# Analytical and nutritional evaluation of rye grain in diets for growing pigs

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**Meinen Eltern**

# SUMMARY


## Analytical and nutritional evaluation of rye grain in diets for growing pigs

Climate change and increasing consumer awareness of environmental and animal welfare issues are constantly challenging the animal nutrition sector to adapt and find improved solutions that are resource efficient, meet the requirements of pigs and promote animal welfare. One such approach is the use of regional feedstuffs such as rye and rapeseed meal to feed pigs. In the past, both feedstuffs were rarely used in conventional pig feeds due to low yields and high concentrations of antinutritive substances. Further development of the varieties; more recent scientific findings; as well as their sensible use from economic, agronomic and animal nutrition points of view require a re-evaluation of rye grain (hybrid rye) and rapeseed meal. For this purpose, compound feeds made from wheat or rye grain were combined with soybean or rapeseed meal and compared regarding several research questions.

The digestibility of phosphorus (P) with and without the supplementation of phytase in the aforementioned compound feeds was investigated, which is important on the one hand because of the finite nature of P and on the other hand because of the negative environmental impact of a surplus. The type of cereal grain had no influence on the P digestibility of the compound feeds; consequently, the high concentration of intrinsic phytase in rye compared with wheat had no influence on P digestibility. In the compound feeds with soybean or rapeseed meal, phytase supplementation produced the same P digestibility: 70.2% and 69.5%, respectively. Rye and rapeseed meal shifted nitrogen excretion from urine to faeces due to the higher fibre concentration compared with wheat and soybean meal, thus contributing to the reduction in ammonia release from manure. The metabolisable energy content in the compound feeds was ≥ 14.2 MJ/kg dry matter, which is suitable for growing pigs.

Rye grain and rapeseed meal are both characterised by a high fibre content. Rye has a relatively high content of soluble dietary fibre, to which positive nutritional and health-promoting characteristics are attributed. Because the soluble and insoluble dietary fibre fractions can vary greatly in their composition and thus in their effect, a practicable procedure should be established to analyse all the individual carbohydrate fractions of the dietary fibres by means of enzymatic photometric, enzymatic gravimetric and chemical gravimetric methods and applied in feed and faecal samples. Due to many interfering factors and complex matrices, the establishment of such a method was not possible. Hence, alternative approaches were considered, whereby in particular sum parameters that subdivide fibre into soluble and insoluble fractions are currently the best practical approach for a differentiated fibre analysis. In the field of dietary fibre analysis, differences in the implementation and description of methods can be observed, which severely limit the comparability, so the scientific community must establish clear rules and definitions in this respect.

The use of compound feeds containing rye grain and rapeseed meal is recommended for growing pigs based on the results obtained, using proper feed formulation and common feed additives such as phytase.

# KURZFASSUNG

## Analytische und ernährungs-physiologische Bewertung von Roggenkorn in Mischfuttermitteln für wachsende Schweine

Der Klimawandel und die zunehmende Sensibilisierung der Verbraucher für Umwelt- und Tierschutzfragen stellen die deutsche Tierernährung kontinuierlich vor die Herausforderung, sich anzupassen und verbesserte Lösungen zu finden, die Ressourcen schonend, bedarfsgerecht und im besten Sinne tiergerecht sind. Ein solcher Ansatz ist der Einsatz von regionalen Futtermitteln wie Roggen und Rapsextraktionsschrot in der Schweineernährung. Beide Futtermittel wurden in der Vergangenheit aufgrund geringer Erträge und einer hohen Anzahl an antinutritiven Stoffen im herkömmlichen Schweinefutter nur in Ausnahmefällen eingesetzt. Aufgrund der Weiterentwicklung von Sorten und neuerer wissenschaftlicher Erkenntnisse war eine Neubewertung von Roggenkorn (Hybridroggen) und Rapsextraktionsschrot in der Schweinefütterung notwendig, um den aus ökonomischen und ackerbaulichen Gründen lohnenden Einsatz aus Sicht der Tierernährung zu bewerten. In diesem Rahmen wurden Mischfutter bestehend aus Weizen- oder Roggenkorn mit Soja- oder Rapsextraktionsschrot kombiniert und hinsichtlich verschiedener Fragestellungen verglichen.

Untersucht wurde die Verdaulichkeit von Phosphor (P) mit und ohne Zusatz von Phytase in den oben genannten Mischfuttermitteln, was einerseits auf Grund der Endlichkeit und anderseits der negativen Umweltauswirkungen eines Überschusses von P relevant ist. Die Getreideart hatte keinen Einfluss auf die P-Verdaulichkeit der Mischfuttermittel, folglich hatte die hohe Konzentration an intrinsischer Phytase in Roggen im Vergleich zu Weizen keinen Einfluss auf die P-Verdaulichkeit. In den Mischfuttermitteln mit Soja- bzw. Rapsextraktionsschrot führt der Zusatz von Phytase zu einer Angleichung der P-Verdaulichkeit mit Werten von 70,2 % bzw. 69,5 %. Roggen und Rapsextraktionsschrot verlagerten die Stickstoffausscheidung aufgrund einer höheren Faserkonzentration im Vergleich zu Weizen und Sojasextraktionsschrot vom Harn in den Kot, was so zur Verringerung der Ammoniakfreisetzung aus der Gülle beiträgt. Der Gehalt an umsetzbarer Energie in den Mischfuttermitteln betrug ≥ 14,2 MJ/kg Trockenmasse, was für wachsende Schweine geeignet ist.

Roggenkorn und Rapsextraktionsschrot zeichnen sich beide durch einen besonders hohen Fasergehalt aus. Roggen zeigt einen relativen hohen Gehalt löslicher Faser, welcher insbesondere positive nutritive und gesundheitsfördernde Eigenschaften zugeschrieben werden. Da die löslichen und unlöslichen Faserfraktionen in ihrer Zusammensetzung und damit in ihrer Wirkung sehr unterschiedlich sein können, sollte eine praxistaugliche Analysemethode aller einzelnen Kohlenhydratfraktionen der Faser mittels enzymatisch-photometrischer, enzymatisch-gravimetrischer und chemisch-gravimetrischer Verfahren etabliert werden, um diese in Futter- und Chymusproben zu untersuchen. Aufgrund vieler Störfaktoren und einer komplexen Matrix war die Etablierung einer solchen Methode nicht möglich. Daher wurden alternative Ansätze in Betracht gezogen, wobei vor allem Summenparameter, die in lösliche und unlösliche Fasern unterteilen, derzeit die besten praktischen Ansätze für eine differenzierte Faseranalyse darstellen.

Im Bereich der Faseranalytik finden sich Unterschiede in der Durchführung und Beschreibung von Methoden, die eine Vergleichbarkeit dieser stark einschränken, so dass die wissenschaftliche Gemeinschaft hier klare gemeinsame Regeln und Definitionen aufstellen muss. Die Verwendung von Mischfuttermitteln, die Roggenkorn und Rapsextraktionsschrot enthalten, ist für wachsende Schweine aufgrund der erzielten Ergebnisse, bei entsprechender Futterformulierung und der Verwendung von Zusatzstoffen wie Phytase empfehlenswert.

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# ABBREVIATIONS

| | |
|---|---|
| AA | Amino acid |
| AD | Acid detergent |
| ADFI | Average daily feed intake |
| ADG | Average daily gain |
| ADF | Acid detergent fibre |
| ADFom | Acid detergent fibre expressed exclusive of residual ash |
| ADFomcp | Acid detergent fibre expressed exclusive of residual ash and crude protein |
| ADL | Acid detergent lignin |
| ADICP | Acid detergent insoluble crude protein |
| AID | Apparent ileal digestibility |
| aNDFom | Neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash |
| aNDFomcp | Neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash and crude protein |
| AOAC | Association of Official Agricultural Chemists |
| ATTD | Apparent total tract digestibility |
| AX | Arabinoxylan |
| A/X | Arabinose to xylose ratio |
| BCFA | Branched chain fatty acids |
| BLE | Federal Office for Agriculture and Food;<br>Bundesanstalt für Landwirtschaft und Ernährung |
| BMEL | Federal Ministry of Food and Agriculture;<br>Bundesministerium für Ernährung und Landwandwirtschaft |
| BR | Basal ration |
| BW | Body weight |
| CER | Cereal grain |
| CF | Crude fibre |
| CP | Crude protein |
| CPF | Compound feed |
| CSI | Cranial small intestine |
| CV | Coefficient of variation |
| d | Day |
| DF | Dietary fibre |
| DM | Dry matter |

| | |
|---|---|
| DMI | Dry matter intake |
| dP | Digestible phosphorus |
| DP | Degree of polymerisation |
| EC | European Comission |
| EE | Ether extract |
| EIR | Ethanol insoluble residue |
| EIRomcp | Ethanol insoluble residue expressed exclusive of residual ash and crude protein |
| e.g. | exempli gratia |
| FM | Fresh matter |
| EIR | Ethanol insoluble residue |
| EPL | Endogenous phosphorus loss |
| EU | European Union |
| etc. | et cetera |
| FOS | Fructooligosaccharides |
| FTU | Phytase activity unit |
| GfE | Gesellschaft für Ernährungsphysiologie<br>Society of Nutrition Physiology |
| GLC | Gas-liquid chromatography |
| GODPOD | Glucose oxidase/peroxidase |
| h | Hour |
| HPAEC-PAD | High-performance anion exchange chromatography with pulsed amperometric detection |
| HMWDF | High-molecular weight dietary fibre |
| HPLC | High-performance liquid chromatography |
| IDF | Insoluble dietary fibre |
| i.e. | id est |
| LMWDF | Low-molecular weight dietary fibre |
| ME | Metabolisable energy |
| $ME_m$ | Metabolisable energy for maintenance |
| mfN | Metabolic faecal N |
| min | Minute |
| N | Nitrogen |
| n.a. | Not analysed |
| NCP | Non cellulosic polysaccharides |
| n.d. | Not detectable |
| ND | Neutral detergent |

| | |
|---|---|
| NDF | Neutral detergent fibre |
| NDICP | Neutral detergent insoluble crude protein |
| NDO | Non-digestible oligosaccharides |
| NDSC | Neutral detergent soluble carbohydrates |
| NDSF | Neutral detergent soluble fibre |
| Non-GMO | Non- ‘Genetically modified organism’ |
| NSP | Non-starch polysaccharides |
| PAHBAH | p-hydroxybenzoic acid hydrazide |
| Phyt | Phytase supplementation |
| PM | Protein meal |
| R-TR | Rye containing test ration |
| RE | Relative error |
| Rd | Round |
| RSM | Rapeseed meal |
| SBM | Soybean meal |
| SCFA | Short chain fatty acids |
| SDF | Soluble dietary fibre |
| SDFP | Dietary fibre soluble in water but insoluble in 76% aqueous ethanol (precipitated) |
| SDFS | dietary fibre soluble in water and soluble in 76% aqueous ethanol |
| SEM | Standard error of the means |
| SID | Standardised ileal digestibility |
| STTD | Standardised total tract digestibility |
| TDF | Total dietary fibre |
| TR | Test ration |
| TTTD | True total tract digestibility |
| VDLUFA | Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten e. V;<br>Association of German Agricultural Analytic and Research Institutes |
| v/v % | Volume % |
| vs. | versus |
| W-TR | Wheat containing test ration |
| (+) | With phytase supplementation |
| (-) | Without phytase supplementation |

# CHAPTER 1

## General introduction

European Union (EU) consumers have become more conscious about animal production in terms of environmental impact, animal welfare and production methods; moreover, climate change is leading to more challenging conditions in feed production. Therefore, the focus in animal nutrition is always on feedstuffs that meet today's expectations in terms of environmental, animal and consumer protection. One such approach in pig nutrition is the focus on the regional feedstuffs such as rye grain and rapeseed meal (RSM).

Rye (*Secale cereal* L.) is a cereale traditionally grown in Northern and Eastern Europe on poor and sandy soils. On these soils, rye is more efficient in water and nutrient use and consequently in yield compared with other cereals due to its deeper and hairier root system (Dittmer, 1937; Kamphues et al., 2019). Since the 1960s, a large part of its acreage along with other traditional crops (barley and oats) has been lost to wheat (*Triticum aestivum*) because of the latter's breeding successes. The old rye varieties were no longer competitive, but this changed with the breeding of hybrid rye, which performs better on poor soils and is equal to wheat in most good soils. However, hybrid rye has not achieved a revival in its cultivated area (Kamphues et al., 2019; BLE, 2022). In addition, a problem that has been known for centuries is the higher susceptibility of rye to ergot disease, a fungal infection of the genus *Claviceps*, which severely

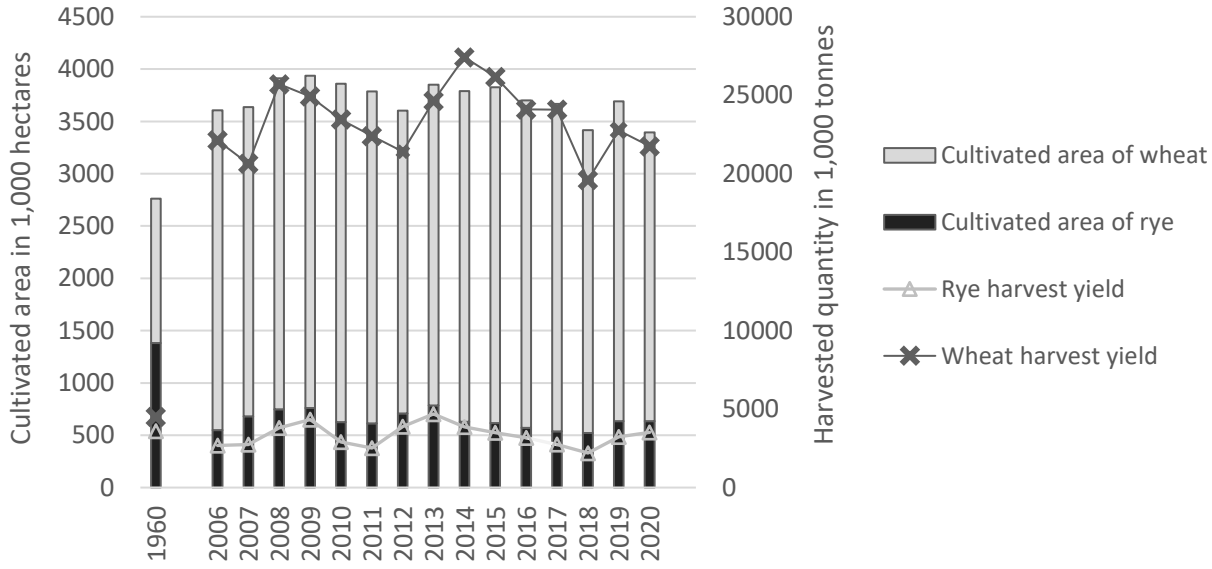


**Figure 1:** Cultivated area and harvest yield of wheat and rye in Germany until 2021 (BLE, 2022)

affects the health of pigs due to its toxic alkaloids (Miedaner and Geiger, 2015).

However, hybrid rye breeding has not only increased the yield of rye, but also improved pollen fertility by introducing effective restorer genes, so ergot infestation is reduced in so-called "Pollen-Plus" varieties (Miedaner and Geiger, 2015). The content of mycotoxins, which cause subclinical and sometimes chronic poisoning in pigs and lead to secondary diseases as well as reduced feed intake, is relatively low in rye and has always been below the maximum permissible levels of the European Commission in studies conducted over several years with various modern hybrid rye varieties (Kosicki et al., 2020).

Rye, compared with other small-grain cereals, has the best overwintering ability, and the highest drought, salt and aluminium stress tolerance (Geiger and Miedaner, 2009). These factors, together with good utilisation at low nutrient levels, make rye a promising cereal that can respond to the challenges of climate change (Kamphues et al., 2019).

In addition to the agronomic benefits of rye, it contains one of the highest dietary fibre contents among common cereal grains (Rodehutscord et al., 2016); this content has been demonstrated to improve intestinal health and well-being in pigs (Bindelle et al., 2008). Numerous health-promoting effects of rye are closely related to dietary fibre and its physicochemical properties, as well as to some bioactive compounds that have already been demonstrated in humans, some of which are also desirable in pigs (Figure 2) (Jonsson et al. 2018). Dietary fibre consists of different carbohydrate fractions, of which the most important in rye are fructan, mixed ß-glucan and arabinoxylan (Rodehutscord et al. 2016). The recognition of the positive aspects of dietary fibre has led to a renewed interest in this feedstuff. In the past, dietary fibre was considered to provide physical structure. In addition, dietary fibre was thought to reduce nutrient content and availability by decreasing the concentration of "valuable" nutrients (digestible carbohydrates [starches and sugars], proteins and fats) and not by providing measurable and performance-enhancing nutritional value to the pig. Dietary fibre is often divided into soluble and insoluble fractions because their effects differ. The soluble fibre fraction is also called rapidly fermentable fibre, which is available to the microbiota of the intestine as a substrate and thus converted into short-chain fatty acids. These serve as a source of energy for the host animal and have a beneficial influence on its intestinal tract and well-being (Jonsson et al., 2018). The main site of microbial activity in pigs is the large intestine, but a small amount of microorganisms may already be present in the caudal parts of the small intestine (Wenk, 2001). Insoluble fibre fractions shorten the faecal transit time and increase faecal bulk, which reduces the risk of constipation and colorectal cancer. Nevertheless, insoluble dietary fibre also decreases nutrient digestibility and is only partly fermentable (Wenk, 2001; Jonsson et al., 2018). Both soluble

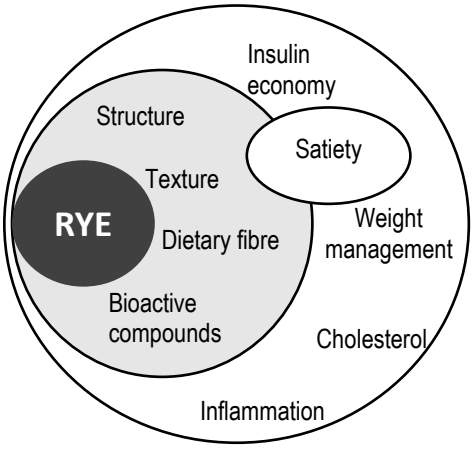


**Figure 2:** An overview of rye and its potential health effects. Adapted from Jonsson et al. (2018)

and insoluble dietary fibre represent a sum parameter, the amount and composition of which can differ considerably not only between feedstuffs, but also between genotypes and crop years (Hansen et al., 2003; Call et al., 2018). Therefore, the composition and thus the individual carbohydrate fractions and their health effects are of great interest and the focus of research.

Rapeseed (*Brassica napus*) has been cultivated as a fuel source for centuries, and its winter cultivar is intensively grown in Central and Eastern Europe (Miedaner, 2014). Since the 1970s, plant breeding has succeeded in developing so-called double-low-rapeseed varieties. These are characterised by less than 2% erucic acid and a low content of glucosinolates (< 30 µmol/g) and used to obtain edible oil for human nutrition. Rapeseed meal is a by-product of oil extraction and a valuable component in animal nutrition (Miedaner, 2014; Mejicanos et al., 2016). In recent years, the use of RSM has increased in Germany, mainly due to the increasing demand for non-genetically modified organism (Non-GMO) feed, especially in dairy farming. Indeed, by 2016/17 (Figure 3) more RSM was used in the German feed industry than soybean (*Glycine max*) (DVT, 2020). The increasing demand is only partly reflected in the German cultivation area and yields (Figure 4). In the 2010s, cultivation declined due to below-average crop yields, but an upward trend has been observed again in recent years (DVT, 2020). To meet the demand, rapeseed and RSM are imported from abroad, currently mainly from Canada, Australia and Eastern Europe (DVT, 2022). Globally, however, rapeseed ranks second in production with 45.0 million metric tonnes, well behind soybean meal (SBM) with 256.9 million metric tonnes (USDA, 2023). The use of SBM is controversial due to the long transport routes (import from outside the EU) but above all the difficult-to-control cultivation conditions (Steinfeld et al., 2006; Gerber et al., 2013). As a result, even in conventional pig farming, SBM, as a high-quality

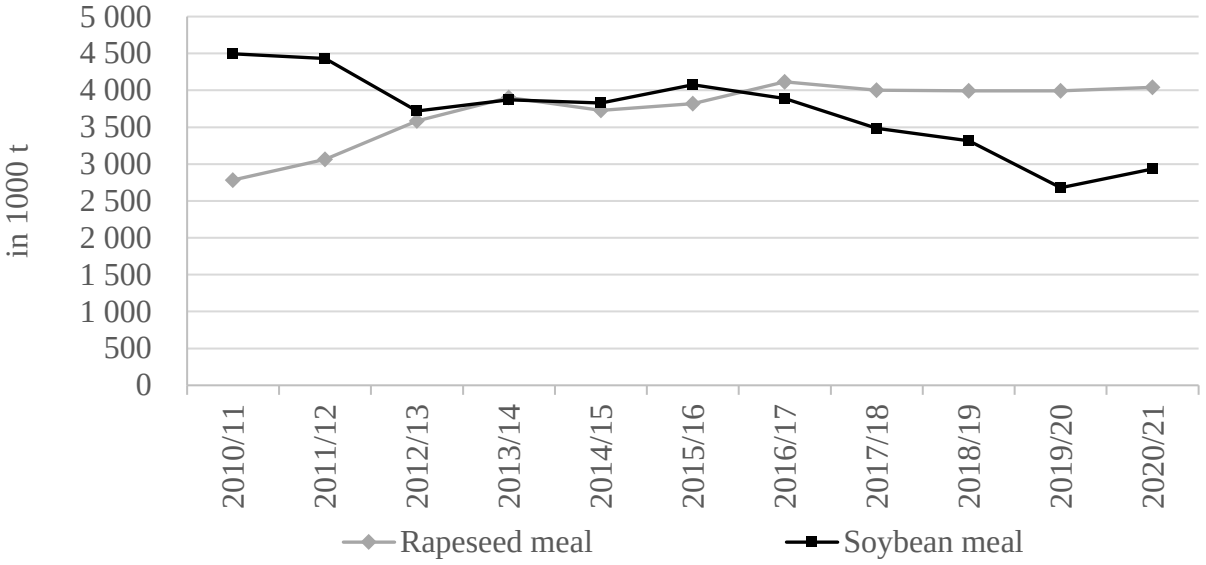


**Figure 3:** Consumption of rapeseed and soybean meal for feedstuff of all species in Germany (BLE, 2022)

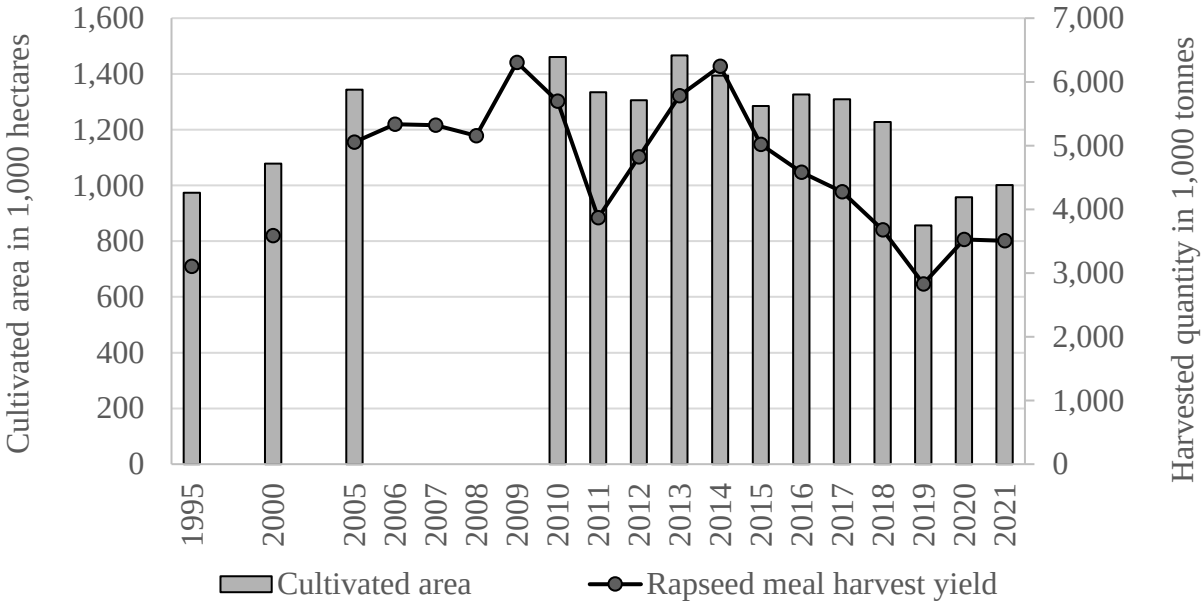


**Figure 4:** Cultivated area and harvest yield of rapeseed in Germany until 2021 (BLE, 2022)

protein source, is being replaced by alternatives such as RSM. The challenges of using RSM are antinutritive substances such as tannins, phytic-P and sinapine (Mejicanos et al., 2016). In addition, the high content of crude fibre and insoluble dietary fibre as well as the amino acid pattern are less suitable for pigs compared with RSM (Bach Knudsen, 1997; Mejicanos et al., 2016). Nevertheless, numerous studies have shown that RSM can be used to feed pigs without disadvantages on pig performance, as long as certain variables (the feeding rate, enzyme

addition, etc.) are considered during feed formulation (Sanjayan et al., 2014; Mejicanos et al., 2016; 2017; Landero et al., 2018). Regional nutrient surpluses in Germany in the form of nitrogen (N) and phosphorus (P) are a widespread environmental problem. On the one hand, finite and already scarce resources such as P are wasted, and on the other hand, nutrient inputs into water bodies and the atmosphere entail diverse negative consequences such as eutrophication and alteration of biodiversity (Suttle, 2010). To counteract these problems, the EU has developed various strategies (Biodiversity and Farm to Fork) and not least introduced the Nitrates Directive as a measure to reduce and limit nutrient losses and to protect soils and water quality (EC, 2021). Nevertheless, the European limits for N and P losses are exceeded by a factor of 3.3 and 2, respectively, which underlines that improvement is still needed (EEA and FOEN, 2020). As nutrition is a key factor in reducing the negative environmental impact of pig production Aarnink and Verstegen (2007), knowledge about the nutrient requirements of animals as well as the nutrient availability of feedstuffs is essential to ensure sustainable resource use not only monetarily, but also to minimise N and P excretion via manure.

Cereal grains contain a lot of P and oilseeds, and even more by-products (DLG, 2014). In addition, some of the P in plants may be bound to phytate, making it inaccessible to the digestion of pigs, so less P is retained and correspondingly more is excreted. RSM in particular has a higher phytate-P content and absolute P content than SBM, which, enhanced by the less favourable ratio of digestible to total P, ultimately increases P excretion (Suttle, 2010; DLG, 2014). Phytate-P can be cleaved by phytase, which can be added as an exogenous supplement (fungal and/or microbial origin) or is already present in the plants (Suttle, 2010; Rodehutscord et al., 2016). Even though wheat and rye have similar P contents, they differ considerably in their intrinsic phytase activity (Rodehutscord et al., 2016). Whether the intrinsic phytase activities of cereals can improve the P digestibility of other ingredients such as RSM and SBM remains unclear. However, a suitable ration formulation may help to ensure that RSM can be used in rations with the same effectiveness as SBM and to support regional production of feedstuffs towards a more independent and sustainable agriculture, thereby improving consumer protection and acceptance.

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# CHAPTER 2

## Scope of the thesis

The research presented in this thesis was part of a collaborative research project on the utilisation of rye grain and rapeseed meal in pig feeding (6-R Project: "Regional renaissance of rye and rapeseed aiming at reducing problems in crop and animal production by re-evaluation of rye constituents and their use for sustainable production in terms of environment, animal welfare and consumer protection") that was funded by the Federal Ministry of Food and Agriculture (BMEL). The reasons for the re-evaluation, in addition to the lack of studies on the combination of rye and rapeseed meal to feed pigs, have already been outlined in the general introduction (Chapter 1) for the individual components. Chapter 3 is a manuscript that has been submitted to a scientific journal, but it has not yet been published. The second manuscript (Chapter 4) is published exclusively as part of this thesis.

The first manuscript (Chapter 3) studies the combination of rye and rapeseed meal in pig regarding phosphorus digestibility and metabolisable energy. The hypothesis was that the combination of hybrid rye and rapeseed meal is a suitable alternative to wheat and soybean meal regarding metabolisable energy concentrations and may show even better phosphorus digestibility without supplementation of additional phytase due to high intrinsic phytase activity in rye. Thus, the aim of this study was to compare the effects of wheat versus hybrid rye combined with either soybean meal or rapeseed meal and either supplemented or not supplemented with phytase on phosphorus digestibility and metabolisable energy. In addition, total nitrogen excretion was measured and the nitrogen balance was estimated to obtain an overview and to assess possible environmental impacts.

The second manuscript (Chapter 4) deals with the determination of the carbohydrate fractions in feed and digesta samples and its analytical challenges. The aim of this work was to develop a method suitable for routine laboratories that represents the individual carbohydrate fractions of dietary fibre and can thus be used to improve nutrient supply recommendations. For this purpose, enzymatic-photometric, enzymatic-gravimetric and chemical-gravimetric methods were applied to analyse feed and digesta samples from the 6-R project in order to localise the disappearance of the specific dietary fibre components from the digestive tract of pigs and to discuss the potential health effects. The hypothesis was that the combination of hybrid rye and rapeseed meal provides a balanced dietary fibre pattern that delivers the benefits of soluble and insoluble fibre on digestion and animal health.

In the general discussion (Chapter 5), common scientific methods for determining the digestibility of phosphorus are considered in greater detail to provide clarity and to increase comparability. In addition, other important influencing factors for a holistic evaluation of dietary fibre in pigs are presented. The approach taken in this work and its implications are discussed and suggestions are made for possible approaches in the future. For the re-evaluation of rye and rapeseed meal, their feeding value is compared with other common feedstuffs in pig feeding. Because this is far beyond the research results obtained from this thesis, an assessment is made primarily on the basis of the data obtained in the 6-R Project as well as some similar studies. Finally, the presented results and discussion points are concluded in an overall context with the combined use of rye and rapeseed meal in pig feed.

# CHAPTER 3

## Differences in phosphorus digestibility and metabolizable energy concentrations of rye- or wheat-based compound feeds in pigs

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## 1. Abstract

In this study, two trials were conducted to determine phosphorus (P) digestibility and metabolizable energy (ME) concentrations of compound feeds. The feeds were formulated with either wheat or hybrid rye supplemented with soybean meal (SBM) or rapeseed meal (RSM). The compound feeds were fed with (+) (trial 1) or without (−) (trial 2) phytase supplementation to estimate the effect of intrinsic phytase activity in wheat and rye. In addition, nitrogen (N) balance of the test rations was evaluated. The P content in each test ration, consisting of a basal ration (deficient in P) and a compound feed, was adjusted to keep digestible P below 2.0 g/kg dry matter. All compound feeds were tested in a duplicate 3 × 3 Latin Square design. Pigs were kept in metabolism crates for a 7-day adaptation period and a 5-day collection period during which faeces and urine were quantitatively collected. Phytase supplementation ($P < 0.05$) and the source of protein supplementation ($P < 0.05$) exerted an influence on P digestibility. Phytase supplementation levelled P digestibility, resulting in values of 70.2% and 69.5% for SBM-compound feed and RSM-compound feed, respectively. The type of cereal grain had no effect on P digestibility of compound feeds, indicating that intrinsic phytase did not show differential efficacy. The ME concentration of all compound feeds was high (≥ 14.2 MJ/kg dry matter) and appropriate for growing pigs. Phytase supplementation had no effect on ME concentration of compound feeds. Rye and RSM, containing higher fibre concentration than wheat and SBM, shifted N excretion from urine to faeces, which may help to reduce ammonia release from slurry.

## 2. Introduction

Rye and rapeseed meal (RSM) have emerged in recent years as attractive regional alternatives to conventional wheat and soybean meal (SBM)-based rations for pigs. Hybrid rye, known for its adaptability to challenging climatic conditions, has demonstrated comparable yields to wheat (Geiger and Miedaner, 2009). Ergot contamination in hybrid rye has been effectively reduced to the levels found in other cereal grain types using molecular breeding techniques (Miedaner and Geiger, 2015). Rye is rich in dietary fibre, offering potential health benefits through components such as arabinoxylans, fructans or β-glucans, as well as bioactive components (alkylresorcinols, lignans, etc.) found in close proximity to or bound to fibre (Jonsson et al., 2018). Since excessive excretion of nitrogen (N) and phosphorus (P) in faeces and urine is a pollutant to the environment, and mineral P is a non-renewable resource, both N and P must be used efficiently and sustainably in animal nutrition. Factors affecting P digestibility, including total P concentration, phytate-P, and phytase activity exhibit considerable variation between and within cereal grain types (Schemmer et al., 2020) and oilseeds, with particularly elevated P concentrations found in co-products of oilseed processing. Despite this, there has been limited efforts to study P digestibility and assess metabolizable energy (ME) values of rye and RSM-based pig rations. Therefore, it appears reasonable to evaluate P digestibility, ME values and N balance of rye, especially hybrid rye, and RSM – considered regional feedstuffs in Central Europe – to comprehensively evaluate their environmental impact and production methods. The aim of this experiment was to compare the effects of compound feeds with wheat (W) or hybrid rye (R) combined with either SBM or RSM, and further supplemented with phytase (+) or non-supplemented (−), on P digestibility and ME concentrations. The hypothesis posited that hybrid rye and RSM could serve as viable alternatives to wheat and SBM, exhibiting comparable energy values. Furthermore, due to high endogenous phytase content in rye, it was anticipated that P digestibility might be even higher without phytase supplementation.

## 3. Material and methods

### 3.1. Rations

The compound feeds used in this study consisted of W, R, SBM and RSM. Each feed formulation comprised 70% cereal grain (CER) and 30% protein meal (PM), with (+) or without (−) phytase supplementation. These compound feeds were mixed proportionally with basal ration (BR) to obtain the test rations (TR), which were eventually fed to the animals. Throughout the formulation process, samples of the compound feed ingredients were

systematically collected, both before and during the creation of the BRs and TRs. Before mixing the TRs, W and R were ground in a hammer mill using a 3.0 mm screen, and SBM and RSM were used as supplied. To determine P digestibility in compound feeds, it is crucial to maintain a suboptimal P supply in the fed rations, thereby minimising the regulatory excretion of P via faeces. Consequently, a BR was formulated (Table 1) low in P and supplemented with all other minerals and vitamins meeting the requirements. The concentration of digestible P (dP) in the TRs was adjusted to a maximum of 2.0 g/ kg DM, following the recommendations of the Committee for Requirement Standards of the Society of Nutrition Physiology in Germany (GfE, 1994). This adjustment was based on the declared P content of the BR and the analysed P content and digestibility of ingredients, as outlined in DLG (2014).

The TRs were formulated by blending each compound feed into the BR at rates ranging from 390 g/kg to 600 g/kg DM. The BR, supplied in two parts by AGRAVIS Raiffeisen AG (Münster, Germany) as premix and other ingredients already mixed as a meal, underwent a final blending process at our institute. All rations (BR, TR) were prepared in one batch for each trial and stored in dry barrels at barn temperature until fed. For the first trial, a commercial phytase (6-Phytase (EC 3.1.3.26); Ronozyme HiPhos, 37500 FTU/kg; DSM, Heerlen, Netherlands) was provided in the premix on limestone as a carrier. In the second trial, an equivalent amount of limestone without phytase was added to the premix. Each BR and TR was offered as a meal to avoid heat effects of pelletisation-induced heat on endogenous phytase activity.

### 3.2. Animals and experimental procedure

The experiments conducted in this study received approval from the State Office for Nature, Environment and Consumer Protection (LANUV), North Rhine-Westphalia, Recklinghausen, Germany, under the file No. 81–02.04.2020.A055. The experiment was split in two trials with phytase supplementation (+) and the other without supplementation (−) due to the limited availability of metabolism crates. A total of 24 healthy male castrated crossbred pigs (German Landrace × Piétrain) were purchased from Campus Frankenforst, University of Bonn (Königswinter, Germany), with 12 pigs designated for each trial. The pigs in trial 1 had an initial mean (± standard deviation) body weight (BW) of 28.2 kg (± 6.0 kg) and age of 63 days (± 2 day), and 34.2 kg (± 5.8 kg) and 72 days (± 2 day) in trial 2. The health status of each pig was assessed daily.

For each trial, a new batch of the BR was mixed. Groups of six pigs were allotted to duplicate 3 × 3 Latin Squares, and three different rations were tested within each Latin Square. To ensure complete sets of Latin Squares, a BR, either (+) or (−), was assigned to each square. This design

aimed to minimise the effects of age or BW of the pigs within each square. Each period consisted of a 7-day adaptation period and a 5-day collection period in metabolism crates. During the adaptation period, the pigs were housed pairwise in an indoor pen of 1.1 m × 1.7 m on sawdust bedding. Individual feeding was provided. Following this period, the pigs were transferred to metabolism crates (height = 55 cm; length = 95 cm; width = 52 cm) equipped with slatted floors, stainless steel troughs and separate collection trays for faeces and urine. Crates were oriented to allow visual contact between pigs. Room temperature was maintained at 22 ± 2 °C and a 10-h lighting programme was utilised. Throughout the whole experiment, the pigs were fed twice daily at 07:30 and 15:30. Meals were mixed with water immediately before feeding. Feed refusals were completely collected, weighed and dried to allow accurate determination of dry matter (DM) intake. After feeding, the pigs had free access to drinking water for at least 30 min. The rations were allocated based on the BW measurements of the pigs, taken at both the initiation and conclusion of each collection period. The feeding amounts corresponded to 2.0 to 2.5 times the maintenance requirement for ME (GfE, 2008). Feed samples for DM determination and calculation of DM intake were taken during preparation of meals, which were weighed at the beginning of each period and stored in polyethylene bags until feeding. Throughout a given period, the daily feed quantity offered in two meals was adjusted to the BW during the previous adaptation period, maintaining a constant during the subsequent collection period. Urine and faeces were systematically collected in a quantitative manner. Urine was collected in a refrigerated plastic container containing 10% (v/v) sulphuric acid to ensure acidification to a pH ≤ 3.0. Each morning after feeding, urine was weighed and subsamples collected. The plastic containers were subsequently emptied and prepared for the next collection cycle. Faeces were collected twice daily following feeding. Faeces and urine were promptly frozen at −18 °C for the 5-day collection period as a pooled sample and stored until analyses.

### 3.3. Chemical analyses

All feedstuffs, including ingredients for both the BR and TR, underwent grinding with a centrifugal mill (Type Z100, Retsch GmbH, Haan, Germany) utilising a 1 mm mesh screen for subsequent analyses. After thawing, urine and faeces were homogenised. Faecal samples were lyophilised (P18K-E-6, Piatkowski Forschungsgeräte, München, Germany) and ground following the same procedure as described for feedstuffs. All chemical analyses were conducted in duplicate according to the standards of VDLUFA (2012). The following parameters were determined in feedstuffs: DM (3.1), ash (8.1), crude protein (CP, N · 6.25; 4.1.1), ether extract

**Table 1:** Ingredients [g/kg] and chemical composition of the basal ration [g/kg DM]

| | | Ingredients | |
|---|---|---|---|
| Wheat starch, pregelatinised | | 624 | |
| Beet pulp, dried | | 144 | |
| Potato protein | | 82 | |
| Blood plasma (poultry) | | 63 | |
| Cellulose | | 21 | |
| Soybean oil | | 16 | |
| Vitamin and mineral premix[1] | | 50 | |
| Analysed chemical composition | | Phytase[2] | |
| | | + | - |
| Dry matter | [g/kg] | 914 | 928 |
| Ash | | 67.6 | 78.1 |
| Crude protein | | 191 | 199 |
| Ether extract | | 34.0 | 36.5 |
| Crude fibre | | 42.2 | 34.4 |
| aNDFom | | 219 | 190 |
| ADFom | | 54.4 | 47.0 |
| ADL | | 6.30 | 12,0 |
| Starch[3] | | 533 | 528 |
| Sugar | | 55.4 | 45.3 |
| Calcium | | 10.0 | 12.7 |
| Phosphorus | | 1.35 | 1.50 |
| Digestible phosphorus[4] | | 0.76 | 0.69 |
| Phytate-P | | 0.48 | 0.48 |
| Phytase activity | [U/kg DM] | 3673 | 381 |
| Gross energy | [MJ/kg DM] | 17.6 | 17.7 |
| Metabolisable energy[4] | [MJ/kg DM] | 15.0 | 15.2 |

+ = with phytase supplementation; - = without phytase supplementation; aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash; ADFom = acid detergent fibre expressed exclusive of residual ash; ADL = acid detergent lignin, P = phosphorus.
[1]Premix provided the following per kg diet : 3.8 g Lysin-HCl; 1.3 g Tryptophan; 3.8 g Na; 0.7 g Mg; 5.000 IU. Vitamin A; 500 I.U. Vitamin D; 28 mg Vitamin E; 4.3 mg Vitamin B1; 6.25 mg Vitamin B2; 25 mg Pantothenic acid 870 mg; Cholinchlorid; 38 mg Nicotinic acid; 7.5 mg Vitamin B6; 25 µg Vitamin B12; 2.5 mg Vitamin K; 0.03 mg Biotin; 127.2 mg Zn; 56.8 mg Mn; 183.9 mg Fe; 10.3 mg Cu; 0.38 mg I; 0.50 mg Se.

[2]Ronozyme HiPhos (37,500 FTU/g; 6-Phytase (EC 3.1.3.26) DSM, Heerlen, Netherlands);
[3]polarimetric measurement; [4]calculated following GfE.

after HCl digestion (EE; 5.1.1b), crude fibre (CF, 6.1), neutral detergent fibre treated with amylase and expressed exclusive of residual ash (aNDFom; 6.5.1), acid detergent fibre expressed exclusive of residual ash (ADFom; 6.5.2), acid detergent lignin (ADL; 6.5.3), minerals phosphorus (10.6) and calcium (Ca; 10.3), and reducing sugars (7.1.1). Ingredients were also analysed for ND insoluble CP (NDICP) and AD insoluble CP (ADICP), and TRs were analysed for NDICP, following the method described by Licitra et al. (1996). Starch (7.2.1) and phytase activity (27.1) were determined at LUFA Nord-West (Oldenburg, Germany) in samples that were refrigerated until shipment to preserve phytase activity. Phytate was analysed at the Institute of Animal Science, University of Hohenheim, Stuttgart, Germany, following Zeller et al. (2015) and using high-performance ion exchange chromatography (Dionex ICS-3000, using Dionex CarboPac® PA 200 column, Idstein, Germany). An adiabatic bomb calorimeter (C 200, Ika-Werke GmbH & Co. KG, Staufen, Germany) was used to analyse the heat of combustion of feedstuffs, faeces and urine (in triplicate after lyophilisation). Ash, N, P and Ca contents were analysed in thawed urine samples as described above. Additionally, urea and ammonia were analysed using an urea/ammonia assay (R-Biopharm AG, Arc. No. 10542946035; Darmstadt, Germany). DM, ash, N, P and Ca contents in fresh faeces samples were analysed as described above. Moreover, the previously specified methods were also applied to lyophilised samples: CF, aNDFom, ADFom, ADL, NDICP and ADICP.

### 3.4. Calculations and statistical analyses

P digestibility in the TRs was calculated according to GfE (1994) as follows:

$$digestibility\ of P_{TR} = \frac{P_{intake} - P_{output}}{P_{intake}}$$

with $P_{intake}$ represents total P intake (g) and $P_{output}$ total faecal P output (g) during the 5-d collection period. P digestibility of the compound feed was determined by difference GfE (1994):

$$digestibility\ of P_{compound\ feed} =$$

$$\frac{digestibility\ of\ P_{TR} - [digestibility\ of\ P_{BR} * (1 - a)]}{a}$$

with $a = \frac{analysed\ P\ content_{compound\ feed}\left(\frac{g}{kg}DM\right) * inclusion\ level\ of\ compound feed\ in\ TR}{analysed\ P\ content_{\ TR}\ (g/kg\ DM)}$

The ME of the corresponding compound feed was calculated by proportionally subtracting the ME of the corresponding BR from the ME value of the TR. Following Mason and Frederiksen

(1979), NDIN (= NDICP/6.25) in faeces was considered as indigestible dietary N and subtracted from total faecal N, leaving metabolic faecal N (mfN).

Data analysis was conducted using the MIXED procedure of SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA). The normal distribution of the results was checked using the Kolmogorov-Smirnov and Shapiro-Wilk tests. If necessary, outliers were identified using a boxplot and eliminated before statistical analysis to ensure normal distribution. In this model, the treatment was divided into its factors, with CER (n = 2), phytase supplementation (n = 2), PM (n = 2) and period (n = 3) included as fixed effects and analysed separately. The animal was considered as a random effect. The level of significance was set at $P < 0.05$. The results of the treatments are presented as least squares means.

## 4. Results

### 4.1. Animals

All pigs were healthy throughout the experiment. However, some animals, particularly those fed the BR, refused up to 20% of their daily ration during a single collection period.

### 4.2. Chemical composition

The planned P and dP contents of the BRs were 0.6 g P/kg and 0.3 g dP/kg, respectively; however, the analysed P and dP concentrations were higher (Table 1). Consequently, the analysed P and dP contents in the TRs were also higher than calculated (Table 2). Both BRs were prepared using the same formulation except for the phytase supplementation, yet CF, aNDFom, and sugar contents, as well as phytase activity of the (+)BR were higher compared to the (−) BR. Differences between the CER and PM were due to their belonging to different species (Table 3); therefore, only the respective types were compared among themselves. The TRs were calculated for a concentration of 2.0 g dP/kg DM, leading to different inclusion levels of the compound feed in individual TRs. The CP was lower in the (+)TRs compared to the (−)TRs (Table 2). All (+)TRs or R-TRs had a phytase activity greater than 1000 FTU/kg DM, while the (−)W-TR remained below 500 FTU/kg DM (Table 2). The GE content in all TRs was similar.

### 4.3. Phosphorus and ME

Phosphorus digestibility values and ME concentrations of compound feeds are shown in Table 4. P digestibilities of the (+) compound feeds were 11.8 percentage units higher ($P$ <0.05) compared to the (−) compound feeds, and 3.0% higher ($P < 0.05$) in the compound feeds with

**Table 2:** Chemical composition of test rations in g/kg DM

| | | **Basal ration** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Cereal grain** | | | | | | | |
| | | Wheat | | | | Rye | | | |
| | | **Phytase** | | | | | | | |
| | | + | | - | | + | | - | |
| | | **Protein meal** | | | | | | | |
| | | SBM | RSM | SBM | RSM | SBM | RSM | SBM | RSM |
| Dry matter | [g/kg] | 892 | 898 | 904 | 911 | 903 | 906 | 915 | 915 |
| Organic matter | | 951 | 943 | 950 | 942 | 951 | 943 | 950 | 949 |
| Ash | | 48.8 | 57.3 | 49.5 | 57.6 | 48.6 | 56.6 | 50.1 | 51.0 |
| Crude protein | | 216 | 196 | 223 | 208 | 208 | 195 | 216 | 193 |
| Ether extract | | 29.4 | 32.4 | 30.3 | 32.9 | 27.2 | 30.1 | 28.0 | 30.1 |
| Crude fibre | | 38.7 | 46.6 | 38.4 | 52.2 | 33.4 | 44.5 | 30.9 | 67.0 |
| aNDFom | | 213 | 231 | 249 | 204 | 223 | 225 | 245 | 249 |
| ADFom | | 66.1 | 73.7 | 72.8 | 70.1 | 51.9 | 72.4 | 57.5 | 72.0 |
| ADL | | 15.1 | 19.0 | 11.0 | 23.0 | 23.6 | 22.6 | 11.0 | 27.0 |
| NDICP | | 76.9 | 63.4 | 74.9 | 70.0 | 80.0 | 81.8 | 68.2 | 64.5 |
| Starch[1] | | 511 | 510 | 518 | 512 | 502 | 493 | 498 | 495 |
| Sugar | | 56.8 | 56.9 | 50.4 | 51.7 | 67.3 | 65.5 | 63.6 | 62.8 |
| Calcium | | 5.90 | 7.73 | 4.84 | 7.11 | 5.16 | 7.11 | 4.86 | 5.49 |
| Phosphorus | | 2.83 | 3.23 | 3.07 | 3.63 | 2.88 | 3.34 | 3.09 | 4.07 |
| Digestible P[2] | | 2.05 | 2.26 | 1.85 | 1.92 | 1.93 | 2.26 | 1.75 | 2.23 |
| Phytate | | 5.68 | 5.94 | 6.20 | 6.80 | 5.54 | 5.81 | 5.74 | 7.66 |
| Phytate-P | | 1.60 | 1.67 | 1.75 | 1.91 | 1.56 | 1.64 | 1.62 | 2.16 |
| Phytate-P of P | [%] | 56 | 52 | 57 | 53 | 54 | 49 | 52 | 53 |
| Phytase activity | [U/kg DM] | 2169 | 1875 | 422 | 458 | 2664 | 2555 | 1466 | 1454 |
| Gross energy | [MJ/kg DM] | 18.1 | 17.9 | 18.1 | 18.0 | 18.0 | 17.9 | 18.0 | 18.0 |

aNDFom − neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash, ADFom − acid detergent fibre expressed exclusive of residual ash, ADL − acid detergent lignin, NDICP − neutral detergent insoluble crude protein, SBM − soybean meal, RSM − rapeseed meal; [1] polarimetric measurement; [2] calculated following GfE (1994)

**Table 3:** Chemical composition of raw components [g/kg DM]

| | | Wheat | Rye | SBM | RSM |
|---|---|---|---|---|---|
| Dry matter | [g/kg] | 877 | 907 | 892 | 889 |
| Ash | | 17.6 | 17.6 | 68.2 | 77.6 |
| Crude protein | | 119 | 94.0 | 512 | 413 |
| Ether extract | | 25.9 | 21.9 | 27.3 | 33.5 |
| Crude fibre | | 30.7 | 21.4 | 43.0 | 128 |
| aNDFom | | **129** | 130 | 212 | 340 |
| ADFom | | 46.0 | 51.0 | 179 | 224 |
| ADL | | 11.0 | 11.0 | 27.0 | 9.00 |
| NDICP | | 19.5 | 17.4 | 152 | 107 |
| ADICP | | 1.42 | 11.4 | 37.0 | 36.0 |
| Starch[1] | | 698 | 657 | 66.0 | 61.0 |
| Sugar | | 36.1 | 63.5 | 108 | 103 |
| Calcium | | 0.16 | 0.19 | 2.11 | 7.96 |
| Phosphorus | | 3.38 | 3.19 | 6.99 | 12.6 |
| Phytate | | 8.32 | 7.26 | 12.0 | 28.3 |
| Phytate-P | | 2.34 | 2.04 | 3.38 | 7.97 |
| Phytate-P of P | [%] | 69 | 64 | 48 | 63 |
| Phytase activity | [U/kg DM] | 505 | 3278 | n.a. | n.a. |
| Gross energy | [MJ/kg DM] | 18.0 | 17.6 | 19.3 | 19.5 |

aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash; ADFom = acid detergent fibre expressed exclusive of residual ash; ADL = acid detergent lignin; NDICP = neutral detergent insoluble crude protein; ADICP = acid detergent insoluble crude protein; P = phosphorus; SBM = soybean meal; RSM = rapeseed meal; n.a. = not analysed.

[1]polarimetric measurement.

**Table 4**: Phosphorus digestibility (%) and metabolizable energy (MJ/kg dry matter (DM) in compound feed presented as least squares means

| Item | Cereal grain | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wheat | | | | rye | | | | | P-value | | | |
| | Phytase | | | | | | | | | | | | |
| | supplemented | | unsupplemented | | supplemented | | unsupplemented | | | | | | |
| | Protein meal | | | | | | | | | | | | |
| | SBM | RSM | SBM | RSM | SBM | RSM | SBM | RSM | SEM | CER | Phyt | PM | Rd |
| Phosphorus digestibility | 73.4 | 71.1 | 62.6 | 55.0 | 67.0 | 67.9 | 58.8 | 55.9 | 2.13 | 0.09 | < 0.05 | < 0.05 | < 0.05 |
| Metabolizable energy | 15.6 | 14.8 | 16.0 | 14.9 | 15.2 | 14.5 | 15.2 | 14.2 | 0.19 | < 0.05 | 0.86 | < 0.05 | 0.10 |

SBM - soybean meal, RSM - rapeseed meal, SEM - standard error of the means, CER - cereal grain, Phyt - phytase supplementation, PM - protein meal, R - round; $P < 0.05$ indicates that data are significantly different

SBM compared to those with RSM. Phosphorus digestibility of the (−) compound feed containing SBM or RSM was 60.7% and 55.4%, respectively, whereas the (+) compound feed showed similar values of 70.2% and 69.5%, respectively. The ME concentration was higher ($P < 0.05$) in wheat-based compound feeds and those with SBM compared to rye-based and RSM-containing compound feeds, respectively. Phytase supplementation had no effect on ME concentration.

### 4.4. Nitrogen balance

There was no effect found of CER on N intake (Table 5). However, urinary N excretion was higher (1.96 g/day; $P < 0.05$) in pigs fed the W-TR compared to the R-TR. In contrast, faecal N excretion in the W-TR-fed pigs was lower (1.47 g/day; $P < 0.05$) compared to pigs fed the R-TR (Table 5); the same phenomenon was observed for mfN, which was lower (1.29 g/day; $P < 0.05$) in pigs fed W-TR (Table 6). An effect of PM on N intake was observed, resulting in higher (1.50 g/day; $P < 0.05$) intake recorded for pigs fed the SBM-TR compared to pigs fed the RSM-TR. This was reflected in higher (1.65 g/day; $P < 0.05$) urine N excretion of the SBM-TR fed animals compared to the RSM-TR group. Conversely, faecal N excretion was lower (1.16 g/day; $P < 0.05$) in pigs fed the SBM-TR than in pigs fed the RSM-TR, which was also reflected in lower mfN (0.89 g/day; $P < 0.05$) and NDIN (0.15 g/day; $P < 0.05$) excretion in pigs fed RSM-TR. Nitrogen balance was higher (1.26 g/day) in pigs fed SBM-TR compared to RSM-TR. An effect of phytase supplementation on N intake was detected, resulting in lower (4.22 g/day; $P < 0.05$) intake of pigs fed the (+)TR compared to pigs fed the (−)TR, as reflected in lower (5.00 g/day; $P < 0.05$) urinary N excretion of the (+)TR-fed animals. However, no effect of phytase supplementation was observed on total faecal N excretion or mfN excretion (Table 6), which, combined with lower N intake, resulted in an 8.7% higher ($P < 0.05$) N utilisation efficiency of pigs fed the (+)TR compared to the (−)TR.

**Table 5:** Nitrogen balance (g/day) and efficiency of N utilisation (%) of test rations presented as least squares means

| | **Basal ration** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Cereal grain** | | | | | | | | | | | | |
| | wheat | | | | rye | | | | | | | | |
| | **Phytase** | | | | | | | | | | | | |
| | supplemented | | unsupplemented | | supplemented | | unsupplemented | | | | | | |
| | **Protein meal** | | | | | | | | | P-value | | | |
| | SBM | RSM | SBM | RSM | SBM | RSM | SBM | RSM | SEM | CER | Phyt | PM | Rd |
| N intake | 35.8 | 34.8 | 40.7 | 38.8 | 35.1 | 35.8 | 41.4 | 37.6 | 0.95-1.04 | 0.94 | < 0.05 | < 0.05 | < 0.05 |
| Urinary N excretion | 12.8 | 11.7 | 18.3 | 17.3 | 11.4 | 10.4 | 17.1 | 13.5 | 0.875 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| Faecal N excretion | 3.97 | 4.85 | 3.75 | 5.15 | 4.98 | 5.88 | 5.63 | 7.08 | 0.335 | < 0.05 | 0.12 | < 0.05 | 0.49 |
| N balance | 19.2 | 18.3 | 19.4 | 16.3 | 18.8 | 19.5 | 18.7 | 16.9 | 1.02 | 0.84 | 0.20 | < 0.05 | < 0.05 |
| Efficiency of N utilisation | 53 | 52 | 47 | 42 | 54 | 55 | 45 | 45 | 2.2 | 0.63 | < 0.05 | 0.32 | 0.30 |

N ‑ nitrogen, CER ‑ cereal grain, Phyt ‑ phytase supplementation, PM ‑ protein meal, SBM ‑ soybean meal, RSM ‑ rapeseed meal, SEM ‑ standard error of the means, R ‑ round; SEM is stated as a range due to different n for test rations (n = 6), when a correction for outliers was made if the whole data set was not normally distributed; P < 0.05 indicates that data are significantly different

**Table 6:** NDIN and ADIN intake and faecal excretion and metabolic faecal nitrogen [g/d] by the group fed the test ration presented as least squares means

| | **Basal ration** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Cereal grain** | | | | | | | | | | | | |
| | Wheat | | | | Rye | | | | | | | | |
| | **Phytase** | | | | | | | | | p-value | | | |
| | Supplemented | | Unsupplemented | | Supplemented | | Unsupplemented | | | | | | |
| | **Protein meal** | | | | | | | | | | | | |
| | SBM | RSM | SBM | RSM | SBM | RSM | SBM | RSM | SEM | CER | Phyt | PM | R |
| Intake | | | | | | | | | | | | | |
| NDIN | 15.0 | 11.3 | 13.9 | 13.0 | 13.5 | 15.0 | 13.1 | 12.5 | 0.125 | 0.52 | 0.10 | <0.05 | <0.05 |
| ADIN | 0.502 | 1.26 | 0.400 | 1.17 | 0.541 | 1.36 | 0.800 | 1.23 | 0.025 | <0.05 | 0.51 | <0.05 | <0.05 |
| Faeces | | | | | | | | | | | | | |
| NDIN | 0.393 | 0.517 | 0.351 | 0.620 | 0.492 | 0.642 | 0.457 | 0.503 | 0.0397 | 0.12 | 0.40 | <0.05 | 0.52 |
| ADIN | 0.558 | 0.512 | 0.525 | 0.682 | 0.480 | 0.575 | 0.542 | 0.704 | 0.0424 | 0.85 | <0.05 | <0.05 | <0.05 |
| mfN | 3.58 | 4.33 | 3.40 | 4.53 | 4.49 | 5.24 | 5.18 | 6.10 | 0.306-0.319 | <0.05 | 0.19 | <0.05 | <0.05 |

NDIN=neutral detergent insoluble nitrogen; ADIN=acid detergent insoluble nitrogen; mfN= metabolic faecal nitrogen; CER=cereal grain; Phyt=phytase supplementation; PM=protein meal; SBM=soybean meal; RSM=rapeseed meal; SEM=standard error of the mean; R=round.

SEM is given as a range due to different n for test rations (n=6) when correction was made for outliers if the entire data set was not normally distributed. P<0.05 indicates that data are significantly different.

## 5. Discussion

### 5.1. Animals and experimental procedure

Deviating from the recommendations of GfE (1994), limestone, as a phytase carrier, was mixed in both BRs, so that the only difference between the two BRs was phytase activity. The BR, as suggested by GfE (1994), was formulated to provide no more than 6 g Ca/kg DM and approx. 1 g P/kg DM. In the present experiment, the Ca:P ratio in the (+)BR was 7.4:1, and in the (−)BR, it was 8.5:1. Recommendations for P supply for growing pigs in Germany are based on dP, and the Ca:dP ratio in pig rations should be between 2:1 and 3:1 (DLG, 2010). In our study, the Ca:dP ratio of the TRs ranged from 2.5 to 3.7. The (−)W-RSM-TR and (+)R-RSM-TR rations had Ca:dP ratios of 3.7 and 3.1, respectively, which was slightly higher than the recommended level. An unbalanced ratio of Ca:dP may negatively affect P digestibility, as it may cause formation of mineral complexes with phytate (Dersjant-Li and Dusel, 2019). Klein et al. (2022) demonstrated that adding limestone corresponding to 8.5 g Ca/kg DM instead of 5.4 g Ca/kg DM (Ca:dP: 3:1 vs. 1.9:1) reduced phytate degradation in the hindgut, but without affecting P digestibility. As the actual Ca:dP ratio did not affect the efficiency of P utilisation, the addition of limestone evidently had no negative effect on P digestibility.

The dP content in three TRs was above the targeted 2 g/kg DM, with a maximum value of 2.26 g/kg DM. Since there was no regulatory excretion via urine (data not shown), these concentrations still ensured a suboptimal supply. Using a BR as the control group and calculating P digestibility of the compound feed, it was assumed that the results were corrected for endogenous losses. Thus, further corrections, as described by She et al. (2018), by estimating the standardised total tract digestibility (STTD) values, were not considered beneficial.

### 5.2. Chemical composition

#### 5.2.1. Ration

The differences in chemical composition between the two BRs could be due to the feed manufacturing processes, given that the BRs were produced in two different batches. The variation and composition between the two BRs were consistent with those of Schemmer et al. (2020), except for the Ca content, and consequently, ash, which were higher in the present experiment due to the inclusion of limestone.

### 5.2.2. Urine

The influence of P intake with drinking water on P digestibility values can be neglected because P concentration in drinking water was below 0.01 mg/l (Stadtwerke Bonn, personal communication, 2021). Daily urinary P excretion was low (<0.04 g/day; data not shown) and consistent with values recommended by GfE (1994) and Schemmer et al. (2020), indicating adequate dP concentrations in the diets, which allowed almost complete utilisation of absorbed P by the pigs and consequently did not affect the measured P digestibility values.

## 5.3. Phosphorus digestibility

Phytate-P must be enzymatically cleaved to be available to animals. While the activity of phytase plays a pivotal role in determining the extent of this effect, factors related to animals, diet and measurements may exert additional influences. The intrinsic phytase activity of CER varied significantly. Interestingly, no discernible effect of CER on P digestibility was observed. The differences in P digestibility could be attributed to the phytate-P content and its proportion in total P, especially in PM. This aligns with the findings of Rodehutscord et al. (1996), who tested P digestibility of wheat, SBM and their combination without phytase supplementation. The latter authors demonstrated the additivity of P digestibility of individual components and suggested no effect of internal wheat phytase on SBM P digestibility. Similar studies on other cereal grains also found no correlation between intrinsic phytase activity and P digestibility values (Hovenjürgen et al., 2003; Schemmer et al., 2020; Klein et al., 2021). Nevertheless, the findings of Archs Toledo et al. (2020) showed a positive effect of endogenous phytase of hybrid rye on P digestibility of maize grain-SBM rations. A possible explanation for this discrepancy could be attributed to the location of intrinsic phytase and phytate-P. Phytase tends to accumulate near its substrate until it is hydrolysed during germination. Notably, phytate is primarily stored in other tissues, such as the aleurone layer of wheat and rye, soybean cotyledon or maize germ (Madsen and Brinch-Pedersen, 2020). Klein et al. (2021) postulated that endogenous phytases might not be able to hydrolyse phytate from other sources in feed. However, grinding may increase the accessibility to softer parts such as the germ or endosperm. The observation by Archs Toledo et al. (2020) could potentially be linked to the accessibility of rye internal phytase to phytate-P in the germ of maize, compared to phytate-P in more resistant cotyledons of granulated SBM or RSM.

Studies have shown that the effect of microbial phytase supplementation increases almost linearly up to 1000 FTU/kg, reaching an asymptote at approx. 1800–2000 FTU/kg (Dersjant-Li and Dusel, 2019; Rosenfelder-Kuon et al., 2020). In our study, phytase activity in the (+)TR

was >1875 FTU/kg DM, implying the attainment of the maximum phytase effect. The difference in P digestibility of the (+)TR and (−)TR suggests that the endogenous phytase, compared to its commercial microbial counterpart, may not have been sufficiently resistant to pH or proteases active in the stomach or other factors affecting its activity (Dersjant-Li et al., 2015; Dersjant-Li and Dusel, 2019). For instance, dietary fibre components can exert a confining effect on phytate-P (Pettersson and Pontoppi, 2013) or lead to a higher viscosity of digesta, hindering phytases from reaching their substrate and impeding their efficiency.

The phosphorus digestibility of wheat and rye in the (−) compound feed was 59% and 57%, respectively, while in the (+) compound feed, it was 72% and 67%, respectively. These values were consistent with Düngelhoef et al. (1994), who tested wheat as a single component and found P digestibility of 62% (± 3%) in rations without phytase supplementation, and 74% (± 3%) in rations with phytase addition (750 FTU/kg). Schemmer et al. (2020) reported a mean P digestibility of 59% in wheat without phytase supplementation, but a significantly lower value for rye of only 45%. McGhee and Stein (2019) tested three different hybrid rye and wheat grains, both supplemented with phytase (1000 FTU/kg) and unsupplemented. They obtained STTD values of P for unsupplemented hybrid rye ranging from 49% to 56%, 37% for wheat, and 62–71% and 58% for supplemented grains, respectively. The values for rye were similar to the P digestibility of the R-compound feed analysed in this study. Generally, a substantial variability exists in phytase activity, phytate-P content and P digestibility between and within different cereal grains. This variability is partly attributed to disparities in the methods employed for digestibility determination and the genotype of cereal grains within a species (Schemmer et al., 2020). Rodehutscord et al. (1997) investigated P digestibility of SBM and RSM, finding 37% for SBM in the ration without phytase supplementation, and 76% in the phytase-supplemented (750 FTU/kg) ration; the corresponding values for RSM amounted to 24% and 73%, respectively. Consistent with these observations, our study also demonstrated that phytase supplementation exerted a stronger effect on RSM-compound feed, particularly at a higher phytate-P concentrations. Consequently, both supplemented compound feeds achieved a P digestibility of approximately 70%. Nevertheless, the digestibility of the (−) compound feed containing PM (61% in SBM, and 55% in RSM) was relatively high compared to most values reported in the literature (Rodehutscord et al., 1997; DLG, 2014; Mejicanos et al., 2016). This suggests that the inclusion of the grain ingredient enhanced the overall P digestibility of the compound feed. The overall outcomes of the experiment were consistent with the findings of She et al. (2017; 2018), who analysed STTD of P in SBM (57% and 66%, respectively) and

RSM (39% and 45%, respectively) without supplements. The latter study also investigated different levels of microbial phytase (500, 1000

and 1500 FTU) supplementation, resulting in even higher STTDs (SBM: 82%, 90% and 90%; RSM: 70%, 72% and 77%, respectively). Similar to CER, the variability in PM values could be due to different concentrations of phytate-P and their proportion relative to total P, enzyme-substrate relationships or the selected method of P digestibility determination. Nevertheless, PM in the (+) compound feed reached a P digestibility of 70%, a remarkably high value for a mixed dry ration.

### 5.4. Energy concentration

Differences in ME concentrations were influenced by individual chemical composition of CER or PM. Wheat contained more starch and CP compared to rye, leading to a higher ME concentration (0.6 MJ/kg DM). Likewise, SBM contained more CP and less fibre than RSM, contributing to a higher ME concentration (0.9 MJ/kg DM). The lowest ME concentration among the compound feeds tested was obtained for the (−)R-RSM compound feed (14.2 MJ/ kg DM), which was still a high value suitable for pig rations. McGhee and Stein (2020) observed no effect of hybrid rye rations on ME concentration, while Arredondo et al. (2019) found no effect of an increase in phytase activity from 0 to 2550 FTU/kg on ME of a ration based on maize grain and soybean meal. The results of both of these studies were consistent with the present findings. Wilke (2020) reported that feed intake and daily weight gain in weaned piglets were neither influenced by the substitution of wheat grain with rye grain nor by SBM and RSM, even when high proportions of rye grain (60%) and RSM (28%) were incorporated. Notably, the feed conversion ratio was predominantly influenced by the elevated proportion of rapeseed meal in the compound feed, a factor that can be further attributed to the lower ME content determined in this study.

### 5.5. Nitrogen balance

The TRs were not intended to be isonitrogenous or isoenergetic. The adjustment of the dP content to 2 g/kg DM in the TR, resulted in varying proportions of the BR to compound feed. Generally, across all TRs, higher N intakes in the SBM-TRs and (−)TRs correlated with elevated urinary N excretion.

Although pigs fed the R-TR or W-TR had equal N intake, urinary N excretion was 1.93 g N/day higher in the latter; the difference can be entirely attributed to 2.00 g/day urea-N and interpreted as excess excretion. Conversely, in pigs fed the W-TR, faecal excretion was 1.46 g N/day lower,

which was further reflected in a 1.29 g N/day lower mfN compared to the R-TR. The higher content of fermentable fibre (aNDFom) in the R-TR likely led to increased fermentation in the large intestine. Consequently, blood urea-N was transferred to the large intestine and utilised to support microbial growth (Bindelle et al., 2008). The shift of N excretion from urine to faeces, bound in microbial biomass, was reflected by higher mfN excretion of animals fed the R-TR.

The elevated CP concentration in SBM and the greater proportion of SBM than RSM in the TRs (on average 18% SBM vs. 14% RSM) resulted in a 1.50 g N/day higher N intake by pigs fed the SBM-TR compared to pigs fed the RSM-TR. Fibre, i.e., cell-wall material, can encapsulate nutrients, and thus hinder their digestion (Agyekum and Nyachoti, 2017). In addition, RSM contains polyphenols, such as tannins, which can bind protein, thereby potentially reducing protein digestibility in RSM compared to SBM (Choi et al., 2015). The higher N intake of the SBM-TR and its better availability due to the lower fibre concentration and different fibre composition compared to RSM was reflected, on the one hand, in a 1.65 g N/day higher urinary N excretion, and a 1.88 g N/day higher urea-N excretion in pigs fed the SBM-TR compared to the RSM-TR. On the other hand, the 1.16 g N/day lower faecal N excretion of pigs fed the SBM-TR compared to RSM-TR, and especially the 0.15 g N/day lower ($P < 0.05$) faecal NDIN, reflected improved digestibility due to the lower amount of undigested dietary N. The higher NDIN digestibility (calculated from Table 6) of the SBM-TR, in addition to the factors mentioned above, was also related to the higher phytate-P content in RSM than SBM, with phytate-bound protein being recovered in the NDIN fraction and unavailable to the animal. The lower mfN excretion in pigs fed the SBM-TR compared to the RSM-TR group as primarily associated with the total intake of fermentable fibre. All these factors resulted in lower nutrient utilisation of the RSM-TR in the small intestine, with more N and carbohydrates entering the large intestine, where they could serve as substrates for microbial digestion and fermentation.

The reduced intake of 4.22 g N/day in animals fed the (+)TRs compared to the (−)TRs was reflected in a 4.98 g N/day lower urinary N excretion, of which 4.12 g N/day was urea-N. However, no effect of phytase supplementation was observed on total faecal N excretion or on mfN, which, in addition to lower N intake and even lower urinary N excretion, resulted in a higher N utilisation of pigs fed the (+)TRs compared to the (−)TRs. Once again, the evident oversupply of nitrogen resulted in direct excretion in the urine and was not conducive to optimal N metabolism. In addition to other fibre-related factors (e.g., pH reduction in the large intestine), feeding fermentable fibre in combination with a reduction in N intake, especially

precaecally indigestible N fractions, can effectively reduce ammonia emissions in manure, as urinary urea N is more susceptible to rapid decomposition (Bindelle et al., 2008).

### 6. Conclusion

The combination of rye and rapeseed meal proved to be a valuable alternative with regard to P digestibility, metabolizable energy content and N excretion, to a wheat-soybean meal-based ration for growing pigs and can be recommended. Supplementation with phytase is essential from the perspective of good agricultural practice, demonstrating benefits both ecologically and economically.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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# CHAPTER 4

# Enzyme-based characterisation of carbohydrate fractions in rye grain diets and digesta of pigs

## 1. Introduction

Fibre is composed of different carbohydrate fractions and associated substances and known to have a positive effect on the digestive tract and health of pigs. However, the positive effects depend on the fibre composition, which varies widely among feedstuffs. Rye is a cereal that contains a high proportion of fermentable fibres, which are available as substrates for the microbiota of the large intestine, thus provoking favourable effects on health (Jonsson et al., 2018). Fibre analyses has evolved from a vague parameter, crude fibre (CF) (Henneberg and Stohmann, 1860; 1864), to a more detailed analysis or estimation of specific fibre components, such as fructan, pectins, arabinoxylan and many more. Currently, a well-established method is the non-starch polysaccharides (NSP) analysis as described by Bach Knudsen (1997) based on Englyst et al. (1982) and the Uppsala method (Theander and Åman, 1979). In this analytical method taken from the field of human nutrition, the fibre components are hydrolysed into their monomers before being analysed using cost-intensive gas chromatography. The content of the original fibre components is then estimated based on the monomer content. This approach requires a uniform or regular structure of all fibre components, which is not always given as these structures can be very heterogeneous (Navarro et al., 2019). Therefore, an alternative approach to measure the fibre components using specific enzymes in combination with generally approved methods was elaborated. Additionally ethanol-insoluble residues (EIR), a simple sum parameter described by Hall et al. (1999), was measured in the feed samples. The aim of this study was to implement a method suitable for routine laboratory use that can be used to improve nutrient supply recommendations.

## 2. Material and methods

### 2.1. Rations

Eight different compound feeds (Table 1) and the corresponding pooled caecal digesta samples of pigs from the experiment of Wilke (2020) were used as reference samples. In the first trial, wheat was gradually replaced by rye in pig feeds 1–4; the other ingredients were kept similar. The proportion of wheat and/or rye in each ration was 69%; soybean meal (SBM) was used as additional protein source and rolled barley was the physical “structural” component. In the

**Table 1:** Composition of the compound feeds in % (Wilke, 2020)

| Component | Pig feed | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | Wheat → Rye | | | | SBM → RSM | | | |
| Wheat | 69.0 | 46.0 | 23.0 | 0 | | | | |
| Rye | 0 | 23.0 | 46.0 | 69.0 | 60.0 | 60.0 | 60.0 | 60.0 |
| Soybean meal | 11.5 | 11.5 | 11.5 | 11.5 | 18.1 | 13.6 | 8.10 | 0 |
| Rapeseed meal | | | | | 0 | 6.70 | 16.1 | 28.4 |
| Barley | 10.0 | 10.0 | 10.0 | 10.0 | 15.1 | 13.6 | 10.0 | 6.50 |
| Lignocellulose | | | | | 2.00 | 1.50 | 1.00 | 0 |
| Potato starch | 5.10 | 4.95 | 4.90 | 4.90 | | | | |
| Calciumcarbonate | 1.00 | 1.00 | 0.95 | 0.90 | 0.80 | 0.75 | 0.75 | 0.70 |
| Monocalciumphosphate | 0.90 | 0.90 | 0.95 | 1.00 | 0.90 | 0.80 | 0.60 | 0.45 |
| Soybean oil | 0.50 | 0.50 | 0.50 | 0.50 | 0.65 | 0.70 | 1.00 | 1.50 |
| Sodium chloride | 0.35 | 0.40 | 0.40 | 0.40 | 0.45 | 0.45 | 0.45 | 0.45 |
| Feed additives | 1.65 | 1.75 | 1.80 | 1.80 | 2.00 | 2.00 | 2.00 | 2.00 |

SBM = soybean meal; RSM = rapeseed meal

second trial, based on the results of the first trial, a high rye content of 60% was established and, in addition, SBM was gradually replaced by rapeseed meal (RSM) in pig feeds 5–8. Barley and lignocellulose were also added to maintain equal nutritional value of these pig feeds regarding crude protein (CP), metabolisable energy (ME) and structure. All pig feeds were offered dry (no additional water), pelleted and for *ad libitum* consumption. Each trial was repeated twice, each time with 20 female or castrated male pigs with a mean initial body weight of 16.2 ± 3.5 kg over all trials. The animals of each trial were equally assigned among the four feeding groups according to weight, sex and maternal descent, resulting in five pigs per feeding group. The animals were singly housed during each trial. On days 29, 30 and 31 after the 4-week experimental period, animals were first anaesthetised using Ketamidor® (100 mg/ml; Fa. Richer Pharma; Wels, Austria) and Stresnil® (40 mg/ml, Fa. Lilly, Bad Homburg, Germany) and euthanised using T61® (Fa. Intervet, Unterschleißheim, Germany) after neuroleptanalgesia. Then the blood was drained from the heart. Digesta samples were collected from the subsequent section. The entire small intestine minus the last 5 m is referred to as the cranial small intestine (CSI). Additional information on the procedure is given in Wilke (2020).

## 2.2. Chemical analysis

The original feed samples and the frozen digesta were provided by the University of Veterinary Medicine Hannover (Germany). The following feed analyses were carried out according to VDLUFA methods: Weender analysis (dry matter [DM], ash, CP, ether extract [EE] and CF), polarimetric starch analysis and sugar according to Luff-Schoorl (Wilke, 2020). In the pig feeds 1, 4, 5 and 8, NSP, uronic acid and lignin according to Klason were analysed at the Aarhus University (Department of Animal and Veterinary Sciences, Tjele, Denmark) following Bach Knudsen (1997).

Unless otherwise stated, all subsequent treatments and analyses were conducted at the University of Bonn (Germany). The feed samples were ground with a centrifugal mill (Type Z100, Retsch GmbH, Haan, Germany) using a 1 mm mesh screen. Digesta samples were freeze-dried (P18K-E-6, Piatkowski Forschungsgeräte, München, Germany) and ground similarly to the feedstuffs. Caecal samples were pooled for every feeding group and CSI sample was investigated as individual samples per animal. All samples were tested in the milling stage just as described, unless explicitly stated otherwise and independently of instructions in the still following method specifications.

In feedstuffs, neutral detergent fibre (NDF) treated with amylase and expressed exclusive of residual ash (aNDFom; 6.5.1), acid detergent fibre (ADF) expressed exclusive of residual ash (ADFom; 6.5.2) and acid detergent lignin (ADL, using sulfuric acid; 6.5.3) were analysed according to VDLUFA (2012) methods. Moreover, neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were analysed following Licitra et al. (1996). Additionally, starch was analysed enzymatically following Brandt et al. (1987); this analysis required milling through a 0.5 mm mesh.

Total dietary fibre (TDF) was analysed using the Integrated Total Dietary Fibre Kit (K-INTDF 09/18, Megazyme, Wicklow, Ireland; AOAC method 2011.25). The assay protocol provided was followed, except that round filters (MN 640 w, Macherey-Nagel, Düren, Germany) were used instead of fritted crucibles. The sample was first treated with pancreatic α-amylase and amyloglucosidase for 16 h at physiological pH and 37°C. After stopping this reaction with heating and adjusting the pH to about 8.2, the sample was treated with protease so that finally soluble starches and proteins were removed. Insoluble dietary fibre (IDF) was determined gravimetrically as the dried residue after filtration of the sample solution. Dietary fibre soluble in water but insoluble in 76% aqueous ethanol (SDFP), dissolved in the filtrate of IDF, was precipitated with four volumes of 95% v/v ethanol (resulting in 76% v/v ethanol in final

solution) and was determined gravimetrically after filtration. The resulting filtrate containing dietary fibre soluble in water and soluble in 76% aqueous ethanol (SDFS) was discarded. The residues were corrected for protein and ash and the sum of both results was declared as high-molecular-weight dietary fibre (HMWDF) (Megazyme, 2018c).

The following three enzymatic analysis for the determination of specific carbohydrate fractions originate from food sector and were adapted to feed and digesta samples in order to remain within the specified analytical range.

Pectin was analysed using the D-Glucuronic Acid and D-Galacturonic Acid Kit (K-URONIC 12/19, Megazyme) with $NAD^+$ as an oxidant and spectrophotometric measurement of the reduced form as NADH at 340 nm using a microplate reader (BioTek Synergy HTX multi-mode reader, BioTek, Winooski, VT, USA) before and after addition of uronate dehydrogenase. The provided assay protocol was followed using the sample preparation for polysaccharides and fibrous plant materials (Instruction C for sample preparation). Moreover, the sample clarification step was integrated using Carrez clarification (Carrez I solution = $K_4[Fe(CN)_6]\cdot 3H_2O$; Carrez II solution = $ZnSO_4\cdot 7H_2O$), followed by increasing the pH to 11 and leaving the sample solution at room temperature for 15 min to solubilise lactones (Megazyme, 2016) and a subsequent neutralisation of the sample solution with NaOH straight before the actual analysis was carried out (Megazyme, 2019).

For ß-glucan analysis, the Mixed-Linked Beta-Glucan Kit (K-BGLU 08/18, Megazyme; AOAC Method 995.16) was used following the assay procedure "for oat and barley flour and fibre samples – streamlined methods" (Instruction A). This procedure is an application of the McCleary method (McCleary and Glennie-Holmes, 1985). The ß-glucan in the sample was dissolved in an ethanol-containing alkaline buffer solution and incubated successively with lichenase and ß-glucosidase. Then, the glucose oxidase/peroxidase (GODPOD) method was applied, which results in a pink colour depending on the amount of released D-glucose; it was measured photometrically at 510 nm (Ultrospec 2100 pro, Biochrom, Cambridge, UK) (Zhi et al., 2016; Cooper, 1973).

Fructan was analysed using the Fructan Assay Kit (K-FRUC 10/18, Megazyme; AOAC Method 999.03), following the extraction procedure for samples low in fructan. Fructan samples were dissolved in distilled water before successive treatment with amylase/sucrase solution and alkaline borohydride, which breaks down starch, reducing sugars and sucrose into their monomers and then into sugar alcohols that might otherwise interfere with the fructan analysis. Then, fructanase solution (an enzyme mix of endo-levanase, exo- and endo-inulinase) was

added to free D-glucose and D-fructose. Later, the working reagent p-hydroxybenzoic acid hydrazide (PAHBAH) was added, which produces a simple colour response to D-fructose and D-glucose. Finally, the absorbance at 410 nm was measured photometrically (Megazyme, 2018a).

EIR was determined following Hall et al. (1999) by direct incubation of the sample in 80% v/v ethanol for 4 hours with constant shaking at room temperature followed by filtration. The resulting residue was corrected for CP and ash (EIRomcp). Starch and NDF corrected for CP (aNDFomcp) were determined from separate sample weighings and subtracted from EIR to obtain soluble neutral detergent fibre (NDSF); this approach differs from the method described by Hall et al. (1999), where aNDFomcp and starch are analysed in the residue. In the digesta samples, analyses of the enzymatic methods for TDF, ß-glucan, pectin and fructan were carried out as detailed above, yet restricted to trial 1.

### 2.3. Calculations

Difference calculations were used to capture all fibre fractions individually. The indirectly recorded fractions and their calculation methods are listed in Table 2. The relative analytical tolerance was calculated as:

$$\text{Relative analytical tolerance (\%)} = \frac{|\text{measurement 1} - \text{measurement 2}|}{\frac{\text{measurement 1} + \text{measurement 2}}{2}} * 100.$$

**Table 2:** Origin and derivation of the specific fibre fractions

| Direct determination via analysis | | Indirect determination | |
|---|---|---|---|
| Fraction | Applied method | Fraction | Calculation |
| aNDFom | VDLUFA 6.5.1 | aNDFomcp | aNDFom – NDICP |
| ADFom[1] | VDLUFA 6.5.2 | ADFomcp | ADFom – ADICP |
| NDICP | Licitra et al. (1996) | | |
| ADICP[1] | Licitra et al. (1996) | | |
| | | Hemicellulose (insoluble pentosane) | aNDFom – ADFom |
| | | Cellulose[1] | ADFom – ADL |
| ADL | VDLUFA 6.5.3 | | |
| IDF | AOAC 2011.25 (McCleary) | | |
| SDFP | AOAC 2011.25 (McCleary) | | |
| | | "TDF" (HMWDF) | IDF + SDFP |
| | | NSP | TDF – Lignin |
| | | TDF | NSP + Lignin |
| Pectin | K-URONIC KIT | | |
| | | Insoluble pectin | IDF – ADL – hemicellulose |
| | | Soluble pectin | Pectin - insoluble pectin |
| ß-glucan | McCleary (1985) | | |
| | | Soluble pentosane (arabinoxylan) | SDFP – ß-glucan – soluble pectin |
| Fructan | AOAC 999.03 | | |
| EIR | Hall et al. (1999) | | |
| | | NDSF | EIRomcp – starch – aNDFomcp |

aNDFom = neutral detergent fibre treated with amylase and expressed exclusive of residual ash; ADFom = acid detergent fibre expressed exclusive of residual ash; NDICP = neutral detergent insoluble crude protein; ADICP = acid detergent insoluble crude protein; ADL= acid detergent lignin; IDF = insoluble dietary fibre; SDFP = precipitated soluble dietary fibre; EIR= ethanol-insoluble residue; EIRomcp = ethanol-insoluble residue expressed exclusive of crude protein and residual ash; aNDFomcp = neutral detergent fibre treated with amylase and expressed exclusive of residual ash and crude protein; ADFomcp = acid detergent fibre expressed exclusive of residual ash and crude protein; TDF = total dietary fibre; HMWDF = high-molecular-weight dietary fibre; NSP = non-starch polysaccharides; NDSF = neutral detergent soluble fibre; [1] = analyses were not carried out sequentially

## 3. Considerations regarding the analytical methods

### 3.1. General methodology

A considerable amount of dried sample material is required to be able to conduct all the necessary analyses. This is not a problem with feed, but it is a problem with digesta samples. For example, it may not always be possible to obtain a sufficient amount of sample from each intestinal section to carry out all analyses or, if necessary, replicates. Therefore, pooled caecal samples were prepared into a homogeneous and representative group for the respective analytical method before analysis. The digesta samples were also needed for other investigations within the scope of the joint project.

All samples were milled using a 1.0 mm sieve, as required for feedstuffs according to the VDLUFA (2012) instructions, even though the requirements for enzymatic and dietary fibre analyses are specified using a 0.5 mm sieve for milling. The reason for this was the small amount of digesta available and that further losses should be avoided by subdividing or further milling the sample. Moreover, it is known from laboratory practice that material that is too fine sometimes escapes filtration during analysis (Fahey et al., 2018). The pelleted pig feed and digesta samples were ground through a 1.0 mm sieve, which resulted in a very fine powder. There were no observable difference between the 0.5 and 1.0 mm sieve size, which was verified by further milling of single samples. McCleary et al. (2013) stated that, for the TDF analysis in particular, mills other than a rotor mill (centrifugal mill) equipped with a 0.5 mm screen may be used with sieve sizes from 0.5 to 0.7 mm, which also demonstrates that minor variations in particle size distribution are acceptable. The particle size can undoubtedly affect the result of analysis (Fahey et al., 2018). However, as long as the basic information about the particle size achieved is defined only by the sieve size and disregards the type of mill (rotor mill, hammer mill, etc.), the type of sample (meal, pelletised), the milling speed (e.g. rotation per minute) and other factors, unknown variations will occur. Therefore, it is assumed that the material used through a 1.0 mm sieve is adequate for the conducted analyses.

### 3.2. Enzymatic colourimetric analysis

#### 3.2.1. Methodological aspects

Enzymatic analyses have several advantages: they are specific, sensitive, fast and do not require extensive sample preparation (Matissek and Fischer, 2021a). In this project, enzymatic assays were used to determine specific compounds, and for this purpose, certain fractions had to be isolated. This is certainly a challenge due to the overlapping solubility properties of the fractions

and the resulting preparation effort (Van Soest, 1994). The optimal temperature and pH range of the enzymes are well declared and must be followed to obtain valid results, and these conditions may not be fully compatible with the requirements of the substrate to be analysed. Many established reference methods currently have very stable and well-developed enzyme preparations – for example, starch analysis. Nevertheless, enzymatic analyses determined photometrically have to be adapted to the sample matrices due to turbidity, colouring, intrinsic enzymes, et cetera, and are not universally applicable (Matissek and Fischer, 2021a). As enzymatic analyses are based on the sole effect of the specifically added enzyme, standardised procedures must and indeed do demand high and specific quality regarding purity and enzyme activity; otherwise, reproducible results could not be obtained. In addition to these individual enzymatic factors, one must always consider that every analysis has an analytical error, which is usually disproportionately large for small results.

The enzymatic assays of pectin, fructan and ß-glucan were repeated with increased initial sample weights or aliquots until the sample material corresponded to a concentration that was within the specified analytical range. The results obtained for feed and digesta samples are shown in Table 3. In the applied ß-glucan assay, despite an increased sample weight, there was no increase in gelatinisation and clumping, a finding in contrast with other published observations (McCleary and Codd, 1991). Thus, impairment of the effect of lichenase in the cleavage of ß-glucan leading to an underestimation of the ß-glucan content can be excluded (McCleary and Codd, 1991).

In all enzymatic assays, the enzymatic application step itself did not cause any problems. This was shown by the integration of a standard curve for D-glucuronic acid in the pectin assay with a coefficient of determination of at least 0.89, as well as the standard deviation of at most 0.01 for the fructose standard in the fructan assay and 0.03 for the glucose standard in the ß-glucan assay over the replicates performed.

Various duplicate determinations were carried out to assess measurement uncertainties. Duplicate determination from one sample solution was defined for the pectin and fructan assays (absorbance difference of 0.005–0.010). In the feed sample, this was achieved for pectin in most cases, but only rarely for fructan. However, these duplicate determinations, as is often the case in enzymatic analyses, deviate from “true duplicate determinations”, meaning two initial weights as usually applied in animal nutrition analyses. For verification, true duplicate determinations were investigated, which showed great variability (coefficient of variation [CV]

**Table 3:** Enzymatic colourimetric analysed pectin, ß-glucan and fructan content [g/kg DM] in the pig feeds and the corresponding digesta

| Sample origin | | Trial 1 | | | | Trial 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pig feed | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | | Wheat → Rye | | | | SBM → RSM | | | |
| Feed | Pectin | 2.9 | 2.3 | 2.6 | 1.9 | 3.2 | 4.0 | 5.9 | 6.5 |
| | ß-glucan | 10.6 | 10.9 | 14.5 | 17.1 | 17.2 | 15.5 | 13.3 | 13.6 |
| | Fructan | 2.06 | 2.75 | 2.93 | 3.39 | n.a. | n.a. | n.a. | n.a. |
| CSI[1] | Pectin | 4.0 (n = 1) | 3.7 (n = 1) | 8.9 (n = 1) | 4.4 (n = 1) | n.a. | n.a. | n.a. | n.a. |
| | ß-glucan | 13.6 (n = 1) | | 29.7 0.27 (n = 3) | 36.0 0.15 (n = 4) | n.a. | n.a. | n.a. | n.a. |
| | Fructan | 1.30 (n = 1) | 1.54 0.12 (n = 2) | 2.05 0.36 (n = 3) | 1.75 0.21 (n = 4) | n.a. | n.a. | n.a. | n.a. |
| Caecum | Pectin | 5.2 | 5.3 | 5.4 | 4.5 | n.a. | n.a. | n.a. | n.a. |
| | ß-glucan | 0.50 | 1.33 | 1.28 | 2.99 | n.a. | n.a. | n.a. | n.a. |
| | Fructan | n.d. | n.d. | n.d. | n.d. | n.a. | n.a. | n.a. | n.a. |

SBM = soybean meal; RSM = rapeseed meal; CSI = cranial small intestine; [1] CSI has been determined from individual samples; therefore, the coefficient of variation (CV) is given in cursive underneath and the underlying sample number is in parenthesis; n.d. = not detectable; n.a. = not analysed

in feed of 0.7–0.8 for pectin and 0.1 for fructan), but they were not integrated as a standard due to the low sample throughput and the general poor comparability.

The purification steps, such as removal of interfering substances, also entailed a considerable potential for error (CV = 0.06), as demonstrated in the fructan assay by duplicate determinations from aliquots of a sample and an extraction, which were subsequently treated individually. One explanation for these differences could be an error caused by improper selection during weighing, which occurs especially at a low initial weight (80–400 mg), as in the enzymatic assays. Another explanation could be that colouring and turbidity, although visually imperceptible at low concentrations, lead to deviations in the results.

In all feed samples, the sample solution became turbid, which may interfere with the photometric measurement. The feed sample in the pectin assay also showed strong colouring after sulphuric acid treatment. To prevent interfering substances, the sample solution for the fructan and ß-glucan assays was centrifuged. In the ß-glucan test, the use of different sample volumes in the final solution led to differences (CV = 0.09) in the results of the feed samples, which should have been corrected by the calculation. This indicates the presence of turbidity, even if is not visually perceptible at low concentrations. This problem did not occur in the fructan assay for feed samples as the CV of different volumes (CV = 0.04) was less than that of true duplicate determinations (CV = 0.03–0.07) from one sample obtained in the same analysis.

In the pectin assay, the sample solution was filtered and because there was a cloudy precipitate in the final assay solution, an additional Carrez clearing step was added before the enzymatic analytical step. Millipore filters (pore size 0.45 µm) were tested, but they did not improve the results and were discarded. Moreover, additional internal results from another project carried out later, dealing with the interference of pectin in photometric analysis, indicate that pectin may get caught in the Millipore filter and thus escape analysis. Despite adding clearing steps, there was a precipitate in the sample solution after it was left for a few hours at room temperature at neutral or alkaline pH; it was not possible to identify the cause.

The samples were also examined using an extraction method similar to Bach Knudsen (1997). Starch, water-soluble and 80% v/v ethanol-soluble carbohydrates were removed first; then, the dried sample residue was dissolved in concentrated sulphuric acid for 2 hours. However, the shorter concentrated sulfuric acid extraction and removal of digestible carbohydrates did not result in any obvious improvement, so this method was not pursued further.

As the values obtained from the pectin analysis were low, an additional step was carried out to convert the lactones back into uronic acids. Therefore, the pH was raised to 11 for at least 15 min, and then lowered again to a slightly alkaline pH between 7 and 8 (ideally 7.4) immediately before the addition of uronate dehydrogenase in order to meet the specifications (Megazyme, 2016). It was difficult to achieve the same pH changes even though the same volumes of sample solution and NaOH were transferred in each replicate, as the turnover point was very fast and seemed to vary. Nevertheless, the pH in the microtiter plate of the final solution including buffer was always within the acceptable range, which was confirmed with pH indicator paper. A benefit of this procedure could not be determined.

To assess the quality of the results, the assays offered various control options. The ß-glucan assay was developed for fibre products, especially oats and barley, and was therefore assumed to be suitable for this high-fibre piglet-rearing feed. To control the success of the ß-glucan assay, control meals are provided in the form of the barley and oat meal samples, for which a standard error of ± 3% was indicated. In the present analysis, deviations of 1.5%–47% from the indicated contents of the control meals were obtained. The values were in the desired range only once. The mean value was 18.5%, and even if the two values with the greatest deviation were removed, the mean value of the remaining deviation was 11%. Certainly, these errors can be caused by pipetting and handling, but because the experiments were always carried out by the same person with the same equipment and according to the same procedure, the difference emerging from analysis to analysis was still noteworthy and questionable. As another check of the quality of the ß-glucan assay, the absorbance of the blank solution containing the GODPOD reagent (p-hydroxybenzoic acid) is measured against distilled water, which is intended to be less than 0.05 absorbance units read at 510 nm. This reading was verified.

In the fructan assay, control flour was also provided in the form of inulin and levan. For the inulin content, some replicates showed a deviation of 6% from the target value, but in other replicates the difference was 60%. For levan, the differences were not as pronounced, indicating that especially fructan with a higher degree of polymerisation (DP) may not have been completely released during extraction. The pectin assay provides no additional controls other than free glucuronic acid, which was used as the standard and in the present assay to generate the standard curve.

The plausibility of the obtained results was checked by comparison with data from the literature for the main components of the respective feed rations. Rodehutscord et al. (2016) analysed the

pectin content according to Scott (1979), reporting a uronic acid content of 2.2–2.9 g/kg DM and a mean value of 2.6 g/kg DM for rye, and 2.5–3.4 g/kg DM and a mean value of 2.9 g/kg DM for wheat. At first glance, these values seem to agree with the results obtained for the uronic acid content feed in trial 1 (Table 3), which fluctuated between 1.9 and 2.9 g/kg DM and contained 69% cereal grain. However, based on other compounds in the pig feeds such as barley (3.5 g/kg DM; Rodehutscord et al. (2016), RSM and SBM (39 and 23 g/kg DM, respectively; (Bach Knudsen, 1997), the predicted uronic acid content would be 4–5 g/kg DM, or approximately twice the amount determined by the analysis. Moreover, within the project of Wilke (2020), but unpublished, the uronic acid content was 8 g/kg DM for pig feed 1 and 7 g/kg DM for pig feed 4, also determined according to Scott (1979). In trial 2, where SBM was exchanged stepwise by RSM, the uronic acid content increased from pig feed 5 to 8, from 3.2 to 6.5 g/kg DM. While this increasing trend is in line with expectations, even these results seem too low.

For ß-glucan, Rodehutscord et al. (2016) used the same method and found concentrations from 16.9 to 26.4 g/kg DM and a mean value of 20.1 g/kg DM for rye and from 4.6 to 7.8 g/kg DM and a mean value of 6.1 g/kg DM for wheat. Other published ß-glucan concentrations based on this method in rye and wheat grain are in general agreement with this range (Bach Knudsen, 1997; Hansen et al., 2003; McCleary, 2018). Our feed samples, which ranged from 10.6 to 17.1 g/kg DM, also match these values, as the diet contained 69% of these specific cereal grains and an additional 10% barley, which is known to have highest concentration of ß-glucan (mean value: 46.7 g/kg DM; (Rodehutscord et al., 2016) among the common cereal grains. The fructan content determined according to Call et al. (2018), hydrolysing fructan to its monomers and analysing those using a two-step procedure with high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), was 2.0–2.3 g/kg DM for wheat grain and 9.4–11 g/kg DM for rye grain (McGhee and Stein, 2018; 2020). Rodehutscord et al. (2016) reported even higher fructan contents with mean values of 9.8 g/kg DM for wheat grain and 29.1 g/kg DM for rye grain, analysed according to Bach Knudsen (1997). Especially considering the proportion of wheat or hybrid rye grains in the feed, the analysed fructan contents of trial 1 ranging from 2.06 to 3.39 g/kg DM do not match any of these results.

Although the enzymatic analyses were repeated several times, trustworthy results could rarely be obtained for feed samples. While the results of the ß-glucan analysis are promising, the deviating standard errors are problematic for reliable interpretations. Comparisons with other

methods, with the exception of ß-glucan, show differences that indicate that the extraction of the respective fractions was not completely accomplished.

All digesta samples showed strong colouration and turbidity, which led to more interferences in the assays. Thus, the digesta samples exceeded the permissible absorbance differences of a duplicate determination from one sample solution in the pectin and fructan assays even more than the feed samples. This phenomenon also occurred in the pectin assay by increasing the sample volume in the final solution by substituting with distilled water. There was visible turbidity, indicating that not all interfering factors were removed by clarification and filtration. An additional influence of interfering substances in the ß-glucan assay may be reflected in the CV of 0.11 for a caecal digesta sample, which was higher than for the feed samples, as different sample volumes in the final solution were used.

Despite the general statement made at the beginning regarding matching the analytical range, this was not possible for the caecal sample submitted to fructan analysis. Despite significantly increasing the amount weighed in and the sample aliquot used in the final solution, the analytical range was not reached. Because the sample was not fundamentally different (colouration, turbidity and other interfering substances) from the CSI sample in which the analytical range was reached, it was assumed that no fructan is present in the caecal sample. CSI samples were analysed based on individual animals, in contrast to the pooled caecal sample. As there was usually not enough sample material available, there were different numbers of individual samples used to calculate each mean or CV (Table 3). The CV varies from 0.12 to 0.36 for the fructan assay and from 0.15 to 0.27 for the ß-glucan assay. It is not possible to judge whether these CV values are related primarily to the interferences or to the individual differences of the samples.

These assays are not suitable for analysis of the digesta at this stage. The overall performance was already suspect for feed samples, and there are uncertainties regarding the digesta material due to an even greater variability in the results and the lack of comparisons. As the chosen approach would require a computational combination of methods, the results are not used any further for feed and digesta samples in order avoid carrying the analytical errors further. Consequently, the data obtained are not representative. They must not and will not be interpreted any further with respect to the research question on the disappearance of carbohydrate fractions and their fermentability.

### 3.2.2. Pectin

Regarding additional pectin analysis, it is worth noting that the D-Glucuronic Acid and D-Galacturonic Acid Kit analyses hexa-uronic acids and not pectin. This method originates from the nutrition sector, where purified pectin, pectin-rich or pectin-enriched foods are used (Megazyme, 2019). Pectin is a generic term for a group of high-molecular-weight polysaccharides characterised by a linear α-(1→4)-D-galacturonic acid backbone partially esterified with methanol and branched with various neutral sugars on the side chains, such as rhamnose, galactose, arabinose and xylose (Flutto, 2003). Thus, galacturonic acid is the main molecule and other quantitative methods for a more direct analysis of pectin have not yet been established due to the heterogeneity of this group. Measuring uronic acids as a representation of pectin is an oversimplification and leads to errors (Van Soest, 1994). Undefined misestimates of the absolute quantities of pectin due to the heterogeneous and complex structure (especially with respect to rhamnogalacutron I and II) and the uronic acids from other carbohydrate fractions (such as arabinoxylan) occur. Apparently, there is no generally approved (i.e. standardised), internationally recognised method for the hydrolysis and analysis of pectin or uronic acids. Hence, researchers have used a large number of methods and, consequently, there is a lack uniform and comparable result. As early as 1950, there were already various approaches to analyse uronic acids or pectic substances based on the "weight of alcohol precipitate, titration of acid carboxyls plus saponification of methyl esters, weight of calcium pectate, decarboxylation by heating in concentrated mineral acids, and optical rotation" (McComb and McCready, 1952). Dische (1947) introduced a simple and specific colourimetric method to determine uronic acid, based on the reaction of released sugars after treatment with concentrated mineral acids and carbazole as a colouring agent; it is still recommended in food analysis (Matissek and Fischer, 2021b). This method (Dische, 1947) has since been modified several times and improvements have been made in terms of specificity, sensitivity, reducing interferences, throughput and work safety (McComb and McCready, 1952). One such improvement was the use of meta-hydroxydiphenyl as colouring agent by (Blumenkrantz and Asboe-Hansen, 1973), with which a higher specificity and sensitivity was achieved and which, according to van den Hoogen et al. (1998), was the most widely used method for uronic acid analysis. Scott (1979) developed a method for hexa-uronic acid analysis, which is embedded in the NSP analysis by Bach Knudsen (1997) and was considered for comparison; it utilises 3,5-dimetylphenol as the reagent, which is selective for 5-formyl-2-furancarboxylic acid formed by uronic acid in concentrated $H_2SO_4$. In addition, this method uses differential measurements at 450 and 400 nm to eliminate interference from neutral sugars and to reduce interference from

lignin. Wagner and Hollman (1976) were the first to describe a stoichiometric method based on the $NAD^+$-linked oxidation of free uronic acids in the presence of uronic acid dehydrogenase, and the $NADH+H^+$ formed is measured spectrophotometrically at 334 nm against a reagent blank. The assay applied in this research is based on this principle but combines different chemical and enzymatic approaches for extraction and hydrolysis. A control flour, besides the D-glucuronic acid standard, as included in other enzyme kits, would be desirable to facilitate the implementation and to validate the recommended steps of the extraction and their effect in the D-Glucuronic Acid and D-Galacturonic Acid Kit (K-URONIC 12/19; Megazyme, (2019).

Methods for the analysis of pectic substances can only be compared and validated based on free uronic acids. This is not satisfactory for the actual objective of pectin quantification, as the extraction process of pectin is not considered by using uronic acids.

To the author's knowledge, alternative enzymatic approaches for the quantification of pectin are not yet available due to the complexity of the pectin structure and the lack of knowledge about specific enzymes. Enzymatic approaches using pectinases are currently being carried out to identify pectin in the food sector, but not yet for quantification.

### 3.2.3. ß-glucan

The used method by McCleary and Glennie-Holmes (1985) was established as an AOAC method (955.16) and also by other international associations for the analysis of ß-glucan, and it is still state of the art and applied often. Nevertheless, some aspects need to be considered during the analysis: McCleary and Codd (1991) stated that the quantitative measurement of ß-glucan requires complete hydration of the samples, for which a particle size of < 0.5 mm is specified as essential, which deviates from the 1.0 mm sieve used in the present study for grinding. The reasons why no negative effects that influence hydration were expected are mentioned in section 3.1 of this chapter. Still, for other approaches, milling using a 0.5 mm sieve should be reconsidered and tested in favour of a finer milling, if this is allowed by the available sample quantity.

### 3.2.4. Fructan

The applied fructan assay is straightforward and its validity was checked by using the internal standard and control flours, although validity was not always achieved. The assay showed good robustness during single-laboratory validation (McCleary et al., 2019), which may indicate that the handling process carried out in this study may not have been sufficiently precise. Muir et al. (2007) showed that a comparable enzymatic assay (Fructan HK; K-FRUCHK 10/18,

Megazyme; a modification of AOAC Method 999.03) was not sensitive at low levels; they stated that fructan is unreliable at < 1 g/100 g DM. This Fructan HK assay was not used in this study because it is not suitable for food or feedstuff containing high amounts of D-glucose, D-fructose, sucrose or maltose (Megazyme, 2018b). However, the results of the applied assay are also very low, so they may also be unreliable when there are low amounts of fructan. Hence, this assay is not recommended for analysing digesta samples, as the same mechanisms are assumed to be at work as described in Muir et al. (2007) regarding the Fructan HK Kit.

In their research on 20 different wheat varieties, Call et al. (2018) analysed the fructan content by using a two-step procedure with HPAEC-PAD, in which fructan is specified based on fructose equivalents and corrected for the free sugar content prior to hydrolysis. Moreover, six of these wheat varieties were also analysed with the Fructan HK Assay (Megazyme, 2018b). The resulting fructan contents showed large differences within the wheat varieties but also between the analyses. Using the two-step procedure described by Call et al. (2018), fructan contents of 9.45–69.21 mg/g and a mean value of 21.95 mg/g were determined. Enzymatically, the fructan contents were lower at 8.50–15.02 mg/g. These differences show very clearly that, despite using the generally approved methods, there are no uniform results in fructan analysis and that the type of analysis must always be considered as it clearly affects the result. Although fructan is considered to be completely soluble in water, the low fructan content could be due to an incomplete extraction process of the applied assay. In contrast, more fructan might have been extracted by other methods, such as using 2.5 M trifluoroacetic acid (Call et al., 2018) or an acetate buffer (65°C) followed by sulphuric acid hydrolysis (80°C). In addition, despite incubation of the sample with distilled water directly in a boiling water bath, the intrinsic enzymes might not be deactivated immediately, thus reducing the fructan content. Moreover, handling errors may have occurred but they did not affect the included controls.

### 3.3. Enzymatic gravimetric methods

The analysed results using enzymatic gravimetric methods for feed and digesta are shown in Table 4. The principle of both analyses is the stepwise partly enzymatic extraction of different fractions and the gravimetric measurement of the remaining residue after alcohol precipitation. In both methods, the results are corrected for ash and protein, so two initial weighings are performed for each set.

**Table 4:** Enzymatic gravimetrically analysed carbohydrate or fibre fractions [g/kg DM] in the pig feeds and the corresponding digesta

| Sample origin | | | Trial 1 | | | | Trial 2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pig feed | | | | | | | |
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | | | Wheat → Rye | | | | SBM → RSM | | | |
| Feed | DM | g/kg | 910 | 922 | 914 | 917 | 896 | 913 | 910 | 901 |
| | IDF | | 144 | 157 | 164 | 167 | | | | |
| | SDFP | | 11.3 | 18.4 | 37.5 | 66.9 | | | | |
| | EIRomcp | | 679 | 664 | 670 | 688 | 668 | 651 | 674 | 669 |
| CSI[1] | DM | g/kg | 837 | 847 | 856 | 855 | | | | |
| | | | 0.05 (n = 2) | 0.07 (n = 4) | 0.03 (n = 3) | 0.01 (n = 4) | | | | |
| | IDF | | 222 | 151 | 269 | 230 | | | | |
| | | | 0.53 (n = 2) | 0.04 (n = 4) | 0.28 (n = 3) | 0.16 (n = 5) | | | | |
| | SDFP | | 29.3 | 32.2 | 50.3 | 72.1 | | | | |
| | | | 0.99 (n = 2) | 0.03 (n = 4) | 0.48 (n = 2) | 0.33 (n = 4) | | | | |
| Caecum | DM | g/kg | 907 | 911 | 919 | 929 | | | | |
| | IDF | | 392 | 407 | 492 | 376 | | | | |
| | SDFP | | 10.3 | 7.55 | 3.27 | 5.74 | | | | |

SBM = soybean meal; RSM = rapeseed meal; DM = dry matter; CSI = cranial small intestine; IDF = insoluble dietary fibre; SDFP = precipitated soluble dietary fibre; EIRomcp = ethanol insoluble residue corrected for protein and ash.

[1] CSI has been determined from individual samples and therefore the coefficient of variation is given in cursive underneath and the underlying sample number in parenthesis.

### 3.3.1. Dietary fibre

#### 3.3.1.1. Methodological aspects

The K-INTDF 08/18 Assay (Megazyme, 2018c) specifies an accurate sample weighing of 1 g with a tolerance of ± 0.005 g. This range is very narrow, resulting in probable separation of the sample during weighing, which can result in errors, similarly to the enzymatic assays. Therefore, after a few repetitions, weighing within this tolerance range was discontinued, especially because the initial weight is considered when calculating the results.

The results are corrected for the blank based on its protein and ash contents. The blank determinations carried out and used for protein analysis always resulted in 0, so the variations in the blank values resulted from the ash of filter paper. The effects of filter papers on the IDF and SDFP results were determined using the maximum and minimum amounts of ash and dried filter residue of the blanks, resulting in a range of -6.3 to 2.5 mg for IDF and -4.9 to 6.3 mg for SDFP. Because the range of the blank value of SDFP is wider compared with IDF, and SDFP has low values, the correction for the blank value has a strong influence on the SDFP results. This adds to the general weaknesses of gravimetric measurement being unspecific at low analytical concentrations in the sample (Bach Knudsen and Glitsø, 1997). This was also shown in interlaboratory studies of older versions of the TDF analysis (Prosky et al., 1992). Consequently, this deviation has a greater impact on the SDFP and digesta samples, as their contents are lower compared with IDF and feed samples, respectively.

Because ethanol and acetone are strong solvents, the potential influence of their handling on the filter papers was checked – that is, whether they could have increased or even caused the variations in the blank sample results. No effect was found. Humidity and handling during drying and weighing is known to influence the filter weight, which was also observed in this trial, as during weighing the results were found to be constant only after a few minutes (Fahey et al., 2018). Therefore, the same handling was applied when weighing blank and residue filters, taking the filter weight that was first displayed as the true dry value. Moreover, the filters require special handling, as they are susceptible to sample loss, especially dried, compared with crucibles. Filtration problems due to covering the filter pores with fine sample material were sometimes encountered with SDFP; these problems were time consuming but had no further negative effect. Note that the delay never exceeded 10 min, which has been stated to lead to inaccurate results (Fahey et al., 2018).

Improved handling through the use of crucibles with celite, as suggested in the K-INTDF 08/18 Assay for the filtration process, could simplify some of the aforementioned problems and be beneficial for subsequent routine analyses. However, it must be recognised that each system has its advantages and disadvantages. When using crucibles, sample transfer for nitrogen measurement and removal of filtration media, among other things, are potential sources of error (Fahey et al., 2018).

Within the assay, capable glass vessels are required for enzymatic digestion. Moreover, the assay suggests using "wide-necked soda glass bottles" (Megazyme, 2018c), whereby special care must be taken that these do not have a bottle neck or bulges, as this makes the quantitative transfer of the sample more difficult. The amount of distilled water for rinsing and transferring the total sample is precisely defined (with two times 10 ml for the IDF fraction) to obtain the correct amount of filtrate for the following SDFP determination, which also limits the quantitative transfer. The quantity used in the subsequent washing steps with alcohol are also defined (15 ml for each step). This specification could only be achieved with a Kipp dispenser head, which did not allow the entire sample to be wetted. Therefore, the washing steps were carried out with as little alcohol per step as possible, irrespective of the quantity, resulting in complete wetting of the sample.

The results obtained are presented in Table 4 and reference values with the respective sources are listed in Table 5. As the reference methods were common but older than the method applied, they only provide soluble dietary fibre (SDF) in TDF analysis. In this case, the SDFP obtained is comparable to SDF as there was no subdivision of SDF at that time. Furthermore, of all the listed methods, only Andersson et al. (2009) included fructan in SDF and TDF, which was reflected in their high content compared with the other methods. The method by Bach Knudsen (1997) analyses all previously hydrolysed non-starch carbohydrates using gas-liquid chromatography (GLC) in the monomeric state and sums them all to obtain NSP. ß-glucan has to be analysed by using the same methods as in this study. To obtain dietary fibre, lignin (Klason) is added to NSP (Bach Knudsen, 1997). Despite the different approaches to analyse dietary fibre, the level of the literature values was in line with the results obtained. Within the feed, the IDF concentration but especially the SDFP concentration increased in trial 1 from pig feed 1 to 4 due to the increasing content of rye compared with wheat. The increase in rye may result from the higher content of dietary fibre in rye compared with wheat. Among common cereals, rye grain has the highest TDF content (Poutanen, 2014). This results in detail from the carbohydrate fractions ß-glucan, arabinoxylan and fructan. Arabinoxylan is the quantitatively

**Table 5:** Literature values for the total dietary fibre content of cereal grains in g/kg DM

| Rye | | | | Wheat | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TDF | IDF | SDF | n | TDF | IDF | SDF | n | Analytical method | Source |
| 184 (173–190) | 156 (150–167) | 27 (20–40) | 3 | 126 | 121 | 6 | 1 | AOAC 991.43 | (McGhee and Stein, 2018) |
| 189 (173–206) | 164 (153–173) | 25 (19–31) | 2 | 129 | 121 | 9 | 1 | | (McGhee and Stein, 2020) |
| 157. (131–190) | 116 | 41.2 (32.7–53.5) | 20 | 109 (96.2–129) | 89.9 | 19.1 (10.6–29.7) | 20 | NSP + Lignin (Bach Knudsen, 1997) | (Rodehutscord et al., 2016) |
| 174 | | | 7 | 138 | | | 5 | NSP + Lignin | (Bach Knudsen, 1997) |
| 147-209 | 108-159 | 34-66 | | | | | | NSP + Lignin (Bach Knudsen, 1997) | (Hansen et al., 2003) |
| 199 (CV = 4.6) | 129 | 70.3 | | | | | | NSP + Lignin + Fructan | (Andersson et al., 2009) |

DM = dry matter; TDF = total dietary fibre; IDF = insoluble dietary fibre; SDF = soluble dietary fibre; NSP = non-starch polysaccharides; CV = coefficient of variation.
Maximum and minimum values are given in brackets unless otherwise stated

most important dietary fibre component of rye and may mostly be found in IDF as it partly forms diferulic bridges and covalent linkages with lignin, but some parts can also be extracted in water (Rybka et al., 1992; Vinkx and Delcour, 1996; Hansen et al., 2003). Fructans with a high molecular weight may only partly be precipitated within SDFP but would mostly be found within the discharged SDFS in form of fructooligosacharides (FOS), similarly to ß-glucans.

No fundamental problems arose when using digesta samples, so that the assay can also be carried out with this type of sample. The high CV found in the analysis of the CSI samples is largely due to the differences between the individual samples. Indeed, when the CV is large, there were "outliers" with the same tendencies in both the IDF and SDFP fractions. This phenomenon, together with the fact that it is difficult to obtain sufficient sample material for digesta sample analysis, underlines the advantage of using pooled samples.

Due to the lack of comparison, some logical assumptions were made regarding plausibility. Dietary fibre is not digested by endogenous enzymes in the small intestine, so an increase in the concentration of fibre fractions is expected because other nutrients (protein, starch, fat, etc.) are already digested in these sections. For SDFP, the concentration increases only in the CSI and is lower in the caecum compared with the feed. This may be related to the rapid fermentation of SDFP in the already intensively microbially colonised caecum. The IDF and SDFP contents of the different pig feeds in the respective sections no longer showed any tendencies depending on the specific compound feed (1 to 4). The observed variations were large. Due to the lack of absolute data, the aforementioned can only remain a presumption, which could not be evaluated within the scope of this work. Nevertheless, this presumption was considered more likely than a failure of the assay, hence, the values measured in the digesta were considered plausible.

#### 3.3.1.2. General aspects

IDF and SDFP were measured in feed and digesta samples using the K-INTDF 08/18 Assay based on AOAC method 2011.25. This method was chosen because it was simple, cost-effective and analyses soluble and insoluble fractions separately. In addition to the high informative value of the results, the implementation is easy and the workload is moderate, although the first incubation – 16 h – is time consuming. Hence, its acceptance in the scientific environment is very high. For routine use automate analysers are already available.

TDF analysis in form of the Prosky method was standardised in AOAC 985.29, but it has developed over time. Although the results in these analyses may be presented in the same way,

they do not incorporate the exact same fractions. Therefore, caution must be taken when comparing different results and the underlying AOAC method must be specified. The method was originally selected to determine TDF, but SDFS was not measured, so the fraction determined was the sum of IDF and SDFP, which is correctly referred to as HMWDF. Note that HMWDF analysed using the Prosky method (AOAC 985.29) or the Lee method (AOAC 991.43, the first approach to measure IDF and SDF, calculating the TDF [nowadays: HMWDF], otherwise basically following 985.29) still followed an older protocol in which resistant starch may be underestimated due to hydrolysis of the sample in boiling water. Depending on the content of resistant starch in the samples, a comparison of the HMWDF values determined with these different procedures may no longer be valid. The latest AOAC method (2017.16) differs from AOAC 2009.11 (HMWDF directly determined) in that the incubation time has been shortened to 4 h, which better reflects the natural retention time of digesta in the small intestine. In turn, the enzyme concentration is increased. These differences, as well as the switch to a high-performance liquid chromatography (HPLC) column, are reflected in SDFS, so HMWDF is still comparable to the previously used method (McCleary, 2018).

In the current experiment, SDFS was discarded because the focus was on developing a system that could be used in less well-equipped laboratories where HPLC may not be available and, consequently, TDF could not be completely determined. SDFS includes the non-digestible oligosaccharides (NDO) also referred to as low-molecular-weight dietary fibre (LMWDF), which again consist of various oligosaccharides (e.g. FOS, xylooligosaccharides or galactooligosaccharides) (Mussatto and Mancilha, 2007). In the samples evaluated in this trial, FOS are of particular importance given that they occur in rye or wheat, and they were intended to be recorded by an additional fructan analysis discussed above. However, this method still leads to the loss of additional NDO, and although this loss may not have been of quantitative importance in this example, it still does not allow the universal transferability of the method.

Carbohydrate analysis uses 80% v/v ethanol precipitation in practice to divide oligosaccharides and polysaccharides, which by definition have a DP of 3–9 and ≤ 10, respectively. This simple technique makes water-soluble substances gravimetrically measurable by precipitating them with a precipitating agent (alcohol, etc.). However, a clear separation between oligosaccharides and polysaccharides cannot be achieved with this method: besides the DP, branching and the molecular weight also have an influence on what is precipitated. Within this method, HMWDF and LMWDF are separated, which depending on the sample may widely exceed a DP of 3–9. The concentration of 80% v/v ethanol has become common, has often been studied and here,

too, one must always be aware that differences in the precipitating reagent and its concentration as well as the temperature among the procedures influence the results. Johansen et al. (1996) investigated how the extraction solvent and temperature affect the extraction yield of oligosaccharides and sugars from plant materials. They found that the extraction yield is more dependent on temperature (highest at the boiling point) at high ethanol concentrations (80% v/v), while temperature has less of an influence at 50% v/v ethanol or in water. In addition, they compared methanol and ethanol and reported higher results for methanol at 80% v/v than for ethanol at the same concentration and no difference between the two at 50% v/v. Ethanol was finally selected because of its better handling. Hall et al. (1999) tested different alcohol strengths by using 90%, 80% and 70% aqueous ethanol in a sequential and non-sequential procedure to measure soluble low-molecular-weight carbohydrates. They used fructose as an indicator of efficiency. In the sequential procedure, they found most of the fructose in 90% aqueous ethanol and some in the subsequent 80% aqueous ethanol; however, there were only traces of fructose in the 70% aqueous ethanol. In the non-sequential method, they found most fructose in 80% aqueous ethanol extraction, which was not significantly higher than the 90% extraction, but both differed significantly from 70% aqueous ethanol.

Ku et al. (2003), using inulin and oligoglucose as indicators, demonstrated that as the ratio of ethanol to distilled water increases, the DP of the precipitated substances decreases. At an ethanol ratio of 4:1 (v/v), as used in AOAC 985.29, detectable amounts of molecules with a DP of 14–18 are still precipitated, and there are even higher amounts of molecules with a DP of 10–13. Ethanol precipitation follows unclear specifications. While the precipitation ratio of 4:1 for ethanol and distilled water is clearly specified for all methods, this does not seem to apply to the concentration of ethanol used in the AOAC methods (Megazyme, 2018c; McCleary, 2018; McCleary et al., 2013). Although the AOAC methods specify the use of 95% v/v ethanol, which would result in 76% v/v ethanol in the final solution, final concentrations of 78% v/v ethanol appear repeatedly in the methods, which result from the usage of 99% v/v ethanol. Researchers, such as Ku et al. (2003), have demonstrated that these differences affect the results, so greater diligence and more precise specifications are needed.

### 3.3.2. Ethanol-insoluble residue

#### 3.3.2.1. Methodological aspects

In the method developed by Hall et al. (1999) “for partitioning neutral detergent soluble carbohydrates” “(NDSC) a sample is directly incubated in 80% v/v ethanol for 4 h with constant

shaking at room temperature followed by filtration, leaving the EIR. “In the applied method, 2 g of the sample was weighed and the samples were stirred instead of shaken. The procedure was simple and there were no problems. Precipitation in 80% v/v ethanol was used to separate NDSC into a water and ethanol-soluble fraction and an ethanol-insoluble fraction containing NDSF, the fraction of interest, aNDFomcp and starch. EIR was corrected for protein (cp) and ash (om). In the original method, the EIR obtained was further used for starch analysis to obtain NDSF. In the present study, starch was determined with a separate sample and NDSF was derived by subtracting the respective fractions (Hall et al., 1998).

$$NDSF = EIRomcp - starch - aNDFomcp$$

The utilisation of polarimetric starch was assessed, as they are part of the legally required feed declaration and their use could facilitate the calculation of the NDSF. However, it was rejected because resistant starch otherwise would be subtracted twice because it is also recovered in aNDFom. This would clearly lead to an underestimation as negative results were found. Although a method for enzymatic starch analysis is given in the method (Hall et al., 1999), the usual enzymatic starch analysis in this laboratory according to Brandt et al. (1987) was carried out. Because starch and aNDFom make up a large part of EIRomcp, only a few conclusions can be drawn from this directly analysed fraction and its fluctuations. The calculated NDSF and aNDFomcp are the informative fraction containing the soluble, readily fermentable carbohydrates and the insoluble carbohydrates including lignin, respectively.

#### 3.3.2.2. General aspects

This method, as well as the dietary fibre analysis, is based on alcohol precipitation and is therefore subject to the inaccuracies mentioned in section 3.3.1.2 of this chapter regarding ethanol precipitation and the convention of using 80% v/v ethanol. The strength of this method is primarily the use of values that are analysed and declared in a legally binding manner within the framework of the German feed declaration. The additional fast and simple analyses enable the evaluation of health-promoting fibre contents in the animal.

### 3.4. Chemical gravimetric methods

Chemical gravimetric measurements as part of the Weender feed analysis are the basis of the legally binding feed declaration. Currently, the term extended Weender analysis is used when the cell wall components are evaluated using the detergent analysis according to Van Soest (1963). This analysis was carried out within this work following VDLUFA (2012). Because this method has been standard in feed and fibre analysis for many years, the detergent analysis

itself is not addressed here. Only the differences resulting from changes in the procedure and the use of digesta samples in this analysis are discussed. In addition, the neutral or acid detergent insoluble nitrogen was determined as an extension of the Van Soest analysis following Licitra et al. (1996).

### 3.4.1. Detergent analysis

Within the detergent analysis of Van Soest (1963), feed samples are boiled in neutral or acid detergent solution leaving NDF (hemicelluloses, celluloses and lignin) and ADF (celluloses and lignin), respectively, as a residue which is determined gravimetrically. To improve the comparability between the results of the detergent analysis and those of the dietary fibre analysis, round filters were used for the aNDFom and ADF analysis instead of fibre bags, as is common in this laboratory. The results for the detergent analysis are the mean value of the duplicate determinations (Table 6). The relative analytical tolerance, referred to as the relative error (RE), is also is specified in this table. An exception are the results of the CSI analysis, in which only single determinations were carried out due to the small amount of sample available. The analysis scope is defined with ± 10% relative deviation for high determined concentrations of aNDFom (350–580 g/kg) and ADFom (220–381 g/kg). For low concentrations of ADFom (53–123 g/kg) and aNDFom (160–350 g/kg), a relative deviation of 18% and 35 g/kg, respectively, is acceptable (VDLUFA, 2012).

In the feed, the aNDFom results of trial 2 and all ADFom results were higher when using fibre bags compared with round filters. The aNDFom values of trial 1 showed the opposite outcome. The former could be due to the differences in permeability and handling of round filters and fibre bags. Handling of fibre bags is easier, more routine and sample losses occur less frequently. For both trials, the relative analytical tolerance, referred to as RE, was calculated and within analysis scope for both variants performed, but remarkably higher in pig feeds 4 and 5 using fibre bags during analysis. Because the RE in aNDFom in feeds analysed with round filter was smaller than with fibre bags, the results seemed more reliable. Regarding the feed composition and literature values for the main components aNDFom contents between the results obtained were expected (DLG, 2014). A similar ration based on wheat or rye with either SBM or RSM as used in Ellner (2022) were more in line with the values obtained using the fibre bags. The ADFom analysis of the feed showed a contrasting picture. The RE when using round filters was outside the analytical range, while the error when using fibre bags was within this range. Due to greater plausibility, the permissible RE of the ADFom results and the consistency of the values from the fibre bags were used for the feeds in the following results;

**Table 6:** Detergent analysis results in g/kg DM and relative error in % using different filters.

| Sample origin | | Trial 1 | | | | | | | | Trial 2 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pig feed | | | | | | | | | | | | | | | |
| | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 8 | |
| | | Wheat → Rye | | | | | | | | SBM → RSM | | | | | | | |
| | | RF | FB | RF | FB | RF | FB | RF | FB | RF | FB | RF | FB | RF | FB | RF | FB |
| Feed | aNDFom | 265 | 177 | 261 | 179 | 283 | 181 | 292 | 183 | 135 | 225 | 136 | 228 | 172 | 200 | 197 | 224 |
| | RE | 10.7 | 1.39 | 5.63 | 10.1 | 5.33 | 18.3 | 10.5 | 29.9 | 4.62 | 31.3 | 0.15 | 12.9 | 3.14 | 0.08 | 0.47 | 6.64 |
| | ADFom | 23.1 | 44.8 | 28.0 | 37.5 | 29.6 | 45.9 | 25.9 | 37.3 | 42.2 | 77.1 | 55.9 | 74.2 | 69.1 | 80.2 | 84.4 | 99.8 |
| | RE | 98.6 | 15.1 | 94.4 | 6.68 | 20.4 | 5.14 | 126 | 2.35 | 4.44 | 8.44 | 6.55 | 5.73 | 6.91 | 8.07 | 2.99 | 29.1 |
| | NDICP | 17.2 | | 17.3 | | 21.4 | | 20.5 | | 12.4 | | 16.3 | | 23.7 | | 34.7 | |
| | RE | 5.12 | | 7.52 | | 5.10 | | 11.7 | | 5.41 | | 5.37 | | | | 1.92 | |
| | ADICP | 4.7 0 | | 3.00 | | 3.90 | | 3.70 | | 3.63 | | 3.61 | | 4.56 | | 7.49 | |
| | RE | 115 | | 67.9 | | 25.2 | | 47.5 | | 70.8 | | 18.2 | | 2.41 | | 7.41 | |
| CSI | aNDFom | 225 | | 156 | | 272 | | 238 | | n.a. | | n.a. | | n.a. | | n.a. | |
| | ADFom | 54.0 | | 52.6 | | 93.5 | | 74.4 | | n.a. | | n.a. | | n.a. | | n.a. | |
| | NDICP | 11.7 | | 8.73 | | 15.5 | | 15.0 | | n.a. | | n.a. | | n.a. | | n.a. | |
| | ADICP | 1.36 | | 3.40 | | 3.92 | | 3.18 | | n.a. | | n.a. | | n.a. | | n.a. | |
| Caecum | aNDFom | 316 | | 339 | | 376 | | 313 | | n.a. | | n.a. | | n.a. | | n.a. | |
| | RE | 2.79 | | 5.32 | | 4.19 | | 4.17 | | n.a. | | n.a. | | n.a. | | n.a. | |
| | ADFom | 136 | | 119 | | 138 | | 116 | | n.a. | | n.a. | | n.a. | | n.a. | |
| | RE | 4.87 | | 3.18 | | 1.87 | | 4.61 | | n.a. | | n.a. | | n.a. | | n.a. | |
| | NDICP | 24.9 | | 31.9 | | 31.0 | | 28.9 | | n.a. | | n.a. | | n.a. | | n.a. | |
| | RE | 15.9 | | 4.81 | | 11.6 | | 5.59 | | n.a. | | n.a. | | n.a. | | n.a. | |

SBM = soybean meal; RSM = rapeseed meal; DM = dry matter; RF = round filter; FB = filter bag; aNDFom = neutral detergent fibre treated with amylase and expressed exclusive of residual ash; RE = relative error; ADFom = acid detergent fibre expressed exclusive of residual ash; NDICP = neutral detergent insoluble crude protein; ADICP = acid detergent insoluble crude protein; CSI = cranial small intestine; n.a. = not analysed

hence, the aNDFom results using round filters in trial 1 were discarded. The digesta samples were tested using only round filters. For the caecal digesta samples, the RE was within the analytical variation range given for feed samples. This, together with the fact that this method has already been used in rumen or faecal samples, shows that the method should be applicable to such matrices (Van Soest, 1994). The values determined for CSI and caecal samples seem reliable because aNDFom and ADFom are considered indigestible in the small intestine and the concentration of each was higher than in the previous digestive part. However, this eventuality could not be verified within this work due to the lack of quantitative data. NDICP and ADICP were determined according to Licitra et al. (1996) and represent the resistant nitrogen × 6.25, which is not directly available to the animal's metabolism because it is associated with lignin, tannins or Maillard reaction products (Van Soest, 1965; Licitra et al., 1996; Van Soest and Mason, 1991). These values were used to correct the results from contamination with CP, which otherwise results in overestimation of the total cell wall compounds given that a considerable amount of protein is part of the analysed NDF (Theander and Åman, 1980).

### 3.5. Conclusion of the analytical consideration

The analyses performed and their quality have already been discussed in the individual sections above. Briefly, the enzymatic colourimetric methods for the samples used, in particular the digesta samples, are currently not valid. From now on, the results obtained will be ignored and excluded from the calculations. The enzymatic gravimetric methods, on the other hand, provided solid results, which are interpreted below together with the standard chemical gravimetric methods with respect to the research question. The comparability of composite but independent analyses to classify the data and their validity is an important factor, regardless of feasibility, to find hidden errors. Therefore, future implementation of such an approach should first apply the individual components from the area of interest to ensure reliable data beyond possible standardised control flours. Moreover, the additivity of the components could be evaluated if a single component could be tested in compound feeds.

## 4. Results

The chemical composition and energy content of the pig feeds carried out at the University of Veterinary Medicine Hannover (Germany) are shown Table 7 and reflect the specific characteristics of the exchanged components. In trial 1, the chemical composition of the diets was similar, except starch decreased and sugar increased as the rye content increased (from pig feed 1 to 4), which was also reflected in the ME. Trial 2 showed more differences as the amount

of RSM increased. As the RSM content increased (from pig feed 5 to 8), CF and EE increased and starch and nitrogen-free extract decreased, which was also reflected in a decrease in ME.

Additional fibre fractions evaluated in the extreme pig feed variants (1, 4, 5 and 8) showed an increase from pig feed 1 to 8 for total NSP, total arabinoxylan and dietary fibre. Insoluble NSP increased from pig feed 1 to 8, in contrast to soluble NSP, which were highest in pig feeds 4 and 5, both based on rye and SBM. The insoluble arabinoxylan content was lower in pig feed 1 (wheat based) than in pig feeds 4, 5 and 8 (rye based), which showed the same content. The soluble arabinoxylan content was higher in pig feed 8 (RSM based) than in pig feeds 1, 4 and 5 (SBM based), which had a similar content. The Klason lignin content was higher in trial 2 than in trial 1, as lignocellulose was added to SBM-containing feed. In trial 1, the Klason lignin content of pig feed 1 was higher than in pig feed 4. In trial 2, the Klason lignin content of pig feed 5 (SBM based) was lower than pig feed 8 (RSM based). Further information on the used raw components – especially rye and wheat grain, RSM and SBM – were not available.

**Table 7:** Chemical composition and energy content [g/kg DM] of the pig feeds (Wilke, 2020)

| | | Trial 1 | | | | Trial 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pig feed | | | | | | | |
| | | Wheat → Rye | | | | SBM → RSM | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| DM | g/kg | 897 | 897 | 894 | 899 | 890 | 890 | 889 | 888 |
| Crude ash | g/kg DM | 48.4 | 53.2 | 46.2 | 51.3 | 53.6 | 54.2 | 54.8 | 57.3 |
| Crude protein | | 205 | 205 | 198 | 198 | 194 | 196 | 195 | 188 |
| Ether extract | | 27.4 | 28.1 | 32.6 | 24.5 | 28.1 | 27.8 | 34.2 | 42.3 |
| Crude fibre | | 26.2 | 24.9 | 29.9 | 22.0 | 43 | 44.1 | 49.8 | 54.7 |
| Nitrogen-free extract | | 625 | 622 | 624 | 637 | 610 | 607 | 594 | 588 |
| Starch | | 530 | 514 | 493 | 491 | 436 | 432 | 417 | 412 |
| Sugar | | 41.3 | 46.5 | 52.1 | 60.0 | 66.6 | 67.3 | 70.9 | 69.5 |
| Metabolisable energy [1] | | 15.8 | 15.9 | 15.7 | 15.7 | 14.9 | 14.9 | 14.7 | 14.6 |
| NSP (total) | | 123 | | | 140 | 157 | | | 170 |
| NSP (insoluble) | | 88 | | | 93 | 109 | | | 140 |
| NSP (soluble) | | 35 | | | 47 | 48 | | | 30 |
| Arabinoxylan (total) | | 63 | | | 74 | 74 | | | 83 |
| Arabinoxylan (insoluble) | | 18 | | | 27 | 26 | | | 26 |
| Arabinoxylan (soluble) | | 45 | | | 47 | 48 | | | 57 |
| Lignin (Klason) | | 20 | | | 16 | 30 | | | 38 |
| "Dietary fibre" | | 143 | | | 156 | 187 | | | 208 |

SBM = soybean meal; RSM = rapeseed meal; DM = dry matter; NSP = non-starch polysaccharides. [1] Metabolisable energy was calculated from the specified contents

The fibre or feed components analysed (University of Bonn) and calculated are shown in Table 8. The calculated fractions are highlighted in bold. The aNDFom contents in trials 1 and 2, except pig feed 7, were at the same level within each trial, with overall higher values in trial 2. The same was seen for aNDFomcp in trial 1, whereas trial 2 showed a decreasing trend from pig feed 5 to 8 for aNDFomcp. In trial 1, ADFom and ADFomcp each showed similar values for the pig feeds. In trial 2, there was an outlier for ADFom (pig feed 8), while the other pig feeds were at a similar level; the CP correction of ADFomcp reduced the outlier gap. ADL showed equivalent values in trial 1, but an increase from pig feed 5 to 8 with increasing RSM content in trial 2. This increase is consistent with the results for Klason lignin shown in Table 7, although the slope was significantly greater for ADL due to the lower ADL content in pig feed 5 compared to Klason lignin. The hemicellulose contents of trial 1 were all similar, while the contents of trial 2 were slightly higher for pig feeds 5 and 6 and slightly lower for pig feeds 7 and 8 than those of trial 1. The undirected variations in the detergent analysis displayed in the calculated hemicellulose and cellulose content and clear differences within the trials were not observed. The analysis of dietary fibre was carried out in trial 1, with both IDF and SDFP increasing from pig feed 1 to 4. The subsequently calculated fractions HMWDF “TDF” and NSP showed higher contents than those of dietary fibre given in Table 7 analysed according to Bach Knudsen (1997). Notably, the contents of pig feed 4 showed a larger discrepancy. The EIR varied at a similar level in trials 1 and 2. The NDSF content of pig feeds 1 and 2 was at the same level as pig feed 5. Pig feed 6 had the lowest NDSF content. Pig feeds 3 and 4 as well as pig feeds 7 and 8 were each at the same level above the aforementioned pig feeds, with the latter having the highest NDSF content. The direct analysed and calculated carbohydrate fractions of digesta samples are shown in Table 9.

**Table 8:** Analysed and calculated fibre or feed components [g/kg DM] in pig feeds 1 to 8

| | | Trial 1 | | | | Trial 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pig feed | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | | Wheat → Rye | | | | SBM → RSM | | | |
| DM | g/kg | 910 | 922 | 914 | 917 | 896 | 913 | 910 | 901 |
| aNDFom | | 177 | 179 | 181 | 183 | 225 | 228 | 200 | 224 |
| NDICP | | 17.2 | 17.3 | 21.4 | 20.5 | 12.4 | 16.4 | 23.7 | 34.5 |
| **aNDFomcp** | | 160 | 162 | 160 | 163 | 213 | 212 | 176 | 190 |
| ADFom | | 48.8 | 37.5 | 45.9 | 37.3 | 77.1 | 74.2 | 80.2 | 99.8 |
| ADICP | | 4.50 | 3.00 | 3.90 | 3.70 | 3.68 | 3.63 | 4.59 | 7.43 |
| **ADFomcp** | | 44.3 | 34.5 | 42.0 | 33.6 | 73.4 | 70.6 | 75.6 | 92.4 |
| ADL | | 7.96 | 6.18 | 9.10 | 8.02 | 16.5 | 19.8 | 29.7 | 37.4 |
| **Hemicellulose** | | 128 | 142 | 135 | 146 | 148 | 154 | 120 | 124 |
| **Cellulose** | | 40.8 | 31.3 | 36.8 | 29.3 | 60.6 | 54.4 | 50.5 | 62.4 |
| | | | | | | | | | |
| IDF | | 144 | 157 | 164 | 167 | n.a. | n.a. | n.a. | n.a. |
| SDFP | | 11.3 | 18.4 | 37.5 | 66.9 | n.a. | n.a. | n.a. | n.a. |
| **"TDF" (HMWDF)** | | 155 | 175 | 202 | 234 | | | | |
| NSP | | 147 | 169 | 192 | 226 | n.a. | n.a. | n.a. | n.a. |
| | | | | | | | | | |
| EIRomcp | | 679 | 664 | 670 | 688 | 668 | 651 | 674 | 669 |
| **NDSF** | | 56 | 54 | 84 | 81 | 56 | 36 | 134 | 131 |

SBM = soybean meal; RSM = rapeseed meal; DM = dry matter; aNDFom = neutral detergent fibre treated with amylase and expressed exclusive of residual ash; CP = crude protein; NDICP = neutral detergent insoluble crude protein; aNDFomcp = neutral detergent fibre treated with amylase and expressed exclusive of residual ash and crude protein; ADFom = acid detergent fibre expressed exclusive of residual ash; ADICP = acid detergent insoluble crude protein; ADFomcp = acid detergent fibre expressed exclusive of residual ash and crude protein; ADL = acid detergent lignin; IDF = insoluble dietary fibre; SDFP = precipitated soluble dietary fibre; TDF = total dietary fibre; HMWDF = high-molecular-weight dietary fibre; NSP = non-starch polysaccharides; EIRomcp = ethanol-insoluble residue corrected for protein and ash; NDSF = neutral detergent soluble fibre; n.a. = not analysed

**Table 9:** Analysed and calculated fibre or feed components [g/kg DM] in the digesta of pigs fed pig feeds 1 to 4

| | | | Trial 1 | | | |
|---|---|---|---|---|---|---|
| | | | Pig feed | | | |
| Digesta section | | | 1 | 2 | 3 | 4 |
| | | | Wheat → Rye | | | |
| CSI[1] | DM | g/kg | 837 | 822 | 856 | 855 |
| | aNDFom | | 225 | 156 | 272 | 238 |
| | | | 0.53 (n = 2) | 0.10 (n = 4) | 0.26 (n = 3) | 0.09 (n = 4) |
| | NDICP | | 11.7 | 9.47 | 15.5 | 15.2 |
| | | | 0.58 (n = 2) | 0.19 (n = 4) | 0.33 (n = 3) | 0.16 (n = 4) |
| | **aNDFomcp** | | 213 | 147 | 257 | 223 |
| | ADFom | | 54.0 | 52.6 | 93.5 | 74.4 |
| | | | (n = 1) | 0.13 (n = 3) | 0.29 (n = 3) | 0.22 (n = 4) |
| | ADICP | | 1.36 | 3.40 | 3.92 | 3.18 |
| | | | (n=1) | 0.26 (n = 3) | 0.16 (n = 3) | 0.19 (n = 4) |
| | **ADFomcp** | | 52.6 | 49.2 | 89.6 | 71.2 |
| | **Hemicellulose** | | 171 | 103 | 179 | 164 |
| | IDF | | 221 | 151 | 269 | 225 |
| | | | 0.54 (n = 2) | 0.12 (n = 4) | 0.28 (n = 3) | 0.16 (n = 5) |
| | SDFP | | 29.2 | 32.2 | 50.2 | 72.1 |
| | | | 9.9 (n = 2) | 0.31 (n = 4) | 4.8 (n = 2) | 3.45 (n = 4) |
| | **"TDF" (HMWDF)** | | 250 | 183 | 319 | 297 |
| Caecum | DM | g/kg | 918 | 919 | 936 | 921 |
| | aNDFom | | 484 | 505 | 336 | 243 |
| | NDICP | | 24.5 | 29.2 | 32.7 | 32.3 |
| | **aNDFomcp** | | 460 | 476 | 303 | 211 |
| | ADFom | | 149 | 151 | 169 | 111 |
| | ADICP | | 15.1 | 11.8 | 12.5 | 9.7 |
| | **ADFomcp** | | 134 | 139 | 157 | 101 |
| | **Hemicellulose** | | 335 | 354 | 167 | 132 |
| | IDF | | 392 | 407 | 492 | 376 |
| | SDFP | | 10.3 | 7.55 | 3.27 | 5.74 |
| | **"TDF" (HMWDF)** | | 402 | 415 | 495 | 382 |

SBM = soybean meal; RSM = rapeseed meal; CSI = cranial small intestine; DM = dry matter; aNDFom = neutral detergent fibre treated with amylase and expressed exclusive of residual ash; aNDFomcp = neutral detergent fibre treated with amylase and expressed exclusive of residual ash and crude protein; CP = crude protein; NDICP = neutral detergent insoluble crude protein; ADFom = acid detergent fibre expressed exclusive of residual ash; ADICP = acid detergent insoluble crude protein; ADFomcp = acid detergent fibre expressed exclusive of residual ash and crude protein; IDF = insoluble dietary fibre; SDFP = precipitated soluble dietary fibre; TDF = total dietary fibre; HMWDF = high-molecular-weight dietary fibre; NDSF = neutral detergent soluble fibre

The caecal digesta were analysed as a pooled sample, whereas the CSI digesta were analysed as individual samples, so the CSI digesta results are presented as the means of all analysed single samples. The respective number of samples as well as CV are indicated. Due to the available amount of each CSI sample, not all samples could be used; therefore, the number of samples and the CV are given for each mean value. The CV ranged from 0.09 to 0.53. For the majority of the samples, there were no trends based on the presented concentrations, as the contents varied. However, it was clear that the CSI digesta of pig feed 2, except for the ADF-related fractions and SDFP, deviated downwards compared with the other pig feeds. ADFom and ADFomcp showed that pig feeds 1 and 2 were at the same level below the others, with the highest content in pig feed 3. Ignoring pig feed 2, all other pig feeds showed a similar hemicellulose content. IDF and NDF content of pig feed 4 was higher compared to pig feed 1 for but highest in pig feed 3. In the pooled caecal digesta samples, there were no clear trends. An exception was the AD fractions (ADFom, ADFomcp and ADICP), which each had similar contents. To be able to compare the concentrations in feed and the respective digestive sections, they were related to each other (Table 10). In CSI, the observation that pig feed 2 deviated from the other pig feeds confirmed, as it has disappeared (< 100%) compared with the feed, while all other pig feeds showed enrichment (> 100%). NDICP disappeared for all pig feeds compared with the feed. ADICP showed strong fluctuation; it is very low in pig feed 1, a phenomenon that can be attributed to the individual animal digesta sample as only one sample was used. SDFP showed enrichment in the CSI compared with feed for all specific pig feeds. However, this is contrary to the trend of decreasing concentrations. Of note, the relative fibre or carbohydrate fractions in the CSI samples increased by ~150% compared to the feed. There was even greater enrichment in the caecal samples, ~ 150%–300% compared to the feed, although with some exceptions. One exception was SDFP, which disappeared relative to the feed and decreased in the caecum at different rates from pig feed 1 to 4. Hemicellulose also showed a decrease in the caecum from pig feed 1 to 4 and was enriched relative to feed in digesta, with the exception of pig feed 2 in the CSI and pig feed 4 in the caecum. However, pig feeds 3 and 4 were higher in the CSI compared with the caecum.

Table 11 shows the ratios of different fractions to each other. The ratio of SDFP to IDF clearly shows that in both feed and the CSI, the ratio of pig feed 1 to 4 increased and the two values are relatively close, with pig feeds 1 and 2 being lower in feeds than in the CSI, which is reversed for pig feeds 3 and 4. In the caecum, the ratio was consistently low in all feeds. The

**Table 10:** Disappearance or enrichment of the fibre and carbohydrate fractions in the course of the digestive tract, expressed as the ratio of the concentration of the digestive sections to the concentration in the feed (feed = 100%) in %.

| | Trial 1 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CSI | | | | Caecum | | | |
| | Pig feed | | | | | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| | Wheat → Rye | | | | SBM → RSM | | | |
| aNDFom | 127 | 87.2 | 150 | 130 | 273 | 282 | 186 | 133 |
| NDICP | 68.0 | 54.7 | 72 | 74.1 | 142 | 169 | 159 | 158 |
| aNDFomcp | 133 | 90.5 | 160 | 137 | 287 | 294 | 190 | 129 |
| ADFom | 121 | 140 | 204 | 199 | 333 | 393 | 368 | 298 |
| ADICP | 30.2 | 113 | 101 | 85.9 | 336 | 403 | 321 | 262 |
| ADFomcp | 131 | 143 | 213 | 212 | 332 | | 373 | 301 |
| Hemicellulose | 139 | 77 | 141 | 118 | 281 | 265 | 124 | 85 |
| | | | | | | | | |
| IDF | 153 | 96 | 164 | 135 | 272 | 259 | 300 | 225 |
| SDFP | 258 | 175 | 134 | 108 | 91.2 | 41.0 | 8.7 | 8.6 |
| "TDF" (HMWDF) | 161 | 105 | 158 | 127 | 259 | 237 | 245 | 163 |

SBM = soybean meal; RSM = rapeseed meal; CSI = cranial small intestine; aNDFom = neutral detergent fibre treated with amylase and expressed exclusive of residual ash; aNDFomcp = neutral detergent fibre treated with amylase and expressed exclusive of residual ash and crude protein CP = crude protein; NDICP = neutral detergent insoluble crude protein; ADFom = acid detergent fibre expressed of exclusive of residual ash; ADICP = acid detergent insoluble crude protein; ADFomcp = acid detergent fibre expressed exclusive of residual ash and crude protein; IDF = insoluble dietary fibre; SDFP = precipitated soluble dietary fibre; TDF = total dietary fibre; HMWDF = high-molecular-weight dietary fibre

ratio of ADFomcp to aNDFomcp remained at a constant level for all feed and was the same for the CSI for pig feed 1. The ratio in the CSI of pig feeds 2 to 4 increased compared with the ratio in the feed. The ratio of ADFomcp to aNDFomcp in the caecum was lower for pig feeds 1 and 2 compared with pig feeds 3 and 4. The ratio of IDF to aNDFomcp showed a similar value for the feed and CSI, but in the caecum the values changed from relatively low in pig feeds 1 and 2 to very high in pig feeds 4 and 3.

**Table 11:** Ratios of analytical fibre fractions to each other in feed and digestive sections

| | Trial 1 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pig feed | | | | | | | | | | | |
| | 1 | | | 2 | | | 3 | | | 4 | | |
| | Wheat → Rye | | | | | | | | | | | |
| | Feed | CSI | Caecum | Feed | CSI | Caecum | Feed | CSI | Caecum | Feed | CSI | Caecum |
| SDFP : IDF | 0.08 | 0.13 | 0.03 | 0.12 | 0.21 | 0.02 | 0.23 | 0.19 | 0.01 | 0.40 | 0.32 | 0.02 |
| ADFomcp : aNDFomcp | 0.25 | 0.25 | 0.29 | 0.21 | 0.34 | 0.29 | 0.26 | 0.35 | 0.52 | 0.21 | 0.32 | 0.48 |
| IDF : aNDFomcp | 0.90 | 1.04 | 0.85 | 0.97 | 1.03 | 0.86 | 1.03 | 1.05 | 1.62 | 1.02 | 1.01 | 1.78 |

SBM = soybean meal; RSM = rapeseed meal; CSI = cranial small intestine; SDFP = precipitated soluble dietary fibre; IDF = insoluble dietary fibre; ADFomcp = acid detergent fibre expressed exclusive of residual ash and crude protein; aNDFom = neutral detergent fibre treated with amylase and expressed exclusive of residual ash and crude protein

# 5. Discussion

## 5.1. General remarks

The primary aim of this work was to quantify all specific carbohydrate fractions with as little effort as possible by enzymatic and chemical methods that are applicable in routine analysis. Enzymatic photometric analyses were not sufficiently reliable and the methods from the field of human nutrition were also not completely suitable for the analysed samples. The conflict of goals of establishing a method that is as simple as it is complex came to the fore. Therefore, the relevance of the analysis itself was questioned. Mertens (2003) defined the relevance of a fibre analysis based on several factors: "reproducibility, repeatability, labour efficiency, timeliness, personnel requirements, cost, and use of the results". In this project, it was not possible to derive the health effects of the individual fractions on pigs from the results. This issue is detailed in chapter 3.

Irrespective of the feasibility, the conflict of objectives raised the question of whether a continuation of such a complex method in animal nutrition is promising or whether it would be used at all in practice. Even today, CF analysis is still used in animal nutrition because of its simplicity ("cost and expediency"), which can no longer be justified due to the lack of sufficient alternative, high-quality data and thus its role as a comparative parameter (Van Soest, 1994). Historically, fibre had been known to reduce the energy content of feed, but the positive effects of fibres have been known for a long time and still have not become part of standard feed analysis. Therefore, complexity, which is justified in terms of fundamental research (along human medicine and nutrition), was abandoned and a stronger focus was placed on simple sum parameters. This approach has a realistic chance of implementation in routine analyses. Sum parameters have the advantages of simplicity and bundling fractions that show similar behaviour – for example, water solubility and fermentability (Van Soest, 1994) – thus reducing misjudgements of the individual fractions.

The criticism that sum parameters do not represent a chemically uniform entity is certainly justified (Hall et al., 1999; Mertens, 2003), as is the fact that the individual carbohydrate fractions are digestible to different degrees and provide distinct physicochemical behaviours. However, even the individually defined carbohydrate fractions often have different chemical structures, as noted for pectin in section 3.2.2, namely its different forms such as homogalacturon, rhamnogalacutron I and others. Grouping without taking the structure into account leads to some fractions being classified as digestible or indigestible, even though some of them do not dissolve due to their DP and cannot be digested in the posterior small intestine

or caecum, or vice versa. In addition to the individual structure, the position in the grain (endosperm, aleurone), the cell wall structure and the interaction with other fractions influence these properties (Glitsø et al., 1999; Bach Knudsen et al., 2023). The joint project aimed to represent the positive properties of the individual carbohydrate fractions and to utilise the respective advantages in the long term. Fructan was a focus of special attention because it – more precisely, inulin – promotes the growth of butyrate-producing bifidobacteria in the large intestine. Increased butyrate production has a particularly positive effect on colonocytes and health (Roberfroid, 2005). However, this positive property is not only shown by fructan. Bach Knudsen et al., 1993; 2005) demonstrated that arabinoxylan but not ß-glucan increases the short-chain fatty acid (SCFA) pattern in favour of butyrate. This finding nevertheless shows that individual performance cannot be attributed to a single carbohydrate fraction and that sum parameters are therefore suitable. However, it must be reemphasised that TDF cannot provide an accurate understanding of its effects on the digestive physiology of pigs (Wenk, 2001). A subdivision into the fractions designated as soluble and insoluble is necessary for interpretation regarding health.

Despite the combination of different analyses foreseen in the approach, difference calculations would need to be performed in order to obtain all fractions. Based on general assumptions about the analysis of dietary fibre or carbohydrates (Figure 1) and the complete determination of the specific fraction, the approach for the calculations in Table 2 was developed in relation to potentially applicable enzymatic methods, which were then selected for this study. The scheme in Figure 1 only represents highly simplified assumptions that are influenced by the respective method, the specific fraction, its structure and DP as well as the component itself. Clear separations as in this and many other overviews do not correspond to reality, as the boundaries are fluid. Table 2 also includes general assumptions regarding how to convert different fractions into one another. This approach is justified by the definition of dietary fibre by CODEX Alimentarius as “carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans” (FAO/WHO, 2021). Other compounds that are associated with these polymers, such as lignin, are also included. Whether oligosaccharides, carbohydrates with 3–9 monomeric units, are included within this definition is determined by the national authorities. In the European context, the oligosaccharides are included so that dietary “fibre” is defined as “carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine” (EC, 2008). This latter definition also applies to the present thesis with the exception that it refers to pig digestion, which is very similar to human digestion.

The approach of Bach Knudsen (1997) and his predecessors (Theander and Åman, 1979; Englyst et al., 1982) relates to the chemical part of the definition of dietary fibre (Mertens, 2003). Thus, after degradation of starch and extraction of sugars, the remaining indigestible carbohydrates (NSP) are broken down into their monomers and are then analysed using gas chromatography. In addition, lignin is analysed as Klason lignin. NSP and Klason lignin are added together to obtain TDF. The approaches known as the AOAC methods, based on Prosky et al. (1985) and its further developments, are based on the physiological part of the definition. This analysis attempts to capture everything that is not digested by mammalian enzymes in the small intestine, with the exception of protein and ash (Mertens, 2003). A more detailed description of these analyses follows.

As already explained in the discussion of the analyses, the comparability among the AOAC dietary fibre methods is reduced because they continue to be developed. This phenomenon has also led to overlap among the terms. The two aforementioned approaches have their justification and clearly show that analyses only try to represent the "truth" as much as possible. Despite many similarities in the various enzymatic gravimetric and enzymatic chemical approaches, the respective analyses show differences, which Bach Knudsen and Glitsø (1997) examined, but they provide comparable results for TDF. However, one must be aware of what one is comparing as TDF. Lignin, for instance, was recorded separately in the NSP method and in the specific case analysed as Klason lignin, which includes not only lignin but a considerable amount of Maillard reaction products, heat treatment products, tannins and others by sulphuric acid–solubilised compounds (Theander, 1987; Bach Knudsen, 1997). These substances may also be present in ADL, but at lower concentrations, as the sample is pre-treated with diethyl ether, ethanol and hydrochloric acid during ADL extraction before being hydrolysed with 72% sulphuric acid (VDLUFA, 2012). This is why the ADL results are smaller than those of Klason lignin (Tables 7 and 8). Therefore, summing NSP and ADL would prevent fibre-associated compounds, as part of the physiological definition, from being included in TDF, although NSP and lignin are added together. Consequently, these conversions often lead to incorrect estimates that are carried forward by the quotations.

Another term that remains problematic is solubility. It should always be stated in "what" a fraction the compound is soluble. The type of solvent (water, ethanol), the respective concentration and the temperature should be specified. While differences between the methods are sometimes only marginal, the resulting differences have not yet been investigated due to the complexity caused by differences in the composition of the sample as well as their structure.

Because the conventional method evaluated soluble fibre after ethanol precipitation, deviating from the defined process is problematic in terms of comparability. In the NSP method, the more precise terms water extractable and water unextractable have sometimes been used instead of soluble and insoluble (Hansen et al., 2003). In the latest AOAC dietary fibre methods, soluble dietary fibre is divided into SDFP and SDFS based on ethanol precipitation, which again underlines how strongly the concept of TDF depends on the method regarding the terms used (McCleary, 2018).

The number of publications and the approaches to analyse dietary fibres is immense and the author is currently not aware whether a comparison of AOAC 2011.25 with the NSP method (Bach Knudsen, 1997) exists – although the author doubts it exists. The reason for this assumption is that the acceptance and application of the NSP method (Bach Knudsen, 1997) is due to its complexity, lack of further development and its European origin, meaning that it is much more limited than the AOAC methods. However, even indirect approaches are used in which dietary fibre is estimated by subtracting moisture, protein, ash, lipids, available starch and free sugars from the initial sample weight. While this approach facilitates the determination (Nyström et al., 2008), the cumulative error of the individual analyses may affect the results. Besides, the differentiation between soluble and insoluble fractions is lacking in such an approach.

The use of indirect determinations again underlines that simplification is an important aspect, which is why the EIRomcp approach was used as an alternative approach to determine the dietary fibre fractions within this project. In Germany, it is now standard to use aNDFom in feed formulation. Based on relevance and reproducibility, Mertens (2003) rated aNDFom as a reasonable choice to measure IDF. Errors in the complete coverage of IDF using aNDFom are known due to the partial dissolution of pectin and lignin in the neutral detergent solution. Lignin can be dissolved in the presence of sulphite, which was used in the analysis of anterior aNDFom, although sulphite is eliminated to counteract this phenomenon; thus, only pectin is dissolved (Van Soest, 1994). Furthermore, aNDFom is not usually corrected for protein, but it was in this experiment (aNDFomcp). Mertens (2003) considered this correction to be disadvantageous, as it has only a small influence on the result but requires considerable additional effort. If the focus is only on fibre, then this approach may be correct, but in modern agriculture, resource efficiency as well as the reduction in excretions, such as nitrogen, which may be present in fibre-bound form, is crucial. In this respect, recording NDICP results in more

accurate recording and increases comparability with other dietary fibre analytical methods through the use of aNDFomcp, so the effort is worthwhile.

### 5.2. Discussion of the results

The composition of the pig feeds, apart from the gradual substitution of cereal grains (trial 1) or protein meal (trial 2), was isoenergetic, isonitrogenous and structured. Lignocellulose, potato starch and rolled barley were used for this purpose. The influence of barley, known to have a high content of dietary fibre and especially insoluble arabinoxylans (Bach Knudsen, 1997; Rodehutscord et al., 2016), was evident in the analysed fibre fractions. In trial 1, the amount of barley increased the results by the same amount, so the changes in the trial can be attributed to the respective substitution of rye and wheat. In trial 2, however, the amount of barley decreased from pig feed 5 to 8. This decrease was reflected in the constant aNDFom and hemicellulose values, as the NDFom contents and the proportion in the RSM ration were higher than that of SBM (DLG, 2014). The latter should have led to an increase in the contents, but it was balanced by the barley. The increase in ADL from pig feed 5 to 8 was mitigated by the addition of lignocellulose, which explained the higher values of trial 2 compared with trial 1, but the higher lignin content of RSM compared with SBM (Bach Knudsen, 1997; DLG, 2014) is still evident. The EIRomcp content in the feed was variable across all pig feeds in trials 1 and 2 and is not informative due to the influence of starch and aNDFom. In trial 1, NDSF showed an increasing trend due to the higher fructan and ß-glucan contents of rye compared with wheat (Bach Knudsen, 1997; Rodehutscord et al., 2016). In trial 2, NDSF also increased, which was unexpected as the proportion in the ration of barley and SBM decreased. SBM contains more soluble NSP than RSM, while barley has similar contents (Bach Knudsen, 1997). A possible explanation could be that pectins, present at higher concentrations in RSM compared with the aforementioned feedstuffs (Bach Knudsen, 1997), are removed from aNDFom due to the lack of covalent bonding and thus remain part of NDSF (Van Soest, 1994). Because the difference between pig feed 5 and 8 is high – 75 g/kg DM – it can be assumed that this fraction contains additional fibre-associated substances such as tannins, Maillard reaction products and phytate, but their amounts were not monitored. An analysis of the raw components would be helpful to interpret the results. As this information was not available, it should be emphasised that the interpretations regarding the compound feeds are speculations and not definitive conclusions.

The trial 1 results for the dietary fibre fractions (HMWDF, IDF and SDFP) showed, as expected, an increase as the amount of rye increased. Rye is generally richer in dietary fibre than wheat – a characteristic that is one of its best nutritional features (Bach Knudsen, 1997; Rodehutscord

et al., 2016; Kamphues et al., 2019). The higher SDFP slope was due to the almost twice as high SDFP content in rye compared with wheat, whereas IDF was only around 30% higher in rye compared with wheat (McGhee and Stein, 2018; McGhee and Stein, 2020). The increase in IDF and SDFP is logically reflected by HMWDF (TDF), which is calculated as the sum of both in the chosen approach.

For the fractions analysed in the feed and the calculated NSP content, the results reported by Wilke (2020) (Table 7) were used for comparison. As already briefly stated, due to differences in the analysis, these results are comparable but not identical. Because NSP is derived from HMWDF in this case, the slope between pig feeds 1 and 4 is logical. However the values published by Wilke (2020) are lower than the results obtained at the University of Bonn. One possible explanation regarding the soluble parts may be that ß-glucan and fructan were not detected in this specific NSP analysis, as Wilke (2020) did not declare additional separate enzymatic analyses. Therefore, the difference between the dietary fibre results from the University of Bonn and the University of Hannover of pig feed 1, which contains only wheat, is smaller than that of pig feed 4, which contains more SDF-rich rye. This difference again illustrates the problems that arise when different methods represent the same fractions and imprecise terms are used. However, the use of this value in this particular case will not fully represent the strengths of rye regarding its dietary fibre fractions.

Because SDFP (gravimetric, precipitation: 76% ethanol in the final solution), soluble NSP (HPLC, precipitation: 80% ethanol in the final solution) and NDSF (gravimetric, precipitation: 80% ethanol in the final solution) are all supposed to represent the rapidly fermentable fibres, their differences were directly compared in the extreme pig feed variants, namely 1 and 4. Pig feed 1 had the lowest SDFP value, which may also be due to the described weaknesses of the enzymatic gravimetric methods at low content levels. However, the low content is reasonable considering the low SDF values of SBM, barley and wheat when using AOAC 991.43 (McGhee and Stein, 2020; Lopez et al., 2020). Moreover, when using a lower ethanol concentration, less precipitate and consequently SDFP is obtained. NSP was in the middle range at 35 g/kg DM and NDSF was higher. The difference despite the same ethanol concentration when precipitating LMWDF results from fibre-associated substances, which are determined in the gravimetric method, but not when measuring monomers using HPLC. In addition, substances such as pectin, which are soluble in neutral detergent solution, are part of the NDSF. The slope from pig feed 1 to 4 was highest for the SDFP method, which may again be due to more fibre-associated compounds being captured with the gravimetric method compared with the NSP

method. The difference between the NDSF and SDFP methods could be due to the different ethanol concentration and treatment of starch in the methods (SDFP and soluble NSP: dissolution by amylase and amyloglucosidase; NDSF: almost complete dissolution by neutral detergent solution and deduction of separately analysed enzymatic starch), which might not be insignificant when using about 5% potato starch as in pig feeds 1–4. The extreme variants of trial 2 for pig feeds 5 and 8 are not discussed further, as the feed composition differed in too many fibre-relevant variables (barley, lignocellulose, SBM and RSM).

Given that no markers were used in the feed conception and that feed restriction was used before sectioning to answer other research questions, the samples, although completely collected, were not suitable to convert the measurable concentrations into quantitative data. This should be done in subsequent approaches to assess individual digestibility, which is necessary to evaluate the effect on animal health and well-being. It would also be interesting to examine the colon and lower ileum, which unfortunately was not possible in this study due to errors in sample handling and low sample volumes. All these factors are important to understand to what extent the soluble and insoluble fibres are fermented and to allow a holistic interpretation of rye and RSM diets. Due to the great effort and the failed intention, the focus was only on the digesta samples from trial 1. When determining the digestibility of the individual sections in the future, there should also be a focus on the influence of endogenous losses in the form of mucins of the gastrointestinal tract in order to avoid possible misinterpretations (Lien et al., 2001).

The number of individual samples that could be used was too small to be able to assess the extent to which pooled samples are necessary or whether individual samples could also be representative. However, the CV was very different regardless of the number of samples used, which indicates outliers due to large individual animal differences. The extent to which digestibility gives a different picture needs to be clarified elsewhere. Nevertheless, pooled samples are more suitable in such a case, given the small sample quantities.

The results obtained indicate that the fibre fraction, with few exceptions, increases along the digestive tract, which is logical due to the digestion of components, especially sugars, starches, proteins and fats. The fibre fractions, which by definition are not digested by endogenous enzymes within the small intestine, accumulate relatively more highly in the digesta. To what extent absolute changes occurred could not be determined due to the aforementioned reasons. However, it is remarkable that the fibre-bound proteins in form of NDICP and some ADICP seem to be partly digested in the CSI, which also indicates that absolute parts of the aNDFom fraction are degraded, but proportionally not noticeable. This can possibly be attributed to

soluble arabinoxylans, whose degradation may leave proteins more accessible for degradation. However, an apparent ileal digestibility of NDF of about 20% is known (Wenk, 2001). In the case of ADICP, the drastic reduction in pig feeds 1 and 4 was due to an error caused by the different number of available individual samples and their representativeness. The same might explain the lower enrichment of aNDFom in the caecal digesta of pig feeds 3 and 4 compared with pig feeds 1 and 2, as those contain more rye and, consequently, more arabinoxylan, especially in the soluble form, compared with wheat. This difference in dietary fibre fractions may be due to the difference of more soluble substances in the NDF or IDF solutions.

Because the concentration and ratio of SDFP act oppositely in the CSI digesta, it can be assumed that part of the SDFP is already digested in the small intestine. This eventuality is in line with results of Jaworski and Stein (2017), who also showed that soluble fibre is mainly fermented in the small intestine and caecum. This breakdown of SDF occurs because the posterior small intestine is already colonised by microorganisms (Wenk, 2001). In the caecum, the concentration in all rations fell to about the same low level, which is possibly related to the respective accessibility of the substrate and the composition of the soluble fibre. The disappearance may be erratic in this case, as it depends on the low starting level in the feed containing primary wheat.

Pig feed 2 in the CSI showed significant downward deviations compared with the other pig feeds, which may indicate that the fibre components of pig feed 2 were already more intensively digested in the small intestine. However, this variation cannot be explained by the components of the feed analysis, which showed no unexpected differences. Consequently, there must be a physiological difference, but this cannot be explained without analysing digestibility and/or non-fibre-related feed components. The ratios presented in Table 11 clearly show that the soluble fibres in the rye-containing rations accounted for a higher proportion and were rapidly fermented. The ADFomcp:aNDFomcp ratio showed that the proportion of lignified or cellulosic structures in the rye containing rations in the CSI and caecal digesta was significantly higher than in those containing wheat, but this also indicates a higher proportion of fermented components in aNDFomcp of rye-containing rations. The methodological comparison of IDF and aNDFomcp demonstrated that these fractions can be used analogously in feed if the pectin content does not deviate too much and in the CSI before these fractions are digested. However, the proportion in the caecum is no longer comparable because of the different substances released in NDF and IDF.

The informative value of the data obtained is lower than that of the methodological suggestions. However, given the higher butyrate concentration especially at the end of the small intestine and the caecum that has been reported (Gdala et al., 1997; Wilke, 2020), the rapidly fermentable fibres (SDFP and NDSF), which were especially higher in rye, could be responsible for this outcome. Rapid fermentation has two main advantages. First, the pH is lowered, which has a negative effect on some pathogenic bacteria (Bederska-Łojewska et al., 2017), as has been demonstrated with higher amounts of rye resulting in a reduction of *Salmonella* shedding (Chuppava et al., 2020) and benefits bifidobacteria (Roberfroid, 2005). Second, the resulting SCFA are available for absorption and, subsequently, various metabolic processes. The SCFA of the entire fermentation contribute to the energy supply (Bach Knudsen et al., 2023). In work by the Joint Project, a rye-rich diet compared with wheat did not show any change in phyla (Ellner, 2022).

### 5.3. Comparison of alternatives

This section briefly explains the three methods that were most frequently referred to in the course of the project.

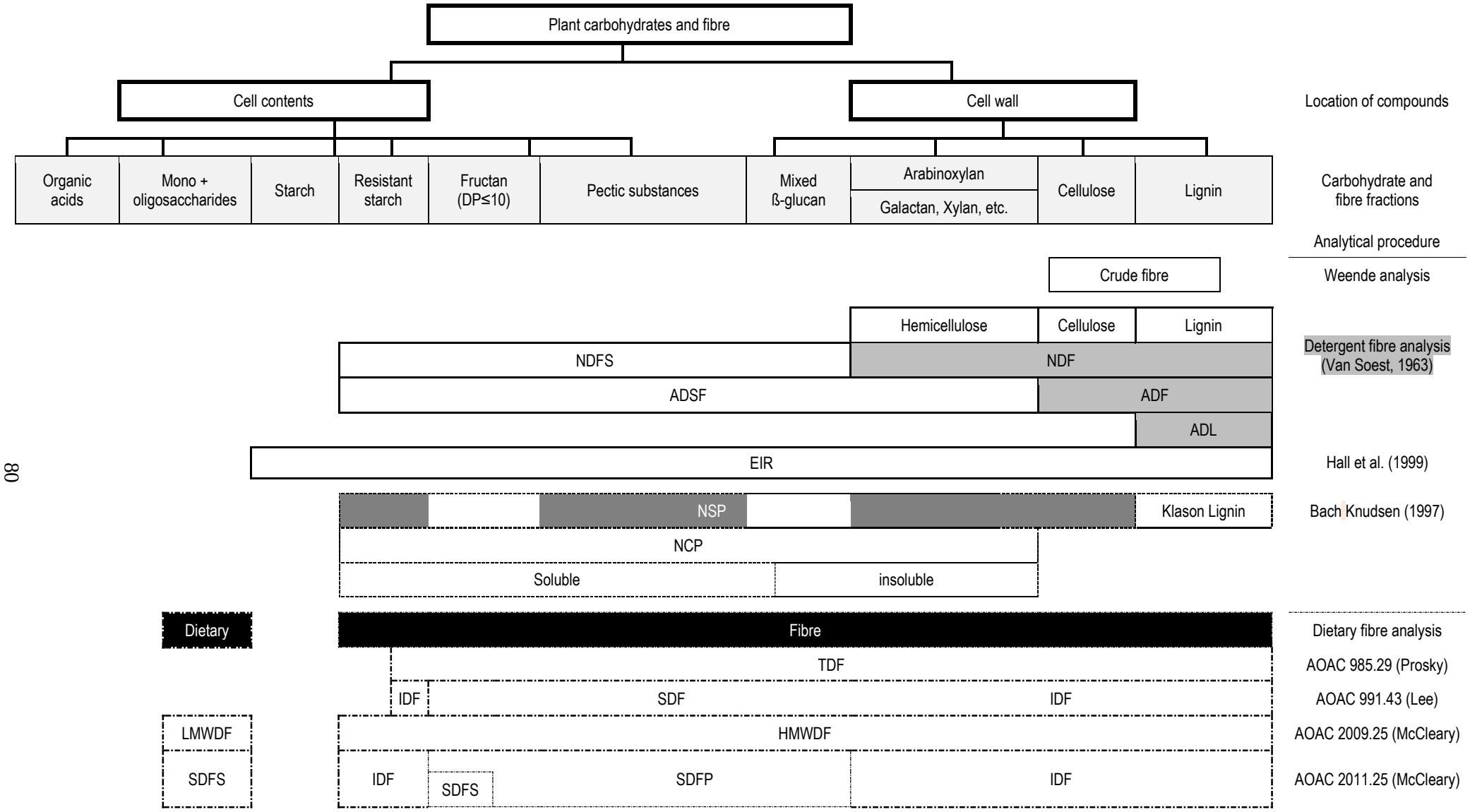
The NSP analysis described by Bach Knudsen (1997) is based on Englyst et al. (1982) and the Uppsala method (Theander and Åman, 1979; 1982; Theander and Westerlund, 1986). Total, soluble and insoluble NSP as well as their constituent sugars, with an additional colourimetric method for uronic acids and lignin (in form of Klason Lignin), are determined using GLC. This method employs three parallel samples utilising different yet complementary procedures (A, B and C), all starting with amylase and amyloglucosidase treatment to remove all sugars and starch. In procedures A and B, soluble NSP were precipitated for 1 h by using four volumes of 99% v/v ethanol (resulting in an end concentration of 80% ethanol) on ice and in procedure C, soluble NSP were removed by boiling the solution in a sodium phosphate buffer. The supernatants were discarded after centrifugation and the residues washed with 85% v/v ethanol and acetone. The dried residues were treated with sulphuric acid to hydrolyse insoluble NSP. In procedures A and C, 12 M $H_2SO_4$ was used to degrade cellulose to its monosaccharides, and in procedure B, only 2 M $H_2SO_4$ was used – and thus the associated cellulose microfibrils were not affected. The solutions of all procedures were filtered. Subsamples for uronic acid analysis were taken and the remaining sample solutions were prepared for gas chromatography by adding an internal standard in form of D-allose. Aldehydes were reduced to alcohols, which were then acetylated and analysed. The three processes differ slightly: procedures A and B differ in the content of glucose released by the hydrolysis of cellulose. Procedures A and C

differ in the content of water-soluble NSP, so the difference calculation for the amount of cellulose; soluble NSP; and total, insoluble and soluble non-cellulosic polysaccharides (NCP) can be determined (Englyst et al., 1982). Uronic acids were analysed according to Scott (1979). Specifically, the samples were pre-treated with $H_3BO_4$ before incubation in $H_2SO_4$ at 70°C for 10 min. Subsequently, the samples were cooled to room temperature and 3,5-dimethylphenol was added as a colourimetric reagent, which selectively forms a chromagen with uronic acids. Absorbance was measured at 400 nm and 10–15 min later at 450 nm, an approach that is necessary due to interference with neutral sugars and lignin (Scott, 1979). To represent all carbohydrate fractions, ß-glucan and fructan are often also analysed, but caution is advised, as this is not always the case when procedures based on Bach Knudsen (1997) are carried out. ß-Glucan was analysed using the McCleary and Glennie-Holmes (1985) method and fructan was evaluated enzymatically after extraction with acetate buffer and further hydrolysis with sulphuric acid. The determined total fructose was corrected by the factor 0.92 (Bach Knudsen, 1997).

Prosky et al. (1985) elaborated the first enzymatic gravimetric procedure that was accepted as a reference method by AOAC to measure dietary fibre – the so-called Prosky method (AOAC 985.29). This method analyses homogenised dry samples using heat-stable α-amylase (thermamyl), protease and amyloglucosidase to degrade substances digested *in vivo* within the small intestine, followed by ethanol precipitation using four volumes of 95% v/v ethanol and gravimetric measurement of the residues with correction for ash and protein. In the original procedure, the corrected residue was declared as TDF (Prosky et al., 1985), but there was criticism due to methodical modifications, so the analysed fraction should be correctly designated as HMWDF (McCleary et al., 2013). AOAC 991.43 is an extension of the Prosky method in which the steps for determining TDF/HMWDF were divided so that SDF and IDF are analysed – the so-called Lee method. HMWDF is then determined as the sum of the two (Prosky et al., 1988). The dividing step is particularly important as these fibre fractions maintain different physiological and nutritive function. Further modification, such as changing the buffer from a phosphate to a MES-TRIS buffer and thereby increasing the pH to 8.2, improve precision and reduce analysis time. Significant methodological changes occurred with the development of AOAC 2009.11 and 2011.25, the McCleary method partly used in this study, which started to include resistant starch as well as NDO, the latter of which may also be referred to as LMWDF.

This method uses 76% aqueous ethanol to precipitate the HMWDF and LMWDF fractions separately. The assumption that this ethanol concentration separates saccharides with a DP of 9, which by definition are oligosaccharides, from those with a DP of ≥ 10, which belong to the polysaccharides, is wrong. This fact is one reason why new terms were introduced: SDFP and SDFS (McCleary et al., 2013). SDFS is determined within the filtrate of SDFP by HPLC. This new development also made it clear that some of the defined dietary fibres were not covered by the previous analysis and thus the terms used were inaccurate (McCleary et al., 2013). In addition, thermamyl was replaced by incubating pancreatic α-amylases together with amyloglucosidase under physiological temperature and pH to obtain more accurate results. As with AOAC 985.29 or its extensions, resistant starch is underestimated as it dissolves at 60–70°C (McCleary et al., 2013). As a result, the incubation is significantly extended to 16 h. This starch digestion step is stopped by adding a buffer that raised the pH to 8.2 inactivating amyloglucosidase and a heating process inactivating pancreatic α-amylase. The latter also ensures necessary denaturation of proteins before their hydrolysis. By integrating all fibre fractions within one analysis, the risk of double counting by summing two different methods is eliminated (McCleary et al., 2013). The authors acknowledged overestimation due to production of resistant maltodextrin though hydrolysis starch and underestimation of fructosyl-β-(2-1)-fructosyl-β-(2-1)-fructose (inulinotriose) in the analysis.

The latest AOAC method, 2017.16, differs from 2009.11 in that the incubation time is only 4 h, which better reflects the natural retention time of digesta in the small intestine and obviates the need to use hazardous sodium azide. In turn, the enzyme concentration is increased, which also prevents the formation of resistant maltodextrin. The underestimation of inulinotriose has been solved by using different HPLC columns (TOSOH TSKgel® G2500PWXL gel permeation column instead of Waters Sugar-Pak® columns) (McCleary, 2018). The latter improvement had no consequences for the analyses and samples carried out in this study. A closer look reveals many similarities and differences between these approaches. Bach Knudsen (1997) and Hall et al. (1999) used 80% ethanol to precipitate low-molecular-weight sugars and 78% or 76% ethanol for dietary fibre analysis. For the dietary fibre analysis, Hall et al. (1999) and the AOAC methods used relatively easy gravimetrically approaches, one chemical and one enzymatic, while Bach Knudsen (1997) used GLC. The approach of Bach Knudsen (1997) can only give a complete overview of all carbohydrate fractions if the extended procedure with ß-glucan and fructan analysis is used. It should be emphasised again that monomers are obtained with GLC and that a derivation of the respective fractions from monomers does not do sufficient justice to the often heterogeneous structures and can lead to




DP = degree of polymerisation; NDSF = neutral detergent soluble fibre; NDF = neutral detergent fibre; ADSF = acid detergent soluble fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; EIR = ethanol-insoluble residue; NSP = non-starch polysaccharides; NCP = non cellulosic polysaccharides; TDF = total dietary fibre; IDF = insoluble dietary fibre; SDF = soluble dietary fibre; LMWDF = low-molecular weight DF; HMWSDF = high-molecular weight soluble DF; SDFP = water-soluble but ethanol-precipitated dietary fibre; SDFS = water- and ethanol-soluble dietary fibre.

**Figure 1:** Scheme of carbohydrate fraction and fibre analytical methods. Based on Hall (2003), Kehraus and Schiborra (2022) and Bach Knudsen et al. (2023)

misinterpretations, although they cannot yet be assessed. This problem should have been avoided in the approach of the present study, but unfortunately this eventuality could not be assessed. The question as to whether such a complex feed evaluation has added value should be raised and the advantages resulting from sum parameters should be considered. The disadvantages and advantages of the three methods are presented in Table 12. Hall's approach is simple and fundamentally promising because it follows the generally approved methods currently used in Germany. However, it still needs further research. The dietary fibre approach is widely accepted in research, but it lacks comparability due to its ongoing development.

## 6. Conclusion

The original goals of this study – to achieve a cost-effective and simple analysis of the individual carbohydrate fractions in different intestinal sections and to determine their disappearance in the sections to make specific and precise statements about the substances available for fermentation and their effect on animal health – were not possible due to a high potential for error for the enzymatic photometric analysis. Nevertheless, this work demonstrates once again that the many differences in analytics exist and that only slightly distinct terms could quickly be equated, although a deep understanding of the individual methods is necessary to ensure comparability. The sum parameters used showed that the differences are mainly due to the soluble fibre fraction, as the insoluble fractions in the feed hardly showed any differences in terms of quantity. The concentration of SDFP largely disappeared until the caecum, which represents a great part of the many positive and health-promoting aspects of rye.

In principle, there is a need for legal German feed analysis to examine which methods are suitable to assess the relevant aspects of (feed) dietary fibre for pigs and other monogastric animals, in order to establish contemporary and profitable fibre-based methods that include both IDF and SDF. This information would allow more accurate recording of nutrients while promoting animal welfare.

**Table 12:** Overview the of advantages and disadvantages of alternative approaches to fibre and carbohydrate analysis

| | Advantages | Disadvantages |
|---|---|---|
| Total (integrated) dietary fibre analysis (AOAC 985.29, etc.) | – Simple, cost-effective, moderate workload<br>– Enzymatic approach, simulating natural digestion<br>– Tailored to the definition of dietary fibre<br>– High acceptance in the scientific environment<br>– NSP and lignin are analysed together<br>– Experienced technicians can obtain reproducible results | – Lack of comparability due to constant development<br>– No information on specific carbohydrate fraction<br>– NSP and lignin are analysed together<br>– Mucopolysaccharides in digesta sample may interfere |
| EIR/NDSF Hall et al. (1999) | – Low workload<br>– No special equipment required<br>– Feed declaration includes most of the required fractions<br>– Simple sum parameter<br>– No enzymatic method | – Not yet introduced for monogastric animals<br>– Evaluation still needed<br>– Does not simulate natural digestion |
| NSP (without extension) | – Individual fractions are displayed<br>– Very routinely mainly chemical process<br>– NSP and lignin are analysed separately | – Expensive equipment<br>– Trained laboratory personnel required for reliable results<br>– High workload<br>– Fractions are generated based on estimates |

Modified following Bach Knudsen (2019). EIR = ethanol-insoluble residue; NDSF = neutral detergent soluble fibre; NSP = non-starch polysaccharides

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# CHAPTER 5

# General discussion and conclusion

This chapter serves to enhance the findings presented in chapters 3 and 4. It presents common scientific methods for the *in vivo* determination of phosphorus (P) digestibility as a supplement to chapter 3 to clarify the respective terminology and thus increase comparability. Regarding chapter 4, this discussion addresses complementary aspects of dietary fibre that should be considered in future approaches or should be considered when interpreting fibre. A critical evaluation of the study design conducted and considerations for the future are outlined. Finally, the feeding value of rye and rapeseed meal (RSM) in pig feeding is outlined, particularly on the basis of the results of this work and the findings of the holistic approach (6-R Project), in order to summarise the re-evaluation of rye and RSM in pig feeding.

## 1. Phosphorus digestibility

P is a vital mineral that is necessary for many biological functions of the body – bone metabolism, energy metabolism and much more – but its excessive excretion harms the environment, especially water bodies. Therefore, methods for determining P digestibility are of particular importance. A number of different approaches exist, some of which vary just slightly, and some of them are briefly described below. The apparent total tract digestibility (ATTD) of P is calculated as follows (NRC, 2012):

$$ATTD\ of\ P\ in\ \% = \frac{\mathrm{P_{intake}} - \mathrm{P_{output}}}{\mathrm{P_{intake}}} * 100$$

$P_{output}$ is the faecal output. This method is easily applicable and can be carried out by using markers without metabolic crates. To determine the digestibility of specific components, the direct and difference methods are common approaches. Using the direct method, the added component is the only source of the nutrient to be tested. The method is simple, but it is only suitable for one nutrient within a feed component, as the test rations must be free of this nutrient, which cannot always be achieved. An alternative is the difference method, which includes substitution and regression methods, where the component to be tested is used together with a defined basal ration. In the substitution method, the component to be tested is fed either on top of or as a substitution for a defined proportion of the basal ration and the digestibility of the component used is determined by the difference in the digestibility relative to the basal ration. In the regression method, in addition to a basal ration, at least two test rations are sampled in

which the component to be tested is exchanged for the respective basal ration. These exchanged proportions differ between the test rations. The differences in digestibility obtained from the test rations are used to extrapolate the sole use (100%) of the component to be tested, thus calculating its digestibility (Zhang and Adeola, 2017). The simplicity of the experimental procedure (*in vivo*) and the determination justifies the use of the ATTD of P. However, one must be aware that this approach ignores endogenous losses and their origins; hence, the digestibility may be underestimated (Zhang and Adeola, 2017; Fan et al., 2001). Therefore, if possible, the true total tract digestibility (TTTD) should be used, which is determined by subtracting the endogenous P loss (EPL).

$$TTTD\ of\ P\ in\ \% = \frac{\mathrm{P_{intake}} - (\mathrm{P_{output}} - \mathrm{EPL})}{\mathrm{P_{intake}}} * 100$$

EPL may result from saliva, gastric and biliary juice; pancreatic and intestinal secretion; as well as sloughed mucosal cells (Fan et al., 2001). EPL can be further divided into basal and specific endogenous losses. Basal endogenous losses ($EPL_{basal}$) are those that do not depend on quality and quantity of the nutrient (here P), but depend on dry matter intake (DMI). Specific endogenous losses ($EPL_{specific}$) are those that depend on the quality and quantity of the nutrient as well as other feed-related factors such as the fibre content and antinutritive substances (McDonald et al., 2011; Alves et al., 2016). A different method had been implemented to determine the TTTD of P as the use of P-free feed, which within a few days leads to detrimental effects on the physiological status of the animals, or the tracer technique with $^{32}$P-labelled phosphate, which is not appropriate in terms of safe handling of radioactive substances (Fan et al., 2001; Petersen and Stein, 2006). A simpler method to determine the TTTD of P and EPL is linear regression analysis. In such an approach, linearity between feed intake and (faecal or ileal) excretion is assumed; consequently, the P flux is expressed in g/kg DMI. To determine EPL, feed intake is extrapolated towards zero (Fan et al., 2001). However, caution is needed as only $EPL_{basal}$ is measured with those approaches. An accurate determination of $EPL_{specific}$ is difficult and there are no reliable routine methods available yet, which is why the standardised total tract digestibility (STTD) was introduced and acquired by NRC (2012). The STTD (%) of P is now calculated as:

$$STTD\ of\ P\ in\ \% = \frac{\mathrm{P_{intake}} - (\mathrm{P_{output}} - EPL_{basal})}{\mathrm{P_{intake}}} * 100$$

The TTTD will result in higher values compared with the STTD because it also captures feed-dependent $EPL_{specific}$ (Almeida and Stein, 2010). In addition, $EPL_{basal}$ is now assumed to be 190

mg/kg DMI. With this estimated $EPL_{basal}$ content, the STTD of P can easily be calculated by determining the ATTD of P without the need to include a P-free diet in each experimental set-up (NRC, 2012). This is a great advance regarding animal welfare, but $EPL_{basal}$ should be directly determined in sensible control intervals.

The method used in this research to measure the P digestibility of the total tract according to the instructions of GfE (1994) is a balance or difference trial. This method is only used in a scientific setting to determine P digestibility, as the animals must be supplied with suboptimal P during the trial and a balance trial with separate faeces and urine collection requires great effort. Moreover, it is currently difficult to conduct such an animal study in Germany because the veterinary authorities classify the stress level of the animals as medium and corresponding authorisation is required, which is not readily granted. The total tract digestibility determined in the applied study (chapter 3) is considered to be close to the true and standardised digestibility, as the animals are fed a basal ration low in P, resulting in a suboptimal supply of the nutrient in each test ration, so regulatory excretion is not expected (GfE, 1994). By subtracting the basal ration proportionally from the test ration, it is assumed that $EPL_{basal}$ is already corrected in the determined P digestibility of the test component, as well as $EPL_{specific}$ of the basal ration. This is the main difference from the STTD method. Consequently, the determined P digestibility still includes $EPL_{specific}$ induced by the test component, which is not part of the TTTD. Using the suboptimal rather than the P-free ration approach is in the best interest of animal welfare, as the test animals are not exposed to such severe P malnutrition and its consequences.

## 2. Dietary fibre related aspects

### 2.1. Dietary fibre associated compounds or phytochemicals

The definition of dietary fibre or "fibre" (EC, 2008) refers to "carbohydrate polymers with three or more monomer units that are neither digested nor absorbed in the 'human' small intestine". This short definition is modified in the Directive by the inclusion of substances closely associated to carbohydrate polymers in plants, such as lignin, and a great variety of other substances: "phenolic compounds, waxes, saponins, phytates, cutin, phytosterols" (EC, 2008). The wide variety of compounds that can fall under this definition is important, because these substances can vary greatly in type and quantity depending on their plant origin. Some of the substances are present only in small amounts but can have large effects, a phenomenon that is particularly true of phytochemicals. Furthermore, the substances detected in the analytical

methods vary from method to method, as explained in chapter 4 – for example, example, the type of determination (gravimetric, photometric and HPLC), the type of solvent, duration and filtration, among others – so these components may not be quantified within dietary fibre analysis. Figure 1 shows which components enter the large intestine together with carbohydrates and are ready for fermentation. The entire content of this fraction may, based on the definition, be part of dietary fibre, but not exclusively, as they may enter the large intestine without attachment to fibre components. The different metabolites resulting from microbial fermentation are thought to have various positive health effects; for example, lignans are thought to act as antioxidants (Hu et al., 2007). However, some substances, especially nitrogen (N)-containing compounds, also show negative effects, such as the release of ammonium in the course of deamination (Bach Knudsen et al., 2017; Diether and Willing, 2019). The majority of knowledge about phytochemicals originates from the field of human nutrition and medicine. Because the pig is often used as a model animal for humans, these observations are also transferable to the pig. Rye is known to contain alkylresorcinol (Landberg et al., 2014), lignans (Peñalvo et al., 2014), phenolic acid (benzoic and cinnamic acid) (Aura, 2014), vitamins and phytosterol (Piironen and Lampi, 2014), which have a variety of functions: antioxidant, antimicrobial and cholesterol lowering (Nyström et al., 2008; Bach Knudsen et al., 2017). However, their value is limited due to the small quantities or the short life span of fattening pigs. In contrast to the positive aspects of these non-nutritive phytochemicals, some substances have antinutritional properties, which may have an influence on the availability or absorption of other essential macronutrients (Van Soest, 1994). In particular, RSM contains many antinutritive substances such as lignin, tannins and phytic acid. In addition to the complexing properties, some are astringent, resulting in reduced palatability and thus feed intake, which in turn may be reflected in lower performance of fed animals (Van Soest, 1994; Mejicanos et al., 2016). However, contrary to their antinutritional properties mainly affecting protein digestibility, tannins may provide some benefits: a tannin-rich chestnut extract exerted antibacterial and antidiarrhoeal effects on piglets and improved performance and feed efficiency when added to the ration (Biagia et al., 2010). Sinapine is a bitter-tasting but natural antioxidant that is abundant in rapeseed and exerts a positive effect on mitochondria by selectively reducing their oxidative stress (Boulghobra et al., 2020). Depending on the type of analysis, these antinutritive substances are detected in the dietary fibre fraction. Nevertheless, carbohydrates do not have any negative effects and are essential for the development and activity of microbes, serving as their main substrates, especially because they still arrive in relatively large amounts in the large intestine

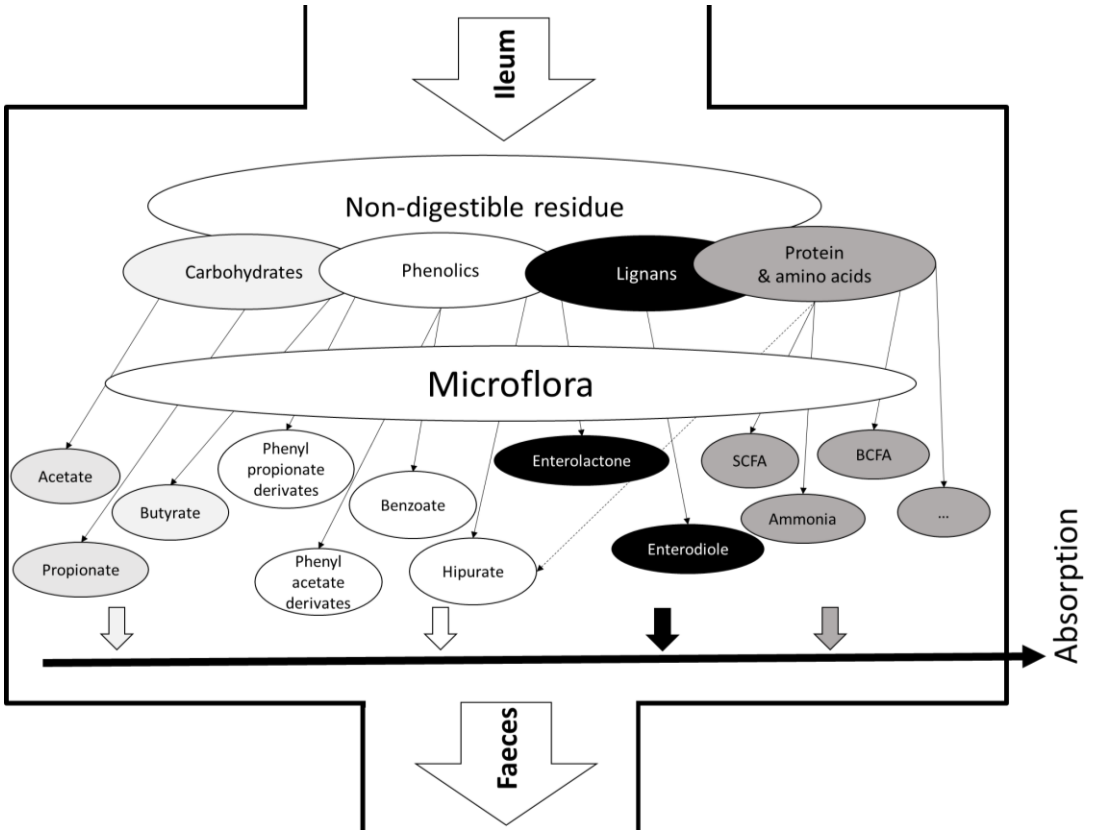


**Figure 1:** Scheme of the main colonic metabolites formed during fermentation of nondigestible carbohydrates, phenolic acids, plant lignans and proteins in the large intestine. Adapted from Bach Knudsen et al. (2017)

### 2.2. Cell wall structure

Dietary fibres in feeds consist almost entirely of cell wall components (Bach Knudsen et al., 2023). In chapter 3, it was emphasised that the structure of the respective carbohydrate fraction has a significant influence on the analysis and an effect on the fibre fraction; this also applies to the cell wall components. The composition of the respective cell wall is strongly dependent on its function in the plant, its tissue and the plant species itself (Loix et al., 2017). Compared with the primary cell wall of the plant, the secondary cell wall has a denser, organised structure with lignin. This also highlights the task of the secondary cell wall to contribute to the stability and mechanical protection of the plant (Zhong and Ye, 2015). This structure leads to a lower accessibility and thus digestibility of the individual fractions in the animal. Table 1 highlights the differences in fibre composition between rye and wheat and between whole grain, flour and bran.

**Table 1:** Dietary fibre composition of whole grain, refined flour and bran from rye and wheat in g/kg DM (Bach Knudsen et al., 2017)

| | Wheat | | | Rye | | |
|---|---|---|---|---|---|---|
| | Whole grain | Flour | Bran | Whole grain | Flour | Bran |
| Resistant starch | 3.0 | 3.0 | 2.0 | 3.0 | 3.0 | n.a. |
| NSP | | | | | | |
| Fructan | 9.0 | 6.0 | 17 | 31 | 23 | 40 |
| Cellulose | 19 | 2.0 | 114 | 13 | 5.0 | 23 |
| ß-glucan | 6.0 | 2.0 | 24 | 20 | 8.0 | 45 |
| AX | 71 | 21 | 337 | 96 | 43 | 216 |
| A/X ratio | 0.62 | 0.53 | 0.62 | 0.61 | 0.78 | 0.39 |
| Others[1] | 19 | 9.0 | 58 | 25 | 17 | 37 |
| Total NSP | 124 | 40 | 550 | 185 | 96 | 361 |
| Lignin | 15 | 0 | 72 | 17 | 2.0 | 51 |
| DF | 142 | 43 | 624 | 205 | 101 | 412 |

[1]Others represent the sum of the non-cellulosic residues galactose, mannose, uronic acid, and glucose not accounted for as ß-glucan or cellulose. NSP = non-starch polysaccharides; AX = arabinoxylan; DF = dietary fibre; n.a. = not analysed; A/X, arabinose to xylose ratio

### 2.3. Physicochemical properties

It is known that the physicochemical properties of dietary fibres influence many functions in the gastrointestinal tract positively (e.g. by improving satiety) as well as negatively (e.g. by reducing ileal digestibility) (McGhee and Stein, 2018; Slama et al., 2019). Numerous studies have been conducted on swelling and water-binding capacity, viscosity as well as buffer capacity, to name but a few, and it is known that physicochemical properties depend on the composition as well as the structure of dietary fibre components (Bach Knudsen et al., 2023; Slama et al., 2019). However, it is problematic that, as with dietary fibre, there are many approaches that often differ only in small details such as the milling, the duration of soaking or centrifugation, but which have a major effect on the final result and thus also on the comparability (Slama et al., 2019). Therefore, it is indispensable and gratifying that there are now approaches in Germany to standardise water-binding, water-holding and swelling capacity through the VDLUFA. In the long term, this would allow correlations between the analysed

feedstuffs and their physicochemical properties and rank the different fibres based on these characteristics as recommended by Slama et al. (2019).

## 3. Critical evaluation of the study design and future research implications

In the study of P digestibility and metabolisable energy (ME), compound feeds, each consisting of two individual components, were compared as one test component, and only compound feeds were used when implementing the fibre and carbohydrate analysis. Both led to a lack of direct comparisons with existing study results. With regard to P digestibility, an investigation of the individual components, especially with regard to the additivity of the components, would have been important and could have provided additional evidence for interpretations. In the context of the dietary fibre and carbohydrate analysis, the use of a known matrix in the form of common individual compounds would have been useful as a starting point for carrying out the analyses in order to gain reliability. Even though the use of a different matrix could also lead to other errors in the use of the actual matrix, the individual components, such as rye or wheat, would have been suitable as comparative variables for analysis of the feed to possibly reveal hidden errors based on comparison with other studies. Examining the individual components used would also help to identify possible interactions between them.

The analytical methods for dietary fibres determination such as neutral detergent fibre (NDF), total dietary fibre (TDF) or non-starch polysaccharides (NSP) were developed for the analysis of food or feed, but are now also being used to analyse digesta or faeces. In doing so, non-dietary interfering substances are often disregarded. Therefore, other approaches should examine whether the determination of nondietary interfering substances would be useful to improve detection of the digestibility of fibre fractions and fermentability. Different approaches for the determination, such as the use of fibre-free diets or regression models similar to those outlined in section 1. of this chapter for P digestibility, might be applicable (Montoya et al., 2016). Furthermore, digestibility is an important parameter that can only be determined if both the feed and the digesta or faeces are collected quantitatively over a certain period of time, as in the determination of P digestibility. If this is not possible in the experimental situation, a marker such as acid-insoluble ash should be used. With such a procedure, the determination of digestibility would also have been possible with the collection method in Wilke (2020). In chapter 3, which focused on P digestibility, N excretion was also briefly addressed on the basis of the N balance. Because an adequate protein supply is essential for the animal, but also the negative effects of N excretion on the environment have been known for many years, a detailed

investigation of N digestibility would be of great interest. Especially in the case of RSM, the effects of the fibres on N digestibility and the form of N excretion should be investigated.

Besides phytase, the effect of other common enzymatic feed additives should also be investigated. In practice, ß-glucosidases or xylanases are used, whose effects were not considered in this project, but they may have an influence on nutrient digestibility. The use of NSP-degrading enzymes may increase nutrient digestibility of fibre-rich ingredients (Zijlstra et al., 2010).

## 4. Feeding value

As already mentioned in the introduction, rye and RSM have rarely been used to feed pigs in the past. Therefore, the feeding value of both is now presented compared with common alternatives (wheat, maize, barley vs rye and SBM vs RSM) based on the results of the 6-R Project and other current work on this topic.

### 4.1. Performance

Alert and Fröhlich (2006) showed that rye is an interesting and underestimated feedstuff in Germany that can be used at high concentrations (70%) during the grower and finisher periods without reducing feed intake and average daily gain (ADG). They made the same observations in piglets, but with only 15% rye in the feed (Alert and Fröhlich, 2006). Within the framework of the 6-R Project, high hybrid rye inclusion levels were tested on young animals. The performance of animals fed compound feeds containing hybrid rye instead of wheat at different concentrations (48%–69%) was not affected by this substitution. The average daily feed intake (ADFI) and ADG of weaned piglets or growing pigs remained unchanged (Chuppava et al., 2020; Wilke, 2020; Ellner et al., 2021). However, even if body weight was not affected, another study showed that ADG and ADFI may be lower when wheat is replaced with rye (15.7%–65.9% rye in the diet), a phenomenon the authors attributed to the higher NSP in rye (Smit et al., 2019). Studies on the substitution and inclusion level of RSM instead of SBM showed that RSM compensates for up to 20% SBM without detrimental effects as long as the ration is balanced by the addition of energy and amino acids (AA). Nevertheless, the ATTD of energy and crude protein (CP) is also reduced by RSM, although to a small extent (Landero et al., 2011). Parr et al. (2015) showed no difference in growth performance of weaned pigs between RSM and SBM (20%–40% in the ration), but ADFI declines as the RSM content increases, a phenomenon they attributed to the lower palatability of RSM. Indeed, Landero et al. (2018) reported that pigs have a strong preference for SBM over RSM when given the choice. In a

meta-analysis, Hansen et al. (2020) showed that RSM significantly reduces ADFI in weaned piglets but has no effect on ADG and feed conversion. In fattening pigs, on the other hand, RSM leads to a small but significant reduction in ADG and feed conversion. The inclusion level of RSM in the diet of fattening pigs appears to have little or no negative effect on the ADG and feed conversion. The authors concluded that RSM in a balanced ration is a good source of protein for fattening pigs (Hansen et al., 2020). Similar results were obtained within the 6-R Project when SBM was replaced by RSM at a maximal inclusion level of 28% (Wilke, 2020) or 30% (Ellner et al., 2021) in the feeds. Moreover, Wilke (2020) found no effect of substitution, whereas Ellner et al. (2021) observed that the animals fed with RSM had a low ADFI and ADG. One reason for this difference may be that Wilke (2020) used a more balanced piglet rearing feed that was isonitrogenous and isoenergetic, whereas Ellner et al. (2021) only used an isonitrogenous feed in which the ME in feeds containing SBM was higher compared with feeds containing RSM.

### 4.2. Protein

McGhee and Stein (2018) investigated the apparent ileal digestibility (AID) and standardised ileal digestibility (SID) of CP and AA of hybrid rye and other cereal grains used to feed pigs. The term ileal digestibility is applied here because it was used in the references, which are of American origin. However, it should be noted that this term actually refers to precaecal digestibility as defined by GfE (2008). The three hybrid ryes tested had a lower CP content compared with wheat and barley, but more than maize. The results for hybrid rye and wheat are in line with the results obtained in the study described in chapter 3; however, the CP content of hybrid rye is lower than the common CP values for rye (DLG, 2014; Rodehutscord et al., 2016) and also other studies within the 6-R Project (Ellner, 2022). The AA pattern also differs among cereal grains (Table 2), so the AA pattern in particular must be adapted to the respective component used. In contrast to wheat and maize, rye is very rich in lysine, similarly to barley, which is good considering the fact that lysine is often the limiting AA in pigs. The AID and SID of CP of hybrid rye was lower compared with maize, barley and wheat due to the higher proportion of SDF, which led to a higher viscosity in rye and thus a reduced the endogenous peptidase efficiency. Although the AID and SID of AA in hybrid rye were lower than those of maize, the CP and AA concentrations were higher in rye, so it is assumed that at least maize can be replaced by hybrid rye without changing the supply of digestible AA (McGhee and Stein, 2018). However, this does not apply to wheat, which performs better in terms of quantity and digestibility of CP and AA.

The CP content of RSM is generally 36%–39% fresh matter (88% DM), although there is high-protein RSM that reach up to 47%, comparable to that of SBM, which varies from 40% and 48% CP depending on dehulling and processing (Newkirk, 2011; DLG, 2014; Berrocoso et al., 2015). The AA pattern of RSM is considered well balanced and has less lysine but more methionine and cysteine compared with SBM (Newkirk, 2011). The AA digestibility was similar to that of CP, so for RSM with 37% CP, the corresponding essential AA (lysine, methionine, threonine and tryptophan) showed an AID of 65%–82% and a SID of 71%–87% (Maison and Stein, 2014; Berrocoso et al., 2015). SBM with about 48% CP showed AID and SID of essential AA of 89%–92% and 89%–95%, respectively (Berrocoso et al., 2015). Newkirk (2011) stated that the true ileal AA digestibility for pigs is generally 10% lower compared with SBM. The lower AID of CP of rye and RSM also transfers to compound feeds, so that compound feeds containing wheat and SBM showed an AID of 81.3%, whereas rye and RSM only showed an AID of 65% (Ellner et al., 2021).

### 4.3. Energy

McGhee and Stein (2020) showed that the ME of different cereal grains was higher in maize and wheat compared with hybrid rye and similar to barley. The AID of starch was > 90% and its ATTD was close to 100% in all cereal grains, which relates the main differences in ME to the starch content of the cereal grains (McGhee and Stein, 2020). The latter underlines the importance of fibre fermentation for energy utilisation, by offering short-chain fatty acids (SCFA) to the host animal (Lancheros et al., 2023).

Although the gross energy of RSM and SBM is quite high, RSM has a significantly lower ME than SBM, which is often attributed to its high fibre content. The already mentioned protein-rich RSM varieties have a slightly lower fibre content and an increased ME up to 15.0 MJ/kg DM (Berrocoso et al., 2015). As shown in chapter 3, there was not a significant difference in the ME of wheat and rye within the compound feeds, but the ME clearly decreased with the use of RSM instead of SBM in compound feeds. This may also be the reason why Wilke (2020) reported a higher ADFI and feed efficiency.

**Table 2:** Crude protein content, digestibility and amino acid pattern of different cereal grains and protein meals

| | Crude protein | | | Amino acids | | | | |
|---|---|---|---|---|---|---|---|---|
| | | AID | SID | Lysine | Methionine | Threonine | Tryptophan | |
| | g/kg DM | % | % | g/100 g CP | | | | |
| Rye | | | | 3.59 | 1.52 | 3.23 | 1.02 | (Rodehutscord et al., 2016) |
| | 98.8–103 | 57.9–62.8 | 75.2–79.6 | 4.06 | 1.69 | 3.30 | 1.01 | (McGhee and Stein, 2018) |
| Wheat | | | | 2.72 | 1.47 | 2.86 | 1.15 | (Rodehutscord et al., 2016) |
| | 129 | 75.7 | 89.5 | 3.26 | 1.59 | 2.91 | 0.97 | (McGhee and Stein, 2018) |
| Barley | | | | 3.49 | 1.57 | 3.55 | 1.41 | (Rodehutscord et al., 2016) |
| | 119 | 67.4 | 89.2 | 3.70 | 1.61 | 3.32 | 1.04 | (McGhee and Stein, 2018) |
| Maize | | | | 2.98 | 2.06 | 3.65 | 0.75 | (Rodehutscord et al., 2016) |
| | 81.7 | 67.4 | 89.2 | 3.75 | 2.08 | 3.75 | 0.83 | (McGhee and Stein, 2018) |
| RSM | 403 | 70.7 | 78.9 | 5.49–5.82 | 1.85–2.03 | 3.87–4.12 | 1.25–1.49 | (Berrocoso et al., 2015) |
| | 409 | | | 5.55 | 2.06 | 4.38 | 1.33 | (Newkirk, 2011) |
| SBM | 552 | 82.3 | 90.0 | 6.36 | 1.43 | 3.77 | 1.41 | (Berrocoso et al., 2015) |

AID = apparent ileal digestibility; SID = standardised ileal digestibility; DM = dry matter; CP = crude protein; RSM = rapeseed meal; SBM = soybean meal

**Table 3:** Energy concentration of different feedstuffs

| | Gross energy | Metabolisable energy | Starch | | |
|---|---|---|---|---|---|
| | MJ/kg DM | MJ/kg DM | g/kg DM | AID in % | |
| Rye | 17.8–18.0 | 14.5–14.6 | 608-628 | 91.2–95.9 | (McGhee and Stein, 2020) |
| | 18.4 | | 643 | | (Rodehutscord et al., 2016) |
| Wheat | 18.0 | 15.2 | 634 | 97.8 | (McGhee and Stein, 2020) |
| | 18.6 | | 713 | | (Rodehutscord et al., 2016) |
| Barley | 18.0 | 14.0 | 559 | 94.4 | (McGhee and Stein, 2020) |
| | 18.7 | | 616 | | (Rodehutscord et al., 2016) |
| Maize | 18.3 | 15.6 | 661 | 95.2 | (McGhee and Stein, 2020) |
| | 19.2 | | 740 | | (Rodehutscord et al., 2016) |
| RSM | 19.0 | 11.4 | 1.69 | | (Berrocoso et al., 2015) |
| | 20.1 | 11.8 | | | (Navarro et al., 2018) |
| SBM | 20.4 | 18.2 | 0.32 | | (Berrocoso et al., 2015) |
| | 19.4 | 18.1 | | | (Navarro et al., 2018) |

DM = dry matter; AID = apparent ileal digestibility; RSM = rapeseed meal; SBM = soybean meal

### 4.4. Fibre

The high dietary fibre content, especially SDF, is considered to be one of the best properties of rye (Kamphues et al., 2019). SDF serves as a rapidly fermentable substrate for microorganisms, leading to the formation of SCFA, which serve as an energy source for the host animal. In particular, the increased formation of butyrate is considered beneficial as it is the preferred energy source of colonocytes. In addition, soluble fibres lead to probiotic effects by strengthening beneficial bacteria and reducing pathogenic bacteria – for example, by lowering the pH. These mechanisms are considered to improve intestinal health and thus animal health (Jha and Berrocoso, 2015; Lancheros et al., 2023).

Moreover, insoluble fibre shortens the transit time in the small and large intestines and increases faecal bulk, which in turn prevents constipation (Bach Knudsen et al., 2023; Johnston, 2023). Rye and barley have the highest TDF content among the cereal grains, as well as a relatively high proportion of SDF (Rodehutscord et al., 2016). This higher proportion of SDF in rye grain–containing compared with wheat grain–containing rations was observed in this study as well as by Ellner et al. (2021). Moreover, Wilke (2020) and Ellner (2022) outlined some of the positive effects due to the increased consumption of rye compared with wheat, such as increased SCFA production in the jejunum and colon and a 35% increase in butyrate in the caecum; the latter might be due to the high amount of fructan in rye (Rodehutscord et al., 2016). In addition, there was increased lactic acid production in the small intestine, leading to a lower pH, which in turn might reduce harmful bacteria in the intestine (Wilke, 2020). Chuppava et al. (2020) also showed a decrease in *Salmonella* shedding by feeding rye grain instead of wheat.

However, as explained in chapter 4, these positive aspects can only be estimated if the SDF and IDF fractions are recorded separately. When considering classical feed parameters such as crude fibre, rye shows a similar content as maize and is below that of wheat and well below that of barley. An interpretation of the positive effects is not possible, only the assumption that negative aspects that are classically attributed to fibre are relatively low in rye (Rodehutscord et al., 2016). Such negative aspects include fibre-encapsulating nutrients in the cell wall, the high viscosity of fibre hindering the access of enzymes to nutrients, both leading to poorer digestibility, and a reduction in "valuable" nutrients due to high fibre content (Bach Knudsen et al., 2023; Agyekum and Nyachoti, 2017). The negative effect of fibre and especially its viscosity has often been demonstrated for protein (McGhee and Stein, 2020; Ellner et al., 2021).

For starch, there are conflicting findings showing that even with high inclusion levels of soluble and insoluble fibre, there is no effect or a reduction in the AID of starch (Rosenfelder-Kuon et al., 2017; Bach Knudsen et al., 2023). This suggests that the individual structure and composition of fibre and its physicochemical properties vary greatly, thus modulating its effects.

RSM contains more dietary fibre, especially due to the high proportion of insoluble fibre compared with SBM. This leads to a lower ATTD of the different fibre fractions (NDF, ADF, TDF, SDF, IDF and NSP) (Berrocoso et al., 2015; Navarro et al., 2018). In addition, the lignin content in RSM is usually higher than that in SBM (Bach Knudsen, 2014; Navarro et al., 2018). Because lignin is not digestible by either endogenous or microbial enzymes, this affects the digestibility or fermentability of IDF and TDF and may also affect other nutrients (Lancheros

et al., 2023). Replacing wheat grain with rye grain as well as SBM with RSM significantly reduced the digestibility of organic matter (78.4% vs 74.7% and 74.3% vs 66.0%, respectively) and N-free extract (81.5% vs 76.8% and 77.5% vs 67.4%, respectively). Consequently, compound feed containing rye and RSM provided more substrates for fermentation in the large intestine. There was more intense fermentation of rye- and RSM-containing ration based on the lack of differences in the total digestibility of the organic matter and the N-free extract (Hartung, 2020). In hybrid rye, the hindgut digestibility of starch, gross energy and TDF is higher compared with wheat, maize and barley, indicating that fermentation provides more energy from rye than from the other cereals (McGhee and Stein, 2020).

Ellner et al. (2022) examined the influence of rye and RSM on the gut microbiota. Replacing wheat with rye did not affect the relative abundance of the microbiota in the large intestine, but its metabolic activity was increased by the higher amounts and more soluble NSP in rye. RSM, in contrast, decreased the metabolic activity of the gut microbiota and resulted in a lower abundance of *Firmicutes*, the most abundant fibre degraders, and an increased abundance of *Actinobacteria* and *Bacteroidetes*, which specifically degrade IDF (Ellner et al., 2022).

## 5. Environmental impact

As shown in chapter 3, the P digestibility of rye and wheat in compound feeds with SBM or RSM is the same despite different contents of intrinsic phytase. Only the protein meal had a significant effect on digestibility due to the higher phytate P content in RSM compared with SBM, resulting in lower digestibility of RSM-containing compound feeds. The use of microbial phytase increased the P digestibility of all compound feeds to a similar level of 70.2% and 69.5% for compound feed containing SBM and RSM, respectively. McGhee and Stein (2019) investigated the ATTD and STTD of P in hybrid rye, wheat and maize with and without supplementation of 1,000 units of microbial phytase/kg feed. As in this trial, they also found no difference between hybrid rye and wheat regarding the ATTD and STTD of P with or without phytase supplementation. However, maize always had a lower ATTD and STTD of P compared with hybrid rye, regardless of whether phytase had been added. (McGhee and Stein, 2019) also did not observe a difference when using intrinsic phytase. Because the addition of microbial phytase had a positive effect on all the compound feeds studied – especially those with a high phytate-P content, due to an improved P utilisation and reduced excretion – it is essential to consider this factor with respect to the environment. Besides P, phytate can also have a negative effect on calcium, other minerals (divalent and trivalent cations: Zn, Fe, etc.) as well as AA, whereby phytase can also improve bioavailability (Dersjant-Li et al., 2015). There was no

apparent effect of phytase on protein or AA in this trial. However the N balance as well as the higher microbial activity reported by Ellner (2022) indicate that due to the increased amount of fermentable fibre from rye, blood urea-N might be required in the large intestine to support microbial growth (Bindelle et al., 2008). Intake of fermentable fibre suggests that N excretion may be shifted from urine to faeces in the form of microbial protein, a phenomenon confirmed by greater microbial faecal N excretion of animals fed rye-containing test ration. RSM, however, increases N excretion, which may be related to the encapsulated proteins (Agyekum and Nyachoti, 2017).

Life cycle assessments are conducted in the agricultural sector to fully consider the environmental impact of feed components. (Riedesel et al., 2022) assessed the production of winter wheat and winter rye in a life cycle assessment (from cradle to farm gate). Specifically, they examined their greenhouse gas emissions and carbon footprint. Compared with wheat, rye production had ~20% lower greenhouse gas emissions per unit area and an ~8% smaller carbon footprint. In principle, the use of hybrid varieties has a positive effect on the carbon footprint due to higher yields. Furthermore, the lower use of chemical plant protection in rye compared with wheat also positively impacts the carbon footprint. Riedesel et al. (2022) concluded that hybrid rye has a greater potential to mitigate climate change compared with wheat.

Wilke et al. (2023) compared balanced pig feeds based on rye grain with an increasing exchange of SBM with RSM as the protein source based on the component as well as on the performance. In the scenario applied, SBM and oil are imported from South America, while RSM and all other components come from Germany. The authors showed that the integration of RSM reduces the carbon footprint and especially the impact on climate change. In addition, there was a less negative impact on freshwater eutrophication and resource use, but at the same time a more negative impact on acidification, terrestrial and marine eutrophication and particulate matter emissions (Wilke et al., 2023).

## 6. Economy

Hybrid rye in particular is characterised not only by its good yield potential and good resistance to fungi and pests, but also by its low production costs, which provide economic benefits from cultivation (Bederska-Łojewska et al., 2017). Alert and Fröhlich (2006) showed that rye can be used in rations up to 15% for piglets and up to 70% during the middle and finishing fattening period without exerting negative effects on feed intake or live weight gain. Combined with the low price of rye compared with wheat, this provides economic advantages, especially when using self-grown rye when market prices are low. Schwarz et al. (2015) showed that rye is

cheaper than barley, but the feed intake and feed conversion are higher. Therefore, the use of rye in dry mixes compared with barley in this trial resulted in higher total costs over the fattening period. Nevertheless, the use of rye was profitable, as better slaughter values were achieved, so the balance was positive (Schwarz et al., 2015). The price of rapeseed and soybeans, as well as their meals, has increased noticeably in recent years, as their growing global consumption has already exceeded supply several times (AMI, 2022). The use of RSM is considered more cost effective than SBM, despite its higher content of dietary fibre and antinutritive substances as well as its lower CP content (McDonnell et al., 2010; Choi et al., 2015). This assumption is based on the common price difference between SBM and RSM (Figure 2). Nevertheless, there are significant market-related fluctuations, especially in recent years, that influence the price difference in the short term. For example, the start of the COVID-19 pandemic in 2020 led to a decline in demand and thus production of biofuel, which reduced the amount of RSM, a by-product of biofuel production, and led to a shortage that caused prices to rise (AMI, 2021). The escalation of the Russo–Ukrainian War in 2022 also caused prices to rise – and even caused the price of RSM to briefly exceed that of SBM for the first time – given

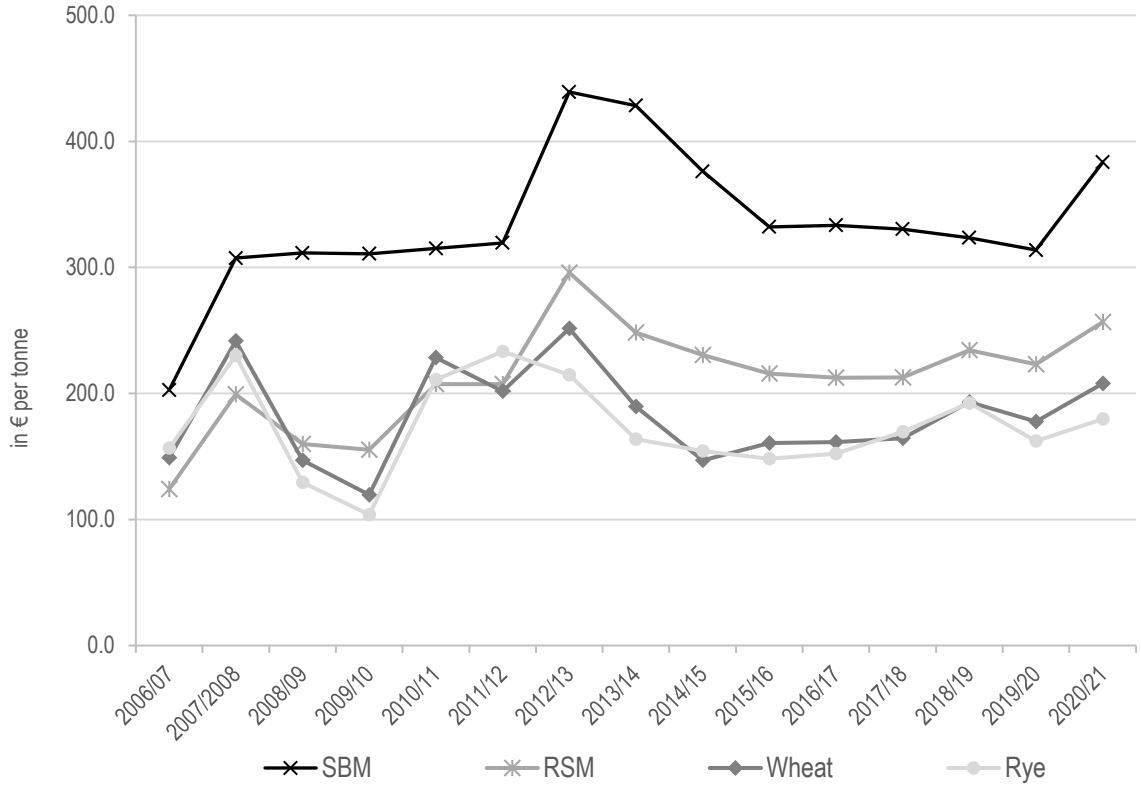


**Figure 2:** Average annual stock market price (AMI, 2010; 2012; 2015; 2019; 2022). SBM = soybean meal; RSM = rapeseed meal

that Ukraine is a leading exporter of non-genetically modified organism (GMO) soybeans and rapeseed (AMI, 2022; DVT, 2022). If RSM is used as an alternative to SBM as a protein source in non-GMO feed, which is required by the food industry in Germany, the price difference between non-GMO SBM and RSM is even greater at the point of purchase (AMI, 2022).

## 7. Conclusion and outlook

In conclusion, the results of this thesis, the 6-R Project and other work outlined in this chapter have shown that the combination of rye grain and RSM to feed pigs is a good alternative to conventional rations. Increased utilisation is worthwhile due to the price, cultivation conditions, environmental protection and the feeding value. Historically negative characteristics of rye (ergot and low yield) and RSM (glucosinolates and erucic acid) have been greatly reduced or eliminated through breeding. Through proper feed formulation, including the use of additives such as enzymes and AA, the weaknesses of the components can be compensated for so that no significant disadvantages arise compared to conventional rations. In addition, advantages regarding animal health and welfare, which result primarily from the dietary fibres in rye, can be exploited.

The implementation of a laboratory method to measure all individual carbohydrate fractions of pig feed based on enzymatic photometric and chemical gravimetric measurement was not successful, but it did reveal a lack of comparability of the fibre methods that determine the soluble or rapidly fermentable fibres as well as TDF. Numerous unaccounted differences in the applied methods as well as imprecise descriptions explained this. It is therefore of undeniable importance that these differences are addressed by feed and animal science committees and associations to raise awareness of this topic on the one hand, but also to establish and standardise procedures and definitions of fibre analysis in order to obtain comparable results that are of use beyond each individual study.

The importance of dietary fibre for pigs in terms of nutrient digestibility and its impact on animal health illustrates why there is still a need for research in the field of dietary fibre analysis. An extension of the classical chemical parameters to include physicochemical aspects is sensible to better understand and evaluate the effects of dietary fibre on the digestive process. At the same time, standardisation must be further promoted and a clear focus placed on practical solutions so that the existing knowledge finds its way into practical (“legally binding”) feed analysis in the long term.

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# APPENDIX

| **Uronsäuren-Fließschema** | | | |
|---|---|---|---|
| Einwaage der Proben | | 100 mg ± 0,005 | |
| 2 M $H_2SO_4$ | | 10,0 ml | |
| 6 Stunden; 100°C | | | |
| Regelmäßiges mischen alle 60 Minuten mit Vortex | | | |
| Abkühlen auf Raumtemperatur | | | |
| 2 M NaOH | | 14,0 ml | |
| Überbringe die Lösung quantitativ in einen 100 ml Kolben | | | |
| dest. Wasser | | Auf 100 ml | |
| In 10 ml Messkolben bei Raumtemperatur | | | |
| Probenlösung | | 5 ml | |
| Carrez Lösung I | | 0,5 ml | |
| Mischen | | | |
| Carrez Lösung II | | 0,5 ml | |
| Mischen | | | |
| 100 mM NaOH | | 1 ml | |
| Mischen | | | |
| dest Wasser | | Auf 10 ml auffüllen | |
| Mischen, kurz stehen lassen, Filtern | | | |
| pH Werte anpassen (Lactone) | | | |
| "Microplate assay procedure" nach Megazyme | | | |
| In 96-wells Platte pipettieren | | | |
| | Blank | Sample | Standard |
| Dest. Wasser | 0,210 ml | 0,200 ml | 0,200 ml |
| Probenlösung | | 0,010 ml | |
| A (= Probenlösung) + B (= dest. Wasser) = 0,210 ml (A und B variabel) | | | |
| Standard-Lösung (Bottle 4) | | | 0,010 ml |
| Pufferlösung (Bottle 1) | 0,020 ml | 0,020 ml | 0,020 ml |
| Lösung 2 (NAD) (Bottle 2) | 0,020 ml | 0,020 ml | 0,020 ml |
| Mische die Lösung und messen die Absorption (bei 340 nm und 37°C) nach zwei Minuten. Anschließend gib Lösung 3 hinzu. → A1 | | | |
| Lösung 3 (UDH) Bottle 3 | 0,002 ml | 0,002 ml | 0,002 ml |
| Mische und messe nach 10-25 Minuten, nach Beendigung der Reaktion die Absorption erneut. →A2 | | | |
| Falls die Reaktion nicht stoppt, dann messe in 2 Minuten Intervallen, bis die Absorption über 2 Minuten konstant ist. | | | |

**Figure 1:** Flow diagram of the uronic acid analysis based on (Megazyme, 2019)

<table>
<tr><th colspan="4">ß-Glucan Fließschema</th></tr>
<tr><td>Probe</td><td colspan="3">Chymus: 200 mg /75 mg<br>Futter: 100 mg<br>B-Std: 50-100 mg<br>abhängig von Aliquot XY</td></tr>
<tr><td>Ethanol</td><td colspan="3">0,2 ml</td></tr>
<tr><td>20 mM<br>Natriumphosphatpuffer</td><td colspan="3">4,0 ml</td></tr>
<tr><td></td><td colspan="3">Mischen (Vortex)<br>Wasserbad (100°C); 60 Sekunden<br>Mischen (Vortex)<br>Wasserbad (100°C); 1 Minute<br>Mischen (Vortex)<br>Wasserbad (50°C); 5 Minuten</td></tr>
<tr><td>Lichenase</td><td colspan="3">0,2 ml</td></tr>
<tr><td colspan="4">Mischen</td></tr>
<tr><td colspan="4">Versiegle die RG Parafilm mit<br>Wasserbad (50 °C); 1 Stunde (Mische alle 15 min (Vortex))</td></tr>
<tr><td>200 mM<br>Natriumazetatpuffer</td><td colspan="3">5,0 ml</td></tr>
<tr><td colspan="4">Mischen</td></tr>
<tr><td colspan="4">Equilibriere Probe 5 Minuten bei Raumtemperatur<br>Anschließend zentrifugiere (1,000 g) für 10 Minuten.</td></tr>
<tr><td colspan="4">Probe durch Milliporfilter filtern!</td></tr>
<tr><td colspan="2">Bilde Aliquote XY ml in neuem RG</td><td>Reagent-BW:<br>XY ml dest. Wasser</td><td><u>4 x</u> K-Glucose:<br>0,1 ml Glucose Std +<br>XY-0,1 ml dest. Wasser</td></tr>
<tr><td>2x 0,1 ml ß-Glucosidase in 50 mM Natriumazetatpuffer</td><td>1x 0,1 ml 50 mM Natriumazetatpuffer</td><td>1x 0,1 ml 50 mM Natriumazetatpuffer</td><td>1x 0,1 ml 50 mM Natriumazetatpuffer</td></tr>
<tr><td colspan="4">Wasserbad (50°C); 10 Minuten</td></tr>
<tr><td>GOPOD Reagenz</td><td colspan="3">3 ml</td></tr>
<tr><td colspan="4">Wasserbad (50°C); 20 Minuten</td></tr>
<tr><td colspan="4">Messe die Absorption bei 510 nm gegen den Blindwert<br>(innerhalb 1 Stunde)</td></tr>
</table>

**Figure 2:** Flow diagram of the mixed-linked ß-glucan analysis based on (Megazyme, 2018c)

| **Fructan-Fließschema** | | | | | |
|---|---|---|---|---|---|
| Probe | Inulin oder Levan | Sucrose | 2 BW | 4 Fructose Standard | D-Fructose-Lsg (Borohydrid-Test) |
| Einwaage 400 mg in Kulturröhrchen | | | | | |
| Zugabe 25 ml dest. $H_2O$ | | | | | |
| inkubieren 100°C; 10 Minuten nach 5 1Minuten Vortex und Deckel schließen | | | | | |
| Zentrifugieren: 13,000 rpm 5 Min. | | | | | |
| 0,2 ml Zentrifugenextakt ins RG (2x) | | | | | 0,2 ml direkt ins RG |
| 0,2 ml Sucrose Amylase-Lsg. | | | | | 0,2 ml Sucrose Amylase-Lsg. |
| inkubieren 30°C; 30 Min. | | | | | |
| 0,2 ml Alkalinesborohydrid | | | | | 0,2 ml Alkalinesborohydrid |
| inkubieren 40°C; 30 Min. | | | | | |
| 0,5 ml Essigsäure | | | | | 0,5 ml Essigsäure |
| = Lösung S; ≈ pH 4 & Schaum | | | | | |
| | | | | 0,2 ml Fructose Lsg. in RG | |
| Lösung S *0,2/0,4/0,8 x* ml in RGs (3x) | | | | 0,9 ml Natriumacetat → 4x 0,2 Aliquote | Lösung S 0,2 ml in RGs (3x) |
| 2x 0,1 ml Fructanase-Lsg; 1x 0,1 ml Natriumacetat | | | 0,3 ml Natriumacetat | 0,1 ml Natriumacetat | 3x 0,1 ml Natriumacetat |
| 5,0 ml PAHBAH-Lsg. in RG geben | | | | | |
| inkubieren 100°C; genau 6 Min. | | | | | |
| Stelle RGs in altes Wasserbad (18 - 20°C) für ca. 5 Minuten | | | | | |
| Messe die Absorption aller Lösungen bei 410 nm gegen die Blindprobe. Direkt durchführen: PAHBAH-Farbkomplex verschwindet mit der Zeit | | | | | |

**Figure 3:** Flow diagram of the fructan analysis based on Megazyme (2018a)

<table>
<tr><th colspan="3">TDF Fließschema</th></tr>
<tr><td colspan="2">Einwaage der Proben</td><td>1 g ± 0,005</td></tr>
<tr><td colspan="2">Ethanol (95 % v/v)</td><td>1,0 ml</td></tr>
<tr><td colspan="2">Maleat-Puffer + Pankreas α-Amylase + Amyloglucosidase</td><td>40,0 ml</td></tr>
<tr><td colspan="3">16 h; 37°C; 150 rpm</td></tr>
<tr><td colspan="2">TRIS-Puffer-LSG</td><td>3,0 ml</td></tr>
<tr><td colspan="3">20 Min; 100°C</td></tr>
<tr><td colspan="3">Abkühlen auf 60°C</td></tr>
<tr><td colspan="2">Protease-LSG</td><td>0,1 ml</td></tr>
<tr><td colspan="3">30 Min; 60°C</td></tr>
<tr><td colspan="2">2 M Essigsäure</td><td>4,0 ml</td></tr>
<tr><td colspan="3">Filtrieren der Probe</td></tr>
<tr><td colspan="3">Auswaschen der Inkubationsflasche mit 60°C dest. Wasser + Überführen</td></tr>
<tr><td colspan="2">Filtrat</td><td>Filter+Probe</td></tr>
<tr><td>Auf 70 ml für SDFP-Bestimmung auffüllen</td><td>226 g EtOH<br>(δ = 0,807 g/ml) einwiegen</td><td>Waschen:<br>je 2x mit 15 ml<br>78% ig EtOH<br>95% ig EtOH<br>Aceton</td></tr>
<tr><td colspan="2">Erwärmen auf 60°C</td><td rowspan="6">→Trockenschrank<br>über Nacht</td></tr>
<tr><td colspan="2">70 ml + EtOH zusammen geben</td></tr>
<tr><td colspan="2">60 Min. warten (Fällung)</td></tr>
<tr><td colspan="2">Auswaschen<br>Inkubationsfalsche mit 78% EtOH um alle Partikel zu überführen</td></tr>
<tr><td>Filtrat</td><td>Filter + Probe</td></tr>
<tr><td rowspan="2">verwerfen</td><td>Waschen:<br>je 2 x mit 15 ml<br>78% ig EtOH<br>95% ig EtOH<br>Aceton</td></tr>
<tr><td>→ Trockenschrank<br>über Nacht</td></tr>
</table>

**Figure 4:** Flow diagram of the Total dietary fibre analysis based on Megazyme (2018b)

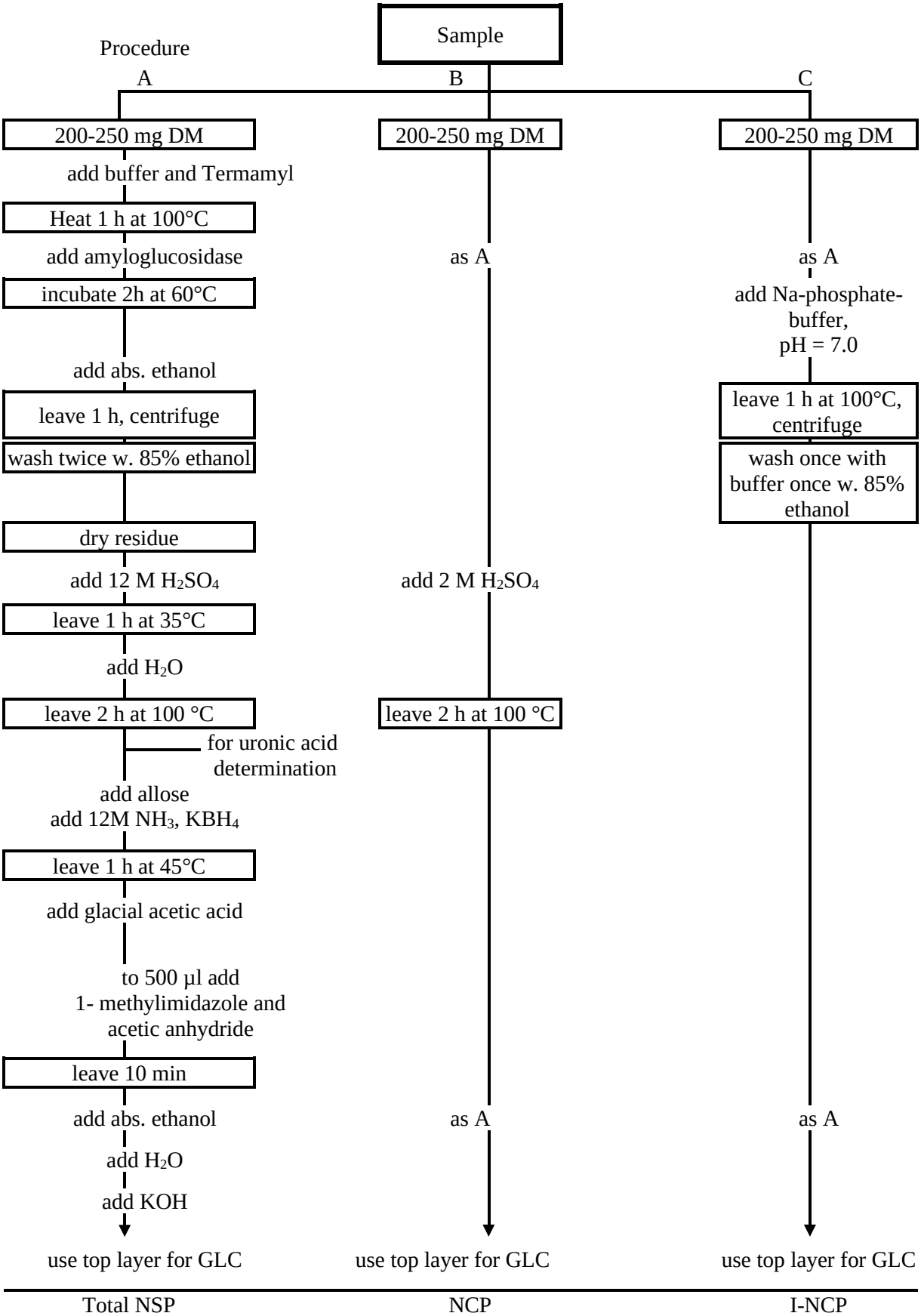


**Figure 5:** Flow diagram of non-starch polysaccharides analysis (Bach Knudsen, 1997; 2019)

**Table 1:** Collected individual animal data

| Round | Pig-No. | Compound feed | | | | Live weight in kg | | Daily gain | Offered feed | Total feed leftover | Total faeces | Total urine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CER | | PM | Phyt | Start | End | g/day | g FM/ feeding | kg DM | g FM | kg FM |
| 1 | 1 | BR | | | - | 30.8 | 31.6 | 160 | 430 | 0.08 | 1128 | 21.8 |
| 1 | 2 | BR | | | - | 31.1 | 32.6 | 300 | 430 | 0.15 | 1533 | 22.9 |
| 1 | 3 | TR | R | SBM | - | 35.4 | 37.3 | 380 | 600 | 0.00 | 2528 | 12.3 |
| 1 | 4 | TR | R | SBM | - | 34.6 | 38.0 | 680 | 600 | 0.00 | 2067 | 16.5 |
| 1 | 5 | TR | R | RSM | - | 37.2 | 38.1 | 180 | 620 | 0.00 | 3238 | 16.0 |
| 1 | 6 | TR | R | RSM | - | 38.8 | 41.3 | 500 | 650 | 0.00 | 3598 | 11.6 |
| 1 | 7 | TR | | | - | 36.2 | 39.0 | 560 | 630 | 0.14 | 1405 | 20.2 |
| 1 | 8 | TR | | | - | 32.3 | 35.6 | 660 | 580 | 0.19 | 1394 | 18.7 |
| 1 | 9 | TR | W | SBM | - | 33.2 | 35.6 | 480 | 550 | 0.00 | 1876 | 10.4 |
| 1 | 10 | TR | W | SBM | - | 33.0 | 36.7 | 740 | 550 | 0.00 | 1441 | 23.1 |
| 1 | 11 | TR | W | RSM | - | 34.8 | 39.4 | 920 | 620 | 0.00 | 2015 | 15.6 |
| 1 | 12 | TR | W | RSM | - | 33.2 | 36.4 | 640 | 620 | 0.00 | 2508 | 11.4 |
| 1 | 13 | BR | | | + | 30.0 | 31.0 | 200 | 470 | 0.21 | 1363 | 14.2 |
| 1 | 14 | BR | | | + | 30.9 | 33.0 | 420 | 550 | 0.00 | 2095 | 12.2 |
| 1 | 15 | TR | R | SBM | + | 25.3 | 26.9 | 320 | 460 | 0.00 | 1439 | 8.5 |
| 1 | 16 | TR | R | SBM | + | 25.5 | 27.9 | 480 | 460 | 0.04 | 1509 | 9.0 |
| 1 | 17 | TR | R | RSM | + | 29.9 | 31.4 | 300 | 550 | 0.01 | 2394 | 8.3 |
| 1 | 18 | TR | R | RSM | + | 30.0 | 32.3 | 460 | 550 | 0.00 | 2670 | 9.2 |

| Round | Pig-No. | Compound feed | | | | Live weight in kg | | Daily gain | Offered feed | Total feed leftover | Total faeces | Total urine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CER | | PM | Phyt | Start | End | g/day | g FM/ feeding | kg DM | g FM | kg FM |
| 1 | 19 | BR | | | + | 24.7 | 27.4 | 540 | 520 | 0.11 | 1782 | 13.0 |
| 1 | 20 | BR | | | + | 24.6 | 27.3 | 540 | 520 | 0.17 | 1230 | 11.9 |
| 1 | 21 | TR | W | SBM | + | 28.2 | 29.9 | 340 | 500 | 0.00 | 2077 | 8.9 |
| 1 | 22 | TR | W | SBM | + | 29.3 | 31.3 | 400 | 500 | 0.00 | 2206 | 12.3 |
| 1 | 23 | TR | W | RSM | + | 28.7 | 30.5 | 360 | 550 | 0.00 | 2638 | 10.4 |
| 1 | 24 | TR | W | RSM | + | 30.9 | 33.3 | 480 | 560 | 0.00 | 1770 | 9.2 |
| 2 | 1 | TR | R | RSM | - | 39.7 | 39.8 | 20 | 620 | 0.13 | 2882 | 31.5 |
| 2 | 2 | TR | R | RSM | - | 40.2 | 41.3 | 220 | 640 | 0.04 | 3888 | 22.5 |
| 2 | 3 | BR | | | - | 41.0 | 42.5 | 300 | 640 | 0.22 | 1842 | 34.1 |
| 2 | 4 | BR | | | - | 41.0 | 43.0 | 400 | 640 | 0.05 | 1759 | 38.6 |
| 2 | 5 | TR | R | SBM | - | 43.0 | 44.6 | 320 | 670 | 0.00 | 2482 | 17.6 |
| 2 | 6 | TR | R | SBM | - | 46.6 | 50.0 | 680 | 700 | 0.00 | 2336 | 25.8 |
| 2 | 7 | TR | W | RSM | - | 40.4 | 45.4 | 1000 | 620 | 0.47 | 1994 | 17.1 |
| 2 | 8 | TR | W | RSM | - | 41.7 | 42.5 | 160 | 600 | 0.01 | 1875 | 17.3 |
| 2 | 9 | BR | | | - | 38.8 | 40.8 | 400 | 600 | 0.00 | 1803 | 26.8 |
| 2 | 10 | BR | | | - | 38.9 | 40.4 | 300 | 600 | 0.00 | 1889 | 32.8 |
| 2 | 11 | TR | W | SBM | - | 42.8 | 44.8 | 400 | 660 | 0.00 | 1797 | 32.7 |
| 2 | 12 | TR | W | SBM | - | 41.4 | 43.4 | 400 | 650 | 0.00 | 2107 | 21.2 |
| 2 | 13 | TR | R | SBM | + | 36.4 | 39.5 | 620 | 600 | 0.00 | 2008 | 10.2 |
| 2 | 14 | TR | R | SBM | + | 37.8 | 40.7 | 580 | 610 | 0.00 | 2551 | 15.3 |

| Round | Pig-No. | Compound feed | | | | Live weight in kg | | Daily gain | Offered feed | Total feed leftover | Total faeces | Total urine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CER | PM | Phyt | Start | End | g/day | g FM/ feeding | kg DM | g FM | kg FM |
| 2 | 15 | TR | R | RSM | + | 32.1 | 34.2 | 420 | 600 | 0.00 | 1795 | 10.6 |
| 2 | 16 | TR | R | RSM | + | 32.2 | 34.9 | 540 | 600 | 0.00 | 2063 | 11.3 |
| 2 | 17 | BR | | | + | 34.7 | 36.6 | 380 | 660 | 0.03 | 1735 | 18.8 |
| 2 | 18 | BR | | | + | 36.9 | 39.3 | 480 | 690 | 0.01 | 1965 | 19.4 |
| 2 | 19 | TR | W | SBM | + | 31.9 | 34.6 | 540 | 550 | 0.00 | 1759 | 11.3 |
| 2 | 20 | TR | W | SBM | + | 30.4 | 33.2 | 560 | 550 | 0.02 | 1516 | 9.0 |
| 2 | 21 | TR | W | RSM | + | 35.5 | 37.3 | 360 | 630 | 0.00 | 2300 | 10.3 |
| 2 | 22 | TR | W | RSM | + | 37.0 | 39.1 | 420 | 640 | 0.00 | 2643 | 15.0 |
| 2 | 23 | BR | | | + | 35.3 | 37.4 | 420 | 670 | 0.01 | 1825 | 15.6 |
| 2 | 24 | BR | | | + | 38.7 | 40.3 | 320 | 700 | 0.04 | 1439 | 17.7 |
| 3 | 1 | TR | R | SBM | - | 46.5 | 48.7 | 440 | 700 | 0.04 | 2499 | 33.1 |
| 3 | 2 | TR | R | SBM | - | 47.5 | 50.7 | 640 | 700 | 0.20 | 2623 | 28.6 |
| 3 | 3 | TR | R | RSM | - | 48.6 | 50.7 | 420 | 740 | 0.00 | 3379 | 24.6 |
| 3 | 4 | TR | R | RSM | - | 48.2 | 50.5 | 460 | 730 | 0.00 | 2955 | 24.7 |
| 3 | 5 | BR | | | - | 50.7 | 52.6 | 380 | 810 | 0.00 | 1995 | 28.6 |
| 3 | 6 | BR | | | - | 54.6 | 58.9 | 860 | 860 | 0.01 | 1664 | 38.4 |
| 3 | 7 | TR | W | SBM | - | 52.7 | 55.9 | 640 | 740 | 0.00 | 1922 | 21.8 |
| 3 | 8 | TR | W | SBM | - | 49.6 | 53.9 | 860 | 710 | 0.01 | 1861 | 20.5 |
| 3 | 9 | TR | W | RSM | - | 46.3 | 48.1 | 360 | 710 | 0.00 | 2298 | 18.4 |
| 3 | 10 | TR | W | RSM | - | 46.1 | 50.3 | 840 | 710 | 0.00 | 2366 | 24.8 |

| Round | Pig-No. | Compound feed | | | | Live weight in kg | | Daily gain | Offered feed | Total feed leftover | Total faeces | Total urine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CER | PM | Phyt | Start | End | g/day | g FM/ feeding | kg DM | g FM | kg FM |
| 3 | 11 | BR | | | - | 48.7 | 49.7 | 200 | 790 | 0.94 | 1809 | 50.3 |
| 3 | 12 | BR | | | - | 48.8 | 49.7 | 180 | 790 | 1.23 | 1752 | 37.1 |
| 3 | 13 | TR | R | RSM | + | 46.1 | 49.1 | 600 | 740 | 0.01 | 2415 | 14.4 |
| 3 | 14 | TR | R | RSM | + | 47.6 | 51.5 | 780 | 760 | 0.00 | 2860 | 15.8 |
| 3 | 15 | BR | | | + | 39.5 | 41.6 | 420 | 670 | 0.27 | 1198 | 23.4 |
| 3 | 16 | BR | | | + | 40.1 | 41.3 | 240 | 670 | 0.34 | 1169 | 26.1 |
| 3 | 17 | TR | R | SBM | + | 43.9 | 46.8 | 580 | 670 | 0.03 | 2805 | 10.7 |
| 3 | 18 | TR | R | SBM | + | 47.0 | 48.8 | 360 | 710 | 0.00 | 2715 | 12.9 |
| 3 | 19 | TR | W | RSM | + | 40.3 | 42.8 | 500 | 680 | 0.00 | 2345 | 15.2 |
| 3 | 20 | TR | W | RSM | + | 39.1 | 41.8 | 540 | 660 | 0.02 | 1910 | 13.1 |
| 3 | 21 | BR | | | + | 43.0 | 46.1 | 620 | 750 | 0.06 | 1491 | 23.9 |
| 3 | 22 | BR | | | + | 44.2 | 44.2 | 0 | 720 | 1.35 | 1775 | 25.4 |
| 3 | 23 | TR | W | SBM | + | 44.5 | 47.6 | 620 | 680 | 0.00 | 2153 | 11.1 |
| 3 | 24 | TR | W | SBM | + | 47.8 | 52.5 | 940 | 720 | 0.00 | 1911 | 12.8 |

CER = cereal grain; PM = protein meal; Phyt = phytase; BR = basal ration; TR = test ration; W = wheat; R = rye; SBM = soybean meal; RSM = rapeseed meal; FM = fresh matter

**Table 2:** Single animal data of energy value, phosphorus and calcium.

| Round | Pig-No. | Compound feed | | | | Energy value | | | Phosphorus | | | Calcium | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Feed | Faeces | Urine | Intake | Faeces | Digestible P | Intake | Faeces | Digestible Ca |
| | | | CER | PM | Phyt | kJ/kg DM | kJ/kg DM | kJ/kg | g/day | g/kg DM | g/kg DM | g/day | g/kg DM | g/kg DM |
| 1 | 1 | BR | | | - | 17658 | 16272 | 93 | 1.17 | 6.97 | 0.70 | 9.94 | 4.5 | 5.4 |
| 1 | 2 | BR | | | - | 17658 | 16184 | 71 | 1.15 | 6.96 | 0.72 | 9.76 | 4.8 | 4.9 |
| 1 | 3 | TR | R | SBM | - | 18009 | 20049 | 137 | 3.39 | 12.00 | 1.46 | 5.34 | 1.9 | 3.5 |
| 1 | 4 | TR | R | SBM | - | 18009 | 20532 | 182 | 3.39 | 12.40 | 1.79 | 5.34 | 1.6 | 3.8 |
| 1 | 5 | TR | R | RSM | - | 18021 | 19464 | 341 | 4.62 | 14.00 | 2.10 | 6.23 | 2.7 | 3.6 |
| 1 | 6 | TR | R | RSM | - | 18021 | 19357 | 267 | 4.84 | 12.50 | 2.08 | 6.53 | 2.3 | 4.3 |
| 1 | 7 | TR | | | - | 17658 | 17828 | 134 | 1.71 | 8.04 | 0.79 | 14.5 | 3.4 | 11.1 |
| 1 | 8 | TR | | | - | 17658 | 16386 | 156 | 1.56 | 7.82 | 0.70 | 13.2 | 4.4 | 8.8 |
| 1 | 9 | TR | W | SBM | - | 18081 | 19319 | 274 | 3.05 | 14.60 | 1.57 | 4.83 | 1.8 | 3.1 |
| 1 | 10 | TR | W | SBM | - | 18081 | 19842 | 119 | 3.05 | 13.50 | 1.83 | 4.83 | 1.3 | 3.5 |
| 1 | 11 | TR | W | RSM | - | 18014 | 18765 | 231 | 4.10 | 14.70 | 1.96 | 8.03 | 2.5 | 5.5 |
| 1 | 12 | TR | W | RSM | - | 18014 | 18272 | 290 | 4.10 | 14.00 | 1.89 | 8.03 | 3.4 | 4.6 |
| 1 | 13 | BR | | | + | 17617 | 17697 | 128 | 1.10 | 5.46 | 0.79 | 8.17 | 2.7 | 5.5 |
| 1 | 14 | BR | | | + | 17617 | 15338 | 235 | 1.36 | 5.07 | 0.73 | 10.1 | 6.2 | 3.9 |
| 1 | 15 | TR | R | SBM | + | 18025 | 20643 | 256 | 2.39 | 8.37 | 2.09 | 4.29 | 0.6 | 3.7 |

| Round | Pig-No. | Compound feed | | | | Energy value | | | Phosphorus | | | Calcium | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Feed | Faeces | Urine | Intake | Faeces | Digestible P | Intake | Faeces | Digestible Ca |
| | | | CER | PM | Phyt | kJ/kg DM | kJ/kg DM | kJ/kg | g/day | g/kg DM | g/kg DM | g/day | g/kg DM | g/kg DM |
| 1 | 16 | TR | R | SBM | + | 18025 | 20914 | 245 | 2.37 | 9.48 | 1.85 | 4.25 | 0.6 | 3.7 |
| 1 | 17 | TR | R | RSM | + | 17945 | 19772 | 285 | 3.32 | 8.71 | 2.08 | 7.07 | 2.1 | 5.0 |
| 1 | 18 | TR | R | RSM | + | 17945 | 19198 | 271 | 3.33 | 8.94 | 2.04 | 7.09 | 3.1 | 3.9 |
| 1 | 19 | BR | | | + | 17617 | 17764 | 210 | 1.25 | 5.32 | 0.82 | 9.29 | 2.6 | 6.7 |
| 1 | 20 | BR | | | + | 17617 | 17607 | 179 | 1.24 | 5.54 | 0.81 | 9.16 | 2.9 | 6.3 |
| 1 | 21 | TR | W | SBM | + | 18094 | 20304 | 289 | 2.52 | 8.59 | 1.84 | 5.26 | 1.0 | 4.3 |
| 1 | 22 | TR | W | SBM | + | 18094 | 20327 | 182 | 2.52 | 8.30 | 1.84 | 5.26 | 1.1 | 4.1 |
| 1 | 23 | TR | W | RSM | + | 17927 | 19162 | 236 | 3.19 | 7.58 | 2.18 | 7.64 | 2.3 | 5.3 |
| 1 | 24 | TR | W | RSM | + | 17927 | 18879 | 302 | 3.25 | 10.00 | 2.16 | 7.77 | 2.2 | 5.5 |
| 2 | 1 | TR | R | RSM | - | 18021 | 19971 | 37 | 4.51 | 13.40 | 2.19 | 6.09 | 1.4 | 4.7 |
| 2 | 2 | TR | R | RSM | - | 18021 | 19746 | 142 | 4.74 | 12.50 | 2.07 | 6.39 | 1.8 | 4.6 |
| 2 | 3 | BR | | | - | 17658 | 16198 | 114 | 1.72 | 6.88 | 0.70 | 14.5 | 7.0 | 7.5 |
| 2 | 4 | BR | | | - | 17658 | 15191 | 83 | 1.77 | 6.88 | 0.72 | 15.0 | 8.4 | 6.6 |
| 2 | 5 | TR | R | SBM | - | 18009 | 19465 | 253 | 3.76 | 13.00 | 1.68 | 5.92 | 2.0 | 3.9 |
| 2 | 6 | TR | R | SBM | - | 18009 | 19893 | 102 | 3.93 | 11.60 | 1.89 | 6.18 | 1.5 | 4.7 |
| 2 | 7 | TR | W | RSM | - | 18014 | 18713 | 245 | 3.76 | 15.10 | 1.70 | 7.37 | 2.2 | 5.1 |
| 2 | 8 | TR | W | RSM | - | 18014 | 18695 | 230 | 3.96 | 14.40 | 2.01 | 7.77 | 2.0 | 5.8 |

| Round | Pig-No. | Compound feed | | | | Energy value | | | Phosphorus | | | Calcium | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Feed | Faeces | Urine | Intake | Faeces | Digestible P | Intake | Faeces | Digestible Ca |
| | | CER | | PM | Phyt | kJ/kg DM | kJ/kg DM | kJ/kg | g/day | g/kg DM | g/kg DM | g/day | g/kg DM | g/kg DM |
| 2 | 9 | BR | | | - | 17658 | 14452 | 150 | 1.67 | 6.65 | 0.53 | 14.1 | 11.1 | 3.0 |
| 2 | 10 | BR | | | - | 17658 | 16979 | 118 | 1.67 | 7.77 | 0.55 | 14.1 | 5.7 | 8.4 |
| 2 | 11 | TR | W | SBM | - | 18081 | 19666 | 123 | 3.66 | 13.60 | 2.07 | 5.80 | 1.0 | 4.8 |
| 2 | 12 | TR | W | SBM | - | 18081 | 19287 | 185 | 3.61 | 14.00 | 1.72 | 5.71 | 1.7 | 4.0 |
| 2 | 13 | TR | R | SBM | + | 18025 | 20472 | 259 | 3.12 | 9.49 | 1.90 | 5.59 | 1.4 | 4.2 |
| 2 | 14 | TR | R | SBM | + | 18025 | 20226 | 192 | 3.17 | 9.07 | 1.79 | 5.68 | 1.7 | 4.0 |
| 2 | 15 | TR | R | RSM | + | 17945 | 19661 | 284 | 3.63 | 7.93 | 2.51 | 7.73 | 1.5 | 6.3 |
| 2 | 16 | TR | R | RSM | + | 17945 | 20209 | 275 | 3.63 | 7.45 | 2.47 | 7.73 | 1.1 | 6.7 |
| 2 | 17 | BR | | | + | 17617 | 17201 | 196 | 1.62 | 6.64 | 0.73 | 12.0 | 4.9 | 7.1 |
| 2 | 18 | BR | | | + | 17617 | 16895 | 203 | 1.70 | 6.47 | 0.66 | 12.6 | 5.8 | 6.8 |
| 2 | 19 | TR | W | SBM | + | 18094 | 20333 | 221 | 2.78 | 6.03 | 2.23 | 5.79 | 0.9 | 4.9 |
| 2 | 20 | TR | W | SBM | + | 18094 | 20191 | 300 | 2.76 | 7.37 | 2.13 | 5.76 | 1.0 | 4.8 |
| 2 | 21 | TR | W | RSM | + | 17927 | 19224 | 314 | 3.65 | 8.28 | 2.26 | 8.75 | 2.4 | 6.4 |
| 2 | 22 | TR | W | RSM | + | 17927 | 19404 | 199 | 3.71 | 7.58 | 2.28 | 8.89 | 2.4 | 6.4 |
| 2 | 23 | BR | | | + | 17617 | 16340 | 216 | 1.65 | 4.78 | 0.87 | 12.2 | 6.5 | 5.7 |
| 2 | 24 | BR | | | + | 17617 | 15788 | 192 | 1.72 | 6.05 | 0.80 | 12.7 | 6.7 | 6.0 |

| Round | Pig-No. | Compound feed | | | | Energy value | | | Phosphorus | | | Calcium | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CER | PM | Phyt | Feed | Faeces | Urine | Intake | Faeces | Digestible P | Intake | Faeces | Digestible Ca |
| | | | | | | kJ/kg DM | kJ/kg DM | kJ/kg | g/day | g/kg DM | g/kg DM | g/day | g/kg DM | g/kg DM |
| 3 | 1 | TR | R | SBM | - | 18009 | 20027 | 118 | 3.91 | 11.50 | 1.81 | 6.14 | 1.2 | 5.0 |
| 3 | 2 | TR | R | SBM | - | 18009 | 19769 | 146 | 3.81 | 11.10 | 1.88 | 5.99 | 0.9 | 5.1 |
| 3 | 3 | TR | R | RSM | - | 18021 | 19896 | 162 | 5.51 | 12.30 | 2.37 | 7.43 | 1.5 | 5.9 |
| 3 | 4 | TR | R | RSM | - | 18021 | 19888 | 182 | 5.44 | 12.10 | 2.57 | 7.33 | 1.5 | 5.9 |
| 3 | 5 | BR | | | - | 17658 | 15567 | 115 | 2.25 | 6.91 | 0.77 | 19.1 | 9.0 | 10.1 |
| 3 | 6 | BR | | | - | 17658 | 16091 | 140 | 2.39 | 7.01 | 0.91 | 20.2 | 6.5 | 13.7 |
| 3 | 7 | TR | W | SBM | - | 18081 | 19834 | 236 | 4.11 | 13.10 | 1.96 | 6.50 | 1.0 | 5.5 |
| 3 | 8 | TR | W | SBM | - | 18081 | 19138 | 207 | 3.94 | 12.80 | 1.98 | 6.23 | 0.9 | 5.3 |
| 3 | 9 | TR | W | RSM | - | 18014 | 18136 | 263 | 4.69 | 14.40 | 1.98 | 9.19 | 2.5 | 6.6 |
| 3 | 10 | TR | W | RSM | - | 18014 | 18149 | 192 | 4.70 | 13.30 | 2.00 | 9.20 | 2.3 | 6.9 |
| 3 | 11 | BR | | | - | 17658 | 15064 | 101 | 1.92 | 8.51 | 0.49 | 16.2 | 9.2 | 7.1 |
| 3 | 12 | BR | | | - | 17658 | 15115 | 129 | 1.83 | 7.14 | 0.65 | 15.5 | 8.9 | 6.6 |
| 3 | 13 | TR | R | RSM | + | 17945 | 19602 | 247 | 4.48 | 9.93 | 2.22 | 9.53 | 2.9 | 6.6 |
| 3 | 14 | TR | R | RSM | + | 17945 | 19497 | 247 | 4.60 | 8.59 | 2.26 | 9.79 | 3.1 | 6.6 |
| 3 | 15 | BR | | | + | 17617 | 16473 | 170 | 1.58 | 6.80 | 0.78 | 11.7 | 5.2 | 6.5 |
| 3 | 16 | BR | | | + | 17617 | 18288 | 149 | 1.56 | 6.31 | 0.84 | 11.6 | 2.1 | 9.5 |
| 3 | 17 | TR | R | SBM | + | 18025 | 20891 | 348 | 3.47 | 8.42 | 1.86 | 6.21 | 0.7 | 5.5 |

| Round | Pig-No. | Compound feed | | | | Energy value | | | Phosphorus | | | Calcium | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Feed | Faeces | Urine | Intake | Faeces | Digestible P | Intake | Faeces | Digestible Ca |
| | | | CER | PM | Phyt | kJ/kg DM | kJ/kg DM | kJ/kg | g/day | g/kg DM | g/kg DM | g/day | g/kg DM | g/kg DM |
| 3 | 18 | TR | R | SBM | + | 18025 | 20437 | 258 | 3.69 | 6.67 | 2.11 | 6.62 | 1.0 | 5.6 |
| 3 | 19 | TR | W | RSM | + | 17927 | 19765 | 259 | 3.94 | 7.70 | 2.28 | 9.44 | 1.9 | 7.6 |
| 3 | 20 | TR | W | RSM | + | 17927 | 19177 | 251 | 3.82 | 7.43 | 2.42 | 9.13 | 2.0 | 7.2 |
| 3 | 21 | BR | | | + | 17617 | 16243 | 204 | 1.83 | 6.39 | 0.81 | 13.6 | 5.9 | 7.6 |
| 3 | 22 | BR | | | + | 17617 | 16923 | 159 | 1.41 | 7.77 | 0.45 | 10.5 | 4.9 | 5.5 |
| 3 | 23 | TR | W | SBM | + | 18094 | 20559 | 324 | 3.43 | 7.45 | 2.08 | 7.16 | 0.8 | 6.4 |
| 3 | 24 | TR | W | SBM | + | 18094 | 20183 | 291 | 3.64 | 7.52 | 2.17 | 7.58 | 1.0 | 6.6 |

CER = cereal grain; PM = protein meal; Phyt = phytase; DM = dry matter; BR = basal ration; TR = test ration; W = wheat; R = rye; SBM = soybean meal; RSM = rapeseed meal; FM = fresh matter

**Table 3:** Neutral detergent insoluble and acid detergent insoluble nitrogen intake and faecal excretion and metabolic faecal nitrogen [g/d] by group fed test ration presented as least square means

| | **Basal ration** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Cereal grain** | | | | | | | | | | | | |
| | Wheat | | | | Rye | | | | | | | | |
| | **Phytase** | | | | | | | | | p-value | | | |
| | + | | - | | + | | - | | | | | | |
| | **Protein meal** | | | | | | | | | | | | |
| | SBM | RSM | SBM | RSM | SBM | RSM | SBM | RSM | SEM | CER | Phyt | PM | Rd |
| Intake | | | | | | | | | | | | | |
| NDIN | 15.0 | 11.3 | 13.9 | 13.0 | 13.5 | 15.0 | 13.1 | 12.5 | 0.125 | 0.52 | 0.10 | < 0.05 | < 0.05 |
| ADIN | 0.502 | 1.26 | 0.400 | 1.17 | 0.541 | 1.36 | 0.800 | 1.23 | 0.025 | < 0.05 | 0.51 | < 0.05 | < 0.05 |
| Faeces | | | | | | | | | | | | | |
| NDIN | 0.393 | 0.517 | 0.351 | 0.620 | 0.492 | 0.642 | 0.457 | 0.503 | 0.0397 | 0.12 | 0.40 | < 0.05 | 0.52 |
| ADIN | 0.558 | 0.512 | 0.525 | 0.682 | 0.480 | 0.575 | 0.542 | 0.704 | 0.0424 | 0.85 | < 0.05 | < 0.05 | < 0.05 |
| mfN | 3.58 | 4.33 | 3.40 | 4.53 | 4.49 | 5.24 | 5.18 | 6.10 | 0.306-0.319 | < 0.05 | 0.19 | < 0.05 | < 0.05 |

Significance level was $p < 0.05$. NDIN = neutral detergent insoluble nitrogen; ADIN = acid detergent insoluble nitrogen; mfN = metabolic faecal nitrogen; CER = cereal grain; Phyt = phytase supplementation; PM = protein meal; + = with phytase supplementation; - = without phytase supplementation; SBM = soybean meal; RSM = rapeseed meal; SEM = standard error of the means; Rd = round.
SEM is stated as a range due to different n for test rations (n = 6), when a correction for outliers was made if the whole data set was not normally distributed.

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