SBH	8	97	2008
Fine SBP	8	89	1937
Pooled SEM		1.9	66.2
P-values[5]		<0.001	0.303

[1] C-SBH = coarse soybean hulls (intact), F-SBH = fine soybean hulls (ground in hammer mill using a 1 mm screen), C-SBH-AX = coarse soybean hulls with additional 50 g/kg arabinoxylans, F-SBH-AX = fine soybean hulls with additional 50 g/kg arabinoxylans, F-SBP = fine sugar beet pulps (ground in hammer mill using 4 mm and 1 mm screen).

[2] Number of replicate pens (3 birds/pen).

[3] Daily feed intake was measured between 22 to 25 d of age.

[4] Model established *P*-values for the fixed effects of soybean hulls particle size, arabinoxylans, or their interaction (PS ×AX).

[5] Model established P-values comparing fine F-SBH and F-SBP diets as fixed effects.

The addition of AX to SBH diets increased EPL in feces (+0.26–0.43 g/kg DM intake, P = 0.020) but not consistently in ileal digesta (+0.01–0.23 g/kg DM intake, P = 0.17, Table 4.4). True ileal protein digestibility was lower in birds fed AX diets compared with SBH diets without AX supplementation (P < 0.001), which seemed to be more pronounced for the F-SBH diets (-3.5%-units) compared with C-SBH diets (-1.4%-units) as indicated by the tendency for a PS × AX interaction (P = 0.077). True total tract protein digestibility was not affected by addition of AX (P = 0.194).

Table 4.4. Total endogenous protein losses (g/kg DM intake) and apparent and true digestibility of protein (%) in female broilers fed diets varying in fiber source (soybean hulls (SBH) vs. sugar beet pulp (SBP)), particle size of SBH, and addition of arabinoxylans (AX), measured at 37 d of age.

Dietary treatment[1]	n[2]	Total endogenous protein losses		Ileal digestibility of protein		Total tract digestibility of protein	
		Ileal digesta	Feces	Apparent	True	Apparent	True
Coarse SBH	8	0.65	1.26	76.2[a]	78.4	78.9	83.1
Fine SBH	8	0.68	1.25	76.4[a]	78.6	80.3	84.4
Coarse SBH + AX	8	0.66	1.69	74.9[a]	77.0	78.4	83.5
Fine SBH + AX	8	0.91	1.51	72.2[b]	75.1	77.4	82.2
Pooled SEM		0.084	0.140	0.62	0.58	0.69	0.69
P-values[3]							
Particle size (PS)		0.105	0.537	0.045	0.165	0.783	0.955
Arabinoxylans (AX)		0.167	0.020	< 0.001	<0.001	0.016	0.194
PS × AX		0.220	0.538	0.024	0.077	0.093	0.063
Fine SBH	8	0.68	1.25	76.4	78.6	80.3	84.4
Fine SBP	8	0.99	2.17	75.7	79.0	79.2	86.5
Pooled SEM		0.162	0.474	1.00	0.91	2.07	1.42
P-values[4]		0.002	0.004	0.474	0.698	0.307	0.173

[1] C-SBH = coarse soybean hulls (intact), F-SBH = fine soybean hulls (ground in hammer mill using a 1 mm screen), C-SBH-AX = coarse soybean hulls with addition of 50 g/kg arabinoxylans, F-SBH-AX = fine soybean hulls with addition of 50 g/kg arabinoxylans, and F-SBP = fine sugar beet pulp (ground in hammer mill using 4 mm and 1 mm screen).

[2] Number of replicate pens (3 birds/pen).

[3] Model established P-values comparing SBH diets (fixed effects of soybean hulls particle size, arabinoxylans, or their interaction (PS × AX)). In case of significant PS × AX interactions, means within a column lacking a common superscript differ ($P < 0.05$).

[4] Model established P-values comparing fine F-SBH and F-SBP diets as fixed effects.

Particle size of SBH did not affect EPL in either ileal digesta nor feces, and did not affect true protein digestibility ($P > 0.1$), other than the above mentioned tendency for PS × AX interaction. The combined effects of diet properties on EPL, on the one hand, and true digestibility on the other, resulted in lower AID in birds fed AX diets compared with SBH diets without AX supplementation (-1.3–4.2%-units), particularly when fed the F-SBH diets (PS $P = 0.045$, AX $P < 0.001$, PS × AX $P = 0.024$). Similar, but less pronounced, patterns were found for ATTD of protein being 0.5–2.9%-units lower in birds fed AX

diets compared with SBH diets without AX supplementation (P = 0.016). The substitution of F-SBH with F-SBP increased EPL in ileal digesta (+0.31 g/kg DM intake, P = 0.002) and feces (+0.92 g/kg DM intake, P = 0.004), but did not significantly influence apparent and true ileal and total tract digestibility of protein.

DISCUSSION

The aim of this study was to investigate how DF sources varying in physicochemical properties, including PS, solubility, and hydration properties, influence EPL and protein digestibility in broilers. To address this, purified wheat AX, a viscous sDF source, was added to maize-based diets containing either C-SBH or F-SBH as the main iDF source. This design enabled evaluation of the effects of adding viscous sDF to iDF differing in PS. A fifth diet was added in which F-SBH were replaced with F-SBP, a DF source with noticeable hydration properties, allowing to compare intrinsic properties between DF sources. We hypothesized that the presence of AX or replacing SBH with SBP would result in (1) increased EPL and (2) reduced true protein digestibility, whereas particle size reduction of SBH was expected to decrease EPL and true protein digestibility. More importantly, it was expected that the adverse effect of AX on EPL and true protein digestibility may depend on PS of the dietary matrix, and thus on PS of SBH.

Role of dietary fibers sources in modulating endogenous protein losses

The addition of AX to SBH diets increased EPL, whereas marginal effects of PS of SBH were found. Endogenous protein losses in ileal digesta observed in the present study were lower than values reported in broilers and pigs (Dänicke et al., 2000; Hodgkinson et al., 2003). However, the observed differences between dietary treatments in our study are in line with previous studies that have consistently shown that viscous sDF and/or fibers with noticeable hydration properties increase EPL (Leterme et al., 1996, 1998a; Souffrant, 2001). In the current study, supplementation of 50 g/kg AX numerically increased ileal EPL, and diets containing F-SBP resulted in higher EPL than those containing F-SBH, which aligns with these earlier findings.

Arabinoxylans consist of a xylose backbone with arabinose side chains, enabling the formation of a viscous gel in the GIT (Bedford and Classen, 1992; Shelat et al., 2015;

Mendez-Encinas et al., 2018; Tiwari et al., 2019). Increased digesta viscosity has been shown to stimulate epithelial cell proliferation in the small intestine (Gee et al., 1996; Wanders et al., 2013, 2014) and to entrap digestive secretions such as bile acids and pancreatic enzymes, thereby limiting their hydrolysis and reabsorption before leaving the small intestine (Souffrant, 2001; Palafox-Carlos et al., 2011; Adeola et al., 2016; reviewed by Jha and Mishra, 2021). These responses could contribute to elevated EPL.

In contrast, EPL associated with SBP may arise primarily from its fermentable fiber fraction. SBP contains higher levels of soluble, highly fermentable components - mainly pectins - compared with SBH (Ivarsson et al., 2014; Feng et al., 2023). Fermentable sDF can stimulate microbial proliferation in the small intestine and hindgut (Ma et al., 2002; Schiavon et al., 2004; Pirman et al., 2007; Lynch et al., 2008; Tan et al., 2016). Indeed, SBP inclusion in pig diets has been associated with increased goblet cell numbers and enhanced mucin production throughout the GIT (Leterme et al., 1998b; Laitat and Philippe, 2015), which may further contribute to elevated EPL.

Contrasting effects of arabinoxylans and sugar beet pulp on true protein digestibility

Although both AX and SBP increased EPL, only AX reduced true ileal protein digestibility, whereas replacing F-SBH with F-SBP had no significant effect. Because the DF sources used in this study contributed negligible amounts of protein, differences in true protein digestibility are likely attributable to interactions between DF physicochemical properties and other dietary components. Formation of viscous gels promoted by the presence of AX may hinder interactions between digestive enzymes and nutrients, reduce nutrient diffusion to the epithelial surface, and ultimately impair nutrient absorption (Maisonnier et al., 2001; Silva et al., 2013; Sadeghi et al., 2015; Lentle and de Loubens, 2015; Henn and Alim, 2025). In contrast, SBP did not influence true protein digestibility, despite this DF source increased EPL, as also reported previously for pigs (Lynch et al., 2008). Although SBP has notable hydration capacity, its relatively low gelling ability (Phatak et al., 1988) may explain the lack of effects on true protein digestibility. Previous studies reported that apparent digestibility of CP between SBP and oat hulls did not differ (González-Alvarado et al., 2010; Jiménez-

Moreno et al., 2013), despite SBP displaying greater water-holding and swelling capacity compared with oat hulls (Jiménez-Moreno et al., 2013).

Impact of particle size of soybean hulls on digestion processes

Although coarse SBH were expected to enhance gizzard contractions and prolong gastric digesta retention time, thereby increasing EPL through stimulation of digestive secretions and physical abrasion of the gastrointestinal mucosa, only marginal differences were observed between diets containing coarse and fine SBH. Relative gizzard weight, retention time of solid and liquid digesta in the proventriculus and gizzard, and apparent nutrient digestibility were not affected by SBH particle size, as previously reported in a paper of the same study (Dorado-Montenegro et al., 2024).

Although grinding reduced GMD of SBH by ~60% and considerably reduced the fraction of coarse particles ≥ 0.63 mm (C-SBH ~60% vs. F-SBH ~23%, w/w), the contrast in PS at diet level was much smaller (Figure 1.4). It may be postulated the difference in PS between the diets was insufficient to markedly influence digestion processes. Remarkably, the effect of AX on AID of protein was found to depend on PS of SBH; where AX reduced AID of protein only when added to the F-SBH diet but not for the C-SBH. Similar patterns, but less pronounced were observed for TID and ATTD of protein, as indicated by the tendencies for PS × AX interactions ($P < 0.1$). Speculatively, subtle differences in PS and morphometry differently modulated digesta viscosity in C-SBH vs. F-SBH diets. Studies on particle suspensions have demonstrated that the viscosity of suspensions is very sensitive to the size of particles present (Senapati et al., 2010; Konijn et al., 2014). These findings indicate that although PS of SBH had a minor effect on protein digestibility, PS of the dietary matrix may modulate the impact of viscous DF on digestion processes, and that coarse particles can partially counteract the adverse effects of AX on protein digestibility.

Limitations of apparent digestibility in understanding dietary fiber effects

Commonly used digestibility assays to assess the bioavailability of proteins and amino acids in poultry feed ingredients primarily rely - largely for methodological convenience - on measurement of apparent digestibility i.e. the net disappearance of ingested nitrogen or amino acids along the gastrointestinal tract (Mateos et al., 2012; reviewed

by Jha and Mishra, 2021; reviewed by Röhe and Zentek, 2021; Vivares et al., 2025). The diverse physicochemical properties of DF ingredients, such as solubility, viscosity, and hydration capacity, can affect apparent digestibility via distinct mechanisms by altering EPL and/or true protein digestibility (Souffrant, 2001; Dégen et al., 2007). Because apparent nutrient digestibility reflects the combined effects of EPL and true digestibility, it provides limited insights into the underlying mode of action and may even mask counteracting DF effects. This was evident in the present study, where interpretation of DF effects based solely on apparent digestibility was clearly compromised. Viscous soluble AX increased EPL and reduced true protein digestibility, resulting in lower apparent protein digestibility, whereas SBP, with high water-binding capacity, increased EPL without affecting true protein digestibility, but also without causing significant reductions in apparent digestibility. Similar limitations of relying solely on apparent digestibility have been reported , for example, in a study with dogs, where apparent total tract digestibility of protein differed between animal- and plant-based diets, while true total tract digestibility did not (Cargo-Froom et al., 2019). Collectively, these observations highlight that apparent protein digestibility does not clearly explain the dietary effects on EPL and/or true protein digestibility, particularly when DF-rich ingredients are included.

In conclusion, our findings confirm that DF can exert heterogenous effects on EPL, as well as on nutrient hydrolysis and absorption (i.e. true protein digestibility) in broilers. The addition of AX to SBH containing diets increased EPL and reduced true protein digestibility. Particle size of SBH marginally affected digestion, but the interaction between PS and AX on ileal digestibility of proteins indicates that the presence of viscous soluble DF depended on PS of the dietary matrix and that, speculatively, coarse particles can partially mitigate the adverse impact of AX on protein digestibility. Substituting fine SBP for fine SBH, elicited EPL in ileal digesta and feces, but did not influence true protein digestibility. Apparent digestibility was only affected by the addition of AX, but not when SBP was substituted for SBH. This illustrates that apparent protein digestibility does not fully capture DF-induced changes in digestion processes. Taken together, these findings highlight the need for further mechanistic studies to elucidate how dietary fibers with different physicochemical properties influence EPL and nutrient hydrolysis and -absorption, enabling better interpretation of observations

at the apparent digestibility level, particularly in the context of the increasing use of fiber-rich by-products as feed ingredients for broiler diets.

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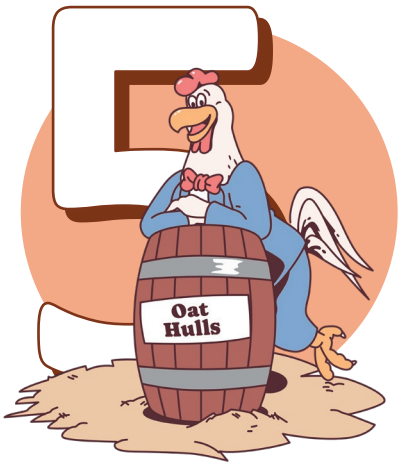
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Oat
Hulls

Chapter 5

Nutrient digestibility, total endogenous protein losses, and digesta mean retention time in broilers fed dietary insoluble fibers varying in particle size and hydration properties

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ABSTRACT

Particle size and hydration properties of insoluble dietary fibers (**iDF**) may influence digestion processes in animals, however, their effects on digesta mean retention time (**MRT**), endogenous protein losses (**EPL**) and nutrient digestibility are poorly understood. The aim of this study was to quantify digesta MRT, EPL, and nutrient digestibility in broilers fed iDF varying in particle size and hydration properties. A total of 300 one-day-old Ross 308 male broilers were allocated to three floor pens (100 birds/pen) and, from d 9, received one of three diets containing 88.6 g/kg iDF derived from coarse oat hulls (**OH**), fine OH, or purified lignocellulose (**LC**), the latter serving as a model iDF with noticeable hydration capacity. At d 13, birds were transferred to individual metabolism cages. Separate subsets of birds were used to evaluate EPL (n = 72), nutrient digestibility (n = 72), and MRT (n = 96). For EPL and digestibility, three adjacent cages constituted one experimental unit (8 replicates per diet), whereas for MRT each bird represented one experimental unit (32 replicates per diet). Between d 16 and 21, birds designated for EPL study received a daily oral dose of a ^{15}N-isotope solution 1.2 g ^{15}N-enriched milk protein concentrate (2.27 atom-%) and 0.12 g $^{15}NH_4Cl$ (≥98 atom-%). Seven days after isotope withdrawal (d 28), excreta were collected, birds were euthanized, and ileal digesta were sampled. For MRT study, birds were euthanized at d 21 and digesta were collected from the crop, proventriculus + gizzard, small intestine, and hindgut. Compared with OH diets, LC reduced apparent total tract digestibility of protein (−4.5 to −4.6%-units, $P = 0.01$), starch (−1.2 to −1.3%-units, $P < 0.001$), and fat (−8.7 to −9.4%-units, $P < 0.001$), decreased true ileal (−3.8 to −3.9%-units, $P < 0.05$) and total tract (−1.2 to −1.3%-units, $P < 0.001$) protein digestibility, and prolonged MRT of digesta in the small intestine (+29–51 min, $P < 0.05$), without affecting EPL. Particle size of OH had minimal effects. In conclusion, LC reduced nutrient hydrolysis and absorption compared with OH, without influencing EPL; whereas particle size of OH mostly did not influence the digestion processes. These findings suggest that physicochemical properties of ingredients, such as particle size and hydration properties should be controlled for in diet formulation, particularly when feeding fiber-rich diets.

INTRODUCTION

Agricultural by-products that are inedible for humans are increasingly incorporated into poultry diets as a strategy to reduce feed–food competition (Sandström et al., 2022). These by-products are typically rich in dietary fibers (**DF**), and depending on the sources, vary in physicochemical properties, such as solubility, particle size, and hydration capacity (Mateos et al., 2012; Slama et al., 2019; Kakhki et al., 2024) These properties have been reported to influence digestion processes in chickens, including digesta passage behavior, endogenous protein losses (**EPL**), and nutrient hydrolysis and absorption (Hetland et al., 2003; Jiménez-Moreno et al., 2013; reviewed by Jha and Mishra, 2021; Tejeda and Kim, 2021; Garçon et al., 2023).

For instance, the inclusion of soluble DF in poultry diets may affect digesta viscosity and result in a longer retention time of digesta in the small intestine and potentially reduce apparent protein, fat, and starch digestibility (Smulikowska et al., 2002; Dorado-Montenegro et al., 2024). In contrast, insoluble DF (**iDF**), due to their structural characteristics, may stimulate gizzard activity and hereby improve nutrient hydrolysis, although these effects may vary depending on the DF sources (Mateos et al., 2012). Notably, the particle size of iDF, has been reported as a key factor influencing its functional impact in improving nutrient digestibility (Naeem et al., 2023). For example, compared to finely ground oat hulls (**OH**), coarse OH may increase relative weight (**RW**) of gizzard due to increased thickness of the gizzard wall (Hetland and Svihus, 2001; Svihus, 2011; Abdollahi et al., 2019). This process may prolong digesta retention time in the gizzard, enhance mixing with digestive enzymes (Svihus, 2011), and ultimately improve digestibility of nutrients (Sacranie et al., 2012; Naeem et al., 2023). The longer digesta retention in that segment, however, may also elevate EPL as a result of stimulated enzymatic secretion from the proventriculus (Svihus, 2011) and induce an abrasive effect on gastrointestinal mucus layer (Leterme et al., 1998; Oryschak et al., 2002).

Beyond the physical structure, hydration properties of iDF, such as their ability to absorb and retain water, exists among iDF sources, which inherently differ in particle size and structural characteristics (Auffret et al., 1994; López et al., 1996; Boulos et al., 2000; Robertson et al., 2000; Brachet et al., 2015; Slama et al., 2019; Wang et al., 2024),

which finally may change the digestion processes in chickens. For example, purified lignocellulose, a fine particle iDF source with high capacity to absorb and retain water in its matrix, has been reported to prolong total digesta retention time and potentially increase EPL by enhancing microbial activity, when compared with almond shells (Rybicka et al., 2024). Another study in laying hens also reported that adding 1% purified lignocellulose increased EPL by stimulating digestive enzymes, such as pepsin, trypsin, and chymotrypsin, when compared with control diet (Yokhana et al., 2016).

Despite these potential increases in EPL, the ability to absorb and retain the water may also exert positive effects on nutrient utilization. The inclusion of purified lignocellulose has been shown to increase apparent digestibility of amino acids and fatty acids in broilers and broiler breeders (Bogusławska-Tryk et al., 2016; Farran et al., 2017).

Physicochemical properties of iDF may influence digestion processes in broilers in various ways. Thus, to accurately predict nutritional value of fiber-rich diets, it is important to understand how these fibers modulate (1) digesta passage behaviour along the gastrointestinal tract (**GIT**), (2) EPL, and (3) nutrient hydrolysis and absorption of other macro nutrients. To address this, the objective of the current study was to investigate the effects of particle size and hydration properties of iDF sources on digesta mean retention time (**MRT**), EPL, apparent nutrient digestibility, and true protein digestibility in broilers. Coarse and fine OH, as well as purified lignocellulose were used to model coarse, medium, and fine particle sizes of an iDF source. The latter is a powdery material with an average particle size of 80 to 300 μm (Zeitz et al., 2019; Röhe et al., 2020a) with noticeable hydration properties (Brachet et al., 2015; reviewed by Röhe and Zentek, 2021; Urban et al., 2024). It was hypothesized that feeding diets with coarse OH would increase the retention time of digesta in the proventriculus and gizzard, increase EPL, and enhance true protein digestibility compared with fine OH. In contrast, substituting LC for OH was expected to prolong digesta retention throughout the GIT, increase EPL, and increase nutrient digestibility.

MATERIAL AND METHODS

Experimental design, birds, and housing

The experimental procedure was approved by the Poultry Research Centre Ethical Committee using the Spanish law Real Decreto RD 53/2013; and performed at the research facility of the Poultry Research Centre of Trouw Nutrition, in Casarrubios del Monte, Spain in June 2022.

A completely randomized design was applied to investigate the effects of iDF varying in particle size and hydration properties on digesta passage behaviour, EPL, and nutrient digestibility. A total of 300 one-d-old Ross 308 male broilers (initial BW: 43.73±0.46) were randomly allocated to 3 collective floor pens. Pens were assigned to 1 of 3 treatments (100 birds per pen per treatment) and were covered with wood shavings. On d 13, in order to measure EPL and nutrient digestibility, 72 birds for each measurement were randomly assigned into 72 individual metabolism cages. Every 3 adjacent individual metabolism cages were considered as 1 experimental unit. This resulted in 8 replicates per dietary treatment with 3 birds each. For the digesta passage behaviour study, a total of 96 birds were randomly allocated to individual metabolism adjacent cages, where each dietary treatment was assigned to 32 birds. In this observation, the individual bird served as experimental unit. All pens and individual metabolism cages were equipped with perches, feeders and nipple drinkers. The rooms were equipped with artificial lighting and an automatic environmentally controlled system for ventilation and temperature maintenance. Birds had *ad libitum* access to feed and water. A summary of the bird housing setting in course of time is depicted in Figure 1.5.

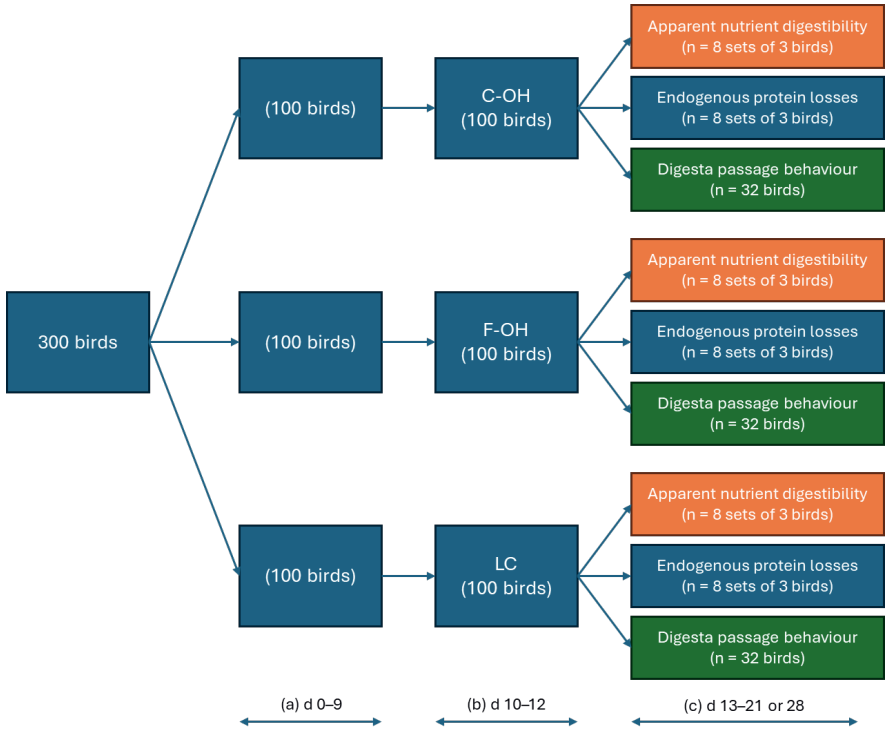


Figure 1.5. Timeline of the experiment. (a) time of all birds received pelleted wheat-based diets (d 0–9) in floor pens, (b) time of all birds started to receive dietary treatments (100 birds/treatment) of intact oat hulls (C-OH), ground fine oat hulls by hammer milling using a 1.5 mm sieve (F-OH), or purified wood lignocellulose (LC, Arbocel® RC Fine J. Rettenmaier & Söhne GmbH + Co KG, Germany) (d 10–12) in floor pens, and (c) time of all birds were distributed to the individual metabolism pens for nutrient digestibility, endogenous protein losses, or digesta passage behavior studies (d 13–21 or 28).

Diets

In the first 9 d, all birds were fed a pelleted wheat-soybean meal-based starter diet. From d 10 until the end of experiment (d 8), pelleted experimental grower diets were offered (Table 1.5). All Experimental diets were formulated to meet or exceed recommended nutrient contents for growing broilers (CVB, 2018), except for standardized ileal digestible lysine which was slightly lower (5%) than recommendations, to avoid excessive growth of birds housed in cages – which would impaired animal welfare - , without causing any nutrient deficiencies. The experimental

diets where contained wheat, soybean meal, and maize as main ingredients, with either 100 g/kg coarse (**C-OH**: intact OH) or fine oat hulls (**F-OH**: ground with a 1.5 mm screen using Full Circle Screen Hammer Mills, ROSAL, Barcelona, Spain), or 89 g/kg purified fine lignocellulose (**LC**, Arbocel® RC Fine, Rettenmaier & Söhne GmbH + Co KG, Germany) as the main insoluble fiber sources (Table 1.5). To match the nutrient contribution of OH in the OH diets, 4.0 g/kg wheat protein and 6.5 g/kg maize starch were added to the LC diet. Titanium dioxide (TiO_2; 2 g/kg) was added to all diets as inert tracer to measure nutrient digestibility and MRT of fine-solid digesta.

Table 1.5. Ingredient and nutrient composition of experimental diets[1,2] (in g/kg as-fed, unless indicated otherwise) fed to female broilers between 10 and 28 days of age.

Item	C-OH/F-OH	LC
Ingredient composition (g/kg)		
Maize	174.9	174.9
Wheat	350.0	350.0
Soybean meal	262.4	262.4
Soya oil	62.2	63.1
Potato protein,	11.2	11.2
Oats hulls	100.0	-
Arbocel RC Fine	-	88.6
Wheat protein	-	4.0
Maize starch	-	6.5
Calcium carbonate	13.1	13.1
Monocalcium phosphate	4.8	4.8
Salt (NaCl)	2.4	2.4
Na bicarbonate	1.4	1.4
Mineral and vitamin premix[3]	5.0	5.0
Dl-Methionine 99%	3.1	3.1
L-Lysine sulphate 70%	3.7	3.7
L-Threonine 98%	1.2	1.2
L-Isoleucine 92.6%	0.1	0.1
L-Arginine 98%	0.6	0.6
L-Valine 96.5%	0.9	0.9
Axtra PHY 500	1.0	1.0
Titanium dioxide	2.0	2.0
Calculated nutrient composition (g/kg)[4]		
AME (MJ/kg)	11.3	11.3
CP[5]	187.0	187.0
Dig lys	10.4	10.5
Dig thr	0.7	0.7

Dig met + cys	0.8	0.8
Ca	8.0	7.9
Available P	4.5	4.3
Analyzed nutritional composition (g/kg)		
DM	894.7	931.9
CP[5]	193.0	185.4
Fat	82.3	84.2
Starch	298.5	301.1
Total non-starch polysaccharides	120.6	123.3
Of which soluble	12.6	11.1
Of which insoluble	108.0	112.2

[1] Oat hulls were either used intact (coarse, C-OH), ground fine by hammer milling using a 1.5 mm sieve (fine, F-OH).
[2] Purified wood lignocellulose (LC, Arbocel® RC Fine J. Rettenmaier & Söhne GmbH + Co KG, Germany).
[3] Premix provided per kilogram of diet: Vitamin A (retinyl acetate), 10,000 IU; Vitamin D_3 (cholecalciferol), 2.500 IU; Vitamin E (dl-a-tocopherol), 50 mg; Vitamin K_3 (menadione), 2.0 mg; Vitamin B_1 (thiamin), 2.0 mg; Vitamin B_2 (riboflavin), 6.0 mg; Vitamin B_6 (pyridoxin-HCl), 4.0 mg; Vitamin B_{12} (cyanocobalamin), 25 µg; Niacin, 40 mg; D-pantothenic acid, 10.87 mg; Choline chloride, 300 mg; Folic acid, 1.0 mg; Biotin, 0.15 mg; Iron, 65 mg, as $FeCO_3$; Copper, 15 mg, as $CuSO_4$; Manganese, 90 mg, as MnO; Zinc, 80 mg, as ZnO; Iodate, 1.0 mg, as KI; Selenium, 0.25 mg, as Na_2SeO_3.
[4] Calculated nutritional composition based on the composition and nutritional value of feed materials (CVB, 2018). Digestible amino acids and metabolizable energy values were calculated based on values of apparent metabolizable energy and standardized ileal digestibility for broilers.
[5] Nitrogen × 6.25.

Oral isotope infusion and sampling procedures

The procedures of ^{15}N-isotope solution administration were adapted from Dänicke et al. (2000) as described by Habibi et al. (2026). In brief, at 16 until 21 d of age, all birds designated to EPL measurement received daily an oral dose of a ^{15}N-isotope solution containing 1.2 g ^{15}N-enriched milk protein concentrate (atom-% = 2.27%) and 0.12 g $^{15}NH_4Cl$ (atom-% ≥ 98%). The ^{15}N-isotope solution was withdrawn at 22 to 28 d of age and representative spot samples of excreta were collected daily at 08:00 and 13:00. Only at 28 d of age, subsamples of excreta of birds used for nutrient digestibility measurement were collected. All collected excreta were pooled per experimental unit, and immediately frozen. At 28 d of age, all birds for EPL and nutrient digestibility measurements were euthanized by cervical dislocation, and ileal digesta were gently rinsed with demineralized water and pooled per experimental unit.

At d 20 or d 21 of age, all broilers designated for the digesta passage behaviour study were euthanized by cervical dislocation. The GIT from esophagus to rectum was

removed and crop, proventriculus + gizzard, small intestine, and ceca + colon (hereafter referred to as hindgut) were separated and weighed. For each segment, digesta contents were collected by gentle-finger stripping and rinsed with demineralized water. Empty GIT segments were dried with paper tissue and weighed. All samples were collected in the plastic tube with lid and stored in -20°C freezer until further analysis.

Sample preparation and analytical methods

Prior to analyses, excreta and ileal digesta samples were freeze-dried and ground using a rotor mill (Retsch ZM200, Haan, Germany) at 12,000 rpm with a 1 mm screen. All chemical and physical analyses were performed in duplicate, unless indicated otherwise.

Samples were analyzed for contents of DM (AOAC, 2016; method 930.15), ether extract (after hydrochloric acid hydrolysis, using Soxhlet apparatus and petroleum ether; AOAC, 2016; method 920.39), and total starch (ISO 15914, 2004). Total non-starch polysaccharides (**NSP**) were measured as neutral sugars and uronic acids, according to the method of Englyst et al. (1994), with some modifications as described by Lannuzel et al. (2024). Titanium (**Ti**), was analyzed after ashing and microwave digestion using an Avio 500 inductively coupled plasma optical emission spectrometer (Perkin Elmer, Waltham), as described by Lannuzel et al. (2024).

Uric acid was extracted from excreta following the procedure of (Baker, 1946) and subsequently quantified using a commercial test kit (Uric Acid liquicolor plus, 10694, Human GmbH, Wiesbaden, Germany). The precipitate remaining after uric acid extraction was collected and considered to represent feces. Ileal digesta, excreta, and fecal samples were re-ground using a mixer mill (Retsch MM200, Germany) for 5 min to obtain finer sample particles. Nitrogen and ^{15}N-enrichment in the samples of digesta, excreta, and feces were analyzed using Dumas method (ISO 16634, 2008) and combustion-isotope ratio mass spectrometry using a Delta C continuous-flow isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany).

Water-binding capacity and particle size distribution

The capacity of diets to absorb and retain the water was assessed by measuring water-binding capacity (**WBC**). All diets were analyzed with the following modifications of the method of Jacobs et al. (2015). Diets and deionized water were mixed in a conical tube (50 mL) using a vortex (2,500 × g) for 5 s to ensure thorough mixing of the components. Values were reported as g water/g dry sample. Particle size distributions of the experimental diets were analyzed by wet sieving method (Wolf et al., 2010). The geometric mean diameter (**GMD**), and geometric standard deviation (**GSD**) of samples were calculated as described by Lyu et al. (2020).

Calculations

^{15}N-enrichment above background enrichment (expressed as atom percentage excess (**APE**)) was calculated from ^{15}N-enrichment in ileal digesta, feces, and excreta minus the background enrichment as measured in the ileal digesta and excreta of birds that did not receive ^{15}N-isotope solution (Supplementary, Table S1). The ^{15}N-enrichment in excess of background enrichment in those samples were used to calculate total EPL proportion as follows (Dänicke et al., 2000):

$$EPL\ proportion = \frac{APE_{Sample}}{APE_{Urine}}$$

where APE_{Sample} is the ^{15}N-enrichments in excess of background enrichment in ileal digesta or feces. ^{15}N-enrichments in excess of background enrichment in urine (APE_{Urine}) was used to represent the ^{15}N-enrichment in endogenous nitrogen pool, which was extensively discussed in a previous study (Habibi et al., 2026) and was calculated as follows (Dänicke et al., 2000):

$$APE_{Urine} = \frac{(APE_{Excreta} \times N_{Excreta}) - (APE_{Feces} \times N_{Feces})}{N_{Uric\ acid} \times 1.2}$$

where $APE_{Excreta}$ and APE_{Feces} are the ^{15}N-enrichment in excess of background enrichment in excreta and feces. Nitrogen content in feces (N_{Feces}; g/kg DM) was calculated as the contribution of feces in excreta:

$$N_{Feces} = N_{Excreta} - (N_{Uric\ acid} \times 1.2)$$

where $N_{Excreta}$, and $N_{Uric\ acid}$ are the nitrogen contents (g/kg DM) in excreta and uric acid. The correction factor of 1.2 was used to account for urinary nitrogen contents others than uric acid, including ammonia, urea, and amino acids (Terpstra and de Hart, 1973; Dänicke et al., 2000). Endogenous protein losses (g/kg DM intake) in ileal digesta and feces were calculated by correcting nitrogen flows (N_{Flows}, g/kg DM intake) in ileal digesta and feces for EPL proportion as previously calculated (Deglaire et al., 2009):

$$N_{flows} = N_{Sample} \times \left(\frac{T_{Diet}}{T_{Sample}}\right)$$

$$EPL = N_{Flows} \times EPL\ proportion$$

where N_{Sample} is the nitrogen contents (g/kg DM) in ileal digesta or feces and T_{Diet} and T_{Sample} are the concentrations of tracers (Ti) (g/kg DM) in the diet and samples of ileal digesta and excreta. Subsequently, the apparent ileal (**AID**) and total tract (**ATTD**) digestibility of protein (%) were corrected for total EPL to determine true ileal (**TID**) and total tract digestibility (**TTTD**) of protein (%).

$$AID\ or\ ATTD\ of\ protein = \left(\frac{N_{Diet} - N_{flows}}{N_{Diet}}\right) \times 100\%$$

$$TID\ or\ TTTD\ of\ protein = \left(\frac{N_{Diet} - (N_{flows} - ENL)}{N_{Diet}}\right) \times 100\%$$

where N_{Diet} is the concentration of nitrogen (g/kg DM) in diet. To calculate the AID and ATTD of other nutrients (fat, starch, and total NSP), the N_{Diet} was changed to the concentration of other nutrients in the diet (g/kg DM) and N_{flows} was changed to flows of other nutrients (g/kg DM intake). The MRT of digesta was calculated from tracer Ti in the diet (T_{Diet}, g/kg DM) ingested during 24 h (intake/1440 min, g) and the pool size of tracer (T_{Sample}, g/kg DM) detected in the digesta sample (W_{Sample}, g) in every GIT segment at dissection, assuming steady state conditions under *ad libitum* feeding strategies (van der Klis et al., 1990).

$$MRT\ (min) = 1440 \times \left(\frac{T_{Sample} \times W_{Sample}}{T_{Diet} \times intake/1440\ min}\right)$$

Statistical analysis

Observations of 3 birds housed individually in adjacent metabolism cages were considered an experimental unit for all statistical analyses of EPL and nutrient digestibility. The individual bird was considered the experimental unit for MRT of digesta and broiler performance. Effects of iDF varying in particle size and hydration properties on EPL, apparent nutrient digestibility, true protein digestibility, MRT of digesta, BW, daily FI, and GIT traits were analyzed by one-way ANOVA (PROC GLM, SAS, version 9.4, SAS Institute Inc., Cary, NC), with diet as fixed effect. Differences among means were tested using type III least squares statistics, with Tukey adjustments for multiple comparisons. Model assumptions were visually verified using histograms, quantile-quantile plots, and studentized residuals. Homogeneity of variances was assessed using Levene's test. Data are presented as estimated means and pooled SEM, unless indicated otherwise. Differences were considered significant at $P < 0.05$.

RESULTS

Diets, performance, and gastrointestinal tract traits

Grinding of OH and LC inclusion reduced GMD of the diet by 8.8%–24.8% (C-OH diet 525±137 µm, F-OH 479±112 µm, and LC diet 395±132 µm, Figure 2.5). The inclusion of LC resulted in a greater WBC by 8.1– 9.2% (Figure 3.5).

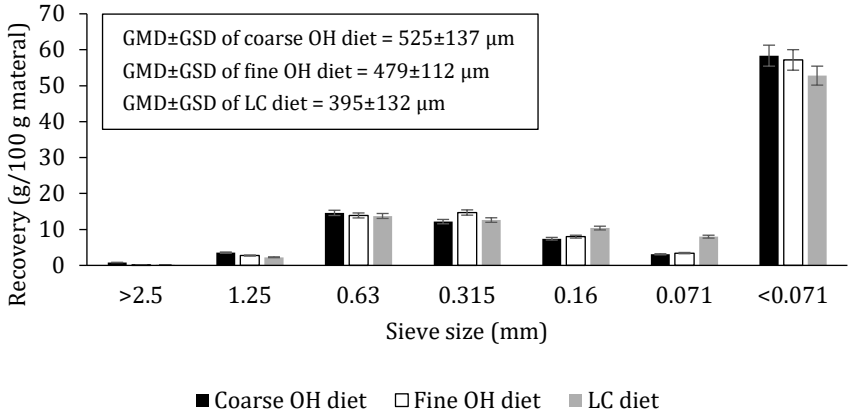


Figure 2.5. Weight fraction of diets including coarse (intact) and fine (ground by hammer milling using a 1.5 mm sieve) oat hulls (OH), or purified lignocellulose (LC, Arbocel® RC Fine J. Rettenmaier & Söhne GmbH + Co KG, Germany) recovered at sieves with various mash sizes during wet sieving analyses. Bars represent mean of 2 replicate measurements. Error bars represent SEM. GMD: geometric mean diameter, GSD: geometric standard deviation.

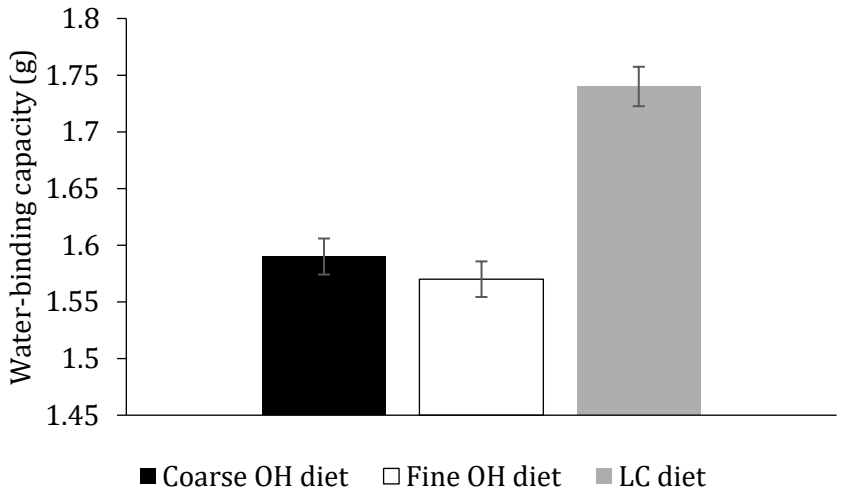


Figure 3.5. Water-binding capacity of diets including coarse (intact) and fine (ground by hammer milling using a 1.5 mm sieve) oat hulls (OH), or purified lignocellulose (LC, Arbocel® RC Fine J. Rettenmaier & Söhne GmbH + Co KG, Germany). Bars represent mean of 2 replicate measurements. Error bars represent SEM.

Body weight at the end of experiment, daily FI, and GIT traits did not differ between C-OH vs. F-OH diets. Body weight of broilers fed LC diet was lower when compared with C-OH diet (-7.3%, $P = 0.033$, Table 2.5). Greater RW of the crop (+9.5–14.3%, $P < 0.05$) and RW of digesta in the small intestine (+14.6–20.2%, $P < 0.05$), but smaller RW of proventriculus + gizzard (-16.4–21.2%, $P < 0.05$), as well as DM content of digesta in crop (-26.6–34.4%, $P < 0.05$) and proventriculus + gizzard (-38.8–41.6%, $P < 0.05$) were found in LC vs. C-OH or F-OH diets. Additionally, RW of the small intestine (+11.0%, $P = 0.040$) was greater in LC vs. C-OH diets.

Table 2.5. Body weight, daily feed intake, digestive organ size, digesta weight, and digesta dry matter in broilers fed diets containing fine vs. coarse oat hulls (100 g/kg) or purified lignocellulose (86 g/kg), measured at 20 or 21 d of age.[1,2]

Item	C-OH	F-OH	LC	SEM	Model established *P*-values[3]		
					C-OH vs. F-OH	C-OH vs. LC	F-OH vs. LC
n[4]	32	32	32				
Body weight (g)	999	983	926	19.3	0.825	0.034	0.112
Daily feed intake (g/d)	98	94	93	1.5	0.129	0.085	0.974
Relative empty organ weight (g/kg BW)							
Crop	3.8	3.6	4.2	0.13	0.934	0.015	0.007
Proventriculus + gizzard	22.6	21.3	17.8	0.75	0.420	< 0.001	0.009
Small intestine	36.4	39.2	40.9	1.21	0.261	0.040	0.579
Hindgut[5]	5.8	5.9	5.5	0.23	0.854	0.660	0.352
Relative digesta weight (g/kg BW)							
Crop	10.2	7.7	8.8	1.42	0.433	0.739	0.866
Proventriculus + gizzard	12.8	15.0	15.2	1.15	0.354	0.312	0.996
Small intestine	38.0	35.5	44.5	1.61	0.528	0.024	0.002
Hindgut[5]	8.5	7.9	7.8	0.42	0.543	0.463	0.990
Dry matter content of digesta (g/kg)							
Crop	298	267	196	18.7	0.468	0.002	0.035
Proventriculus + gizzard	313	298	183	7.9	0.458	< 0.001	< 0.001
Small intestine	167	162	174	3.0	0.407	0.216	0.016
Hindgut[5]	123	134	140	5.8	0.375	0.128	0.783

[1] Oat hulls were either used intact (C-OH) or ground fine by hammer milling using a 1.5 mm sieve (F-OH).

[2] Purified wood lignocellulose (LC, Arbocel® RC Fine J. Rettenmaier & Söhne GmbH + Co KG, Germany).

[3] Model established *P*-values for the fixed effects of diet.

[4] Number of replicate birds.

[5] Relative organ weight, relative digesta weight, and DM of digesta were calculated from collected samples of ceca + colon.

Nutrient digestibility, endogenous protein losses, and digesta passage

While AID of starch (+1.7–2.0%-units, $P < 0.001$), TID of protein (+3.8–3.9%-units, $P = 0.02$), ATTD of DM (+2.5–3.6%-units, $P < 0.05$), ATTD of starch (+1.2–1.3%-units, $P < 0.001$), ATTD of fat (+7.9–8.6%-units, $P < 0.001$), and ATTD of protein (+3.8–3.9%-units, $P = 0.01$) were greater in OH compared with LC diets, TTTD of protein (+2.6%-units, $P = 0.03$) was only greater in F-OH vs. LC diets (Table 3.5). Starch disappearance in the hindgut (+0.8 %-units, $P = 0.04$) was significantly greater in LC compared with C-

OH diets. Endogenous protein losses in ileal digesta and feces did not differ among diets (Table 4.5). Purified lignocellulose diet only increased MRT of digesta in the small intestine (+29–51 min, $P < 0.05$; Table 5.5) when compared with OH diets.

Table 3.5. Apparent ileal (AID), total tract (ATTD) digestibility (% of intake), and disappearance (%) of nutrients in the hindgut in broilers fed diets containing fine vs. coarse oat hulls (100 g/kg) or purified lignocellulose (86 g/kg)[1,2]

Item	C-OH	F-OH	LC	SEM	Model established *P*-values[3]		
					C-OH vs. F-OH	C-OH vs. LC	F-OH vs. LC
n[4]	8	8	8				
AID							
Dry matter	62.5	64.8	61.4	1.11	0.312	0.785	0.101
Protein	80.0	78.7	75.0	1.46	0.992	0.161	0.197
Starch	98.6	98.3	96.6	0.20	0.552	< 0.001	< 0.001
Fat	96.2	93.7	89.0	2.30	0.721	0.092	0.340
ATTD							
Dry matter	69.1	70.6	68.1	0.64	0.243	0.467	0.024
Protein	84.9	84.8	81.0	0.85	0.998	0.011	0.013
Starch	98.8	98.9	97.6	0.18	0.826	< 0.001	< 0.001
Fat	91.3	92.0	83.7	1.13	0.877	< 0.001	< 0.001
Total NSP	3.7	10.6	5.4	2.31	0.114	0.858	0.281
Nutrient disappearance in the hindgut							
Protein	5.9	6.1	6.0	1.87	0.998	0.999	0.999
Starch	0.2	0.6	1.0	0.23	0.370	0.040	0.437
Fat	-4.9	-1.6	-5.2	2.73	0.672	0.996	0.617

[1] Oat hulls were either used intact (C-OH) or ground fine by hammer milling using a 1.5 mm sieve (F-OH).
[2] Purified wood lignocellulose (LC, Arbocel® RC Fine J. Rettenmaier & Söhne GmbH + Co KG, Germany).
[3] Model established *P*-values for the fixed effects of diet.
[4] Number of replicate pooled samples of 3 birds individually housed in adjacent metabolism cages.

Table 4.5. Total endogenous protein losses (EPL) estimates in ileal digesta and excreta (g/kg DMI), true ileal (TID) and total tract digestibility (TTTD) of protein (%), and protein disappearance in the hindgut (%) in broilers fed diets containing fine vs. coarse oat hulls (100 g/kg) or purified lignocellulose (86 g/kg)[1,2]

Item	C-OH	F-OH	LC	SEM	Model established *P*-values[3]		
					C-OH vs. F-OH	C-OH vs. LC	F-OH vs. LC
n[4]	8	8	8				
EPL in ileal digesta	1.53	1.57	1.42	0.205	0.992	0.918	0.864
TID of protein	83.4	83.3	79.5	0.91	0.999	0.018	0.019
EPL in feces	2.04	2.19	2.42	0.121	0.642	0.086	0.389
TTTD of protein	90.7	91.2	88.6	0.64	0.853	0.077	0.025
True protein disappearance in the hindgut	7.4	7.9	9.1	1.26	0.951	0.587	0.771

[1] Oat hulls were either used intact (C-OH) or ground fine by hammer milling using a 1.5 mm sieve (F-OH).
[2] Purified wood lignocellulose (LC, Arbocel® RC Fine J. Rettenmaier & Söhne GmbH + Co KG, Germany).
[3] Model established *P*-values for the fixed effects of diet.
[4] Number of replicate pooled samples of 3 birds individually housed in adjacent metabolism cages.

Table 5.5. Mean retention time (MRT) (min) of digesta in broilers fed diets containing fine vs. coarse oat hulls (100 g/kg) or purified lignocellulose (86 g/kg)[1,2,3]

Segment	C-OH	F-OH	LC	SEM	Model established *P*-values[4]		
					C-OH vs. F-OH	C-OH vs. LC	F-OH vs. LC
n[5]	32	32	32				
Crop	54	45	35	8.6	0.776	0.303	0.659
Proventriculus + gizzard	46	55	45	4.2	0.286	0.999	0.267
Small intestine	206	184	235	6.2	0.078	0.024	< 0.001
Hindgut[6]	23	24	23	2.6	0.942	0.996	0.968
Total tract[7]	335	319	330	11.0	0.589	0.935	0.792

[1] Oat hulls were either used intact (C-OH) or ground fine by hammer milling using a 1.5 mm sieve (F-OH).
[2] Purified wood lignocellulose (LC, Arbocel® RC Fine J. Rettenmaier & Söhne GmbH + Co KG, Germany).
[3] Mean retention time was measured using TiO_2 as continuous marker.
[4] Model established *P*-values for the fixed effects of diet.
[5] Number of replicate birds.
[6] Mean retention time was calculated from collected samples of ceca + colon.
[7] Total tract MRT was calculated by summing MRT in all segments.

DISCUSSION

The aim of this study was to evaluate how iDF sources differing in physicochemical properties, including particle size and hydration properties, influence nutrient digestibility, EPL, and digesta retention time throughout the GIT of broilers. To address this, coarse and fine OH, as well as purified LC were used to obtain a contrast in particle size of iDF, where LC also has noticeable capacity to bind and retain water. We hypothesized that feeding a coarse OH diet would result in a longer MRT of digesta in the proventriculus + gizzard, greater EPL, and higher apparent nutrient digestibility, as well as true protein digestibility, when compared with feeding a fine OH diet. In contrast, substituting LC for OH was expected to prolong digesta retention throughout GIT, enhance EPL, and increase nutrient digestibility in broilers.

Physicochemical properties of insoluble dietary fibers and their consequences for digesta transit and nutrient absorption

Grinding of OH showed only marginal effects, whereas the inclusion of purified LC decreased nutrient digestibility and only increased MRT of digesta in the small intestine. Overall, nutrient digestibility, digesta MRT, and RW of GIT segments were in line with previous studies in chickens fed diets containing OH or LC (Hetland and Svihus, 2001; Röhe et al., 2020a; Habibi et al., 2024). Although coarse iDF were expected to enhance grinding activity in the gizzard, increase digesta retention time in the proventriculus + gizzard, and improve nutrient hydrolysis (Hetland and Svihus, 2001; Hetland et al., 2003; Svihus, 2011; Sacranie et al., 2012; reviewed by Kheravii et al., 2018), only marginal differences were found between diets containing coarse and fine OH in our study. Despite formulating diets to achieve a clear particle size contrast of iDF sources, wet-sieving analysis showed that only ~3–4% (w/w) of particles ≥ 0.63 mm in all diets (Figure 2.5).

Nevertheless, compared with OH, feeding LC diets resulted in smaller RW of proventriculus + gizzard, decreased apparent nutrient and true protein digestibility, without influencing MRT of digesta in the proventriculus + gizzard, as also observed in previous studies (Jiménez-Moreno et al., 2009; Moradi et al., 2021; Mens et al., 2022). Other work likewise demonstrated that inclusion of ≥ 5% purified LC decreased

apparent digestibility of protein, fat, and organic matter in chickens and pigs (Cao et al., 2003; Son and Kim, 2015; Röhe et al., 2020a). Compared with OH, LC contains higher WBC, as well as swelling capacity (Slama et al., 2019), which may promote formation of a digesta network structure and limit 140tilizsibility of endogenous enzymes to macronutrients (Williams et al., 2019; Meldrum and Yakubov, 2025). Therefore, it may reduce nutrient hydrolysis and absorption (Meldrum and Yakubov, 2025).

Furthermore, although LC only significantly affected digesta MRT in the small intestine, LC numerically increased RW of digesta in the crop and proventriculus + gizzard, but reduced the content of DM in these segments. In contrast, both RW and DM of digesta in the small intestine were greater in LC- than OH-fed birds. These findings indicate that the high WBC of LC indeed results in more incorporation of water in the digesta matrix, as previously reported by Takahashi et al. (2005, 2008). This suggests that fibrous particles from the LC diet may be retained shorter in the crop and proventriculus + gizzard compared with OH diets, but the liquid fraction may be retained longer. In the small intestine, however, both fibrous and liquid fractions may be retained longer with LC than OH diets. In the present study, however, digesta MRT was evaluated using TiO_2 as a marker, which is most representative for the fine-particle digesta fraction (Garçon et al., 2023). Taken together, these findings indicate that MRT based solely on TiO_2 may not fully capture differences in phase-specific digesta behavior in birds fed fiber-rich diets. Additional approaches, such as the use of other markers (e.g., Chromium-mordanted fibers for fibrous fraction and ytterbium–EDTA for liquid fraction), would allow characterization of the behavior of distinct digesta fractions in chickens (Dorado-Montenegro et al., 2023; Dorado-Montenegro, 2024).

Contrasting effects of physicochemical properties of insoluble dietary fibers on endogenous protein losses

In the present study, dietary treatments did not result in significant differences in EPL, suggesting that the iDF sources evaluated may equally influence EPL, albeit through different mechanisms. Inherent to the study design and objectives, no low-fiber control diet was included, and the absolute contribution of iDF to increased EPL could not be quantified.

The Influence of OH on EPL may be associated with enhanced secretion of digestive enzymes and physical abrasion of the gastrointestinal mucus layer, driven by the coarse structure of this fiber source. Previous studies have reported that inclusion of structurally rigid iDF sources, such as OH or rice husk, increases HCl secretion and reduces gizzard pH (Jiménez-Moreno et al., 2010, 2013; Itani et al., 2024; Kardel et al., 2025), reduces intestinal mucus thickness (Mateos et al., 2012; Sadeghi et al., 2015; Han et al., 2017), and stimulates secretion of pancreatic enzymes such as amylase, lipase, and trypsin and bile acids in the small intestine (Hetland et al., 2003; Svihus et al., 2004; Husvéth et al., 2015).

Additionally, although LC has been shown to increase pancreatic activity (Bogusławska-Tryk, 2005; Yokhana et al., 2016), its influence on EPL may also arise from its capacity to bind and retain water in the digesta matrix, thereby enhancing microbial activity and biomass production (Tan et al., 2017). Supporting this interpretation, several studies demonstrated that purified LC increased microbial fermentation and bacterial populations in GIT of broilers and dual-purpose hens (Bogusławska-Tryk et al., 2016; Kheravii et al., 2017; Röhe et al., 2020a; b).

In conclusion, our findings demonstrate that iDF differing in particle size and hydration properties influence nutrient hydrolysis and absorption in broiler chickens. Purified LC, representing a fine insoluble fiber source with high water-binding capacity, reduced apparent nutrient and true protein digestibility compared with OH, without influencing EPL in ileal digesta and feces. Purified LC also prolonged the retention time of digesta in the small intestine without affecting retention in other gastrointestinal segments. These results highlight the importance of considering the hydration properties of iDF when incorporating fiber-rich ingredients in feed formulation, as certain iDF source may adversely affect hydrolysis and absorption utilization of other nutrients in broilers.

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SUPPLEMENTARY MATERIAL

Table S1. Background ^{15}N-enrichment (atom percentage) as measured in non-infused male broiler chickens fed diets containing coarse oat hulls, fine oat hulls, or purified lignocellulose at 25 days of age[1,2].

Item	C-OH	F-OH	LC
n[3]	2	2	2
Ileal digesta	0.3676	0.3674	0.3692
Excreta	0.3659	0.3658	0.3658

[1] Data are presented as means.

[2] Oat hulls were either used intact (C-OH) or ground fine by hammer milling using a 1.5 mm sieve (F-OH). Purified wood lignocellulose (LC, Arbocel® RC Fine J. Rettenmaier & Söhne GmbH + Co KG, Germany).

[3] Number of replicate observations (individual birds), unless indicated otherwise.


Oat
Hull

Chapter 6

Gastrointestinal tract morphology, nutrient digestibility, and digesta mean retention time in laying hens of two different breeds fed with fine or coarse oat hulls

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ABSTRACT

The inclusion of fiber-rich ingredients in poultry diets is expected to increase due to societal-, ecologic-, and economic developments. Particle size of dietary fiber sources, such as oat hulls (**OH**), has been shown to play a key-role in nutrient digestibility and digestion process, but the response may depend on the genetic background of the bird. This study aimed to test the hypothesis that laying hens varying in genetic background respond differently to the particle size of OH regarding gastrointestinal tract development, apparent ileal (**AID**) and total tract digestibility (**ATTD**), and digesta mean retention time (**MRT**). A total of 224 29-week-old Dekalb White (**DW**) and Bovans Black (**BB**) laying hens were assigned to one of two dietary treatments (fine vs. coarse OH). The diets were formulated to contain 150 g/kg OH as the main dietary fiber source. Titanium dioxide (3 g/kg) and cobalt-EDTA (2 g/kg) were added as inert tracers. Bovans Black hens had greater relative weights of the gizzard (+3.9% of BW, $P = 0.015$), ceca (+14.3% of BW, $P < 0.001$), and colon (+9.1% of BW, $P < 0.001$) compared with DW hens. Moreover, AID of protein (+3.5%; $P < 0.001$) was greater in BB vs. DW hens, whereas AID of starch was only greater in BB when fed the fine OH diet (breed × diet, $P = 0.037$). In contrast, ATTD of NSP (-11.2%, $P = 0.035$) was lower in BB vs. DW hens and digesta MRT was longer in proventriculus + gizzard (solids: +25 min, $P < 0.001$; liquids: +5 min, $P < 0.001$) and ileum (solids: +53 min, $P = 0.001$; liquids: +38 min, $P < 0.001$). Birds fed coarse OH only had a greater relative weight of the gizzard (+3.9%, $P < 0.001$) compared with those fed fine OH. In conclusion, our study indicates that gastrointestinal tract traits, nutrient digestibility and digesta transit behavior differed between breeds, regardless of the particle size of OH in the diet.

INTRODUCTION

For decades, laying hens have been selected for productivity traits, resulting in modern breeds with high egg production (Kidd and Anderson, 2019). At the same time, diets have been adjusted to meet the production requirements of these modern breeds, which typically contain highly digestible ingredients and low amounts of fibers (Barros de Carvalho et al., 2012; Desbruslais et al., 2021). However, due to societal-, ecologic-, and economic constraints, the poultry industry is urged to increasingly include agricultural

by-products that cannot be used for human consumption, in poultry diets. These products characteristically contain high levels of dietary fibers (**DF**), which can affect digestion processes and digestibility of other nutrients (Hetland and Svihus, 2001; Jimenez-Moreno et al., 2013; Jha and Mishra 2021). For instance, various soluble DF sources have been found to influence digesta viscosity, digesta passage rate, and microbial activity in the upper gastrointestinal tract (Owusu-Asiedu et al., 2006; Bedford and Cowieson, 2012; Konieczka and Smulikowska, 2018), thereby often reducing digestion of protein and starch (Dorado-Montenegro et al., 2024). However, insoluble DF sources may improve nutrient digestibility (Gonzalez-Alvarado et al., 2007), generally ascribed to the physical structure (Naeem et al., 2023). In this respect, oat hulls (**OH**), rich in insoluble DF, have been extensively studied and shown to improve grinding activity of the muscular gizzard and stimulating enzyme secretion, resulting in improved digestion of starch (Svihus et al., 2002; Hetland et al., 2003). These properties make insoluble DF-rich by-products an attractive and competitive ingredient to include in poultry diets, despite the resulting dietary energy dilution. To optimally use DF in poultry diets and exploit their physical structure, particle size is an important factor to consider. Coarsely ground OH, were found to increase gizzard weight by 46 to 92% (Hetland and Svihus, 2001; Svihus, 2011; Sacranie et al., 2012), thereby enhancing the gizzard's grinding capacity due to increased muscular contractions and a prolonged retention time (Hetland and Svihus, 2001; Svihus, 2011; Sacranie et al., 2012; Singh et al., 2014). Eventually, a longer MRT of digesta results in longer exposure to digestive juices, and this may improve nutrient absorption (Ngoc et al., 2013; Kheravii et al., 2018).

In response to the inclusion of DF-rich sources in a diet, previous research suggested that chickens have the ability to cope with DF, but that this may vary genetically. Broiler chickens, selected for high nutrient digestion, had heavier GIT segments (proventriculus, gizzard, and ileum), longer digesta mean retention time (**MRT**) in the gizzard, and higher protein digestibility compared with chickens selected for low nutrient digestion (Rougière and Carré, 2010). Moreover, other authors found that broiler chickens representative for the broiler population in 1957, did not differ in BW and FCR when offered a diet reflecting formulation practices from 1957 with the inclusion of DF (alfalfa meal and wheat middlings), compared with a modern (high-

energy and low-fiber) diet, whereas modern-type broilers grew less fast and had impaired FCR when such a diet was fed (Havenstein et al., 2003). Similarly, fast-growing broilers (Cobb500) had higher CP digestibility and metabolizability of acid detergent fiber compared with slow-growing broilers (Label Rouge) only when they were fed with a low-fiber (3 g/kg) diet, but not when fed a high-fiber (5 g/kg) diet (Krás et al., 2013).

Based on the above-mentioned findings, it may be postulated that the response of chickens to fiber particle sizes on the development of the digestive tract, nutrient digestibility, and digesta retention time, may vary among breeds. Although previous studies clearly indicated that (artificially selected) broiler breeds varying in production or digestion efficiency, differ in their ability to respond to high-fiber diets, little is known about the variation in responses among modern commercial breeds, particularly in laying hens. Therefore, this experiment aimed to investigate whether two commercial laying hen breeds, Dekalb White and Bovans Black, which selected for different production traits, respond differently when fed with fine or coarse OH, as an insoluble DF source, regarding digestive tract development, nutrient digestibility, and digesta MRT. It was hypothesized that coarse OH will increase GIT weight, especially of the gizzard, improve nutrient digestibility, and prolong digesta MRT in proventriculus + gizzard compared with fine OH, but that the responses may differ between the two breeds.

MATERIALS AND METHODS

Birds, housing, and diets

This study was conducted at the research facilities of Wageningen University & Research, and all experimental procedures were approved by the Animal Welfare Body of Wageningen University & Research and the Dutch Central Committee of Animal Experiments, the Netherlands, under the authorization number AVD1040020197324.

The effect of coarseness of OH (fine vs. coarse) was tested in two laying hen breeds (Dekalb White vs. Bovans Black; Hendrix Genetics, Boxmeer, the Netherlands) using a 2×2 factorial arrangement of treatments. A total of 112 29-week-old Dekalb White (**DW**) (BW: 1.53±0.107 kg) and 112 29-week-old Bovans Black (**BB**) (BW: 1.79±0.127 kg)

laying hens were housed in 32 pens (1.85 x 1.10 m, 7 birds/pen) with floating plastic slatted floors, covered with rubber matting and wood shavings. Pens were located in three rooms, with 10 or 12 pens per room. Breed- and diet treatment combinations were balanced over rooms. Within the room, pens were assigned alternately to one of the two breeds. Within breed, pens were randomly assigned to one of two experimental diets. Diets were formulated to contain 150 g/kg fine (ground using a hammermill (LHM20/16, 1.5 kW, Condux International, Mankato, United States of America) at 1500 rpm using a 1.5 mm screen) or coarse (intact) oat hulls (**OH**) as the main DF source. Diets were fed as pellets and formulated to meet or exceed nutrient requirements for laying hens (Table 1.6; CVB, 2018) and fed as pellets. Titanium dioxide (TiO_2; 3 g/kg), and cobalt-ethylenediaminetetraacetic acid (Co-EDTA; 2 g/kg) were included in the diet as inert tracers for fine-solid (hereafter referred to as solid) and liquid digesta, respectively. Birds had *ad libitum* access to feed and water. Room temperature was controlled at 20ºC and relative humidity at 60 to 70%. The photoperiod was 15L:9D and the maximum light intensity was 10 lux at bird level. At d 24 of the experiment, bedding and rubber matting were removed and birds were housed on slatted floors.

Table 1.6. Ingredient and nutrient composition of experimental diets containing oat hulls varied in particle size[1] (g/kg, as-fed basis)

Item	Diet
Ingredient	
Maize	525.5
Oat hulls[1]	150.0
Wheat gluten	100.0
Toasted soybeans	50.0
Soy oil	42.0
Mineral and vitamin premix[2]	5.0
$CaCO_3$	90.0
Dicalcium phosphate	18.0
Salt	1.0
$NaHCO_3$	3.6
MgO	1.0
L-Lysine HCl 79%	5.0
DL-Methionine	1.4
L-Threonine	1.3
L-Valine	0.2
L-Tryptophan	0.4

L-Isoleucine	0.6
TiO_2	3.0
Co-EDTA	2.0
Calculated nutrient composition[3]	
AME[4] (kcal/kg)	2916
Digestible lysine[5]	7.6
Digestible methionine + cysteine[5]	6.9
Digestible threonine[5]	5.3
Digestible tryptophan[5]	1.6
Calcium	39.1
Phosphorus	3.0
Analyzed nutrient composition	
Dry matter (g/kg)[6]	902.2
CP[7] (g/kg in DM)	169.6
Fat (g/kg in DM)	53.8
Starch (g/kg in DM)	390.8
Total Non-Starch Polysaccharides (g/kg in DM)	128.5
Of which soluble (g/kg in DM)	14.3
Of which insoluble (g/kg in DM)	114.2

[1] Oat hulls were either used ground fine by hammer milling using a 1.5mm sieve (fine) or intact (coarse).
[2] Provided per kilogram of diet: Vitamin A (retinyl acetate), 10,000 IU; vitamin D (cholecalciferol), 2,000 IU; vitamin E (dl-a-tocopherol), 25 mg; vitamin K_3 (menadione), 1.5 mg; vitamin B_1 (thiamin), 1 mg; vitamin B_2 (riboflavin), 3.5 mg; vitamin B_6 (pyridoxine-HCl), 15 µg; niacin, 30 mg; D-pantothenic acid, 12 mg; choline chloride, 350 mg; folic acid, 0.8 mg; biotin, 0.1 mg; iron, 50 mg; copper, 10 mg; manganese, 60 mg; zinc, 54 mg; iodine, 0.7 mg; selenium, 0.1 mg.
[3] Calculated using data from (CVB, 2018).
[4] Apparent metabolizable energy for laying hens (12.2 MJ/kg).
[5] Standardized ileal digestible amino acids for laying hens.
[6] The DM of the fine OH diets was 908.2 g/kg.
[7] Nitrogen × 6.25.

Sample collection

Excreta were quantitatively collected at d 28, 29, 30, and 31 per pen. Subsamples of clean excreta were taken and frozen immediately in the freezer at -20°C. At d 34, 35, or 36, birds were euthanized, weighed, and dissected according to a dissection schedule balancing treatments and timepoints for cecal digesta transit measurements over days and time of the day. Individual BW of birds was recorded prior to dissection. Subsequently, each individual bird received an injection of 0.5 mL sodium pentobarbital (20% or 500 mg/mL) administered at the base of the posterior edge of the skull. The abdominal cavity was opened and, if present, fully developed eggs in the distal oviduct were removed and weighed to correct BW. Then the GIT from esophagus to rectum, was

removed and crop, gizzard + proventriculus, duodenum + jejunum, ileum, and ceca + colon were dissected and weighed. For each segment, digestive contents were collected on ice, by gentle-finger stripping, and frozen at -20°C. Empty GIT segments were flushed with water, dried with paper tissue, and weighed. The length of small intestine, ceca, and colon was measured using measuring tape. Before freeze-drying, excreta and digesta samples were thawed (4°C) and pooled per pen.

Cecal digesta transit

To assess cecal digesta transit, a pulse dose of Cr-mordanted OH (1 g) and ytterbium acetate (250 mg) markers was provided to individual birds between d 34 to 36. Coarse and fine OH were mordanted with Cr_2O_3 according to the procedure described by Dorado-Montenegro et al. (2023). Ytterbium acetate is soluble in water, but tends to associate with particulate matter (Siddons et al., 1985). The markers were incorporated into a small amount of feed mixed with water and rolled into spheric boluses of ~1.5 cm diameter (~ 5 g / pulse dose, divided into 4 to 6 boluses). Each set of two pens of the same treatment within a block of 8 pens, was considered one experimental unit (4 replicates/treatment). The fourteen birds of 1 experimental unit were randomly assigned to one of the 14 timepoints. At t = 0 (d 34, d 35, or 36), the marker pulse dose boluses were gently dosed into the oropharyngeal cavity by hand. Birds were subsequently euthanized at after 30, 60, 120, 180, 240, 300, 360, 480, 600, 720, 960, 1200, 1440, and 1800 min after the pulse dose.

Analytical methods

Wet sieve analysis

Particle size distribution of OH and OH diets was analyzed by wet sieving in duplicate (Wolf et al., 2010) using sieve sizes 2.5, 1.25, 0.63, 0.315, 0.160, and 0.071 mm. The geometric mean diameter (**GMD**) and geometric standard deviation (**GSD**) of the OH diets were calculated as described by Lyu et al. (2020). The GMD±GSD of fine OH was 474±0.16 µm and of coarse OH was 541±0.28 µm. This resulted in a GMD±GSD of 493±0.18 µm for the fine OH diet and 631±0.25 µm for the coarse OH diet (Figure 1.6).

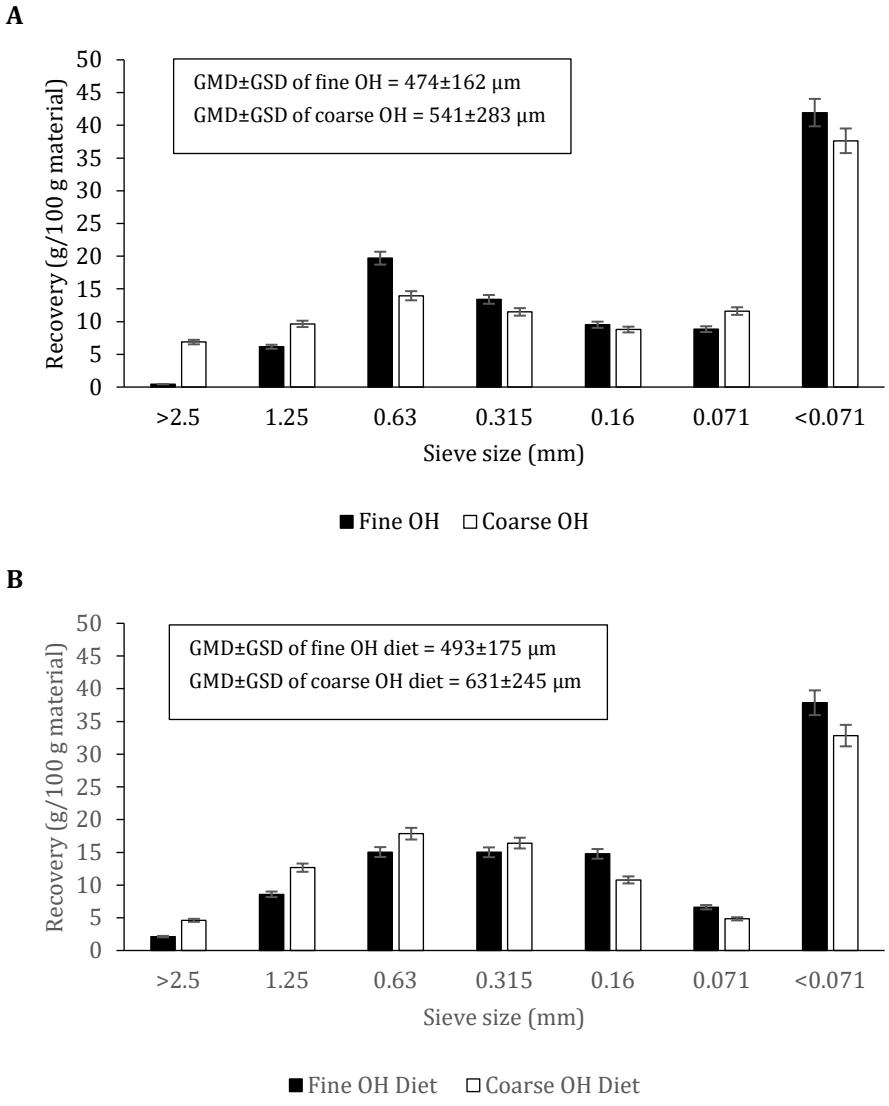


Figure 1.6. Weight fraction of fine and coarse oat hulls (OH) (A) and OH diets (B) recovered at sieves with various mash sizes during wet sieve analyses. Bars represent mean of two replicate measurements. Error bars represent SEM. GMD: geometric mean diameter, GSD: geometric standard deviation.

Chemical analyses

Prior to chemical analyses, digesta and excreta samples were freeze-dried and moisture loss was recorded. Feed-, digesta-, and excreta samples were ground by a rotor mill

(Retsch ZM200, Haan, Germany) at 12,000 rpm using a 1 mm screen. All analyses were performed in duplicate. Samples were analyzed for contents of DM (AOAC, 2016; method 930.15 for feed and excreta; due to limited sample quantity, DM of digesta was derived from the moisture loss during freeze-drying), nitrogen (ISO 16634, 2008), ether extract (after hydrochloric acid hydrolysis, using Soxhlet apparatus and petroleum ether; AOAC, 2016; method 920.39), and total starch (ISO 15914, 2004). Total non-starch polysaccharides (**NSP**) were measured as neutral sugars and uronic acids, according to the method of Englyst et al. (1994), with some modifications as described by Lannuzel et al. (2024). For feed samples, starch was gelatinized and enzymatically degraded prior to NSP extraction. Inositol and allose were used as internal standards. Uronic acid concentration was analyzed according to the colorimetric m-hydroxydiphenyl assay (Blumenkrantz and Asboe-Hansen, 1973), using a spectrophotometer (Evolution 201, Thermo Scientific, Waltham, MA). Galacturonic acid was used for calibration. Titanium (**Ti**), cobalt (**Co**), chromium (**Cr**), and ytterbium (**Yb**) were analyzed after ashing and microwave digestion using an Avio 500 inductively coupled plasma optical emission spectrometer (Perkin Elmer, Waltham, USA), as described by Lannuzel et al. (2024).

Calculation of digestibility and mean retention time

Apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) of DM, protein, fat, starch, and NSP were calculated, as follows (de Vries and Gerrits, 2018):

$$AID_X \text{ or } ATTD_X(\%) = \left(1 - \frac{[T]diet \times [X]sample}{[T]sample \times [X]diet}\right) \times 100\%$$

where [T]diet, [T]sample, [X]diet, and [X]sample (g/kg) are concentrations of the tracer (T) (titanium) and nutrient (X) (DM, nitrogen, starch, NSP) in the diet, digesta, or excreta samples. Digesta MRT was calculated from ingestion of tracer T during 24 h (intake/1440 min, g) and the pool size of tracer T detected in the digesta sample at dissection, assuming steady state conditions under *ad libitum* feeding strategies (van der Klis et al., 1990):

$$MRT\ (min) = 1440 \times \left(\frac{[T]sample \times\ Wsample}{[T]diet\ \times\ intake/1440min} \right)$$

where [T] is concentrations of the tracer Ti (g/kg) and Co (mg/kg) in the diet and digesta sample, and Wsample (g) is the digesta pool size. The marker intake was calculated from the average of feed intake from d 11 to 31 for each bird.

Time of peak of marker concentration in the ceca

Time of peak of Cr and Yb concentrations in the ceca after providing a pulse dose, was estimated by fitting (PROC NLIN, SAS, version 9.4, SAS Institute Inc., Cary, NC) a generalized Michaelis-Menten equation based on van Den Borne et al. (2007):

$$y = \frac{[a\ \times c\ \times t^{(-c-1)}\ \times\ b^c]}{[1 + (\frac{b}{t})^c]^2}$$

where y = the marker concentration (chromium or ytterbium) in cecal digesta (mg/kg) at time t (min); a, b, and c are parameters that define the curve. Then, the time of peak (min) was calculated as $t_{max} = [c^b \cdot (1\text{-}b)/(\text{-}b\text{-}1)]^{(1/b)}$. The concentration at time of peak (maximum marker concentration, mg/kg) was then calculated as $y_{max} = a \cdot b \cdot t_{max}^{(\text{-}b\text{-}1)} \cdot c^b/[1+(c/t_{max})^b]^2$.

Statistical analysis

The pen was the experimental unit for statistical analyses of GIT traits, nutrient digestibility, digesta MRT, and time of peak. These data were analyzed by two-way ANOVA (PROC GLM, SAS, version 9.4, SAS Institute Inc., Cary, NC), with breed (BB vs. DW), diet (fine vs. coarse), and their interaction as fixed effects. In case of significant breed × diet interactions, differences among means were assessed using Tukey adjustments. The set of two pens containing 14 birds dissected at 14 consecutive timepoints after marker pulse dose was considered the experimental unit for statistical analysis of cecal digesta transit. Effects of breed, diet, and their interaction on the estimated time of peak of Cr and Yb concentrations were analyzed using the model described above. Subsequently, differences in the estimated time of peak between markers (Cr vs. Yb) were evaluated. Preliminary analyses revealed no interactions between breed, diet, and marker and hence data were pooled over breeds and diets.

Differences in estimated time of peak and estimated concentration at time of peak between markers were tested using a paired t-test (PROC GLM, SAS, version 9.4, SAS Institute Inc., Cary, NC) with marker (chromium vs. ytterbium) as a fixed effect. Relations among AID of protein and MRT in proventriculus + gizzard and small intestine were evaluated with Pearson correlation coefficients (PROC CORR, SAS, version 9.4, SAS Institute Inc., Cary, NC), where pen was considered as experimental unit. For all statistical analyses, homogeneity and normality of model assumptions were visually verified using histograms, QQ-plots, and studentized residuals. Data are presented as means and pooled SEM. Differences were considered significant at $P < 0.05$.

RESULTS

Body weight, feed intake, and gastrointestinal tract traits

Body weight of BB hens was greater than that of DW hens (1,624 vs. 1,541 g, $P < 0.001$), whereas relative daily feed intake was smaller (41 vs. 47 g/d per kg BW, $P < 0.001$) (Table 2.6). Diet did not influence BW nor feed intake. BB hens had smaller liver (-2.1 g/kg BW, $P < 0.001$), pancreas (-0.4 g/kg BW, $P < 0.001$), crop (-0.3 g/kg BW, $P = 0.007$), and proventriculus (-0.1 g/kg BW, $P = 0.046$) than DW hens. Empty relative weights of gizzard (+1.4 g/kg BW, $P = 0.015$), ceca (+0.7 g/kg BW, $P < 0.001$), and colon (+0.2 g/kg BW, $P = 0.019$) were greater for BB than for DW hens. Moreover, BB hens had shorter ileum (37.4 vs. 40.1 cm/kg BW, $P < 0.001$) and longer ceca (10.6 vs. 9.1 cm/kg BW, $P < 0.010$) compared with DW hens. Relative digesta weights of proventriculus + gizzard (9.8 vs. 8.3 g/kg BW, $P < 0.001$), ileum (5.4 vs. 4.8 g/kg BW, $P = 0.026$), and ceca (1.1 vs. 0.8 g/kg BW, $P = 0.005$) of BB hens were larger, but the relative weight of digesta in the crop (10.9 vs. 15.5 g/kg BW, $P < 0.001$) was smaller, compared with DW hens. When fed the fine OH diet, empty relative weights of the gizzard (19.6 vs. 20.8 g/kg BW, $P < 0.001$) and digesta in ileum (4.8 vs. 5.4 g/kg BW, $P = 0.040$) were smaller compared with the coarse OH diet. In contrast, particle size had no effect on empty relative weights and lengths of other empty GIT tract segments, nor on relative weights of digesta contents.

Apparent ileal and total tract digestibility

Bovans Black hens had higher AID of DM (62.7 vs. 60.7%, $P = 0.033$) and protein (81.9 vs. 78.4%, $P < 0.001$) than DW hens (Table 3.6). The AID of starch was only different between breeds when the fine OH diet was fed (breed × diet, $P = 0.037$). Contrastingly, BB hens had a lower ATTD of DM (62.8 vs. 64.9%, $P < 0.001$), protein (39.1 vs. 47.1%, $P < 0.001$), fat (83.2 vs. 87.4%, $P = 0.001$), and NSP (6.4 vs. -2.4%, $P = 0.035$) than BB hens. No differences in AID nor ATTD between diets were observed.

Digesta passage behavior

In BB hens, solid digesta retained longer in proventriculus + gizzard (55 vs. 30 min, $P < 0.001$), duodenum + jejunum (52 vs. 33 min, $P < 0.001$), ileum (79 vs. 57 min, $P = 0.001$), and total GIT (311 vs. 255 min, $P = 0.021$) compared with DW hens (Table 4.6). Liquid digesta retained longer in proventriculus + gizzard (14 vs. 9 min, $P < 0.001$), duodenum + jejunum (26 vs. 15 min, $P < 0.001$), ileum (36 vs. 26 min, $P < 0.001$), ceca + colon (137 vs. 77 min, $P < 0.001$), and total GIT (256 vs. 169 min, $P < 0.001$) in BB hens compared with DW hens. No differences in MRT of solid and liquid digesta between birds fed coarse vs. fine OH were observed in any of the GIT segments. The estimated time of peak of marker concentrations (chromium and ytterbium) in the ceca of birds (Table 5.6) did not vary among treatments.

Table 2.6. Body weight, feed intake, digestive organ size, and digesta weight in Dekalb White or Bovans Black laying hens fed diets containing fine vs. coarse oat hulls (150g/kg)[1]

Item	Dekalb White		Bovans Black		SEM	Model established *P*-values[2]		
	Fine	Coarse	Fine	Coarse		Breed	Diet	B×D
n[3]	8	8	8	8				
Body weight (g)[4]	1,547	1,534	1,647	1,600	18.5	<0.001	0.115	0.380
Relative feed intake (g/kg BW/d)	47	47	40	41	1.5	<0.001	0.705	0.899
Relative organ weight (g/kg BW)								
Liver	27.0	26.7	24.6	25.0	0.51	<0.001	0.895	0.478
Pancreas	2.3	2.3	1.9	1.8	0.05	<0.001	0.449	0.556
Crop	3.7	3.8	3.4	3.6	0.10	0.007	0.101	0.632
Proventriculus	3.8	3.8	3.6	3.7	0.06	0.046	0.259	0.616
Gizzard	19.1	20.5	20.1	21.0	0.31	0.015	<0.001	0.499
Duodenum + Jejunum	22.0	21.9	21.8	22.3	0.48	0.434	0.289	0.229
Ileum	8.6	8.5	8.0	8.4	0.27	0.211	0.436	0.324
Ceca	4.3	4.1	4.8	4.9	0.14	<0.001	0.971	0.213
Colon	2.0	2.0	2.1	2.2	0.06	0.019	0.188	0.394
Relative organ length[5] (cm/kg BW)								
Duodenum + Jejunum	57.5	57.4	57.4	58.7	0.90	0.622	0.445	0.373
Ileum	39.6	40.6	36.5	38.2	0.75	<0.001	0.071	0.568
Ceca	8.8	8.7	10.1	9.8	0.25	<0.001	0.468	0.629
Colon	5.1	5.4	5.3	5.4	0.23	0.812	0.430	0.522
Relative weight of digesta (g/kg BW)								
Crop	14.8	14.3	8.6	11.3	1.19	<0.001	0.171	0.365
Proventriculus + Gizzard	8.3	8.3	9.6	10.0	0.30	<0.001	0.391	0.555

Duodenum + Jejunum	5.9	6.6	6.4	6.8	0.29	0.262	0.053	0.719
Ileum	4.5	5.0	5.0	5.7	0.27	0.026	0.040	0.588
Ceca	0.8	0.7	1.1	1.0	0.09	0.005	0.336	0.972
Colon	1.7	1.6	1.8	1.7	0.24	0.612	0.519	0.982

[1] Oat hulls were either used ground fine by hammer milling using a 1.5mm sieve (fine) or intact (coarse).

[2] Model established *P*-values for the fixed effects of breed, oat hull particle size, or their interaction (B×D).

[3] Number of replicate pens (5 to 7 birds/pen, unless indicated otherwise).

[4] Body weight was corrected for egg weight inside the body during the dissection day.

[5] Three birds/pen.

Table 3.6. Apparent ileal (AID) and total tract (ATTD) digestibility (% of intake) of dry matter, protein, fat, starch, and non-starch polysaccharides (NSP) in Dekalb White or Bovans Black laying hens fed diets containing fine vs. coarse oat hulls (150 g/kg)[1]

Item	Dekalb White		Bovans Black		SEM	Model established *P*-values[2]		
	Fine	Coarse	Fine	Coarse		Breed	Diet	B×D
n[3]	8	8	8	8				
AID								
Dry matter	59.7	61.6	62.4	62.9	0.89	0.033	0.189	0.457
Protein	77.8	78.9	81.8	82.0	0.88	<0.001	0.467	0.644
Fat	86.4	85.8	85.3	84.8	1.75	0.551	0.744	0.986
Starch	96.5[b]	97.5[ab]	98.2[a]	97.3[ab]	0.44	0.101	0.940	0.037
ATTD								
Dry matter	64.1	65.7	62.9	62.6	0.65	<0.001	0.120	0.274
Protein[4]	46.5	47.6	39.2	38.9	1.30	<0.001	0.735	0.600
Fat	87.3	87.5	82.1	84.3	1.14	0.001	0.284	0.392
Starch	97.4	97.9	97.7	97.9	0.25	0.426	0.172	0.524
NSP[5]	4.9	7.9	-3.8	-0.9	3.80	0.035	0.450	0.989

[1] Oat hulls were either used ground fine by hammer milling using a 1.5mm sieve (fine) or intact (coarse).
[2] Model established *P*-values for the fixed effects of breed, oat hulls particle size, or their interaction (B×D).
[3] Number of replicate pens (7 birds/pen).
[4]Apparent total tract retention of protein, calculated as the difference between nitrogen intake and (urinary + fecal) nitrogen in excreta.
[5] Number of replicate pens for coarse diet in both breeds = 7 and SEM = 4.06.

Table 4.6. Mean retention time (MRT) (min) of solid and liquid digesta in Dekalb White or Bovans Black laying hens fed diets containing fine vs. coarse oat hulls (150 g/kg)[1]

Item	Dekalb White		Bovans Black		SEM	Model established *P*-values[2]		
	Fine	Coarse	Fine	Coarse		Breed	Diet	B×D
n[3]	8	8	8	8				
Solid digesta (min)								
Crop	108	118	88	106	11.9	0.199	0.260	0.708
Proventriculus + Gizzard	30	31	56	56	4.3	<0.001	0.943	0.984
Duodenum + Jejunum	34	34	53	53	3.3	<0.001	0.931	0.962
Ileum	56	62	79	82	5.8	0.001	0.508	0.777
Ceca + Colon	28	24	37	29	4.0	0.105	0.161	0.773
Total	256	268	312	324	22.8	0.021	0.592	0.945
Liquid digesta (min)								
Crop	84	81	78	84	4.7	0.871	0.882	0.645
Proventriculus + Gizzard	16	18	25	27	0.9	<0.001	0.439	0.977
Duodenum + Jejunum	26	28	47	50	1.9	<0.001	0.489	0.868
Ileum	47	52	68	69	2.5	<0.001	0.609	0.658
Ceca + Colon	155	137	284	238	12.3	<0.001	0.179	0.551
Total	328	316	503	467	17.3	<0.001	0.462	0.729

[1] Oat hulls were either used ground fine by hammer milling using a 1.5mm sieve (fine) or intact (coarse).

[2] Model established *P*-values for the fixed effects of breed, oat hulls particle size, or their interaction (B×D).
[3] Number of replicate pens (7 birds/pen).

Table 5.6. Estimated time of peak (min) of marker concentrations in the ceca of Dekalb White or Bovans Black laying hens fed diets containing fine vs. coarse oat hulls (150 g/kg)[1]

Item	Dekalb White		Bovans Black		SEM	Model established *P*-values[2]		
	Fine	Coarse	Fine	Coarse		Breed	Diet	B×D
n[3]	4	4	4	4				
Chromium	1276	1173	1605	1245	278.1	0.485	0.422	0.653
Ytterbium	1123	1185	1357	1235	124.2	0.274	0.815	0.472

[1] Oat hulls were either used ground fine by hammer milling using a 1.5mm sieve (fine) or intact (coarse).
[2] Model established *P*-values for the fixed effects of breed, oat hulls particle size, or their interaction (B×D).
[3] Number of replicate timepoints (4 birds/timepoint).

DISCUSSION

This experiment was conducted to examine the effects of feeding high-fiber diets containing 150 g/kg OH varying in particle size (fine and coarse) on GIT traits, nutrient digestibility, and digesta MRT in two laying hen breeds (Dekalb White and Bovans Black). We hypothesized that coarse OH would increase gizzard weight, improve nutrient digestibility, and prolong digesta MRT in proventriculus + gizzard compared with fine OH, but that the responses would differ between the two breeds. Unlike our expectations, marginal diet effects were observed, despite the notable particle size contrast of the OH. Interestingly, considerable breed-related variation in gastrointestinal traits, digesta retention time, and digestibility were observed, regardless of OH particle size.

Digesta passage throughout the gastrointestinal tracts

Although digesta transit behavior in chickens is limitedly understood, it is clear that various digesta fractions may separate as they pass through the gastrointestinal tract (de Vries et al., 2014; Garçon et al., 2023; Dorado-Montenegro et al., 2024). For instance, the grinding activity in the gizzard of chickens facilitates particle size reduction of digesta before entering the small intestine (Hetland et al., 2002) and in this process, liquid digesta is expected to flow more rapidly into the small intestine than solid digesta (Garçon et al., 2023). In the hindgut, retrograde peristaltic contractions may provoke digesta segregation and are responsible for transporting liquid digesta from the cloaca to the ceca (Björnhag, 1989; Vergara et al., 1989; Clench and Mathias, 1995; Braun, 1999; Dorado-Montenegro et al., 2024). Although the filling and emptying processes of the ceca have been scarcely studied, it has been identified that solid digesta, particularly larger particles, only limitedly enter the ceca, as illustrated by the presence of 12-fold higher concentrations of liquid- vs. solid digesta tracers typically found in this segment (Vergara et al., 1989; de Vries et al., 2014). In addition, it seems that only a fraction of the liquid digesta is transported into the ceca, with provisional estimates ranging from 30 to 50% (Vergara et al., 1989; Garçon et al., 2023). Those findings are in line with the results in our study, where Co concentrations in the ceca – originating from the soluble Co-EDTA tracer - were ~6 times higher than Ti concentrations – originating from the solid digesta tracer TiO_2 (data not shown).

Most studies quantifying digesta retention time in chickens considered only solid digesta by using TiO_2 or ^{103}ruthenium phenanthroline as tracers (Shires et al., 1987; Dänicke et al., 1999; Rougière and Carré, 2010; van Krimpen et al., 2011; Duve et al., 2011; Moquet et al., 2018; Dorado-Montenegro et al., 2024). Only a few studies have looked into the MRT of liquid digesta throughout the gastrointestinal tract of broilers (Garçon et al., 2023; Dorado-Montenegro et al., 2024). Additionally, it was revealed that the cecal retention times of liquid digesta that actually enter the ceca can be considerable (~500 to 1500 min; Garçon et al., 2023). To our knowledge, the current study is the first investigation into the retention time of not only solid, but also liquid digesta fractions throughout the GIT of laying hens. Overall, the MRT of solid digesta observed in our study was in the same range as previously reported (Shires et al., 1987; van Krimpen et al., 2011) and the MRT of liquid digesta throughout GIT was also consistent with the range expected, based on previous studies in broilers (Garçon et al., 2023; Dorado-Montenegro et al., 2024).

Digestion processes in two laying hen breeds

In the current study, considerable differences in prececal digestion processes between breeds were found. Bovans Black hens showed a higher AID of protein, coinciding with a lower feed intake and longer MRT of solid and liquid digesta in the proventriculus + gizzard and small intestine, compared with Dekalb White hens. The negative correlation between feed intake and MRT of digesta in the gizzard and small intestine (Table 6.6), supports suggestions of previous authors that lower feed intake facilitates more and prolonged contact between digesta, enzymes, and intestinal mucosa and may lead to a higher digestibility of protein (Guixin et al., 1995; González-Alvarado et al., 2008; Svihus, 2011; Ngoc et al., 2013; Svihus and Itani 2019).

Table 6.6. Pearson correlation coefficients of AID of protein and MRT of prececal digesta [1,2,3]

Parameters	AID of protein	MRT of solid digesta in proventriculus + gizzard	MRT of liquid digesta in proventriculus + gizzard	MRT of solid digesta in duodenum + jejunum	MRT of liquid digesta in duodenum + jejunum	MRT of solid digesta in ileum	MRT of liquid digesta in ileum	Relative feed intake
AID of protein	1.00							
MRT of solid digesta in proventriculus + gizzard	0.36*	1.00						
MRT of liquid digesta in proventriculus + gizzard	0.52†	0.89†	1.00					
MRT of solid digesta in duodenum + jejunum	0.44†	0.84†	0.79†	1.00				
MRT of liquid digesta in duodenum + jejunum	0.52†	0.81†	0.83†	0.94†	1.00			
MRT of solid digesta in ileum	0.50†	0.75†	0.79†	0.81†	0.83†	1.00		
MRT of liquid digesta in ileum	0.51†	0.75†	0.78†	0.76†	0.81†	0.97†	1.00	
Relative feed intake	-0.24	-0.74†	-0.13	-0.18	-0.61†	-0.19	-0.62†	1.00

[1] Parameters n = 32 pen observations.

[2] AID: apparent ileal digestibility; MRT: mean retention time.

[3] * $P \leq 0.05$; †$P \leq 0.01$

Surprisingly, BB hens had a lower ATTD of NSP, despite their heavier ceca and colon, and a longer MRT of liquid digesta in those segments compared with DW hens. The ATTD of total NSP was low as expected from the dietary composition. Nevertheless, interesting differences between breeds were observed, as fermentation of NSP was virtually zero in BB hens, but reached 5 to 8% of NSP in DW hens. Although OH was the major source providing dietary NSP (~56% of total NSP in the diet), NSP also originated from maize (~7% of total NSP in the diet) and soybeans (~37% of total NSP in the diet). Considering the limited cecal access of OH observed in our study - as indicated by the low concentrations of Cr found in the ceca (Table 7.6) - and the poor fermentability of NSP from OH, the difference in the ATTD of NSP between breeds should thus most likely be ascribed to degradation of NSP from maize and soybeans.

Table 7.6. Estimated time of peak (T_{max}, min) and maximum concentration (Y_{max}, mg/kg DM) between markers in the ceca of laying hens fed diets containing oat hulls (150 g/kg)

Item	Chromium	Ytterbium	SEM	Model established *P*-values[1]
n[2]	16	16		
T_{max} (min)	1325	1225	102.2	0.495
Y_{max} (mg/kg DM)	1022	4099	224.4	<0.001

[1] Model established *P*-values for the fixed effects of marker.
[2] Number of replicate pens.

Differences in DF fermentation speculatively may point out differences in the cecal microbiome between the two breeds of birds. However, it should be noted that differences in ileal nutrient digestibility and feed intake between the breeds also imply that the quantity and composition of digesta flowing into the hindgut, which were different, thereby presumably affecting amount and type of substrate available for the cecal microbiome and most likely microbial activity. The lower AID of protein and numerical lower AID of fat observed for BB hens may thus indirectly have contributed to differences in NSP digestibility observed. Provisional calculations indicate that in BB hens, substrate flow into the hindgut was ~3-4 g/ day less compared with DW hens, almost exclusively explained by a difference in protein. The selective entry of only certain digesta fractions into the ceca complicates quantitative estimates of substrate

flow into the ceca. Nevertheless, considering that the ratios between markers for the various digesta fractions in cecal contents did not notably vary between the two breeds, it seems reasonable to assume that any differences in substrate passing the ileocecocolic junction reflect differences in cecal substrate flow. The lower inflow of protein in BB hens may have steered fermentation processes, thereby affecting hindgut disappearance of NSP and other nutrients as illustrated by the observed differences in ATTD of NSP and fat between BB and DW hens.

Effects of oat hull particle size on digestion processes

Grinding of OH, as a model for insoluble DF, considerably reduced the fraction of coarse particles; in the coarse OH and OH diet, ~17% (w/w) of the particles was found to be ≥ 1.25 mm, whereas this fraction was reduced to ~7% in fine OH and ~10% in the final fine OH diet (Figure 1.6). Birds fed the coarse OH diet had ~5.8% heavier gizzard weight than those fed the fine OH diet, as also reported by van Krimpen et al. (2011) and Naeem et al. (2023). This increased gizzard weight is assumed to be the result of the accumulation of coarse OH that provokes the grinding activity (Sacranie et al., 2012), thereby resulting in an increased size of the two pairs of gizzard muscles (Jha and Mishra, 2021; Svihus, 2011). It could be expected that an increased gizzard weight in response to coarse OH would also result in a prolonged MRT of digesta, especially of coarse particles, in that segment. However, we did not observe differences in MRT in the proventriculus and gizzard for solids, nor liquids, between diets, which is in accordance with the findings by van Krimpen et al. (2011). Furthermore, the estimated time of peak of OH-particles in the ceca, as estimated from the pulse-dosed Cr-mordanted OH (Table 7.6), as well as the area under curve (data not shown), did not differ between chickens fed fine or coarse OH. Moreover, in line with a previous broiler study (Abdollahi et al., 2019), AID of DM, protein, starch, and fat were not affected by OH particle size. It should be noted that both our study and the study of Abdollahi et al. (2019) considered, high OH-inclusion levels when compared to typical commercial practices for laying hens (Ben-Mabrouk et al., 2022).

In conclusion, the results of our study indicated that in high-fiber diet with 150 g/kg oat hulls, the particle size of OH had only marginal effects on digestion processes. Coarse OH increased gizzard weight as expected, but did not affect nutrient digestibility nor

digesta retention time throughout the gastrointestinal tract segments. Nevertheless, interesting variation between breeds was observed. Bovans Black hens had greater relative gizzard weight than Dekalb White hens, coinciding with greater ileal protein digestibility and longer retention of solid and liquid digesta in the foregut (proventriculus and gizzard) and small intestine. Bovans Black also had higher AID of starch, but only when fed the fine OH diet. However, the total tract digestibility of NSP in Bovans Black hens was smaller, whilst having larger ceca and colon and longer MRT of liquid digesta in those segments compared with Dekalb White hens. These findings suggest that when formulating diets containing fiber-rich ingredients, regardless of their particle sizes, the variability among laying hen breeds should be taken into consideration.

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Chapter 7

General discussion

AIM AND MAIN FINDINGS

The main aim of this thesis was to investigate how dietary fibers (**DF**) varying in physicochemical properties (solubility, particle size, viscosity, and hydration properties) influence digestion-related processes, including digesta passage behavior, endogenous protein losses (**EPL**), and nutrient digestibility in chickens. Overall, physicochemical properties of DF modified digesta characteristics and behavior, thereby influencing the passage of solid and liquid digesta through the gastrointestinal tract (**GIT**) of chickens, the secretion of endogenous enzymes, and nutrient hydrolysis and absorption. For example, coarse insoluble DF (**iDF**) were hypothesized to prolong the mean retention time (**MRT**) of solid digesta in the proventriculus and gizzard, potentially stimulating digestive enzyme secretion and enhancing nutrient hydrolysis. In contrast, soluble DF (**sDF**) were expected to alter digesta properties, delay the passage of both solid and liquid digesta in the small intestine and hindgut, stimulate intestinal cell proliferation, and ultimately reduce nutrient absorption.

The insights gained from this thesis may contribute to improve accuracy of prediction of the nutritional value of fiber-rich ingredients in poultry diets formulation. Such ingredients, often derived from agricultural by-products, are increasingly used as a strategy to reduce competition between animal feed and human food resources (Pinotti et al., 2025).

To address these objectives, several experimental approaches were applied, with MRT of solid digesta measured in **Chapters 2, 5, and 6** as shown in Figure 1.7. In **Chapter 2**, broilers were fed diets containing coarse or fine soybean hulls (**SBH**) as iDF source differing in particle size, with or without purified arabinoxylans (**AX**) as viscous sDF source. In **Chapter 5**, broilers were fed coarse or fine oat hulls (**OH**), or another diet containing purified lignocellulose (**LC**) as fine iDF source with high hydration capacity. In **Chapter 6**, two laying hen breeds (Bovans Black and Dekalb White) were fed coarse or fine OH diets. Mean retention time of liquid digesta was only measured in the **Chapters 2** and **6**. Across studies, particle size of iDF alone did not affect MRT of either solid or liquid digesta along the GIT. In contrast, purified LC diet only prolonged MRT of solid in the small intestine compared with OH diets, while the addition of purified AX to SBH diets prolonged MRT of solid and liquid digesta in the total GIT.

In **Chapter 3**, a minimally invasive ^{15}N-isotope dilution (**ID**) method was evaluated to quantify total (basal + specific) EPL in broilers. The birds were fed the same diets as in **Chapter 2**, as well as a diet containing fine sugar beet pulp (**SBP**) as a substitution for fine SBH, or a semi-synthetic protein source, a highly digestible diet containing enzymatically hydrolyzed casein (**EHC**). It was demonstrated that ^{15}N-enrichment in urine can be used as a proxy for ^{15}N-enrichment in the precursor pool, thereby enabling estimation of total EPL under minimally invasive conditions. As expected, fibrous diets increased total EPL, compared with the EHC diet. Additionally, due to the absence of a gold-standard method for quantifying total EPL, the ID method was compared with the EHC and EHC-ultrafiltration methods. The ID was identified as a suitable method to quantify total EPL and true protein digestibility in broilers fed diets containing a mix of feed ingredients, despite the need for further research. Based on these findings, the ID method was subsequently applied in **Chapters 4** and **5** to investigate the dietary effects on total EPL and true protein digestibility.

The addition of purified AX to SBH diets and the replacement of fine SBH with fine SBP increased total EPL (**Chapter 4**). Purified AX also reduced true protein digestibility, whereas replacing fine SBH with fine SBP did not influence true protein digestibility. Nevertheless, diets containing coarse OH, fine OH, or purified LC (**Chapter 5**) did not differ in total EPL, although the LC diet decreased true protein digestibility in broilers. Furthermore, apparent starch and fat digestibility was not influenced by coarse or fine iDF, while purified AX and LC reduced apparent ileal and total tract digestibility of these nutrients (**Chapters 2, 5, and 6**).

In this final chapter (**Chapter 7**), the findings of the previous chapters are integrated, discussed, and placed in a broader nutritional and physiological context. For each section, limitations of the study designs that may explain differences compared with other studies are highlighted. Finally, implications for nutritional strategies including the use of fiber-rich ingredients in poultry diets, impact of the research to poultry feed industry, and key take-home messages from this thesis are presented.

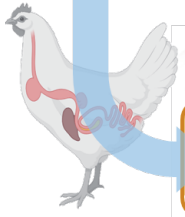


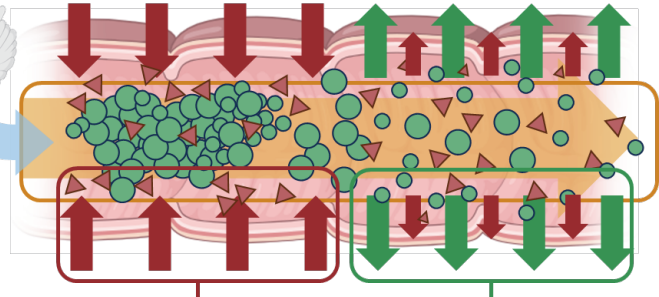
THESIS AIM

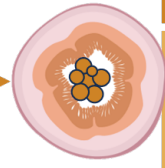
To study how physicochemical properties of fiber-rich ingredients influence digestion processes in chickens

THESIS BACKGROUND

The increased use of fiber-rich ingredients from agricultural by-products in poultry diets.







DIGESTA PASSAGE BEHAVIOR

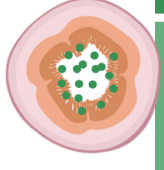
Ch.2:
Viscous soluble fibers slow down digesta passage throughout gastrointestinal tract.

Ch.2,5,6:
Grinding of insoluble fibers does not influence digesta passage.



METHODOLOGY ASSESSMENT

Ch.3 :
Minimally-invasive ^{15}N-isotope dilution is a suitable method to quantify total (basic + specific) endogenous protein losses.

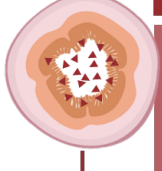


NUTRIENT DIGESTIBILITY

Ch.2,4,5,6:
Grinding of insoluble fibers does not influence appparent nutrient digestibility; however, digestibility varies between laying hen breeds.

Ch.2,4,5:
Viscous soluble fibers and fine insoluble fibers with high water-binding capacity decrease apparent fat and starch digestibility.

Ch.2,4,5:
Viscous soluble fibers and fine insoluble fibers with high water-binding capacity decrease true protein digestibility, as reflected by apparent protein digestibility.



TOTAL ENDOGENOUS PROTEIN LOSSES

Ch.4,5:
Viscous soluble fibers and fibers with high water-binding capacity increase endogenous protein losses.

Ch.4,5:
Grinding of insoluble fibers does not influence endogenous protein losses.

Correcting apparent protein digestibility for total endogenous protein losses

Figure 1.7. Graphical abstract of main findings from different chapters of this thesis to illustrate the influence of dietary fibers varying in physicochemical properties on digestion processes in gastrointestinal tract of chicken. The red arrows, lines, and boxes depict chapters addressing endogenous protein losses (**Chapters 3, 4** and **5**), the green arrows, lines, and boxes depict chapters addressing nutrient digestibility (**Chapters 2, 4, 5,** and **6**), and yellow arrows, lines, and boxes depict chapters addressing digesta passage (**Chapters 2, 5,** and **6**). Images were generated from Biorender.com.

PHYSICOCHEMICAL PROPERTIES OF DIETARY FIBERS AND THEIR ROLE IN DIGESTION PROCESSES IN CHICKENS

Dietary fibers are plant-derived components that differ markedly in physicochemical properties, including solubility, viscosity, and hydration properties. These properties vary according to botanical origin, structural composition, and processing, and therefore cannot be captured by a single chemical analysis such as total DF content. In this thesis, DF sources were characterized and tested based on these physicochemical attributes to enable a mechanistic interpretation of their effects on digestion-related processes in chickens.

Across the experimental chapters, dietary treatments were systematically designed to create contrasts in specific DF properties (Table 1.7). This approach enabled evaluation of how individual characteristics, as well as their combined effects, were associated with changes in digesta passage, EPL, and nutrient digestibility. Importantly, the results indicate that not all DF properties contributed equally to digestion-related responses. Instead, effects were observed more consistently in dietary treatments with high extract viscosity than in those differing in particle size of iDF or water-binding capacity (**WBC**).

Diets that have ability to modulate digesta viscosity altered digesta passage throughout GIT, increased EPL, and reduced nutrient digestibility. In contrast, modifications in particle size of iDF, which were expected to influence mechanical stimulation and digesta retention, resulted in limited or inconsistent effects on the digestion processes. However, DF with a high WBC and low extract viscosity, primarily affected nutrient digestibility, without consistently altering other digestion processes.

Overall, this thesis demonstrates that differences in physicochemical properties of DF are associated with distinct effects on digestion-related processes in poultry. These relations are explored in more detail in the following subsections.

Table 1.7. Change in solubility, extract viscosity and water-binding capacity (WBC) obtained by dietary contrasts across chapters[1,2,3,4,].

Diets	Solubility	Extract viscosity	WBC
C-SBH vs. F-SBH (Chapters 2 and 4)	N/A	0	--
C-OH vs. F-OH (Chapter 5)	0	0	0
C-OH vs. F-OH (Chapter 6)	0	0	--
C-SBH-AX vs. F-SBH-AX (Chapters 2 and 4)	N/A	0	0
SBH-AX vs. SBH (Chapters 2 and 4)	N/A	+++	--
F-SBP vs. F-SBH (Chapters 2 and 4)	N/A	0	+++
LC vs. OH (Chapter 5)	--	0	+

[1] Some information are N/A (not available).

[2] Solubility was expressed as the ratio between water-soluble and total non-starch polysaccharides (NSP).

[3] C-SBH = coarse soybean hulls (intact), F-SBH = fine soybean hulls (ground using a hammer mill with 1 mm screen at 1500 rpm), C-SBH-AX = coarse soybean hulls with additional 50 g/kg arabinoxylans, F-SBH-AX = fine soybean hulls with additional 50 g/kg arabinoxylans, F-SBP = fine sugar beet pulp (ground using hammer mill with 4 mm and 1 mm screen at 1500 rpm). C-OH = coarse oat hulls (intact), F-OH = fine oat hulls (ground in hammer mill using a 1 mm screen), LC = purified lignocellulose.

[4] Change of the second relative to the first in the contrast statements in physicochemical properties of the dietary treatments. Where −−, a decrease of 10–15%; −, a decrease of 5–10%; 0, a change of −5 to 5%; +, an increase of 5–10%; ++, an increase of 10–15%; +++, an increase of >15%.

The impact of viscous soluble dietary fibers on digestion processes

Viscous sDF exert pronounced adverse effects on digestion processes in chickens, including prolonged MRT of digesta, increased EPL and reduced nutrient digestibility, as also showed in **Chapters 2** and **4**. These effects are primarily driven by the formation of gel-like structures by viscous sDF in the intestinal environment, which may result in increased digesta viscosity.

Gel formation induced by viscous sDF involves the establishment of a hydrated three-dimensional polymer network within the digesta, in which water and solutes are entrapped, resulting in a semi-solid structure that is resistant to flow (Khramova et al., 2019). This physical barrier further restricts the diffusion and mobility of nutrients and digestive enzymes, limiting access to brush-border enzymes and slowing macronutrient hydrolysis and absorption (Zijlstra et al., 2012; Slama et al., 2019; reviewed by Karim et al., 2024).

Previous studies in pigs and chickens reported that the inclusion of viscous sDF increase digesta viscosity in the small intestine (Hung et al., 2021; Schop et al., 2020;

Smulikowska et al., 2002; McDonald et al., 2001). As the concentration of viscous sDF increases, long-chain polysaccharide molecules begin to interact and become entangled, forming a high-viscosity network in digesta (Zhang et al., 2021). The increased digesta viscosity may stimulate cholecystokinin (Mir et al., 2000), which is associated with a slowdown of digesta passage in the small intestine (Duke, 1992) and may reduce nutrient diffusion and hamper intestinal contractions along the small intestine (Henn and Alim, 2025). Therefore, this process potentially lowers nutrient hydrolysis, limiting transport through the gut lumen, decreasing interactions with the epithelial surface, and ultimately impairing nutrient absorption. In addition to its effects on nutrient digestion, high digesta viscosity may increase EPL by stimulating mucus secretion, enzyme secretion, and microbial growth along the GIT (Ikegami et al., 1990; Mackie et al., 2016; reviewed by Karim et al., 2024).

Evidence from previous animal studies supports these viscosity-driven effects. In pigs, when compared with control diet, inclusion of 4% carboxymethylcellulose increased crude mucin concentration in digesta by ~33% (Piel et al., 2005). Additionally, adding 6.25% of carboxymethylcellulose increased digesta viscosity by ~86% and amylase activity in the jejunum (by 46%) and ileum (by 50%) of pigs, while reducing apparent ileal digestibility of DM (by ~20%-units) and protein (by ~12%-units) compared with non-viscous cellulose (Hung et al., 2021). In chickens, dietary inclusion of 1–2% guar gum increased ileal digesta viscosity by two- to six-fold and reduced apparent starch digestibility by 12-14%-units (Sacranie et al., 2012). Similarly, supplementation of 1% high-viscosity carboxymethylcellulose almost doubled ileal digesta viscosity, reduced apparent digestibility of protein, starch, and fat, decreased bile salt secretion, and increased both aerobic and anaerobic microbial populations in the small intestine (Smits et al., 1997, 1998, 2000). Together, these effects demonstrate that gel formation by viscous sDF, which manifests as increased digesta viscosity, provides a coherent explanation for the pronounced negative effects on digestion processes observed in this thesis.

Based on the mechanisms discussed above, future studies should also focus on how to mitigate the adverse effects of viscous sDF while maintaining or enhancing nutrient utilization in poultry diets. For example, the use of non-starch polysaccharide–

degrading enzymes that have been reported modifying digesta viscosity and gelling behaviour (Ahmad et al., 2025). Investigating enzyme–fiber interactions in relation to MRT of digesta, EPL, and nutrient digestibility could provide valuable insight into effective strategies for improving nutrient utilization in diets containing viscous sDF.

Limited effects of grinding insoluble dietary fibers on digestion processes

In contrast to the pronounced and consistent effects of viscous sDF, modifications of iDF sources through particle size reduction exerted only limited effects on digestion processes under the conditions studied. Across several chapters of this thesis (**Chapters 2, 4, 5**, and **6**), reducing the particle size of iDF sources did not markedly influence MRT of digesta, EPL, or nutrient digestibility in chickens. These findings are in contrasts with previous studies demonstrating that coarse iDF can influence gastrointestinal function, particularly by stimulating gizzard development, prolonging digesta retention in that segment, and improving nutrient digestibility (Amerah et al., 2007; Hetland et al., 2005; Svihus et al., 2002). The absence of the anticipated effects of grinding iDF sources in the present study indicates that variation in particle size alone may not provide sufficient contrast to influence digestion processes or that its effects may depend on other factors, such as fiber inclusion level and the structural characteristics of the DF source.

At relatively low inclusion levels (e.g. 2–5%), coarse iDF has been shown to stimulate gizzard activity and modulate digesta kinetics (Mateos et al., 2012; Jiménez-Moreno et al., 2010; Hetland et al., 2005). In contrast, the higher inclusion levels used in this thesis (10–15%) may already have strongly stimulated gizzard activity, thereby limiting the additional impact of reduction of iDF particle size on digestion processes, as also reported by (Ghimire et al., 2025). Under conditions of high iDF inclusion, gizzard activity may already be substantially stimulated, resulting in diminished physiological responses to further modifications in particle size (van Krimpen et al., 2011). It may be speculated that the presence of a functional threshold (of an already high DF level in the diet) beyond which an additional physical contrast does not elicit proportional changes in digestion.

In addition to inclusion level, the findings from this study also underscore the importance of structural characteristics of iDF sources, beyond particle size. Structural characteristics such as bulk density and resistance to mechanical breakdown appear to play a critical role in determining the extent of gizzard stimulation and subsequent digestive responses (Mateos et al., 2012; Svihus, 2011; Amerah et al., 2009). A descriptive comparison of particle size distribution, based on reductions in geometric mean diameter (**GMD**) following grinding of different iDF sources–including OH, SBH, and SBP–showed that OH exhibited a smaller reduction in particle size (57%) compared with SBH (59%) and SBP (71%) when ground using a coffee grinder (Akbari Moghaddam Kakhki et al., 2024). These results suggest greater hardness and grinding resistance of OH, whereas SBP appears more elastic and less resistant to mechanical breakdown. It should be noted, however, that particle size distributions derived from sieving methods may not fully capture the true structural characteristics of iDF sources due to their differences in multidimensional structures. For example, ground OH particles, which are often long and thin, may be retained on larger sieves when oriented horizontally, even though they could pass through the same sieve when oriented vertically. This methodological limitation may therefore lead to an overestimation of the structural persistence of iDF sources.

Insoluble DF sources with high mechanical resistance, such as OH or rice hulls, have consistently been shown to increase gizzard weight and muscularity, slow digesta passage in that segment, and improve nutrient digestibility (Kardel et al., 2025; Hetland et al., 2005; Svihus et al., 2004a). In contrast, more elastic DF sources that tend to recover their original shape after deformation may appear to have a limited capacity to stimulate gizzard function, even when included as coarse particles. Therefore, grinding such fibers may not meaningfully alter their functional behavior.

Moreover, the processing of the ingredient may also play an important role in influencing digestion processes in chickens. All experimental diets were provided in pelleted form, and the pelleting process is known to induce secondary grinding, particle fragmentation, and structural modification of fibrous ingredients due to mechanical pressure, shear forces, and thermal treatment (Svihus et al., 2004b; Abdollahi and Ravindran, 2013; Vukmirović et al., 2017; reviewed by Lyu et al., 2020; Bastiaansen et

al., 2024). These processing effects likely partially diminish the initial differences in particle size of iDF at the ingredient level as shown in the analysis of GMD using dry sieve analysis in **Chapters 2 and 4**. Despite the pelleting process may finally limit contrast between coarse and ground iDF sources, this process may change viscosity and hydration capacity of the diet (reviewed by Svihus and Zimonja, 2011).

From a diet formulation perspective, these findings suggest that grinding or inclusion of coarse iDF should not be regarded as a universally effective strategy to manipulate digestion processes in chickens. Instead, the functional impact of iDF may depend on different factors, including inclusion level, structural characteristics of the fiber source, feed processing, or their combinations. For practical poultry diets, selecting iDF sources with sufficient mechanical resilience and incorporating them at inclusion levels that provide meaningful physical stimulation of the gizzard may be more effective than further only reducing particle size. Future studies should consider for both low-fiber control diets and different inclusion levels of iDF to quantify how increasing fiber inclusion changes digestion processes in chickens, specifically digesta MRT and EPL.

Relations between dietary water-binding capacity and digesta mean retention time in chickens

Across the experimental chapters (**Chapters 2, 4, and 5**), changes in digestion processes in chickens were most consistently associated with extract viscosity, while associations with WBC were less consistent.

To further evaluate the potential contribution of WBC to variation in digestion processes, an exploratory correlation analysis was conducted across all dietary treatments included in this thesis. This analysis, based on data from two experiments comprising 52 replicates (pen of broilers) across 8 experimental diets. No relations were observed between dietary WBC and true ileal digestibility (**TID**) of protein (r = 0.02, P = 0.859), total EPL in ileal digesta (r = −0.07, P = 0.561), or true total tract digestibility of protein (r = 0.05, P = 0.679). However, a negative correlation was found between WBC and apparent ileal digestibility (**AID**) of starch (r = -0.28, P = 0.023), which diminished total tract (r = -0.13, P = 0.291). Interestingly, WBC was negatively correlated with the MRT of solid digesta in the crop (r = −0.35, P = 0.011), proventriculus + gizzard (tendency, r = −0.27, P = 0.051), and small intestine (r = −0.33, P = 0.017),

while no association was observed between dietary WBC and MRT of solid digesta in the hindgut (r = 0.15, *P* = 0.305) (Figure 4.7). These results suggest that although the total extent of nutrient digestion does not seem to be related to dietary WBC in diet, the rate of nutrient absorption may differ, as reflected by the MRT of digesta.

Although the relation between MRT of digesta and dietary WBC in broilers has been limitedly studied, such relations between dietary WBC and digesta transit have also been observed by others. A descriptive comparison from previous study reported that broilers fed SBP diets exhibited shorter MRT of solid digesta in the crop and gizzard, but longer MRT in the small intestine when compared with birds fed OH diets (Garçon et al., 2023). Another study also reported that the inclusion of 5% purified LC in the diet shortened MRT of solid digesta in the gizzard by 48% compared with a diet containing 5% OH, while prolonging MRT in the duodenum and jejunum by 57% and 47% (Mens et al., 2022). Together, these findings support the potential of WBC as a simple and easily measurable dietary property to predict feed passage and digestion processes in chickens.

QUANTIFYING TOTAL ENDOGENOUS PROTEIN LOSSES

Total EPL were quantified using a minimally invasive ID method, in which ^{15}N-enrichment in urine was used to represent the ^{15}N-enrichment in the precursor pool. As extensively elaborated in **Chapter 3**, this methodological approach enables the quantification of total EPL, and thus diet-specific EPL, under close-to-practical conditions and is applicable across a wide range of dietary compositions, including diets containing fiber-rich ingredients. Despite being associated with high costs and substantial demands in terms of time, labour, and technical complexity, the ID method offers important practical advantages. Notably, the measurement of ^{15}N-enrichment in the precursor pool can be done by measuring ^{15}N-enrichment in the urine, thereby eliminating the need for daily blood sampling. This substantially improves animal welfare and experimental feasibility and justified the application of this method in **Chapters 4 and 5** to assess the effects of physicochemical properties of DF on total EPL and true protein digestibility.

Despite these advantages, further optimization is warranted, particularly because this method does not distinguish between host-derived endogenous protein and microbial protein biomass. Separating these protein sources would improve the biological and physiological interpretation of EPL values in broilers. Without separating microbial protein, host-derived EPL may be overestimated, as microbial protein present along the GIT may also become enriched with ^{15}N during isotope infusion due to microbial utilization of available nitrogen sources. Microbial protein undergoes more rapid turnover than host-derived protein. Microbes rapidly degrade and recycle amino acids from undigested dietary protein and host-derived proteins to support their rapid proliferation and high metabolic activity, resulting in isotopic discrimination between microbial and host nitrogen pools (Arike et al., 2020; Reese et al., 2018; Torrallardona et al., 1996).

Evidence from previous studies underscores the importance of this limitation. For instance, in pigs fed an enzymatically hydrolyzed casein–based diet, microbial protein accounted for up to 54% of the total EPL measured in ileal digesta, while porcine cellular protein, soluble free protein, and mucin contributed only 6.7%, 18.2%, and 14.1% (Miner-Williams et al., 2009). Similarly, a recent study from our group, applying an amino acid profile approach in pigs fed ten different protein sources, reported that microbial protein contributed between 37% and 83% of total EPL in ileal digesta (Noorman et al., 2024).

Based on previous studies on estimates of microbial protein contribution to ileal digesta nitrogen by Miner-Williams et al. (2009) and Noorman et al. (2024), the proportion of total EPL in ileal digesta originating from endogenous body protein in broilers fed an enzymatically hydrolyzed casein diet (**Chapter 3**) was re-calculated. Incorporation of these microbial correction factors showed that host-derived EPL were overestimated by 0.16–0.36 g/kg DMI when microbial protein biomass was not considered (Figure 2.7). Therefore, these results highlight the importance of correcting for microbial protein biomass to enhance the accuracy of the minimally invasive ID method. Importantly, distinguishing between microbial protein biomass and host-derived endogenous protein would allow a more precise interpretation of dietary effects, particularly in fiber-rich diets where their physicochemical properties may

differentially stimulate microbial proliferation and endogenous protein secretion (Ravindran, 2021).

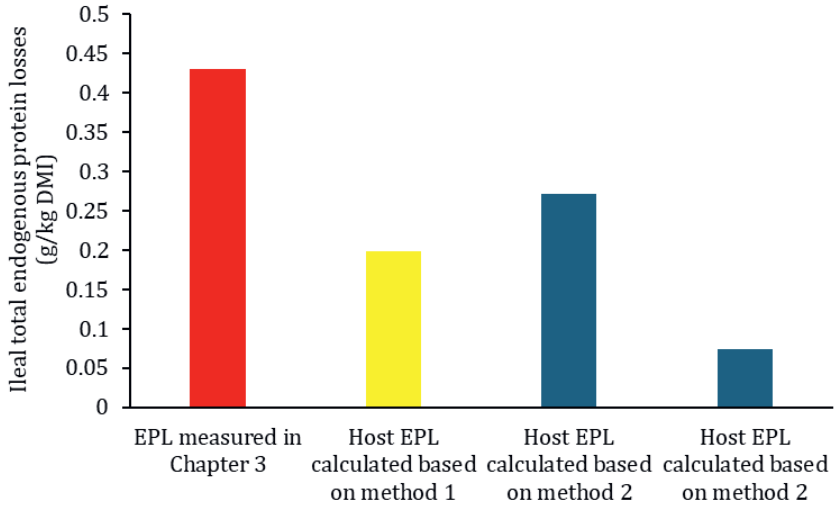


Figure 2.7. Estimates of total endogenous protein losses (EPL) in ileal digesta of broilers fed a diet containing enzymatically hydrolyzed casein (EHC), as the only protein source. The first bar represents total EPL measured in **Chapter 3** of the present thesis without correction for microbial protein biomass (■). The remaining bars show re-calculated host-derived EPL values after correcting total EPL measured in **Chapter 3** data for microbial protein contribution using published proportions of microbial nitrogen reported by Miner-Williams et al. (2009) (■, method 1) and Noorman et al. (2024) (■, method 2).

In addition to methodological considerations, differences total EPL measured in ileal digesta of broilers in **Chapters 3 and 4** were lower than those observed in **Chapter 5** (Figure 3.7). As expected, the lowest total EPL values were observed in broilers fed the EHC diet (**Chapter 3**), while inclusion of fiber-rich ingredients increased total EPL in this study. However, the higher total EPL in diets containing OH and LC (**Chapter 5**) compared with SBH, AX, and SBP–based diets (**Chapter 4**) were found. These higher total EPL values in **Chapter 5** may be influenced not only by iDF sources, but also other ingredients in the diet, such as wheat. This ingredient contains AX and β-glucans, which have been reported to influence diet-specific EPL (Angkanaporn et al., 1994; reviewed by Bartłomiej et al., 2012; McCleary et al., 2012; reviewed by Jacob and Pescatore,

2014). In addition to the dietary factors, practical and non-dietary factors may also have influenced to these differences.

First, differences in the ^{15}N-isotope administration protocol between experiments may have contributed to variation in estimated total EPL. Broilers in **Chapter 4** received a double dose of ^{15}N-labelled milk protein concentrate on the first day of isotope infusion, whereas withdrawal of the ^{15}N-isotope in **Chapter 5** was extended by one additional day. Although excreta were pooled over the final four days of isotope withdrawal in both studies, such differences in ^{15}N-isotope administration and withdrawal protocols may have influenced ^{15}N-enrichment in host and microbial protein that are reflected by ^{15}N-enrichment in urine. Indeed, urinary ^{15}N-enrichment in **Chapter 5** was two- to three-fold lower than in **Chapter 4**. Because urinary ^{15}N-enrichment is used as the denominator in the calculation of total EPL proportion, variation in urinary ^{15}N-enrichment could contribute to differences in estimated EPL. Notably, ^{15}N-enrichment in ileal digesta remained similar between those chapters, suggesting that the methodological differences mainly affected the precursor pool estimation rather than the enrichment of nitrogen in digesta.

Second, age-related differences in basal EPL may also have played an important role. Total EPL in **Chapters 3** and **4** were measured in broilers at 28 days of age, whereas measurements in **Chapter 5** were conducted at 37 days of age. Previous studies have shown that basal EPL or endogenous amino acid losses decrease with increasing age in broilers (An and Kong, 2022; Barua et al., 2021). For example, basal EPL in broilers fed nitrogen-free diets at six weeks of age were reported to be 29% lower than those at four weeks of age (Barua et al., 2021). Because total EPL reflects the combined contribution of basal and diet-specific losses, age-related reductions in basal EPL may partially mask dietary effects in older birds. This highlights the importance of accounting for both methodological factors and bird age when comparing and interpreting the effects of fiber-rich ingredients on total EPL in broilers.

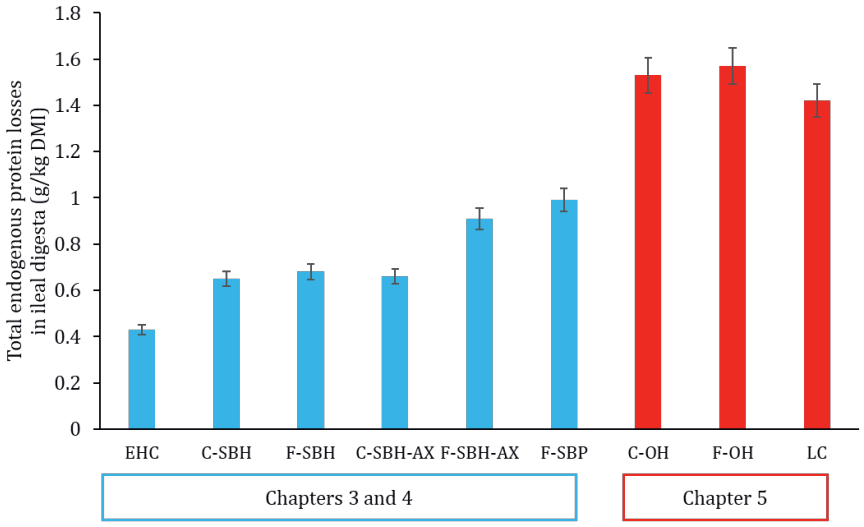


Figure 3.7. Total endogenous protein losses in ileal digesta of broilers fed different diets from **Chapters 3, 4,** and **5**.

THE ROLE OF CHICKEN BREEDS IN COPING FIBER-RICH INGREDIENTS

In addition to dietary physicochemical properties, non-dietary factors such as chicken breeds may play an important role in determining digestion processes and the effective utilization of fiber-rich ingredients. Chicken breeds differ in growth rate, feeding behavior, microbiome, and gastrointestinal capacity, which can lead to divergent physiological responses to DF and, consequently, differences in digesta passage and nutrient digestibility (Nafady et al., 2025; Rougière and Carré, 2010; Péron et al., 2006; Havenstein et al., 1994, 2003). Available evidence indicates substantial inherent physiological variation among poultry breeds. For example, a high body-weight-selected chicken breed fed *ad libitum* diet containing 20% CP and 2,685 of ME/kg of feed exhibited higher amylase, trypsin, and chymotrypsin secretion compared with a low body-weight–selected chicken breed (O'sullivan et al., 1992). In addition, high egg-producing chickens have been reported to exhibit greater abundance of *Firmicutes*, *Proteobacteria*, and *Lactobacillus* in the ceca, compared with low egg-producing chickens (Wu et al., 2025). Similarly, comparisons between two native slow-growing breeds showed that Sasso chickens had a higher abundance of *Clostridium* but a lower abundance of *Escherichia coli* in the ceca than Green-legged Partridge chickens

(Sztandarski et al., 2022). Such differences suggest that digestive capacity, microbiome, and adaptive responses to DF may differ among chicken breeds, potentially influencing their ability to cope with fiber-rich diets.

In this context, it is hypothesized that chicken breeds that are more adaptable to less intensive environment, such as backyard production systems, may better tolerate fiber-rich diets. In **Chapter 6**, two laying hen breeds fed OH-based diets differed markedly in gastrointestinal organ development, digesta retention time, and apparent nutrient digestibility. At 21 weeks of age, Bovans Black hens exhibited larger gizzard weight, shorter MRT of solid and liquid digesta, and higher apparent ileal nutrient digestibility compared with Dekalb White hens. Although both breeds was developed for high egg production type breeds, Bovans Black has been selected for broader adaptability to diverse housing systems, including backyard chicken farming system, whereas Dekalb White has been optimized for high productivity under controlled commercial conditions (Hendrix Genetics, n.d). This indicates that breeds selected for robustness and adaptability may possess a greater adaptability in coping with fiber-rich ingredients compared with breeds primarily selected only for maximal production efficiency.

Further evidence for bird-type-related differences in digestion processes was observed when comparing laying hens and broilers. Mean retention time of solid digesta differed markedly between these chicken breeds fed diets with high WBC (**Chapters 2, 5, and 6**; Figure 4.7). Overall, solid digesta was retained on average 1 h longer in the crop of laying hens than in broilers, whereas retention in the proventriculus + gizzard (~1 hour) and small intestine (~2 hours) was shorter in laying hens. In contrast, retention of solid digesta in the hindgut of laying hens was approximately 2.5 hours longer than in broilers. These differences may be partly reflected by differences in relative daily feed intake, as in this study, laying hens consumed approximately 55% less feed than broilers.

Overall, there seems to be no relation between WBC and MRT of solid digesta for laying hens within the range measured in alle GIT segments. For broilers, however, there seems to be a somewhat negative relationship between WBC and MRT of solid digesta

in the crop and small intestine, but this relation seems to be absent in the other segments.

The breed-related differences in digesta passage have been reported previously, with broilers having shorter digesta retention time in the crop but longer digesta retention time in the jejunum compared with White Leghorn chickens when fed soybean- or canola-based diets (Shires et al., 1987). Considering the results from our study and others, digesta passage of different chicken breeds are sensitive to the change of the diet as also showed by previous study of Rougière and Carré (2010).

Collectively, these observations demonstrate that the nutritional value and digestive impact of fiber-rich ingredients cannot be generalized across chicken breeds. Breed-specific differences in gastrointestinal function, feeding behavior, and digestive capacity should therefore be considered key factors when optimizing the inclusion and evaluation of DF in poultry diets.

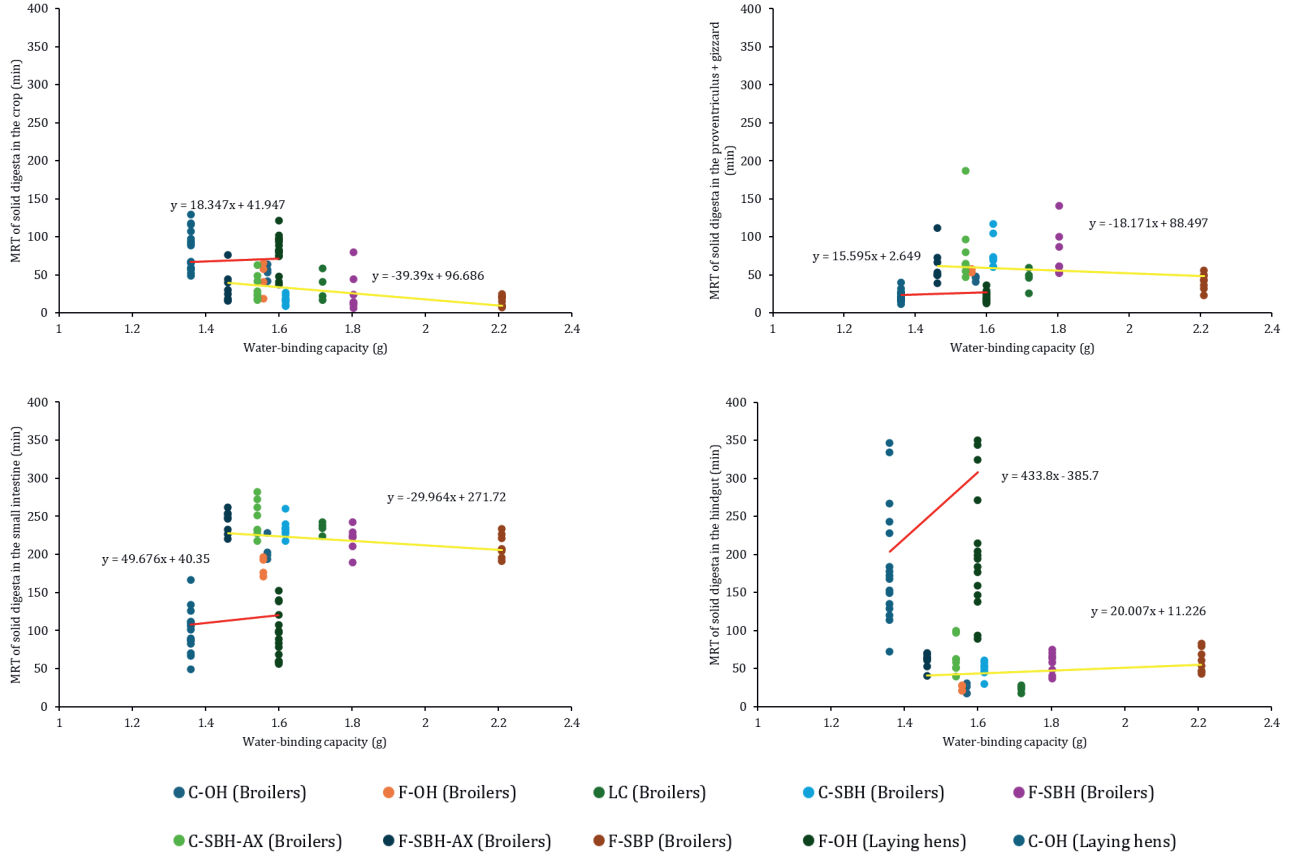


Figure 4.7. Data plots of mean retention time (MRT) of solid digesta throughout gastrointestinal tract of broilers (52 pen replicates) and laying hens (32 pen replicates) fed diets containing coarse (intact) oat hulls (C-OH), finely ground oat hulls (F-OH), purified

lignocellulose (LC), coarse soybean hulls (C-SBH), finely ground soybean hulls (F-SBH), coarse soybean hulls with purified arabinoxylans (C-SBH-AX), finely ground soybean hulls with purified arabinoxylans (F-SBH-AX), or finely ground sugar beet pulp (F-SBP). Points represent individual observations. Red (–) and yellow (–) lines indicate regression lines based on mean values for laying hens and broilers, respectively. Regression equations are shown as y = ax + b.

THE CORRELATION BETWEEN DIGESTION PROCESSES AND PROTEIN DIGESTIBILITY IN CHICKENS

It is hypothesized that nutrient digestibility is influenced by the duration of interaction between digesta and digestive enzymes along the GIT (Svihus and Itani, 2019; reviewed by Jha and Mishra, 2021). Prolonged retention of digesta in the proventriculus, gizzard, and small intestine is often associated with increased endogenous enzyme secretion and enhanced opportunities for nutrient hydrolysis and absorption (Svihus, 2011). However, digestion is a complex, integrated process that is simultaneously influenced by multiple interacting factors, including diet composition, physicochemical properties of DF, and physiological responses of the bird.

To further explore the relations among various parameters that contribute to protein digestibility in chickens, an additional correlation analysis was performed using data across dietary treatments (detailed in Text Box 1.7, Figure 5.7). The main objective of this analysis was to study the relation between EPL, digesta MRT and digestibility of proteins, considering both dietary treatment effects and variation among birds fed the same diet. When dietary effects are present, there were negative correlations between total EPL in ileal digesta and MRT of solid digesta in proventriculus + gizzard ($r = -0.33$, $P = 0.016$) and small intestine ($r = -0.44$, $P = 0.001$), as well as between MRT of solid digesta in the small intestine and AID ($r = -0.44$, $P < 0.001$) and TID ($r = -0.59$, $P = 0.001$) of protein (Figure 5.7A). After correcting for diet effects and other between-experiment variation, only a negative correlation between total EPL in ileal digesta and AID ($r = -0.82$, $P < 0.001$) and TID ($r = -0.58$, $P < 0.001$) of protein was found (Figure 5.7B). These findings indicate that relations between MRT, EPL, and protein digestibility are largely diet-dependent, whereas the association between EPL and protein digestibility appears to be independent of dietary treatment.

Text Box 1.7. Relation of digestion processes of protein in the foregut of broilers.

A database containing data of 52 replicate pens of broilers and 8 different diets coming from 2 different experiments (**Chapters 2, 4, and 5**) was used to study the relation between total ileal endogenous protein losses (EPL), apparent (AID) and

true ileal digestibility (TID) of protein, and mean retention time (MRT) of solid digesta in the proventriculus + gizzard and small intestine.

To check possible experiment effects, the relations between MRT of solid digesta, total EPL in ileal digesta, and AID and TID of protein was analyzed individually by experiment through Pearson correlation (PROC CORR, SAS, version 9.4, SAS Institute Inc., Cary, NC). In two out of the eight diets, increases in EPL in ileal digesta were associated with reductions in TID (r = -0.87 to -0.97, $P < 0.01$) and AID (r = -0.87 to -0.99, $P < 0.01$) of protein. Thus, as no consistent experimental effects were observed, the correlation analysis was subsequently repeated using the pooled dataset.

Subsequently, to extract non-dietary variation, each parameters was analyzed using a general linear model (PROC GLM, SAS, version 9.4, SAS Institute Inc., Cary, NC) with diet (experiment) as fixed effect to remove variation due to dietary treatments applied in the various experiments and other experiment-related variation. Then model residuals were extracted for all parameters and analyzed using Pearson correlation analysis. *P-values* below 0.05 were considered statistically significant, and *P-values* between 0.05 and 0.1 as tendencies.

By definition, AID of protein is the results of EPL accumulated in ileal digesta and the TID of dietary protein, thus, as expected, a strong negative correlation between AID of protein and total EPL in ileal digesta was found (r=-0.82, $P < 0.001$). Notably, the negative correlation observed between total EPL and TID suggests that higher total EPL are biologically associated with reduced true protein digestibility. Endogenous protein losses comprise not only digestive enzymes and sloughed epithelial cells, but also mucus secretion. In particular, increased mucus secretion may impair nutrient digestibility by limiting nutrient absorption. Consistent with this interpretation, previous studies have reported that a thinner mucus layer in the small intestine is associated with improved nutrient absorption efficiency (Leal et al., 2017).

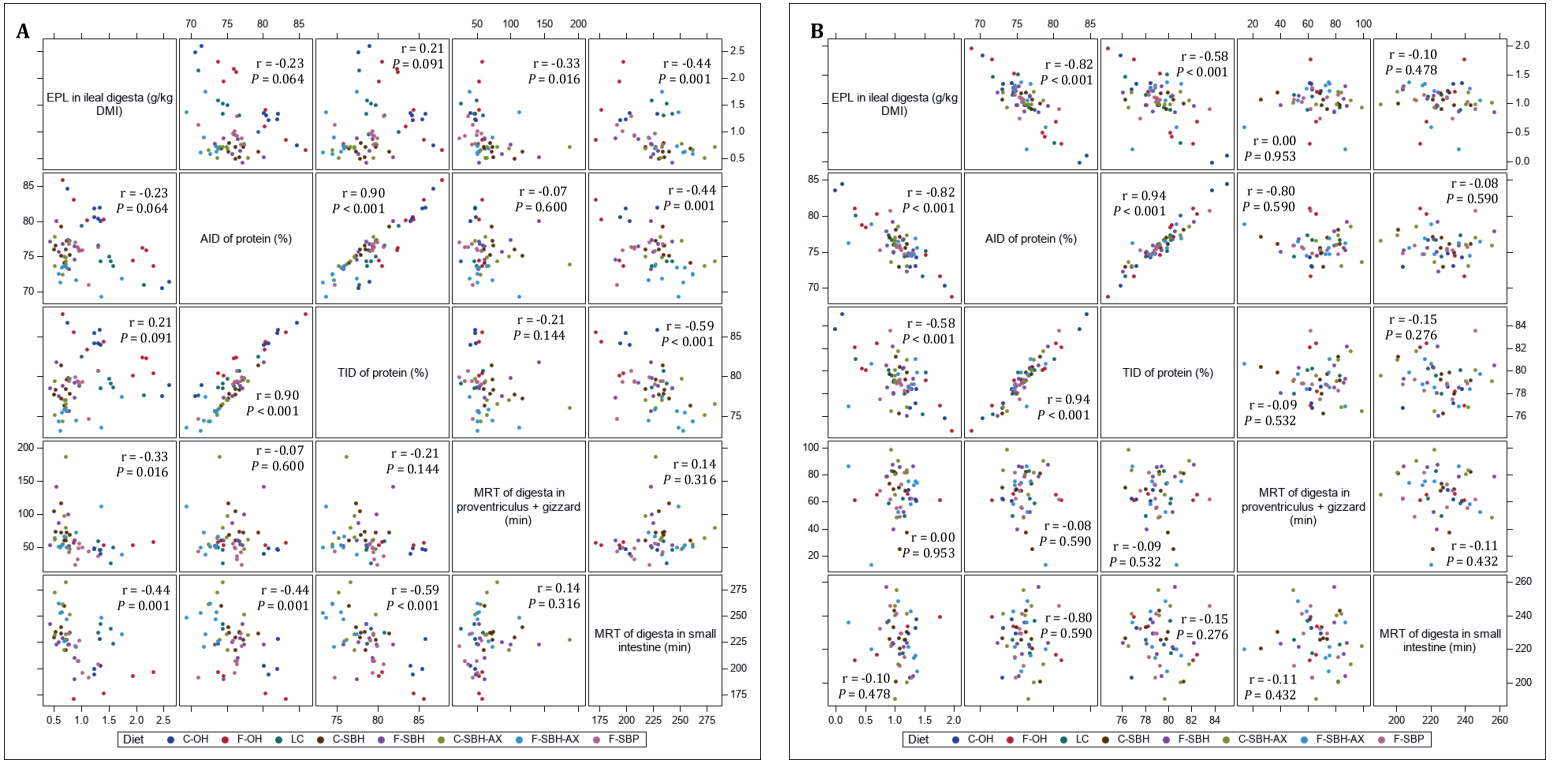


Figure 5.7. Correlations between total endogenous protein losses (EPL) in ileal digesta, true ileal digestibility (TID) of protein, and mean retention time (MRT) of solid digesta in proventriculus + gizzard and small intestine in broilers (A) and correlations between means corrected for residual of total EPL in ileal digesta, TID of protein, and MRT of solid digesta in proventriculus + gizzard and small intestine in broilers (B) from Chapters 2, 4, and 5 fed diets containing coarse (intact) oat hulls (C-OH), finely ground oat hulls (F-OH),

purified lignocellulose (LC), coarse soybean hulls (C-SBH), finely ground soybean hulls (F-SBH), coarse soybean hulls with purified arabinoxylans (C-SBH-AX), finely ground soybean hulls with purified arabinoxylans (F-SBH-AX), or finely ground sugar beet pulp (F-SBP).

Apart from the relations between total EPL and TID of protein, the correlation analysis revealed another notable pattern. Apparent ileal digestibility of protein was strongly and positively correlated with TID of protein, both when diet effects were included ($r = 0.90$, $P < 0.001$) and when diet effects were excluded ($r = 0.94$, $P < 0.001$) (Figure 5.7). As expected, the variation in TID was largely reflected in AID values, indicating that AID may serve as a useful proxy for TID of protein when direct quantification of endogenous protein losses is not feasible. However, the higher correlation coefficient found after correcting for diet effects – particularly driven by viscous sDF diets – demonstrates diets that induce EPL can cause deviations between AID and TID of protein. Under such conditions, reliance on AID alone may mask true dietary effects on protein digestion. Accurate evaluation of diet-specific effects on protein digestibility therefore requires consideration of EPL to distinguish between apparent and true digestibility responses.

IMPACT OF THIS RESEARCH

Dietary fibers in animal nutrition have been conventionally characterized using gravimetrical methods such as crude fiber, neutral detergent fiber, or acid detergent fiber, which primarily quantify inert carbohydrate fractions. Although these metrics are useful for compositional description, the findings of this thesis demonstrate that they have limited value in predicting the physiological and nutritional effects of fiber-rich ingredients in digestion processes of poultry.

Across the experimental chapters, physicochemical properties of DF, particularly viscosity, emerged as key determinants of digestion-related responses, including digesta passage, EPL, and nutrient digestibility. In contrast, particle size of iDF was insufficient to explain the observed variation in digestive outcomes. Other properties such as WBC influenced digesta behavior only in specific GIT segments, but did not consistently influence protein digestion. These findings imply that evaluation of fiber-rich ingredients should focus on functional physicochemical properties rather than conventional fiber classifications. For practical feed formulation, the results from this thesis indicate that accounting for extract viscosity and WBC of diets may improve prediction of the digestive impact of fiber inclusion. Therefore, it is recommended to account for those properties in chicken feed formulation. Incorporating these

properties into ingredient databases and formulation may help to optimize fiber inclusion without compromising protein digestion. In particular, formulating diets with awareness of viscosity-related effects may reduce unintended increases in EPL and/or associated decreases in true protein digestibility.

Importantly, the findings of this thesis indicate that individual digestion parameters should not be interpreted independently, as changes in one process are closely linked to changes in others. Relations between total EPL and true protein digestibility highlight the interconnected nature of protein digestion in chickens. Additionally, when using fiber-rich ingredients in poultry diets, reliance on apparent digestibility alone may lead to misinterpretation of dietary effects, particularly when EPL are elevated. For accurate assessment of protein digestion, measurements of total EPL and true protein digestibility are recommended to consider alongside apparent protein digestibility, especially when comparing diets containing fiber-rich ingredients.

Finally, the observed non-dietary factors, such as breed- and age-related differences, in digestive responses suggest that the nutrient digestion of fiber-rich ingredients cannot be generalized across poultry populations. Differences in gastrointestinal capacity, feeding behavior, microbiome, and physiological adaptation modulate the impact of DF and should be considered when optimizing fiber inclusion strategies. Incorporating these biological factors in the future research and feed formulation work will enable more precise and targeted fiber inclusion across poultry populations.

TAKE-HOME MESSAGES

- Viscous soluble fibers prolong retention time of both solid and liquid digesta along gastrointestinal tract and reduce apparent nutrient digestibility.
- Viscous soluble fibers increase total endogenous protein losses and decrease true protein digestibility.
- In high inclusion level, grinding insoluble fiber sources only marginally influence digesta retention time, endogenous protein losses, and nutrient digestibility.
- Fine insoluble fibers with high hydration capacity may reduce (true) nutrient digestibility, without influencing endogenous protein losses.

- Under the same dietary conditions, higher endogenous protein losses are associated with lower true protein digestibility.
- Correcting apparent protein digestibility for total (basal + specific) endogenous protein losses is essential for an accurate assessment of true digestibility in chicken diets with increasing inclusion of fiber-rich feed ingredients.
- The ^{15}N-isotope dilution is a suitable method to estimate total endogenous protein losses in chickens fed complete diets, including those containing fibrous ingredients, under minimally invasive conditions.
- When using ^{15}N-isotope dilution method, separation of protein originating from endogenous body protein and microbial biomass will improve biological and physiological understanding of endogenous protein losses.

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Summary

Global demand for animal-based protein is rising rapidly, with poultry production playing a key role due to its high efficiency and relatively low production costs. Genetic selection has substantially increased growth rates and egg production in modern poultry breeds, enhancing productivity but also increasing nutritional requirements and reliance on highly digestible feed ingredients. At the same time, sustainability challenges—particularly competition for high-quality feed ingredients and the environmental impacts of feed production—have intensified interest in using agricultural by-products as alternative feed ingredients. These by-products are typically rich in dietary fiber (**DF**), whose diverse physicochemical properties can markedly influence gastrointestinal function, nutrient digestibility, and overall performance in poultry. This highlights the need for a deeper understanding of the role of DF in poultry nutrition. The main aim of this thesis was therefore to investigate how DF with differing physicochemical properties (solubility, particle size, viscosity, and hydration capacity) influences digestion-related processes in chickens, including digesta passage behavior, endogenous protein losses (**EPL**), and nutrient digestibility.

Chapter 2 investigated the effects of coarse or fine soybean hulls (**SBH**), as insoluble DF sources, with or without purified arabinoxylans (**AX**), as viscous DF source, on digesta mean retention time (**MRT**) and nutrient digestibility in broilers. The addition of AX markedly influenced gastrointestinal physiology and digestion by increasing digesta retention time in several segments of the gastrointestinal tract and reducing the apparent digestibility of dry matter, starch, and protein. In contrast, the particle size of SBH had relatively minor effects on digestion processes. However, an interaction was observed whereby AX reduced ileal protein digestibility only when combined with finely ground SBH. This finding suggests that coarse fiber particles may partly counteract the negative effects of soluble viscous fibers, potentially through changes in digesta viscosity.

Chapter 3 focused on quantifying total endogenous protein losses (basal + diet-specific) in broilers fed the same diets as in **Chapter 2**, as well as diets in which fine soybean hulls were replaced by fine sugar beet pulp (**SBP**), which DF with a high hydration capacity or enzymatically hydrolyzed casein (**EHC**). A minimally invasive ^{15}N-isotope dilution (**ID**) method was studied using urinary ^{15}N-enrichment as a proxy for

the enrichment of the precursor pool involved in endogenous protein synthesis. Additionally, because no gold standard method exists for measuring EPL, the ID method was compared with the commonly used EHC and EHC-ultrafiltration methods. Although the ID approach resulted in lower estimates of EPL than the other methods, the results showed that the ID method can reliably quantify EPL under near-practical feeding conditions across diets differing in ingredients, including those varying in fiber composition.

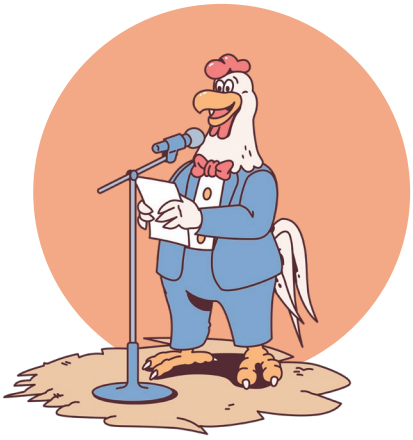
Chapter 4 further investigated total EPL and protein digestibility in broilers fed the same diets as in **Chapter 3**, excluding the EHC diet. it was found that purified AX increased EPL in feces and reduced true ileal protein digestibility, regardless of the particle size of SBH. However, apparent ileal protein digestibility decreased only when AX was combined with finely ground SBH, again highlighting interactions between viscous soluble DF and particle size of insoluble DF. In addition, replacing fine SBH with fine SBP, increased endogenous protein losses without affecting true protein digestibility. These findings demonstrate that different DF properties influence endogenous losses and protein digestibility in distinct ways.

Chapter 5 examined the effects of coarse or fine oat hulls (**OH**), as insoluble DF sources, or purified lignocellulose, a fine insoluble with notable hydration capacity on digesta MRT, EPL, and nutrient digestibility. Diets containing purified lignocellulose reduced apparent and true protein digestibility as well as digestibility of starch and fat compared with OH diets. Purified lignocellulose also prolonged digesta retention time in the small intestine, without influencing EPL. In contrast, the particle size of oat hulls had minimal influence on digestion processes. These findings indicate that hydration properties of insoluble fiber ingredients may play a more important role than particle size in determining digestion responses in broilers.

Chapter 6 investigated whether laying hen breeds differing in genetic background respond differently to insoluble DF particle size. Two breeds, Dekalb White and Bovans Black, were fed diets containing either fine or coarse OH. Clear differences between breeds were observed in gastrointestinal tract development, digesta retention time, and nutrient digestibility. Bovans Black hens showed larger gastrointestinal organs, longer digesta retention time, and greater ileal protein digestibility compared with Dekalb

White hens. However, the particle size of OH had limited effects on most digestion parameters. These results suggest that genetic background can influence digestive physiology and nutrient utilization, potentially affecting the response to DF characteristics.

Overall, this thesis demonstrates that the physicochemical properties of DF, including viscosity and hydration capacity, strongly influence digesta passage, EPL, and nutrient digestibility in poultry. These findings highlight the importance of considering dietary fiber properties in poultry feed formulation. Therefore, incorporating these properties into ingredient databases and formulation may help to optimize fiber inclusion without compromising nutrient digestion. The implications for nutritional strategies, the broader impact of this research to the poultry industry, and the key take-home messages have been extensively discussed in **Chapter 7**.



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About the author

CURRICULUM VITAE



Mochammad Fahmi Habibi was born on 30 July 1990 and grew up in Magetan, East Java, Indonesia. In 2010, he moved to Yogyakarta to pursue a bachelor's degree at the Faculty of Animal Science, Universitas Gadjah Mada (UGM). During his undergraduate studies, he completed an internship at the Sumiyoshi Livestock Centre in Miyazaki, Japan, where he gained experience in the barn management of Wagyu cattle. He obtained his bachelor's degree in 2015.

Following his graduation, he worked as a research assistant at the Centre for National Livestock Development Studies, UGM. In 2016, he was awarded the Stipendium Hungaricum scholarship by the Hungarian Government to pursue a master's degree at the Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, specializing in Animal Husbandry Engineering. He completed his master's degree in 2018 with a thesis entitled "The effects of bioactive compounds on immune responses and behavior of broiler chickens challenged with *Escherichia coli* lipopolysaccharides."

Upon returning to Indonesia, he joined his alma mater, UGM, as a lecturer, working in the Laboratory of Poultry Science. After two years of employment at UGM, he received a letter of acceptance from Wageningen University & Research to pursue a PhD. In 2020, he was awarded a scholarship from the Indonesia Endowment Fund for Education (LPDP). Due to the COVID-19 outbreak, the start of his PhD was postponed, and he officially started his doctoral studies in February 2021.

After completing his PhD, Fahmi will return to Indonesia to continue his academic career at UGM. He can be reached at mochammad.fahmi.h@ugm.ac.id.

TRAINING AND SUPERVISION PLAN

Completed in fulfillment of the requirements for the Education Certificate of the Wageningen Institute of Animal Sciences

The Basic Package (1.7 ECTS[1])	Year
WIAS Introduction course	2021
Scientific Integrity	2021
Ethics in Animal Sciences	2021
Disciplinary Competences (8 ECTS)	
WIAS Research Proposal	2021
Introduction to R	2022
Design of Experiments	2022
Gut Health in Pigs and Poultry	2023
Professional Competences (17.8 ECTS)	
Effective Behaviour	2021
Project and Time Management	2021
Reserach Data Management	2022
Searching and Organising Literature	2022
Efficient Writing Strategies	2022
Un-box Your PhD Process	2022
Scientific Writing	2022
Entrepreneurship In and Outside Science	2022
Organizer of Animal Nutrition Research Forum	2023
Organizer of Wageningen-Indonesia Scientific Exposure	2023
Supervising BSc & MSc thesis students	2023
Scientific Artwork	2023
Adobe Indesign - from Dissertation Layout to Poster Design	2023
Career Perspectives	2024
Scientific Publishing	2024
WIAS Midterm Reflection Course	2024
Presentation Skills (4 ECTS)	
The 7th EAAP International Symposium on Energy and Protein Nutrition. Granada, Spain (Poster)	2022
The 45th Animal Nutrition Research forum. Wageningen, the Netherlands (Oral)	2023

The 23rd European Symposium on Poultry Nutrition. Rimini, Italy (2 posters)	2023
Teaching competences (6 ECTS)	
Supervising 2 BSc. Thesis	2023
Supervising 2 MSc. Thesis	2021/2024
Assisting laboratory practical of principal of animal nutrition	2022/2023
Assisting laboratory practical of animal nutrition and physiology	2024/2025

[1]One ECTS credit equals a study load of 28 hours.

LIST OF PUBLICATIONS

Peer reviewed scientific publications

Hanim, C., Dono, N.D., Ariyadi, B., Habibi, M.F., Al Anas, M., Hanif, M.F., 2023. Effect of protected sodium butyrate on growth performance, carcass traits, relative weight of digestive organs and intestinal histomorphology of broilers. Journal of Animal and Feed Sciences 32, 413–419. doi:10.22358/jafs/166080/2023

Habibi, M.F., Kwakkel, R.P., de Vries, S., 2024. Gastrointestinal tract morphology, nutrient digestibility, and digesta mean retention time in laying hens of two different breeds fed with fine or coarse oat hulls. Poultry Science 103, 104333. doi:10.1016/j.psj.2024.104333

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Oat Hulls
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